

Health Effects of Endocrine-Disrupting Chemicals on Wildlife, with Special Reference to the European Situation*

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ABSTRACT: Many wildlife species may be exposed to biologically active concentrations of endocrine-disrupting chemicals. There is strong evidence obtained from laboratory studies showing the potential of several environmental chemicals to cause endocrine disruption at environmentally realistic exposure levels. In wildlife populations, *associations* have been reported between reproductive and developmental effects and endocrine-disrupting chemicals. In the aquatic environment, effects have been observed in mammals, birds, reptiles, fish, and mollusks from Europe, North America, and other areas. The observed abnormalities vary from subtle changes to permanent alterations, including disturbed sex differentiation with feminized or masculinized sex organs, changed sexual behavior, and altered immune function. For most reported effects in wildlife, however, the evidence for a *causal* link with endocrine disruption is weak or nonexistent. Crucial in establishing causal evidence for chemical-induced wildlife effects appeared semifield or laboratory studies using the wildlife species of concern. Impaired reproduction and development *causally* linked to endocrine-disrupting chemicals are well documented in a number of species and have resulted in local or regional population changes. These include:

- Masculinization (imposex) in female marine snails by tributyltin, a biocide used in antifouling paints, is probably the clearest case of endocrine disruption caused by an environmental chemical. The dogwhelk is particularly sensitive, and imposex has resulted in decline or extinction of local populations worldwide, including coastal areas all over Europe and the open North Sea.
- DDE-induced egg-shell thinning in birds has caused severe population declines in a number of raptor species in Europe and North America.
- Endocrine-disrupting chemicals have adversely affected a variety of fish species. In the vicinity of certain sources (e.g., effluents of water treatment plants) and in the most contaminated areas is this exposure causally linked with the effects on reproductive organs that could have implications for fish populations. However, there is also a more widespread occurrence of endocrine disruption in fish in the U.K., where estrogenic effects have been demonstrated in freshwater systems, in estuaries, and in coastal areas.
- In mammals, the best evidence comes from the field studies on Baltic gray and ringed seals, and from the Dutch semifield studies on harbor seals, where both reproduction and immune functions have been impaired by PCBs in the food chain. Reproduction effects resulted in population declines, whereas impaired immune function has likely contributed to the mass mortalities due to morbillivirus infections.
- Distorted sex organ development and function in alligators has been related to a major pesticide spill into a lake in Florida, U.S.A. The observed estrogenic/antiandrogenic effects in this reptile have been causally linked in experimental studies with alligator eggs to the DDT complex.

Although most observed effects currently reported concern heavily polluted areas, endocrine disruption is a potential global problem. This is exemplified by the widespread occurrence of imposex in marine snails and the recent findings of high levels of persistent potential endocrine-disrupting chemicals in several marine mammalian species inhabiting oceanic waters.

KEY WORDS: endocrine modulator, estrogenic effects, reproduction effects, immunotoxic effects, marine mammals, reptiles, fish, birds, invertebrates, chlorinated compounds, PCBs, PCDFs, PCDDs, DDT, TBT.

I. INTRODUCTION

A. Background

There is growing concern about the possible harmful consequences of exposure to xenobiotic compounds that are capable of modulating or disrupting the endocrine system. This concern for endocrine-disrupting chemicals (EDCs) is directed at both wildlife and humans (Colborn et al., 1993; Sharpe and Skakkebaek, 1993). Several expert working groups (Harrison et al., 1995; Toppari et al., 1995; Weybridge, 1996; Ankley et al., 1998; Crisp et al., 1998; Olsson et al., 1998) have concluded that there is increasing evidence of adverse effects in human and wildlife reproductive health and have discussed the hypothesis that chemicals in the environment have caused these endocrine-mediated adverse effects. This has led a series of stakeholders to consider the topic of endocrine disruption as of sufficient interest/concern to justify action.

Among the stakeholders mentioned are the European Commission, the European Parliament, the US Environmental Protection Agency, OECD, the IPCS, the Commission of the OSPAR Convention, the European Environment Agency, NGOs, and the Chemical Industry. The European Commission took this advice seriously and allowed for the setting up of a Working Group on Endocrine Disrupters under the Scientific Committee on Toxicity, Ecotoxicity, and the Environment (CSTEE) of DG XXIV Consumer Policy and Consumer Health Protection. The working group concluded that, in terms of endocrine-disrupting effects, the main reasons for concern have to do with the effects on wildlife and the environment. This article is a review of the existing literature and scientific opinion on the evidence for chemically induced endocrine disruption, in particular as it relates to European wildlife. In addition, gaps in knowledge are identified and recommendations for future research are made.

The CSTEE working group agreed on the definition of the IPCS Steering Group that met at the joint IPCS/OECD Scoping Meeting on Endocrine Disrupters, March 16 to 18, 1998 in Washington, DC:

An *endocrine disrupter* is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations.

A *potential endocrine disrupter* is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations.

Alteration of endocrine function caused by an endocrine disrupter may be through interference with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior. Industrial chemicals, consumer compounds and chemicals in the environment can be endocrine disrupters that mimic, enhance (an agonist), or inhibit (an antagonist) the action of hormones. Dose, body burden, timing, frequency, and duration of exposure at critical periods of life are important considerations for assessing adverse effects of an endocrine disrupter.

B. Mechanisms of Endocrine Disruption

The biological actions of hormones synthesized within an organism, such as estrogen, progesterone, testosterone, and thyroxin are mediated by high-affinity receptor proteins located within target cells. The interaction of a hormone with its receptor initiates a cascade of events that lead to myriad effects associated with the particular hormone. Also, exogenous chemicals may bind to a

receptor, for example, the estrogen receptor and mimic or block the actions of its natural hormone. Such compounds include some natural products such as coumestrol and genistein, pharmaceuticals such as diethylstilbestrol, 17 α -ethinylestradiol and tamoxifen, and industrial chemicals such as DDT, bisphenol A, and nonylphenol (ECETOC, 1996; Schäfer et al., 1996; DFG, 1996; Weybridge, 1996). Compounds that have been shown to alter estrogen biosynthesis are cyanoketone, ketoconazole, and the fungicide fenarimol (Hirsch et al., 1987). Methoxychlor, chlordecone (kepone), DDT, some PCBs and alkylphenols can disrupt estrogen receptor function (White et al., 1994; Mueller and Kim, 1978). Metabolites of the fungicide vinclozolin and the DDT metabolite *p,p'*-DDE have been found to bind to the androgen receptor and block testosterone-induced cellular responses *in vitro*. *o,p'*-DDT and chlordecone can inhibit ligand binding to the estrogen and progesterone receptors (Laws et al., 1995). Nonylphenol inhibits binding to the estrogen, progesterone, and androgen receptors (Schäfer et al., 1996). Environmental agents have also been shown to affect the thyroid. For example, hydroxylated metabolites of PCBs, as competitive inhibitors of thyroxine binding to transthyretin, can alter thyroid hormone levels and interfere with hormone transport.

The steroidal sex hormones and their receptors are phylogenetically old and may be found in a range of vertebrate and invertebrate species. Their regulatory role in developmental processes such as sex determination and differentiation is of particular interest with respect to endocrine disruption. In mammals, the differentiation of the conceptus into a male phenotype is regulated by testicular hormones, including testosterone. Consequently, the androgen receptor antagonists *p,p'*-DDE and vinclozolin may demasculinize male rat pups following exposure of the pregnant dam (Kelce et al., 1995, 1997). In birds, the differentiation into a female phenotype is regulat-

ed by estrogen. Consequently, injection of an estrogen synthesis inhibitor into the fertilized egg may result in phenotypically sex-reversed females with bilateral testicles, sperm production, and a male copulatory behavior (Elbrecht and Smith, 1992). Feminized male birds with an ovotestis (a gonad containing both ovarian and testicular tissue) and/or reduced male copulatory behavior have been induced by *o,p'*-DDT and 17 α -ethinylestradiol (Adkins, 1975; Schumacher et al., 1989; Berg et al., 1998).

The genetic sex in mammals and birds is defined by genes located on the sex chromosomes. In lower vertebrates and invertebrates, the mechanisms of sex determination and differentiation are less characterized but may be more flexible than in mammals and birds and influenced by environmental factors such as temperature and xenogenous substances with hormonal activity. Experimentally disturbed sex differentiation has also been reported in reptiles, amphibia, fish, and invertebrates such as marine gastropods. There is evidence that malformed sex organs occur in wild populations of birds, reptiles, fish, and marine gastropods (see next sections). The mechanisms of pollutant-induced reproductive toxicity observed in wild mammalian species generally remain unclear but could also involve endocrine disruption.

The present literature overview assesses the currently available evidence on adverse health effects in wildlife populations by exposure to environmental contaminants that manifest effects that may be modulated through the endocrine system. Reports of field and semi-field studies dealing with (suspected) endocrine-mediated abnormalities and ecological well-being in both terrestrial and aquatic (fish eating) populations of mammalian, avian, reptilian, fish, and invertebrate species are tabulated and discussed. A distinction is made between reproductive and nonreproductive disturbances and/or effects. Particular emphasis in the overview is placed on recent reports from European countries.

II. MAMMALS

A. Reproductive Effects

Effects on reproduction in mammals, in particular reported for the aquatic environment, are summarized in Table 1a.

Perhaps the best evidence for cause-effect relationships between organochlorine compounds and reproductive toxicity in a marine mammal comes from the field studies on Baltic gray and ringed seals, and from the Dutch semifield studies on harbor seals, where both reproduction and immune function have been impaired by PCBs in the food chain. Reproduction effects resulted in population declines, whereas impairment of immune functions have likely contributed to the mass mortalities due to morbillivirus infections.

1. *Baltic Seal Disease Syndrome*

A great deal of attention has been given to a disease syndrome in Baltic gray and ringed seals that is firmly linked to high body burdens of PCBs, DDT, and their persistent methylsulfone metabolites (Bergman and Olsson, 1985; Bergman et al., 1994; Jensen and Jansson, 1976; Haraguchi et al., 1992). An investigation, initiated because of a serious reduction of the gray and ringed seal populations, revealed that uterine stenosis and occlusions (Figure 1) had occurred in 30% of the adult gray seals and in 70% of the ringed seals subjected to autopsy (Bergman and Olsson, 1985; Helle et al., 1976a,b; Helle, 1980). In addition, uterine smooth muscle tumors (leiomyomas) were common findings in older gray seals but not in ringed seals (Bergman and Olsson, 1985). Along with decreasing body burdens of organochlorines, the reproduction of Baltic seals has gradually improved during the last 2 decades (Bergman, 1999). In addition to reproductive disorders and sterility, a suite of pathological lesions have been reported, including severe adrenocortical hyperpla-

sia (Figure 2), osteoporosis, intestinal ulcers, and a decreased epidermal thickness in both sexes (Bergman and Olsson, 1985; Bergman et al., 1992; Bergman, 1999; Mortensen et al., 1992; Olsson et al., 1994). Hyperadrenocorticism (Cushing's disease) has been proposed as one major cause of the disease syndrome in the Baltic seal populations (Bergman and Olsson, 1985). The prevalence of some of these lesions has decreased during the last 2 decades, especially lesions related to reproduction. Notably, however, the incidence of intestinal ulcers seems to be increasing (Bergman, 1999).

Despite a strong correlation to persistent organochlorine exposure, the underlying mode of action and the individual PCB and DDT compounds responsible for these toxic effects in Baltic seals are still incompletely understood. Given the diverse pattern of pathological lesions observed and the large number of organochlorines and metabolites identified in the seals, it is conceivable that the disease syndrome is caused by several classes of persistent organohalogenes and several independent mechanisms of action. As there is often a co-variation in the exposure to the different organochlorines, the eco-epidemiological study approach will generally not allow conclusions about the etiological role of individual chemicals. Eco-epidemiology may, however, direct attention to groups of chemicals. Recent studies thus have concluded that high concentrations of DDT compounds were present in reproducing females in the past, whereas females with high PCB concentrations failed to reproduce (Roos et al., 1998). These results support an etiological role of PCB for parts of the disease syndrome, particularly for lesions connected to the reproductive failure among the seals.

Several of the observed pathological lesions are compatible with an increased glucocorticoid production in the seals. Osteoporosis, decreased epidermal thickness, and immunosuppression could result from an increased hormone synthesis in the hyperplastic adrenal

TABLE 1a
Reproductive Effects in Terrestrial and Aquatic (Fish-Eating) Mammals

Effect/disorder	Location	Species	Associated contaminants	Ref.
Cryptorchidism	Florida	Panther (<i>Felis concolor coryi</i>)	Mercury, <i>p,p'</i> -DDE and PCBs	Facemire et al., 1995
Masculinization	Alberta (Canada)	Black and brown bear	Unknown	Cattet, 1988
Masculinization	Spitsbergen	Polar bear	PCBs	Wiig et al., 1998
Reproductive impairment	Nordic countries	European otter (<i>Lutra lutra</i>)	PCBs	Leonards, 1997
Reproductive effects	Great Lakes, U.S.	Mink (<i>Mustela vison</i>) Otter (<i>Lutra canadensis</i>)	TCDD and related compounds	Wren, 1991
Premature pupping	California, USA	Sea lions (<i>Zalophus californias</i>)	DDT-like compounds	Delong et al., 1973
Decreased fecundity, implantation failure Sterility	Wadden Sea, NL Baltic Sea	Harbor seals Ringed seals (<i>Phoca hispida</i>)	PCBs and metabolites PCBs	Reijnders, 1986 Helle, 1980
Decreased population size	Lake Saimaa, Finland	Saimaa ringed seal (<i>Phoca hispida saimensis</i>)	Mercury	Hyvärinen et al., 1998
Decreased fecundity, hermaphroditism	St Lawrence Bay, Canada	Beluga whales (<i>Delphinapterus leucas</i>)	PCBs, dieldrin, 2,3,7,8,-TCDD	Martineau et al., 1988; De Guise et al., 1994a
Reduced testosterone levels	NW Pacific Ocean	Dall's porpoises (<i>Phocoenoides dalli</i>)	PCBs and DDE	Subramanian et al., 1987



FIGURE 1. Uterus from a sterile gray seal showing lesions resulting from a disrupted pregnancy. Arrows depict sites of strictures and occlusions in the uterine horns, originating from aborted early embryos. (Photo courtesy of M. Olsson.)



FIGURE 2. Transsected adrenal glands from a Baltic gray seal (right) and a gray seal from Svalbard (left) showing massive hyperplasia of the adrenal cortex in the Baltic seal. (Photo courtesy of Swedish Veterinary Institute.)

cortex. Notably, however, no information on the functional activity of the adrenal cortex in affected animals has been provided. In conflict with the concept of hyperadrenocorticism, recent experimental data rather suggest that several pollutants in the affected seals are capable of disrupting both glucocorticosteroid hormone synthesis and receptor-mediated action. Several distinct mechanisms of action have been described, that could result in a compensatory hyperplasia of the adrenal cortex. Along with a number of methylsulfonyl-PCBs (MeSO₂-PCBs), the persistent DDT metabo-

lite MeSO₂-DDE was originally identified in the blubber of the Baltic gray seal (Jensen and Jansson, 1976). Studying the biological properties of this novel class of metabolites, it was found that MeSO₂-DDE is a highly potent adrenal toxicant following a site-specific cytochrome P450-catalyzed irreversible binding in the glucocorticoid-producing *zona fasciculata* in mice. This observation suggested a role for MeSO₂-DDE in the etiology of adrenocortical hyperplasia in Baltic seals (Lund et al., 1988; Jönsson et al., 1991; 1992; Brandt et al., 1992). A comparative *in vitro* study with

adrenal tissue of gray seal, mink, and otter showed significant cytochrome P450-catalyzed irreversible binding of MeSO₂-DDE in seal adrenal tissue, while no binding of this compound occurred in mink and otter adrenal tissue (Jönsson et al., 1993; Lund, 1994). Another DDT metabolite, the classic adrenocortolytic agent DDD, showed a marked P450-catalyzed binding in the adrenal cortex of seal, mink and otter. Consequently, DDD could also contribute to adrenal dysfunction, resulting in compensatory hyperplasia of the adrenal cortex (Jönsson et al., 1994; Lund, 1994). In addition, MeSO₂-DDE and certain MeSO₂-PCBs are potent inhibitors of CYP11B1 and corticosterone synthesis in mouse adrenocortical Y1 cells (Johansson et al., 1998a). Finally, as reported recently by the same group, certain MeSO₂-PCBs may also act as glucocorticoid receptor antagonists in CHO cells expressing a human glucocorticoid receptor-responsive element (Johansson et al., 1998b). This novel finding supports a complex etiology of disturbed glucocorticoid hormone homeostasis in Baltic seals, which could also involve a component of physiological stress.

2. Semifield Reproduction Study in Seal

In this study with harbor or common seals (*Phoca vitulina*) (Figure 3), two groups, each consisting of 12 female seals, were fed for 2 years with either a diet of flatfish derived from the Wadden Sea or a diet of mackerel from the open Atlantic. In this prospective study, female harbor seals fed fish from the polluted Wadden Sea displayed a lower reproductive success than seals fed less-contaminated fish from the Atlantic Ocean (Reijnders, 1986). In the same study, the Wadden Sea group seals also had lower concentrations of 17 β -estradiol (Reijnders, 1986). Two possible explanations for the low reproductive success are

given by Reijnders (1996). First, an increased breakdown of steroid hormones and consequently lower plasma concentrations as a consequence of cytochrome P450 (CYP1A) enzyme induction by PCBs/PCB metabolites. This process has been shown in various other studies (Tanabe et al., 1988; Boon et al., 1992). However, because Froixi and Mason found a negative correlation between PCB concentrations and hormone metabolism, this mechanism seems to be a less likely explanation (Reijnders, 1999). Second, interference by PCB or DDE and their metabolites with receptors in target tissues could be involved. PCB-methylsulfones bind with high affinity to a uteroglobin-like protein in the murine and human airways (Brandt et al., 1995; Lund et al., 1995). Uteroglobin is a steroid-binding protein that was originally identified in secretions from the uterus and uterine tubes and probably plays a regulatory role in the reproductive tract. Considering the above reports on uterine strictures and occlusions in Baltic seals, it was of particular interest that phenolic and methylsulfone-containing PCB metabolites are selectively enriched in the uterine luminal fluid of pregnant mice (Brandt et al., 1982).

Seals from the group with the highest PCB uptake had reduced the levels of retinol (vitamin A) and thyroid hormones (Brouwer et al., 1998), both biomarkers generally associated with exposure to PCBs and related compounds (Peakall, 1992). Competition between thyroid hormones and retinol and certain PCB congeners (such as PCB-77) for binding to a transport protein was presumably responsible for the reduced plasma levels (Brouwer et al., 1989). It is known that reduced levels of retinol and thyroid hormones may be of biological significance. Thyroid hormones play an important role in the development of the brain during the early developmental period, and an indirect role in the maturation of the testicular Sertoli and Leydig cells, which are important in spermatogenesis. Thyroid hor-

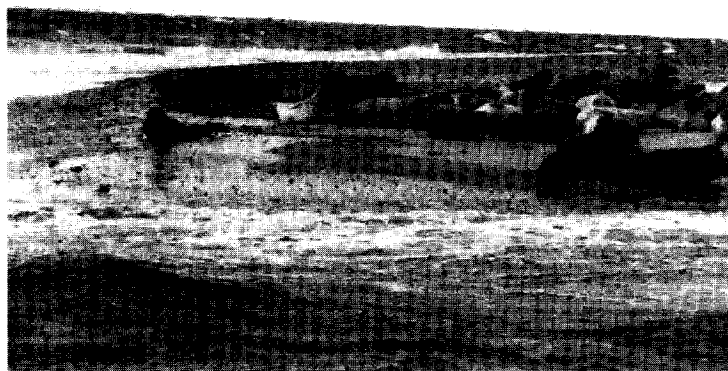


FIGURE 3. Harbor and gray seals at Blakeney Point, Norfolk, United Kingdom.

mones are known to be important in cell differentiation, while vitamin A deficiency may lead to delayed growth and reduced reproductive success. Based on the presence of hydroxylated PCB metabolites in seal blood (Bergman et al., 1994) and the strong accumulation of such metabolites in the feto-placental unit in pregnant mice (Brandt et al., 1982; Darnerud et al., 1986), it has been postulated that fetal accumulation of PCB metabolites occurs also in pregnant seals (Murk et al., 1994b; Brouwer et al., 1998).

The reproductive effects observed in the Baltic seals and the harbor seals exposed to Wadden sea flatfish for 2 years were fairly different. Particularly striking were the reduced fertility, uterine occlusions resulting from abortions, uterine smooth muscle tumors, and a suite of pathological changes, including adrenocortical hyperplasia and osteoporosis that characterized the disease syndrome in the Baltic seal populations. In the two different semifield studies performed with harbor seals, decreased fertility, reduced levels of 17β -estradiol, retinol, and thyroid hormones were found. The differences observed between the field study in the Baltic and the Dutch semi-

field studies may be explained by the different species (gray, ringed, and harbor seals), the different exposure scenarios, as well as the duration of exposure (lifetime in the Baltic, 2 years in the Dutch studies). Also, the study design and the different biological endpoints examined may explain the different effects observed.

Cryptorchidism (90% of the male population), high prevalence of sperm abnormalities, and occasional cases of sterility have been reported in the endangered Florida panther (*Felis concolor coryi*) (Facemire et al., 1995). The involvement of endocrine-disrupting chemicals in the early developmental stages as a contributing cause for reproductive impairment is suspected, but further studies are required to elucidate the observed reproductive failure and disorders in this population of animals (see EPA, 1997).

Masculinization (pseudohermaphroditism) is reported from local populations of black and brown bears in Alberta, Canada (Cattet, 1988). Recently, a parallel case was reported in polar bears in Europe (Spitsbergen): two female yearlings showed a normal vaginal opening and a 20-mm penis containing a baculum.

As neither of the yearlings showed Y chromosomes, they were considered female pseudohermaphrodites (Wiig et al., 1998). Polar bears at Svalbard are highly polluted with organochlorines, particularly PCBs (Bernhoft et al., 1997). The authors emphasize that the mechanisms involved are unclear but suggest that the pseudohermaphrodites may be due to endocrine disruption from environmental pollutants.

In top predators such as the mustelids, the European otter (*Lutra lutra*) and mink (*Mustela vison*) high concentrations of PCBs (ppm range) have been reported (Keymer et al., 1988; Giesy et al., 1994a; Leonards, 1997; Wren, 1991). Non-ortho-substituted PCBs are generally held responsible for adverse reproductive health effects observed in these species (Aulerich et al., 1985; Leonards, 1997). In Scandinavia, as well as in many other European countries, the otter populations were found to decline after the 1950s. Early on PCB was proposed to be responsible for this population decline (Sandegren et al., 1980). The markedly decreased environmental concentrations of PCB recorded in Sweden in the last 2 decades consequently has been followed by a recovery of the otter populations all over the country. This improvement has been most pronounced in the remote, less-contaminated parts of Sweden (Roos et al., submitted).

Today thriving otter populations are found only at the boundaries of Europe where the lowest concentrations of PCBs are present (Leonards, 1997). There is, however, one exception, the Shetland Islands, where relatively high concentrations of PCBs are found in a thriving otter population (Kruuk and Conroy, 1996). A literature review of the presence of PCBs in otters (Smit et al., 1994) concluded that it was neither possible to dismiss the role of PCBs in the decline of otter populations nor to assume the role as completely proven. This was mainly due to the fact that reported concentrations of PCBs in otters are difficult to compare because of the differences in analytical methods.

Several experimental studies have revealed a high reproductive toxicity of PCB

(but not of DDT) in mink (Aulerich et al., 1985; Jensen et al., 1977; Kihlström et al., 1992). Mink exposed to a technical PCB preparation (Clophen A50) for 18 months showed a dose-dependent decrease in the number of whelps born with virtually no reproductive capacity remaining in females exposed to 0.3 mg PCB/day (Brunström et al., 1994). Based on a sensitive EROD bioassay, the highest amounts of 'dioxin-like' compounds (Bio-TEQs, 2,3,7,8-TCDD toxic equivalents) found in Swedish otters were higher than those in the Clophen A50-exposed. Assuming a similar reproductive toxicity of PCB in otter as in mink, these results clearly support the conclusion that PCB exposure may be involved in the decline of European otter populations.

Toxicity data and levels of PCBs observed in wildlife populations of mink (*Mustela vison*) and otters (*L. canadensis*) in the Great Lakes Basin (U.S.) were reviewed by Wren (1991). He concluded that there was little doubt that contaminants had effects on mink and otters from the Great Lakes Basin, but that available data were insufficient to provide final proof of cause-effect linkages between chemical compounds and population status. Data on contaminants in the diet of mink and otter were not available.

A large number of xenobiotics with potential endocrine-disrupting effects have been reported in tissues of many marine mammals (Colborn and Smolen, 1996; Reijnders, 1996; Wagemann and Muir, 1984). In general, these species are related to coastal zones. However, more recently it has been shown that tissue of more oceanic species such as sperm whales (*Physeter macrocephalus*) and minke whale (*Balaena acutorostrata*) show considerable concentrations of these compounds, including toxaphene and polybrominated diphenylethers (PBDE) (De Boer et al., 1998). Reproductive and developmental disorders in these mammals have been associated with certain organochlorine contaminants (mainly DDT metabolites and PCBs, and their metabolites) in California sea lions (*Zalophus californias*) (Delong et

al., 1973; Addison, 1989), ringed seals (*Phoca hispida*) and gray seals (*Halichoerus grypus*) in the Baltic Sea (Helle, 1980; Bergman and Olsson, 1985), harbor seals (*Phoca vitulina*) in the Wadden Sea (Reijnders, 1986), and beluga whales (*Delphinapterus leucas*) from the polluted Gulf of St. Lawrence (Beland et al., 1987; Martineau et al., 1988) (see Table 1a). In most of the above cases, however, it was not possible to confirm a cause-and-effect relationship between a specific chemical and effects found (Reijnders and Brasseur, 1992; Colborn and Smolen, 1996; O'Shea and Brownell, 1998). In all of the above cases, the etiology of the observed effects therefore remains unsolved (Reijnders and De Ruiter-Dijkman, 1995).

Decreased testosterone levels in the blood have been associated with high concentrations of PCBs and DDE in the blubber of Dall's porpoises (*Phocoenoides dalli*) from the Northwest Pacific, but the mechanism for this decrease remains unknown (Subramanian et al., 1987). It can be speculated that PCB-related enzyme induction, as proposed for seals in the Wadden Sea, may have played a role.

High levels of organochlorines found in beluga whales from the polluted Gulf of St. Lawrence in Canada have been associated with the low reproductive rate of the population (Martineau et al., 1988). In addition, a single unique case of true hermaphroditism (presence of ovaries and testes) was described in one out of nine investigated beluga whales from this area (De Guise et al., 1994a). However, based on the available literature and most recent findings, the evidence for a primary effect of contaminants leading to the observed effects in St. Lawrence belugas has been considered largely circumstantial and should be treated with caution (ICES, 1998).

In a very recent report of the Saimaa ringed seal (*Phoca hispida saimensis*) in Lake Saimaa, Finland, it has been suggested that increased mercury levels in the seal pups, possibly due to selenium shortage, may have contributed to the decrease in density of this population

in the 1960s and 1970s (Hyvärinen et al., 1998).

B. Nonreproductive Effects

Besides poor reproductive performance, also mass mortalities by infectious diseases, immunosuppression, thyroid abnormalities, and other nonreproductive disorders have been associated with the presence of organochlorine compounds and other potential endocrine-disrupting chemicals in the body fat of mammals (Table 1b).

The role of chemical-induced immunosuppression in the mass mortality in 1988 in harbor seals due to morbillivirus infections has been investigated in a second semifield study by Dutch research groups. In this long-term feeding study it is shown that ambient levels of environmental contaminants, notably PCBs, are immunotoxic to harbor seals. It is concluded that the morbillivirus infection in harbor seals was exacerbated by these contaminants.

1. Semifield Immune Function Study in Seal

The mass mortality of harbor seals in northwestern Europe in 1988 resulting from infection with a previously unidentified morbillivirus (Osterhaus and Vedder, 1988), being subsequently characterized and named phocid distemper virus 1 (PDV-1), led to speculation about the possible contributing role of environmental contaminant-induced immunosuppression. A similar epizootic among Baikal seals (*Phoca sibirica*) in Siberia in 1987 was later attributed to infection with canine distemper virus (Grachev et al., 1989; Osterhaus et al., 1989). In order to investigate the role of immunotoxic environmental pollutants, including the PCBs, PCDFs, and PCDDs (Vos and Luster, 1989; Vos et al., 1991), a semifield study was carried in which two groups of 11 harbor seals were fed herring (*Clupea*

TABLE 1b
Nonreproductive Effects in Terrestrial and Aquatic (Fish-Eating) Mammals

Effect/disorder	Location	Species	Associated contaminants	Ref.
Skull lesions	Baltic Sea; German, Wadden Sea	Harbor seals (<i>Phoca vitulina</i>)	PCBs DDT/DDE	Mortensen et al., 1992; Stede and Stede, 1990
Uterine stenosis, occlusions	Baltic Sea	Ringed seals (<i>Phoca hispida</i>)	DDE-/PCB-methylsulfones	Helle et al., 1976b; Olsson et al., 1994;
Increased rates of lesions and infections, lowered levels of hepatic retinol or retinylpalmitate	Denmark	European otter (<i>Lutra lutra</i>)	PCBs	Bergman, 1999 Leonards et al., 1996
Lowered levels of vitamin A and thyroid hormones	Netherlands, Wadden Sea	Harbor seals (<i>Phoca vitulina</i>)	PCBs	Brouwer et al., 1989
Skull-bone lesions (osteoporosis)	Baltic Sea	Gray seals (<i>Halichoerus grypus</i>)	PCBs, DDT/DDE	Bergman et al., 1992
Adrenocortical hyperplasia	Baltic Sea	Ringed seals (<i>Phoca hispida</i>) Gray seals (<i>Halichoerus grypus</i>)	PCBs	Bergman and Olsson, 1985; Olsson et al., 1994
Lowered immuno- competence	Netherlands, Wadden Sea	Harbor seals (<i>Phoca vitulina</i>)	TCDD-like	Ross et al., 1995; De Swart et al., 1994
Mass mortalities	U.S., Atlantic Ocean, Mediterranean Sea	Various dolphin species	<i>o,p'</i> -DDE, <i>p,p'</i> -DDE, <i>o,p'</i> -DDT, PCBs	Lahvis et al., 1995; Geraci et al., 1987; Aguilar and Borrell, 1994
Mass mortality	Mediterranean Sea	Striped dolphins (<i>Stenella coeruleoalba</i>)	TBT?	Scott et al., 1988
Pathological disorders	Canada, Gulf of St. Lawrence	Beluga whales (<i>Delphinapterus leucas</i>)	Organochlorines	De Guise et al., 1994b

harengus) from either the highly contaminated Baltic Sea or the relatively unpolluted Atlantic Ocean. A variety of parameters related to immune function were monitored and compared in the two groups over a period of 2 years. Natural killer-cell activity and T-cell mitogen (but not B-cell)-induced proliferative responses in peripheral blood were found to be significantly lower in the seals fed contaminated fish (De Swart et al., 1994). While the results of these nonspecific *in vitro* tests of immune function indicated that contaminants in the Baltic herring were immunotoxic, further evidence was provided when impaired mixed lymphocyte reactions and antigen-specific lymphocyte proliferative responses were observed in the Baltic group (De Swart et al., 1995). In addition, *in vivo* antibody responses and delayed-type hypersensitivity skin reactions were significantly depressed following immunization with ovalbumin, both being T-cell-dependent immune responses (Ross et al., 1995, 1996a). The observed immunosuppression clearly indicates that in harbor seals the resistance to a virus infection is impaired as NK cells and T cells play a major role in defense against viral infections. The impaired immune responses in seals fed the Baltic Sea herring are consistent with the effects observed in studies of laboratory animals exposed to Ah-receptor binding PCBs, PCDFs, and PCDDs (Vos and Luster, 1989; Vos et al., 1991; Vos et al., 1997/98). An additional indication that PCB-like compounds led to a toxic effect in the seals of the Baltic group was the observed decline in vitamin A levels (De Swart et al., 1994). The estimated daily intakes of 2,3,7,8-TCDD toxic equivalents (TEQs) by the Baltic group of seals were 10 times higher than those of the Atlantic group of seals and led to a blubber concentration of 286 ± 17 ng TEQ/kg lipid (mainly derived from mono-ortho PCB) in the Baltic seals compared to 90 ± 6 ng TEQ/kg lipid in the Atlantic seals (Ross et al., 1995). From the comparison of these results and literature data it appears that many free-ranging harbor seal populations

inhabiting polluted areas of Europe and North America have Σ PCB or TEQ blubber concentrations being at or above those observed in the Baltic group of seals of the semifield study (Ross et al., 1996b). In addition to its contribution to the severity and extent of the 1988 morbillivirus outbreak, chemical-induced immunosuppression therefore may affect the immunocompetence of free-ranging populations in many areas of the industrialized world. This is the more true as free-ranging seals are likely to be more vulnerable than the seals of the semifield study, as perinatal TCDD exposure produces more profound immunosuppression, at least in laboratory rodents (Vos and Luster, 1989), and the lifespan of seals allows for long-lasting accumulation (Ross et al., 1996b). Finally, it is likely that hormone disruption is involved in the observed immunosuppression, as TCDD causes thymic atrophy that is linked to an adverse effect on the epithelial cells of the thymus, which cells support thymocyte proliferation and maturation by cell-cell contact and secretion of thymic hormones (De Waal et al., 1997).

The health status of Danish otters (*Lutra lutra*) was investigated in relation to the concentration of PCBs on a TEQ basis (Leonards et al., 1996). The concentration range showed an association with increased rates of various skin and internal lesions, including infections and correlated well with decreased levels of hepatic retinol or retinylpalmitate found in the otter. These results indicate that the concentrations of PCBs found in European otters could be associated with adverse health effects in the individual animal. Any consequences at the population level are still unknown.

Two experimental studies have provided evidence for immune modulation from dietary exposure to bleached-kraft pulp mill effluent (BKME) in mink. Mink exposed for 26 weeks to dietary BKME responded with a significantly greater antibody response following vaccination with mycobacterium than did control mink (Smits et al., 1996). By con-

trast, their cell-mediated immune response was suppressed (Smits et al., 1996).

High levels of organochlorines have been noted in dolphins that died in several mass mortality incidents. Aguilar and Borrell (1994) compared PCB levels in striped dolphins (*Stenella coeruleoalba*) that died during the Mediterranean morbillivirus epizootic in 1990 with biopsies taken from live dolphins in 1987 to 1989. These authors suggested that PCBs left the blubber during mobilization of fat reserves and large quantities reached the liver shortly before the epizootic. This may have increased the dolphins' susceptibility to the morbillivirus.

Mass mortality of bottlenose dolphins (*Tursiops truncatus*) along the east coast of America in 1987 to 1988 has been associated with consumption of fish poisoned with brevetoxin — a naturally occurring algal toxin (Geraci et al., 1987). However, various other authors have disputed this conclusion and suggested that the high concentration of organochlorines found in the carcasses were a major factor in the mortality (Lahvis et al., 1995). The dolphins also showed a number of symptoms indicative of immune system dysfunction (skin and organ lesions, most of them believed to be caused by opportunistic infections by bacteria, viruses, and fungi) that were positively correlated with various organochlorine compounds, including DDT and PCBs (Lahvis et al., 1995).

High concentrations of butyltin residues have been found in harbor porpoise from the Baltic Sea (Poland) and from bottlenose dolphins from the Mediterranean (Kannan and Falandysz, 1997). Organotin compounds (*bis(tri-n-butyltin)oxide*) have been shown to exhibit immunosuppressive and endocrine-modulating activities in rodents (Vos et al., 1984; Krajnc et al., 1984). In particular long-term exposure reduced the resistance to infectious diseases (Vos et al., 1990).

Beluga whales from the Gulf of St. Lawrence in Canada show a high prevalence of

lesions of endocrine glands (hyperplastic and degenerative changes of adrenals) (Lair et al., 1997) and thyroids (in ICES, 1998). Similar changes were not observed in belugas from less-contaminated areas (Lair et al., 1997; St. Aubin and Geraci, 1989) and in other species of marine mammals (Cowan, 1967; Kuiken et al., 1993). In addition to the above-mentioned pathologies, frequent infection by mildly pathogenic bacteria and more importantly a high prevalence of various types of tumors have been observed in these stranded animals (Martineau et al., 1994; De Guise et al., 1994b). The prevalence of malignant tumors in belugas from the St. Lawrence estuary is high and affects mainly older animals (17 years and above).

III. BIRDS

A. Reproductive Effects and Nonreproductive Effects

Reproductive effects have been reported in many species, especially aquatic and terrestrial birds of prey (Table 2).

PCBs were originally identified as environmental pollutants in a white-tailed sea eagle (*Haliaeetus albicilla*) found dead in the Stockholm archipelago (Jensen, 1966, 1972). The reproductive success of the Baltic sea eagle was subsequently shown to be negatively correlated to the levels of persistent organochlorines in their eggs (Helander et al., 1982; Falandysz et al., 1994).

Bird eggs are important matrices for monitoring the levels of persistent pollutants in aquatic and terrestrial ecosystems, and many lipophilic chemicals such as PCBs, PCDFs, PCDDs, chlorinated pesticides are readily taken up and excreted in the egg yolk in laying birds. This efficient route of elimination from the laying female may result in a pronounced exposure of the avian embryo to toxic chemicals from its earliest stages of development.

TABLE 2
Reproductive Effects in Birds

Effect/disorder	Location	Species	Associated contaminants	Ref.
Reproductive failure	Sweden, Baltic Sea	White-tailed sea eagle	PCBs, DDT	Jensen, 1972; Helander et al., 1982
Eggshell thinning	Europe, North America	Numerous species, e.g., peregrines, osprey, bald eagle, sparrow hawk, guillemot	DDE	Ratcliffe, 1967; Koeman et al., 1972; Peakall et al, 1973; Bignert et al., 1995; Odsjö and Sondell, 1982
Reproductive failure	Sweden, Netherlands	Peregrine, sparrow hawk	Pesticides, methylmercury	Borg, 1958; Koeman et al., 1972
Behavioral effects	Sweden	Robin		Ulfstrand et al., 1971
Feminization, ovotestis, persistent Mullerian ducts, female-female pairing	U.S., California, Great Lakes	Western gull	DDT compounds, methoxychlor, OC	Fry, 1995; Fry and Toone, 1981
Reproductive failure, deformities	U.S., Great Lakes	Colonial fish-eating birds	PCDD/PCDF	Gilbertson et al., 1991; Giesy et al, 1994b
Embryotoxicity, impaired adrenal and ovarian function	Oil spill- contaminated seas	Sea birds	PAH fractions	Hoffman, 1990
Decreased hatching success	U.S., Lake Michigan	Forster's tern (<i>Sterna forsteri</i>)	TCDD, PCDD, PCB, DDT, DDE	Kubiak et al., 1989
Reproductive impairment	U.S., Europe	Numerous species	PCDDs, PCDFs, PCBs	Hoffman et al., 1996; Hart et al., 1991
Female-female pairing, eggshell thinning	U.S., California, Great Lakes, Puget Sound	Western gull	DDT compounds	Fry et al., 1987
Goiter and thyroid abnormalities	U.S., Great Lakes	Herring gull (<i>Larus argentatus</i>)	PHAHs?	Moccia et al., 1986

TABLE 2 (continued)
Reproductive Effects in Birds

Effect/disorder	Location	Species	Associated contaminants	Ref.
Developmental impairment	The Netherlands, river Rhine and its tributaries	Cormorant (<i>Phalacrocorax carbo</i>)	Dioxins and related compounds, PCBs, DDE	Boudewijn et al., 1988; Dirksen et al., 1995; Van den Berg et al., 1994
Lower breeding success	The Netherlands, Haringvliet, semifield study	Tufted duck (<i>Aythya fuligula</i>)	Heavy metals and PCBs	Marquenie et al., 1986
poor survival of young	U.S., Great Lakes	Seabirds	DDT/DDE	Cooke, 1973
Eggshell thinning				
breeding failure				
population declines				
Impaired reproduction	San Francisco Bay, Great lakes	Crowned night heron (<i>Nycticorax nycticorax</i>)	PCBs	Hoffman et al., 1996
Reduced hatchability, dead chicks, morphological abnormalities	Dutch and Belgian estuaries, Western Scheldt	Common tern (<i>Sterna hirundo</i>)	PHAHs?	Murk et al., 1994a, 1996; Bosveld et al., 1995

Certain avian top predators therefore may be sensitive targets for these persistent lipophilic pollutants.

Several examples of pollutant-induced toxicity in wild bird populations and reproductive or developmental toxicity following exposure to chlorinated pesticides, PCBs, PCDFs, and PCDDs have been reported (Gilbertson et al., 1991; Fry, 1995, and references therein). In birds as in mammals, it is generally difficult to determine the mechanism of action in the field, where toxic effects may result from co-exposure to complex mixtures of persistent organic pollutants and metals. Based on recent experimental data, those chemicals characterized to date may affect the endocrine system by a number of mechanisms, including estrogen-, androgen-, thyroxin-, glucocorticoid-, and retinoid-regulated pathways. In addition, Ah-receptor agonists such as PCDDs/PCDFs, coplanar PCBs, and polycyclic aromatic hydro-

carbons (PAHs) represent an important group of highly toxic compounds with an array of toxic effects, including reproductive toxicity and teratogenicity (Bosveld and Van den Berg, 1994). Some endocrine disrupters may work by different mechanisms of action in different tissues and species, as demonstrated by the classic DDT-molecule and its persistent metabolites DDE, DDD, and methylsulfonyl-DDE (Brandt et al., 1997). DDE-induced reproductive failure due to eggshell thinning is one of the most serious and well-documented effects of persistent organic pollutants in avian wildlife.

1. DDE-Induced Eggshell Thinning

Ratcliffe (1967) drew attention to the thinning of the eggshells (Figure 4) combined with the occurrence of broken eggs that had been



FIGURE 4. Measuring the shell thickness of osprey egg. (Photo courtesy of G. Frisk.)

observed in nests of the peregrine falcon (*Falco peregrinus*), sparrowhawk (*Accipiter nisus*), and golden eagle (*Aquila chrysaetos*) since about 1946 in Great Britain. He noted that at the same time DDT had come into general use. As shown by numerous subsequent experimental and field studies, eggshell thinning may be produced by several environmental pollutants, including methylmercury and organochlorines such as the persistent DDT metabolite DDE. Notably, *p,p'*-DDE is the dominating DDT residue in most bird species. DDE-induced reproductive failure due to eggshell thinning and broken eggs is one of the most serious and well-documented effects of persistent organic pollutants in avian wildlife (Ratcliffe, 1970; Peakall et al., 1973; Lundholm, 1997). It is probably also the first example of endocrine disruption in wild populations, even though the mechanism of action has not been understood until recently.

Originally reported in peregrines, eggshell thinning has been described in a large number of species, including raptors such as the osprey (*Pandion haliaetus*) and the bald eagle (*Haliaeetus leucocephalus*) (Koeman et al., 1972; Lincer, 1975; Odsjö and Sondell, 1982; Peakall et al., 1973; Ratcliffe, 1970). Bignert et al. (1995) examined eggshell thickness in Baltic guillemot (*Uria aalge*) eggs collected 1861 to 1989. Along with decreasing DDT and PCB levels in the Baltic fauna since the 1970s, the eggshell thickness in Baltic guillemot has gradually improved. Notably, however, guillemot eggshells were still thinner in their recent material than in historical material collected before 1946.

Several hypotheses have been forwarded to explain the mechanism of eggshell thinning. Studying the pigeon (*Columba livia*), Peakall (1970) reported that *p,p'*-DDT decreases both estradiol blood levels and deposition of medullary bone, which is an important source of calcium for the formation of the eggshell. Another proposed mechanism involves inhibition of carbonic anhydrase in the eggshell gland following exposure of Japa-

nese quail (*Coturnix japonica*) to *p,p'*-DDT and *p,p'*-DDE (Bitman et al., 1970). Carbonic anhydrase supplies carbonate ions required for calcium carbonate deposition in the eggshell, and the carbonic anhydrase inhibitor acetazolamide produces experimental eggshell thinning in chicken (*Gallus domesticus*). Despite these early proposals, the mechanism of *p,p'*-DDE-induced eggshell thinning has not been clarified until recently. Using the domestic duck as the test organism, Lundholm (1997) showed that *p,p'*-DDE inhibits prostaglandin synthase and reduces prostaglandin E2 in the eggshell gland mucosa, resulting in a reduced calcium transport across the mucosa into the eggshell gland lumen. The structurally related compounds *o,p'*-DDE, *p,p'*-DDT, *o,p'*-DDT, and *p,p'*-DDD did not cause eggshell thinning or inhibit prostaglandin synthesis in the eggshell gland. The finding that eggshell thinning could also be induced by the cyclooxygenase inhibitor indomethacin suggested that inhibition of prostaglandin synthesis was part of the mechanism of action (Lundholm, 1997).

Experimental studies have shown that *in ovo* exposure to xeno-estrogens such as *o,p'*-DDT, DES, and 17 α -ethinylestradiol may alter male sexual behavior in adult birds (Bryan et al., 1989; Berg et al., 1998). Changed behavioral patterns have been observed also in wild birds, even though the mechanisms are not well understood. Hunt and Hunt (1973, 1977) noted female pairing in gulls in southern California when the sexes of birds producing supernormal clutches were examined. Gulls normally lay three eggs, whereas numbers of eggs in the supernormal clutches were four to six. Supernormal clutches and female-female pairing have been noted in breeding populations of four species of gulls in North America. Western gull (*Larus occidentalis*) and herring gull (*Larus argentatus*) colonies with supernormal clutches have been located in areas contaminated with high levels of organochlorines. Fry and Toone (1981) sug-

gested that female-female pairing among Western gulls in southern California was related to high organochlorine levels. Because gulls seem to be rather resistant to eggshell thinning by DDT, levels of DDT causing eggshell thinning in other species such as the brown pelican (*Pelecanus occidentalis*) did not damage shells of gull eggs. Hence, gull chicks did hatch from eggs containing high levels of organochlorines.

Experimental injection of the DDT derivatives *o,p'*-DDT (an estrogen in rats), methoxychlor (an estrogen following demethylation in rats), and *p,p'*-DDE (an antiandrogen in rats) into embryonated gull eggs resulted in malformed and feminized sex organs in male chicks. Similar effects were observed when estradiol was injected as a positive control (Fry and Toone, 1981). All substances induced primordial germ cell growth in an increased, ovary-like cortex of the left testicle (ovotestis) and retention of the left or both Müllerian ducts. Notably, these changes were observed at environmentally relevant doses. These authors also speculated that reproductive failures, skewed sex ratio, and female-female pairing in breeding populations of Western gulls on the Great Lakes resulted from pollution with organochlorines. Feminization of male gull embryos was suggested to affect reproductive behavior in males leading to reduced migration to the breeding sites. A reason for female-female pairing in a population could be a skewed sex ratio with an increased number of females. An alternative hypothesis to explain the sex ratio skew in contaminated areas is that the low male/female ratio was due to a higher mortality among the males (Conover and Hunt, 1984).

Colonies of glaucous-winged gulls (*Larus glaucescens*) breeding in polluted areas of Puget Sound in 1984 exhibited eggshell thinning and persistent right oviducts in adult females (Fry et al., 1987). Twenty-one of 31 female gulls retained right oviducts of which 13 were longer than 10 mm. Normally, female birds have completely regressed or small (less than 5 mm) right oviducts. The

causes of these effects in the glaucous-winged gulls are unknown. The major contaminants in Puget Sound were heavy metals, PCB, and polycyclic aromatic hydrocarbons, whereas DDT concentrations were not particularly high.

Several species of colonial fish-eating birds nesting in the Great Lakes basin have exhibited chronic impairment of reproduction (Gilbertson et al., 1991; Kubiak et al., 1989). High levels of DDT and its metabolites have caused eggshell thinning in many species. In addition, the reproductive impairment is characterized by high embryonic and chick mortality, edema, growth retardation, and deformities as well as altered incubation behavior contributing to decreased hatchability. The syndrome of fish-eating birds in the Great Lakes has been designated GLEMEDS (Great Lakes Embryo Mortality, Edema, and Deformities Syndrome) and clearly resembles chick edema disease, a condition resulting from unintended exposure of millions of broilers to 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin in the southeastern United States in 1957 (Firestone, 1973). Reproductive impairment related to high PCDD/PCDF exposure has been described in a number of species, including the double-crested cormorant (*Phalacrocorax auritus*), black-crowned night heron (*Nycticorax nycticorax*), herring gull (*Larus argentatus*), ring-billed gull (*Larus delawarensis*), common tern (*Sterna hirundo*), Forster's tern (*Sterna forsteri*), and Caspian tern (*Hydroprogne caspia*). Current concentrations of PCDDs/Fs and PCBs in fish-eating birds in the Great Lakes area are less than those during the 1960s and 1970s, and some bird populations such as double-crested cormorants and herring gulls have made dramatic recoveries since that time (Giesy et al., 1994b). Certain populations of common and Forster's tern continue to decline, however, and double-crested cormorants and Caspian terns in Saginaw and Green Bays still display abnormal rates of developmental deformities and embryo lethality (Giesy et al., 1994b).

No thorough investigation of the possible occurrence of GLEMEDS-like symptoms

in fish-eating bird populations in Europe seems to be available, even though TCDD-receptor binding PCBs, PCDDs, and/or PCDFs have been found to be present in biologically active concentrations in several wild bird populations at the North Sea coast, in the Baltic area, or the Norwegian Arctic (Bosveld and Van den Berg, 1994; Bernhoft et al., 1997; Falandysz et al., 1994). In The Netherlands, reproductive effects have been reported in cormorant (*Phalacrocorax carbo*) (Boudewijn et al., 1988; Van den Berg et al., 1994; Dirksen et al., 1995) and common tern (*Sterna hirundo*) (Murk et al., 1994a, 1996). DDT metabolites in the eggs of cormorants and the food were positively correlated with the occurrence of thin eggshells, while the PCB concentrations were negatively correlated with the percentage of outcome success of the eggs and breeding success (Dirksen et al., 1995). Van den Berg et al. (1994) examined correlations between PCB, PCDD, and PCDF levels in the yolk sac, and a number of biochemical and morphological changes in newly hatched chicks from two Dutch cormorant (*Phalacrocorax carbo*) colonies. Significant concentration-effect relationships for EROD induction, plasma-free thyroxine reduction, yolk sac weight, relative liver weight, and head size were observed. The authors conclude that these alterations may play a role in the observed reduced reproductive success of cormorants in the Dordtse Biesbosch area. Artificial incubation of common tern (*Sterna hirundo*) eggs collected in the main sedimentation area of the Rhine and Meuse rivers resulted in a fourfold increase in EROD activity in the developing chick liver when compared with chick livers from eggs collected in a reference colony (Bosveld et al., 1995). Residual yolk sacs from the hatchlings contained on an average 16 ng TEQ (TCDD toxic equivalents) per g lipid, a concentration five times higher than that in the reference colony, and the average TEQ concentrations in chicks hatched after 23 days of incubation were twice the concen-

trations found in chicks that hatched after 21 days of incubation (Bosveld and Van den Berg, 1994). Murk et al. (1996) examined the relation between yolk sac polyhalogenated hydrocarbon levels and reproductive outcome using embryonated eggs collected from eight Belgian or Dutch common tern colonies. The results showed that higher yolk sac polyhalogenated hydrocarbon levels and liver EROD levels correlated with late egg laying, prolonged incubation period, small eggs and chicks. These parameters also correlated with low yolk sac retinoid and plasma thyroid hormone levels in the embryos. In an experimental study, tufted duck (*Aythya fuligula*) fed contaminated freshwater mussels from the Haringvliet in The Netherlands had lower breeding success (fewer eggs) and poorer survival of the young. The observed effects were attributed to heavy metals and PCBs (Marquenie et al., 1986).

Extractable lipid tissue from glaucous gull (*Larus hyperboreus*) caught in the Norwegian Arctic (Svalbard) has been found to contain PCB in the 10 to 1000 ppm range (Gabrielsen et al., 1995). Using a sensitive bioassay based on cultivated chicken embryo liver to estimate the amounts of 'dioxin-like' compounds in captured gulls fed on polar cod (*Boreogadus saida*), Henriksen et al. (1998) showed that the Bio-TEQs ranged from 5 to 254 ng/g extracted lipid. Similar levels of TCDD or TCDD equivalents in eggs have been associated with embryotoxicity in other bird species (Hoffman et al., 1996).

Bird embryos are highly sensitive to petroleum. Small quantities (1 to 20 µl) of some types of oil on the eggshell are sufficient to cause embryo death, particularly during early life-stages. The PAH fractions of crude and refined oils seem to be responsible for the lethal and sublethal effects on bird embryos caused by eggshell oiling (Hoffman, 1990; Parnell et al., 1984). Crude oil also exerts effects on the adult birds such as ultrastructural changes in the adrenals, diminished adrenal responsiveness to adrenocorticotrophic hormone, and reduced corticosterone secre-

tion, suggesting a direct effect of petroleum on the adrenal gland. Suppression of ovarian function and decreased circulating prolactin levels caused by oil exposure have been associated with impaired reproductive function.

Several phenolic environmental pollutants bind with high affinity to the thyroxin transport protein transthyretin in rodents. This competitive binding provides a mechanism for the disruption of thyroxin transport to the tissues. Recent studies have shown that phenolic metabolites of persistent halogenated hydrocarbons such as PCBs are present in blood plasma of Laysan and black-footed albatrosses (*Diomedea immutabilis*; *Diomedea nigripes*) collected at the Midway islands (Klasson-Wehler et al., 1998). The impact of such phenolic pollutants on thyroxin homeostasis in birds remains to be established.

The ubiquitous DDT metabolites 3-methylsulfonyl-DDE and DDD are metabolism-activated toxicants in the corticosteroid-producing interrenal cells in chicken embryos (Jönsson et al., 1994). Information on the effects of these compounds on interrenal cell function in wild birds is not available.

IV. REPTILES/AMPHIBIANS

A. Reproductive and Nonreproductive Effects

Reproductive and nonreproductive effects have been reported in aquatic reptiles and amphibians (Table 3).

Research has shown that all crocodiles, many turtles, and some lizards lack distinct sex chromosomes and that the sex is not organized until well after fertilization, during organogenesis. In these animals gender is determined during organogenesis by incubation temperature (temperature-dependent sex determination, TSD) (Crews, 1994; Crain and Guillette, 1998). Experimental evidence shows that animals with TSD are sensitive to developmental exposure to exogenous hormones, and temperature-dependent gender

can be altered by hormone exposure (Crain and Guillette, 1997). When 17 β -estradiol is administered to eggs during the temperature-sensitive period, phenotypic females are produced at male-producing temperatures in turtles, lizards, and alligators (see Crain and Guillette, 1998).

Experimental studies in turtles that exhibit TSD show sex reversal following embryonic exposure to various polychlorinated biphenyls. For instance, Bergeron et al. (1994) found that exposure of red-eared slider turtle (*Trachemys scripta elegans*) eggs to artificial hydroxy-PCBs (2,4,6-trichloro-4'-biphenylol and 2,3,4,5-tetrachloro-4'-biphenylol), incubated at a temperature known to produce 100% males, significantly altered sex ratio, producing more females. The results were identical for estradiol-treated eggs. There was no evidence of sex reversal for (combined) nonhydroxylated PCBs. Results of this laboratory study indicate that also hydroxy-PCBs found to be present in the blood of wild mammals, birds, and fish (Bergman et al., 1994; Klasson-Wehler et al., 1998; Asplund et al., 1999) may have the capacity to exert estrogenic effects on the embryonic gonad and thus influence sexual differentiation in wildlife.

Bishop et al. (1991) found a strong statistical association between the presence of organochlorine contaminants (especially the PCB congener 2,3,3',4,4'-pentachlorobiphenyl) and decreased hatching success and increased developmental abnormalities in snapping turtles (*Chelydra serpentina serpentina*). However, the study could not demonstrate that any particular organochlorine chemical analyzed was the responsible agent.

In a field study with common snapping turtles in Ontario, Canada, the hypothesis was tested that snapping turtles from organochlorine-contaminated sites would be feminized when compared with snapping turtles from noncontaminated sites (De Solla et al., 1998). The authors found some evidence that the sexually dimorphic morphology of adult snapping turtles was more feminized at the con-

TABLE 3
Reproductive and Nonreproductive Effects in Reptiles and Amphibians

Effect/disorder	Location	Species	Associated contaminants	Ref.
Decreased hatching success, increased developmental abnormalities	Great Lakes	Snapping turtles (<i>Chelydra serpentina serpentina</i>)	Organochlorine compounds, including PCBs, PCDDs, DDE, and other pesticides	Bishop et al., 1991
Feminization	Canada Lake Ontario	Snapping turtles (<i>Chelydra serpentina serpentina</i>)	Organochlorine compounds	De Solla et al., 1998
Low hatching rates, various abnormalities in males and female, including abnormal gonadal morphology, altered gonadal steroidogenesis, and abnormal sex steroid concentration	Florida, U.S.	American alligator (<i>Alligator mississippiensis</i>)	<i>p,p</i> -DDE	Guillette et al., 1994, 1996
Inhibition of metamorphosis and high prevalences of skin lesions	U.S., lagoon	Salamander (<i>Ambystoma tigrinum</i>)	Pollutants present in sewage, including perylene and possibly estrogenic compounds	Rose and Harshbarger, 1977

taminated sites. However, no evidence was found that contaminants (PCBs/PCDDs/PCDFs and pesticides) had any effect on testosterone or estrogen levels. Sample sizes of testosterone measurements of males from some of the sites were small and therefore may not have been reliable. This study suggests that in adults external sexual development may be more sensitive to exposure to environmental contaminants than testosterone production or metabolism.

One well-documented case is that of the American alligator (*Alligator mississippiensis*) living in a contaminated lake in Florida (U.S.), Lake Apopka. The lake has contaminants derived from agricultural activities and municipal runoff and was also contaminated by a major spill of a pesticide mixture that was primarily composed of dicofol but also contained DDT in 1980 (see Crain et al., 1998). Alligator eggs from this lake contain elevated levels of a variety of persistent bioaccumulated pesticides and their metabolites such as *p,p'*-DDE, *p,p'*-DDD, trans-nonachlor, dieldrin, toxaphene, oxychlordane, and various PCBs and metals (Heinz et al., 1991). Significantly greater concentrations of *p,p'*-DDE have been found in fat and liver of juvenile alligators from Lake Apopka when compared with control lakes (see Guillette et al., 1996). The alligator population in Lake Apopka exhibited a 90% decline in juveniles during the years immediately following the spill. Alligator eggs obtained from Lake Apopka showed poor viability and poor offspring survival under controlled laboratory conditions (Woodward et al., 1993; Guillette et al., 1994). Developmental abnormalities in hatchling alligators included abnormal gonadal morphology, altered gonadal steroidogenesis, and abnormal sex steroid concentration in males and females (Guillette et al., 1994, 1995). Female alligators from Lake Apopka had abnormally high concentrations of 17 β -estradiol in blood plasma for the 6 months after hatching, whereas males had depressed testosterone levels and increased levels of 17 β -estradiol (Guillette et

al., 1994). All neonatal females hatched from eggs collected on Lake Apopka had prominent polyovular follicles and many multinucleated oocytes, whereas polyovular follicles were not observed in control females. Male juvenile alligators 6 months of age born after the spill had poorly organized testes with germ cells undergoing premature spermatogenesis and small phalli (Guillette et al., 1994). Thus, abnormal plasma steroid concentrations in the alligators of Lake Apopka correlated with altered gonadal morphology (Guillette et al., 1994) and smaller penis size (Guillette et al., 1996) in juvenile alligators.

To date the exact causative agents of the endocrine disruption in Lake Apopka alligators has not been identified, although the principal contaminant found in alligator eggs and juveniles is *p,p'*-DDE. Many hypotheses have been proposed to explain the endocrine-disrupting effects in male and female alligators. For example, it is hypothesized that some of the endocrine-disrupting effects (such as polyovular follicles) are a response to embryonic exposure to EDCs caused by the interaction of multiple EDCs with the alligator estrogen receptor (Crain and Guillette, 1997). This is supported by the results obtained with traditional receptor competition assays that have shown that a number of the environmental estrogens, including those identified in the eggs of alligators living in Lake Apopka, have the ability to bind to the alligator estrogen and/or progesterone receptors (Vonier et al., 1996). Polyovular follicles in female alligators from Lake Apopka are very similar to those observed in mice treated with the estrogenic agent DES (see Bern, 1992). The observed developmental abnormalities in male and female alligators have also been linked to the antiandrogenic effects of *p,p'*-DDE. Androgens are essential for normal maturation and growth of the male reproductive system in reptiles (Guillette et al., 1996). It has been shown that *p,p'*-DDE displays antiandrogenic activity both *in vivo* and *in vitro* in rats (Kelce et al., 1995). In alligator eggs from Lake

Apopka levels of *p,p'*-DDE (5.8 ppm wet weight) have been observed that are above the concentrations that block androgen receptor function *in vitro*, although very high doses were used to demonstrate antiandrogenic effects *in vivo* in rats (Kelce et al., 1995). Therefore, it is possible that this contaminant could act as an androgen antagonist in embryonic and juvenile alligators (Guillette et al., 1996). However, to date there are no data available that indicate the ability of *p,p'*-DDE to bind the reptilian androgen receptor. It is, however, conceivable that multiple endocrine mechanisms, including non-receptor-mediated mechanisms, are involved in the Apopka alligator case. *In vitro* studies suggested that the observed changes in reproductive and endocrine systems are the result of modifications in gonadal steroidogenic activity and increased hepatic degradation of steroids (Guillette et al., 1995). Recent field and laboratory studies indicate that induction and suppression of aromatase enzyme activity are potential modes of contaminant-induced endocrine disruption in alligators (Crain et al., 1997). Recent field observations also suggest that the thyroid/gonad axis may be involved in the reported reproductive endocrine disruption (Crain et al., 1998). It is clear that further experimental and field studies are needed to provide causal relationships between pesticide exposure, especially during embryonic development, and the reproductive abnormalities in Lake Apopka alligators.

Laboratory studies have indicated that DDT may be estrogenic in other reptilian and amphibian species. Palmer and Palmer (1995) reported that *o,p*,-DDT was able to induce the estrogen-regulated hepatic synthesis of the yolk-protein precursor, vitellogenin, in male red-eared turtles (*Trachemys scripta*,) and African clawed frog (*Xenopus laevis*). In a follow-up study by the same group (Palmer et al., 1997), vitellogenin induction in frogs was also demonstrated for dieldrin and toxaphene.

Amphibians have been mentioned by several bodies as an important group of wildlife species that deserve more attention. This class

of vertebrates is subject to multiple exposures during different stages in their life cycle and therefore may be particularly at risk. It has been shown that known EDCs, including several pesticides, can alter vitellogenin expression in a model amphibian species, *Xenopus* (Palmer and Palmer, 1995; Palmer et al., 1997; see also above). Further, thyroid hormones play a crucial role in the processes of metamorphosis and osmoregulation in amphibians (Galton, 1992; Shi, 1994) and fish (Norris, 1997) and the migrational behavior driven by changes in buoyancy of amphibians (Reinke and Chedwick, 1939; Dent, 1985). So far, to our knowledge, no studies have been undertaken in Europe to investigate the possible effects of EDCs on wild amphibian populations. This is despite the fact that many amphibian populations are declining in both pristine and polluted habitats throughout the world (Blaustein and Wake, 1990; Carey and Bryant, 1995). Recently, increasing prevalences of amphibian deformities (e.g., malformed or lacking hindlimbs) in species in North America, central America, and Australia have been reported (e.g., Burkhart et al., 1998). However, so far the role of EDCs to the population declines or the deformity phenomenon remains unknown (Vial and Saylor, 1993). Apart from EDC effects in nontargeted amphibian species by pesticide spraying (Hall and Henry, 1992), few studies yet exist on whether reproduction of amphibian populations in the wild has been affected by environmental toxicants. The potential roles of environmental xenobiotics to decline of amphibian populations by impacting growth and development of the young have been examined by Carey and Bryant (1995). Their conclusion was that critical data are lacking in most cases. In another study, Rose and Harshbarger (1977) reported on inhibition of metamorphosis and high prevalences of skin lesions in the salamander *Ambystoma tigrinum* inhabiting a lagoon receiving secondary domestic sewage and perylene (a component of jet fuel). The authors suggested that the observed phenomena could have been caused

by pollutants present in the sewage, including estrogenic compounds. The observation that the observed phenomena were reversed when animals were transferred to unpolluted ponds supported this hypothesis. A possible role of environmental factors, including TPT and pH in growth and development of two toad species (*Rana lessonae* and *Rana esculenta*), in particular during the larval period, has been reported in a recent Swiss study (Fioramonti et al., 1997). The two species have a sympatric and broad geographic distribution in Europe. The authors suggest that interspecies differences in these factors could have played a role in determining the variations of spatial distribution of these species and could have generally contributed to the decline of populations of sensitive amphibians. Recent studies have demonstrated that disturbances of the thyroid hormone system as a result of dosing amphibians with realistic levels of PCBs may result in distorted development of larvae, prolonged or even absent metamorphosis, or increased mortality depending on the life-stage at dosing (Gutleb et al., 1998). It is clear from the above that field and laboratory studies are necessary to determine the true scale of the problem, if any, in amphibian populations especially in Europe.

V. FISH

A. Reproductive Effects

Reproductive effects have been reported in various fish species in association with sewage effluent, paper industry, and habitats (Table 4a).

1. Sewage Effluent

Perhaps the best example of estrogenic contamination in the aquatic environment is that resulting from the discharge of treated sew-

age to rivers. These discoveries have relied on the utility of the egg yolk precursor protein vitellogenin as a sensitive biomarker for estrogen exposure in male fish. Treated domestic sewage discharges have been identified as a major cause of estrogenic effects on fish in the U.K. and U.S. fresh water environments (Purdom et al., 1994; Folmar et al., 1996). The U.K. studies, which are of particular relevance to the European situation, have shown that a large number of sewage treatment plant effluents in this country are estrogenic for fish (Purdom et al., 1994; Sumpter and Jobling, 1995) and that a number of receiving surface waters show estrogenic activity (Harries et al., 1996). These studies revealed that caged male rainbow trout (*Oncorhynchus mykiss*) produce vitellogenin, and further also show a reduction in testicular growth. Although in the first instance only alkylphenol derivatives were identified as most likely causative agents (Jobling et al., 1996), later studies using bio-assay fractionation techniques showed that the estrogenic activity of the water samples could also be explained by the presence of natural hormones (17 β -estradiol and oestrone) and to a lesser degree synthetic hormones deriving from contraceptive agents (17 α -ethinylestradiol) (Desbrow et al., 1998). Recent surveys throughout the U.K. clearly demonstrated increased plasma levels of vitellogenin in wild populations of a freshwater fish, roach (*Rutilus rutilus*), in a number of river systems that receive waste water effluents. Experimental findings indicate that environmentally relevant concentrations of natural steroidal estrogens are sufficient to account for the levels of vitellogenesis observed in caged male fish placed downstream of certain sewage water treatment-effluent discharges in British rivers (Routledge et al., 1998). A large number of the examined roach from each of the surveyed rivers also showed a high prevalence (locally up to 100%) of intersexuality (ovotestis) (Jobling et al., 1998). These findings are proof of a widespread effect of estrogenic compounds in wild fish populations in the U.K. The observed reproductive

TABLE 4a
Reproductive Effects in Fish

Effect/disorder	Location	Species	Associated contaminants	Ref.
Elevated blood vitellogenin in male fish	UK, estuaries semifield study	Rainbow trout (<i>Oncorhynchus mykiss</i> (caged))	Sewage effluents: natural and synthetic estrogenic hormones, nonylphenol	Harries et al., 1996, 1997
Elevated blood vitellogenin in male fish	Sweden semifield study	Rainbow trout (<i>Oncorhynchus mykiss</i> (caged))	Sewage effluents: natural and synthetic estrogenic hormones, i.p. 17 α -ethinyloestradiol	Larsson et al., 1999
Elevated vitellogenin in males, intersex (up to 100%)	UK rivers	Roach (<i>Rutilus rutilus</i>)	Steroids	Purdom et al., 1994; Jobling et al., 1998; Routledge et al., 1998
Vitellogenin induction and reduced serum testosterone concentrations in male fish	U.S.	Carp (<i>Cyprinus carpio</i>)	Metropolitan sewage treatment effluents	Folmar et al., 1996
Inhibition of spawning in female fish	U.K.	Sand goby (<i>Pomatoschistus minutus</i>)	Diluted sewage sludge	Waring et al., 1996
Elevated blood vitellogenin in male fish; ovotestis locally up to 20%	UK, estuaries, and coastal waters	Flounder (<i>Platichthys flesus</i>)	(Xeno)estrogens	Allen et al., 1999; Matthiessen et al., 1998
Elevated blood vitellogenin in female fish; testicular abnormalities	Scotland, estuaries	Flounder (<i>Platichthys flesus</i>)	Sewage and industrial waste: (xeno)estrogens	Lye et al., 1997
Reduced plasma sex hormone levels, reduced gonad growth and delayed sexual maturity	Sweden	Several species	Bleached kraft pulp mill effluents: chlorinated organic chemicals	Andersson et al., 1988; Sandström et al., 1997
Elevated blood vitellogenin in male fish	Sweden	Perch (<i>Perca fluviatilis</i>)	Pulp mill effluents: unknown chemicals	Förlin et al., 1999

Various reproductive, physiological and behavioral disturbances including masculinization of females	U.S., Florida	Poeciid species including musquitto fish (<i>Gambusia affinis affinis</i>)	Pulp mill effluents, e.g., phyosterols (from trees) and organochlorines (PME)	Davis and Bortone, 1992; Drysdale and Bortone, 1989; Howell and Denton, 1989
Altered serum steroid levels	Lake Superior, Canada	White sucker (<i>Catostomus commersoni</i>)	Bleached kraft mill effluent	Van der Kraak et al., 1992
Delayed gonadal maturation, reduced egg size, increased fecundity	Canada, New Brunswick, Miramichi estuary	Mummichog (<i>Fundulus heteroclitus</i>)	Bleached kraft mill effluent	Leblanc et al., 1997
Reproductive impairment	Puget Sound, U.S.	English sole (<i>Parophrys vetulus</i>)	PCBs, PAHs	Johnson et al., 1988, 1997b
Reproductive impairment	San Francisco Bay, U.S.	Starry flounder (<i>Platichthys stellatus</i>)	Organic contaminants	Spies and Rice, 1988
Exposed males lead to decreased hatching success of progeny	Canada, St. Lawrence Bay, semi-field study	American plaice (<i>Hypoglossoides platessoides</i>)	Marine sediments contaminated with organic compounds	Nagler and Cyr, 1997
Decreased hatching success	Baltic Sea	Baltic herring (<i>Clupea harengus</i>)	Chlorinated hydrocarbons	Hansen et al., 1985
Decreased fecundity	Baltic Sea	Cod (<i>Gadus morhua</i>)	Lipophilic xenobiotics	Petersen et al., 1997
Disruption of the normal ovarian cycle	France, coast of Brittany	Plaice (<i>Pleuronectes platessa</i>)	Amoco Cadiz oil spill: petroleum contamination	Stott et al., 1983
Premature vitellogenesis (decreased turnover of steroids)	Netherlands, Texel semifield study	Flounder (<i>Platichthys flesus</i>)	Harbor dredge contaminants	Janssen et al., 1997
Elevated blood vitellogenin in female fish	Boston Harbor, U.S.	Winter flounder (<i>Pleuronectes americanus</i>)	Unknown	Pereira et al., 1992
Decreased age at first maturation (1960 to 1995)	North Sea	Plaice, sole	Unknown	Rijnsdorp and Vethaak, 1997

TABLE 4a (continued)
Reproductive Effects in Fish

Effect/disorder	Location	Species	Associated contaminants	Ref.
Changes in sex ratio (1981 to 1995)	North Sea	Dab (<i>Limanda limanda</i>)	Unknown	Lang et al., 1995
Reproductive disorder M74 Syndrome (environmentally related disease 1974)	Baltic Sea	Atlantic salmon (<i>Salmo salar</i> L.)	Dietary vitamin B1 deficiency, but also chlorinated organic compounds, thyroid hormones and retinoids have been implicated	Akerman et al., in SEPA, 1995; Vuorinen et al., 1997a; Amcoff et al., 1998; Bengtsson et al., 1999
Reproductive disorder EMS Syndrome (Early Mortality Syndrome)	North American Great lakes	Salmonids	Dietary vitamin B1 deficiency, but also chlorinated organic compounds have been implicated	Mac and Edsall, 1991; Fitzsimons et al., 1999; AFS, 1996

disturbances are consistent with experimental findings and show an association with discharges of water treatment plants that are proven to contain estrogenic steroids (Jobling et al., 1998). It appears, however, that low background prevalence of ovotestis in roach can be found anywhere in the U.K., but it is not known whether this is due to natural factors or to the absence of completely pristine freshwater habitats. Whether the observed effects actually do have a negative impact on reproduction is still unknown. However, an experimental study reported by another group in the U.K. has demonstrated inhibition of spawning in female sand goby (*Pomatoschistus minutus*) after exposure to diluted sewage sludge (Waring et al., 1996).

Another series of studies in the U.K. have concentrated on estrogenic effects in the euryhaline flatfish flounder (*Platichthys flesus*) captured in the U.K. estuaries receiving municipal and industrial effluent (Allen et al., 1999). Flounder collected at inner stations on the Thames had moderately induced serum vitellogenin levels, while outer stations had little induction. It was noted that flounder is less sensitive with respect to vitellogenin induction when compared with salmonid species. Similar moderate levels of induction were observed in the Clyde, while low or negligible levels of induction were observed in the Humber, Dee, Tamar, Alde, and Crouch. High levels of induction were observed in the Tees estuary that receives input from several chemical manufacturers, and in particular elevated levels of nonylphenols. Similar high induction was seen in the Mersey and Tyne estuaries where nonylphenol inputs are thought to be smaller. In general, the data tentatively suggest that induction of flounder vitellogenin in the U.K. estuaries is not primarily related to the number of people in the catchment area but rather to the industrial effluent inputs and the chemical characteristics of those effluents. A graded response in vitellogenin induction was observed as samples were taken away from estuarine sources. Estrogenic effects could still

be observed as slightly elevated levels at the offshore spawning areas in the Southern Bight (Allen et al., 1999). Induced vitellogenin persists in the blood of male fish for several weeks, and therefore it is not unlikely that the origin of the exposure to estrogenic compounds of the open sea spawning populations of flounder is largely from the open estuaries. However, the possibility of contamination in open sea cannot be excluded.

Overall, the studies on wild male flounder have provided convincing evidence that estrogenic exposure occurs in U.K. estuarine and marine waters at biologically significant concentrations, but preliminary studies of the possible effects indicate that intersex prevalence does not exceed 20% in fish from the most polluted estuaries (Figure 5). The causes of these phenomena in flounder are unknown, but industrial effluents may play a greater role than seems to be the case in fresh waters (Matthiessen et al., 1998).

As shown in a recent paper by Larsson et al. (1999), endocrine disruption may represent a widespread but largely unrecognized adverse health effect in fish downstream municipal sewage treatment works. Substances found include the synthetic estrogen 17 α -ethinylestradiol, the natural estrogens 17 β -estradiol and estrone, as well as nonylphenol and bisphenol A. Following a 2-week exposure of caged male rainbow trout, elevated blood levels of vitellogenin were observed, and chemical analysis revealed that all the above-mentioned estrogenic compound were present in a conjugated form in the bile of these fish. The results were concluded to indicate that synthetic estrogens (oral contraceptives) give a major contribution to the estrogenic effect.

Laboratory experiments have shown that natural steroids and various xenobiotics are capable of inducing vitellogenin and the development of ovary tissue in male fish, but that interspecies differences clearly exist. For example, exposure of guppy (*Poecilia reticulata*) and medaka (*Oryzias latipes*) during 1 to 3 months to β -HCH caused particularly in

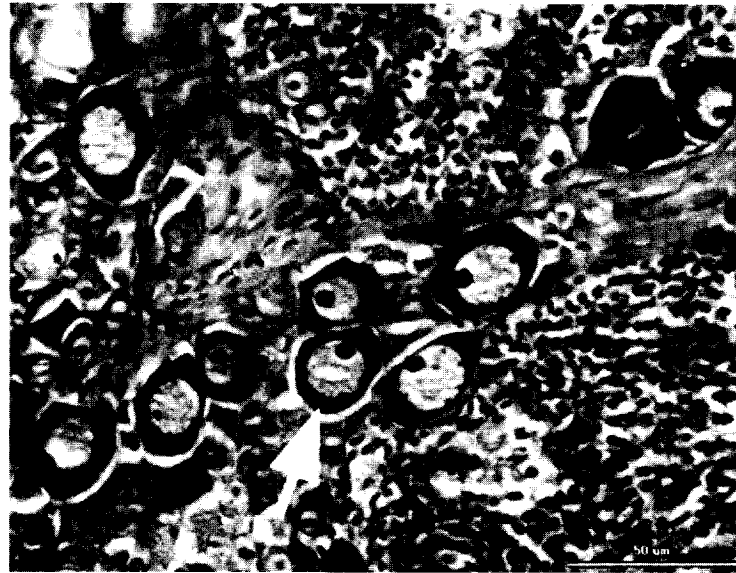


FIGURE 5. Histology of the testis of male flounder with characteristics of intersexuality (hermaphroditism): besides spermatogenesis oocytes are also present (arrow). (Photo courtesy of S.W. Feist.)

guppy and to a lesser extent in medaka induction of vitellogenin, while ovotestis was only induced in medaka (Wester et al., 1985; Wester and Canton, 1986). Further, Gray and Metcalfe (1997) found intersex conditions in medaka exposed from hatch to 3 months of age: in fish exposed to 50 mg/l and 100 mg/l 4-nonylphenol, male fish developed 50 and 86% testis-ova, respectively. The ratio male/female for the 100 mg/l dose showed a significant altered sex ratio (1:2, M:F) in comparison with the control (2:1, M:F). Young rainbow trout i.p. exposed to chlordecone, *o,p*-DDT and *o,p*-DDE showed *in vivo* induction of vitellogenin at relatively high liver residue concentrations, which indicates a lower estrogenicity of these compounds for this particular species (Donohoe and Curtis, 1996). Gimeno et al. (1996) showed that for carp (*Cyprinus carpio*) exposure to 17 β -estradiol, genetically male fish developed female sexual characteristics resulting in a total phenotypic sex reversal of the exposed fish. It is still not clear whether

the observed effects in wildlife species such as the increased and nonfunctional vitellogenin production and ovotestis in males lead to adverse effects at the population level. The induction of vitellogenin in male rainbow trout has been associated with retarded testicular growth (Jobling et al., 1996). Further, it has been demonstrated that the unusual production of vitellogenin in male fish can result in kidney damage (Wester and Canton, 1986), and the suggestion was made that it may reduce metabolic expenditure on growth and spermatogenesis (Herman and Kincaid, 1988; see also Sheahan et al., 1994). An increased plasma level of vitellogenin has been associated recently via a feedback mechanism with reduced estradiol production (Reis-Henriques et al., 1997), which in turn can negatively influence the egg quality. Vitellogenin induction in female fathead minnow (*Pimephales promelas*) exposed long-term (300 days) to 17 α -ethinylestradiol has been associated with a decreased egg production (Laenge et al., 1997).

2. Effluent from the Paper Industry

The reproductive toxicity of bleached kraft pulp mill effluents (BKME) in fish was examined in a research program sponsored by the Swedish EPA. In the middle of 1980s fish caught near Swedish pulp mills frequently showed physiological disturbances, including reduced plasma sex hormone levels, reduced gonad growth, and delayed sexual maturity, suggesting exposure to endocrine-disrupting substances (Andersson et al., 1988; Sandström et al., 1997). These effects were attributed to the chlorine bleaching process, which results in the formation and release of a vast number of chlorinated organic chemicals, many of unknown structure. As a result of these studies, the chlorine bleaching process was successively abandoned. Although reproductive disturbances and other fish health effects have been less frequently observed during the 1990s, reproductive disorders are still considered as a major remaining effect of pulp mill effluents. As determined by vitellogenin measurements in male and female perch (*Perca fluviatilis*) caught near pulp mills, a weak estrogenic effect in male fish and possibly a weak antiestrogenic response in female fish have been indicated recently. The chemicals causing these effects are presently unknown (Förlin et al., 1999).

Endocrine disrupting of reproductive function resulting in masculinization (changes of secondary sex characteristics) of fish from exposure to effluents from kraft pulp mills has been demonstrated in various studies in the U.S. and Canada (Van der Kraak et al., 1992; McMaster et al., 1991; Gagnon et al., 1995; Leblanc et al., 1997). For example, masculinization and a variety of other biochemical, physiological, metabolic, and behavioral disturbances were observed in viviparous poeciliid fishes during or after laboratory or *in situ* exposure to effluents from KME, including a very complex mixture of natural and anthropogenic compounds (Drysdale and Bortone, 1989; Howell and Denton, 1989; Davis and

Bortone, 1992). Laboratory studies also showed lower egg production and delayed reproduction in fathead minnow exposed to BKME (Kovacs et al., 1995). *In vitro* studies of Timothy et al. (1995) demonstrate that pulp and paper mill black liquor and effluent fraction contain estrogen- and dioxin-like compounds. Identified compounds include plant sterols, polychlorinated dibenzofurans and thianthrenes, dibenzothiophenes, and diphenyl sulfides, all of which have been detected in the vicinity of pulp and paper mills. Based on the dose-response curves, black liquor and the effluent fraction contain 10 ± 4 ppb and 20 ± 6 ppt "TCDD equivalents", respectively. Several studies have shown that TCDD and related compounds exhibit dioxin receptor-mediated antiestrogenic activity and can disrupt female reproduction in fish. In another field study, in white sucker (*Catostomus commersoni*), a large number of disturbances of the pituitary/hypothalamus axis at different levels was demonstrated as a consequence of exposure to BKME (Van der Kraak et al., 1992), resulting in a disrupted steroid synthesis by the ovary, a reduction of plasma steroid concentrations, and altered peripheral metabolism of steroids (Van der Kraak et al., 1992). The ecological significance of pollution-induced sex alteration has been discussed by Davis et al. (1992). The combination of various studies and their own observations of the "highly" masculinized females from BKME effluents let them to strongly suspect that reproductive function becomes impaired, if not entirely lost, after continued BKME exposure. Furthermore, the long-term effects of EDCs would include reduced embryo viability and modification, or perhaps neutering of female reproductive function. More recently, Leblanc et al. (1997) observed a modification of the time of onset, intensity and duration of the spawning period in *Fundulus heteroclitus*, a small sentinel fish species exposed to BKME in the Miramichi estuary, New Brunswick, Canada. The onset of gonadal maturation was delayed and egg size was reduced in fish

captured downstream from the mill. However, at this site, fish also demonstrated a marked increase in reproductive investment and increased fecundity. A general problem when dealing with the complex BKME is the identification of the causative chemicals responsible for the observed reproductive effects.

3. Polluted Habitats

A number of field studies suggest a decreased reproductive success in fish as a consequence of exposure to xenobiotics, including known endocrine disrupters in polluted habitats, such as industrialized estuaries and coastal waters (e.g., Johnson et al., 1988, 1997a,b; Spies and Rice, 1988; Nagler and Cyr, 1997; Hansen et al., 1985; Petersen et al., 1997). In most cases the ecological significance of these phenomena on reproductive output on the individual and populations level is not clear. Generally, knowledge on responsible mechanisms is lacking and no clear cause-and-effect relationships between specific chemicals and the reproductive effects observed have been established. For example, Johnson et al. (1988) described the inhibition of gonadal recrudescence and reduced levels of plasma estradiol in female English sole (*Parophrys vetulus*) from a heavily polluted area. Because both conditions were associated with elevated hepatic aryl hydrocarbon hydroxylase activity, it was tentatively concluded that the disposition of estradiol and consequently the ovarian development was influenced via interference of xenobiotics with enzymes involved in steroid metabolism. Of the two classes of xenobiotics suspected, aromatic hydrocarbons (AH) and organochlorine compounds, the AHs appeared to be most closely associated with the inhibited ovarian development and depressed plasma estradiol. Johnson et al. (1997a) also reported, on an individual fish basis, elevated tissue PCB con-

centrations that were significantly correlated with low-plasma alkali-labile protein (an indicator of vitellogenin levels), reduced egg weight, and reduced egg number, whereas elevated biliary fluorescent aromatic compounds were associated with increased ovarian atresia, increased egg weight, and reduced egg number. The authors stated that nutritional and other environmental factors may also contribute to the observed intersite differences in egg weight and fecundity. In a more recent publication more evidence was found for a close association between aromatic hydrocarbons and inhibited gonadal development in adult sole, while both chlorinated (HCB, DDT, and PCBs) and aromatic compounds were identified as potential risk factors for precocious maturation, a phenomenon that was not included in the previous studies (Johnson et al., 1997b). Histological examination of gonads obtained from plaice (*Pleuronectes platessa*) from petroleum-contaminated estuaries following the Amoco Cadiz oil spill (1978) along the coast of Brittany showed evidence of disruption of the normal ovarian cycle, but the underlying mechanisms remain unclear (Stott et al., 1983). Laboratory studies show that a large variety of pollutants, including heavy metals, pesticides, and organochlorine pollutants, may adversely affect reproduction in a large number of fish species and that exposure to 0.001 mg l⁻¹ (1 ppb) of pollutant is generally sufficient to produce harmful effects for long-term exposure (for review of literature up to 1993 see Kime, 1995). In a recent laboratory study, young rainbow trout exposed to environmental relevant concentrations of PCBs (Aroclor 1260) showed altered sex ratios (less females) and serious abnormalities of the gonads (inhibition of the development of the oocytes) (Baker Mata et al., 1998). It is not clear whether the observed phenomena are estrogen receptor mediated or inhibition takes place of the enzymes that convert testosterone into estrogen. The latter has been demonstrated in another

study in which short-term treatment with aromatase inhibitors resulted in the masculinization of female chinook salmon larvae (Piferrer et al., 1994).

Pollution-induced asynchrony in ovarian development (with 3 to 4 months) of flounder has been demonstrated in a 3-year semi-field study performed in The Netherlands. In this study, flounder were chronically exposed to polluted dredged spoil from Rotterdam harbor. Female fish exhibited premature vitellogenin production, which was attributed to enhanced plasma estradiol. The authors also performed laboratory experiments and found indications that the increased estradiol levels resulted from decreased clearance rather than enhanced ovarian production (Janssen et al., 1997). Pereira et al. (1992) have demonstrated enhanced blood vitellogenin in female winter flounder (*Pleuronectes americanus*) from polluted estuarine environments on the east coast of North America, although this also could be the result of impaired ovarian uptake of vitellogenin rather than elevated vitellogenin production.

Long-term data on fishery statistics were retrospectively analyzed (1960 to 1995) to assess a possible effect of environmental contaminants on reproductive status and population size of commercial fish species (Rijnsdorp and Vethaak, 1997). The data on North Sea plaice (*Pleuronectes platessa*) and sole (*Solea solea*) populations showed a decrease in size and age to maturation, but no change in sex ratios were observed. Lang et al. (1995) have shown anomalies in the sex ratio of dab (*Limanda limanda*) from the North Sea, with increased representation of females in some areas, and decreased representation in others. In Canada, in a semifield study, American plaice from the St. Lawrence have also shown a decrease in size and age to maturation (Nagler and Cyr, 1997). It appears unlikely, however, that these changes in North Sea plaice, sole and dab, and American plaice are significantly related to spe-

cific contaminants, but rather may be caused by changes in population dynamics.

4. M74 Syndrome and EMS Syndrome

An intriguing, but still unresolved, case is the M74 syndrome (environmentally related disease 1974) in Baltic salmon and the equivalent EMS (Early Mortality Syndrome) syndrome in salmonids of the North American Great Lakes (AFS, 1996). The M74 syndrome has caused high mortality in fry of sea-run Atlantic salmon (*Salmo salar* L.) from the Baltic Sea. The cause of M74 is largely unknown, but the pathology has been well described and treatment with thiamin (vitamin B1) in the hatcheries has been found to cure afflicted fry (Bengtsson et al., 1999). Reduced hatching success and diminished fry survival have been observed in lake trout eggs experimentally exposed to PCBs and DDTs, and it was found that there was a gradual increase in hatchability and fry survival of Lake Michigan trout since 1984 following a reduction in organochlorine levels in Great Lakes (Mac and Edsall, 1991). The M74 syndrome shows great similarities to EMS in salmonids from the North American Great Lakes (Fitzsimons et al., 1999), a condition also characterized by a diet-related deficiency of thiamin in fry and brood stocks. A combination of factors may contribute to M74, for example, the diet of salmon in the Baltic Sea, the content of thiamin or thiaminase in their prey fish, ecological changes in Baltic food chains, and environmental pollutants. One of the most probable hypothesis for these reproductive disorders is that toxic symptoms are expressed as a result of vitamin B1 deficiency in the early life stages (SEPA, 1995; Amcoff et al., 1999). In a publication by Vuorinen et al. (1997a), dioxin-like compounds, especially certain PCDDs and PCDFs, were found to have a significant connection

to the occurrence of M74 in Baltic salmon. However, there is no firm evidence to causally link these contaminants with the disease development. The same group (Vuorinen et al., 1997b) also suggested that changes in thyroid hormone status and retinoids may play a role in the M74 syndrome, but the results in this respect are still inconclusive. A basic problem is to fully understand the environmental factors influencing the occurrence of this disease syndrome, which affects the few remaining wild, naturally spawning populations of Baltic salmon that are at great risk of extinction. At present, there seems to be no firm evidence to support that endocrine-disrupting or other chemicals are involved in the etiology of M74 and EMS.

B. Nonreproductive Effects

Nonreproductive effects have been reported in several fish species (Table 4b). Suggestions have been made that larval deformities and increased mortality in pelagic eggs of various fish species and demersal eggs of herring in the Baltic Sea are caused by chlorinated hydrocarbons and other highly persistent bioaccumulative compounds (Von Westernhagen et al., 1988). In addition, there have been reports since the early 1990s of a widespread occurrence of embryonic deformities in pelagic eggs of several fish species in coastal waters in the North Sea (Cameron et al., 1992). It is not yet clear, however, whether these fluctuations reflect exposure to endocrine disruptors or other pollutants or may be caused by natural factors such as temperature, as the data also indicated a strong temperature-related correlation (Dethlefsen et al., 1996).

Several studies have shown that potential adverse estrogenic effects on fish can also alter growth in fish. Growth reduction has been observed in catfish exposed to DES (Bulkley, 1972). Johnstone et al. (1978) described a significant suppression of both length and weight in rainbow trout during a period of

dietary administration of 17 β -estradiol. Of particular environmental concern are the recent experiments conducted by Ashfield et al. (1998). They examined in a series of experiments the long-term effects of different alkylphenol ethoxylates (nonylphenol, octylphenol, NP1EC) at environmentally relevant concentrations (1 to 50 mg/l) on growth and ovosomatic index in female juvenile rainbow trout. Exposure to alkylphenol ethoxylates commenced at hatch (day 0) to include the "critical window" of estrogen sensitivity, and was terminated on day 22 or 35 followed by sampling at regular intervals up to 108 and 466 days. From the results of both experiments it was apparent that all the test compounds modified the growth of exposed fish. They often found, however, variable physiological effects in the juvenile fish: no clear dose-related effect was observed. In most cases growth was suppressed, but in some cases growth was accelerated. The authors stated that very similar, rather variable effects on growth have been observed in juvenile rats exposed via their drinking water during pregnancy and lactation to alkylphenol compounds (Sharpe et al., 1995). The similarity between data obtained in the rat and fish study suggest that alkylphenol compounds are capable of influencing growth in both juvenile fish and mammals. Although it is possible that alkylphenol chemicals influence growth in fish and mammals via a direct estrogenic effect, other mechanisms of action are more likely. They concluded that exposure of natural populations of fish to these chemicals at concentrations currently measurable in the aquatic environment may have an impact on the performance of those populations. Survival of fish in the natural environment is strongly influenced by body size and an appropriate ovosomatic index is a crucial factor in successful reproduction. Survival of fish in the natural environment is closely linked to growth rate, primarily because smaller fish compete less successfully for resources, such as food and territory, than do larger fish (Elliott, 1990).

TABLE 4b
Nonreproductive Effects in Fish

Effect/disorder	Location	Species	Associated contaminants	Ref.
Embryonic malformation in pelagic eggs	North Sea coastal waters	Various fish species	Unknown	Cameron et al., 1992
Developmental defects in embryos	Western Baltic Sea	Various pelagic fish species	Unknown	Von Westernhagen et al., 1988
Thyroid hyperplasia (up to 100%), decreased fecundity	Great Lakes, U.S.	Salmonids	Unknown	Leatherland, 1992
Reduced vitamin A levels	Netherlands semi-field study	Flounder (<i>Platichthys flesus</i>)	PHAHs	Besselink et al., 1998

A large number of factors can influence the immune response in fish, including species, age, reproduction, social status, food, stress, and water temperature (Anderson, 1993; Wester et al., 1994). Furthermore, several contaminants, including endocrine disrupters such as PCBs, PCDDs, and TBT, may have a role in immunosuppression in fish that may be associated with an increased prevalence of diseases in wild populations (Vos et al., 1996). In recent decades there have been many reports indicating widespread occurrence of epizootic skin and liver diseases, including cancer, in wild fish populations in connection to polluted habitats (Vethaak and ap Rheinallt, 1992; Sindermann, 1993). However, there is an absence of mechanistic and experimental data elucidating the possible effects of these compounds on fish disease.

Recent evidence demonstrates that estrogens, either natural or xenobiotic, may promote the growth of hepatic preneoplastic lesions and tumors in fish. Cooke and Hinton (1999) have shown that 17 β -estradiol is a tumor promoter in medaka and β -HCH a weakly positive modulator. 17 β -estradiol particularly promoted tumor development in male medaka, indicating xenobiotics with mechanisms of action like that of 17 β -estradiol may promote growth in wild fish of previously initiated cells into tumors (Cooke and Hinton, 1999). Although liver neoplasia in wild fish populations is primarily attributed to the carcinogenic properties of polycyclic aromatic hydrocarbons (De Maagd and Vethaak, 1997; Myers et al., 1990), endocrine-disrupting compounds could be involved as both risk factors for immunosuppression and tumor promotion.

Epizootics of thyroid hyperplasia and hypertrophy (affecting 100% of the population) have been reported in various species of salmon of the Great Lakes (see review of Leatherland, 1992). There is, however, no firm evidence linking thyroid hyperplasia observed in Great Lakes salmon with any specific chemical contamination (LC Forman, personal communication in EPA, 1997). Further, non-

reproductive effects in fish include a significant reduction in retinoid levels in both liver and plasma in flounder in polluted mesocosms, providing a clear indication that retinoid levels are affected by long-term exposure to contaminants (Besselink et al., 1998). Together, these observations suggest that in fish, as in mammals, thyroid function appears to be sensitive to contaminant exposure.

VI. INVERTEBRATES

A. Reproductive and Nonreproductive Effects

Reported studies of both reproductive and nonreproductive effects in various groups of invertebrates are considered in this section (Table 5).

Although invertebrates constitute about 95% of the faunal species and are key components of all ecosystems, relatively little is known about their endocrine systems and their susceptibility to environmental endocrine disruption. Utilization of hormones to control and coordinate biochemical, physiological, and behavioral processes is common to all invertebrate taxa. The majority of invertebrate hormones identified to date are peptide neurohormones, but non-peptide endocrine messengers such as ecdysteroids and juvenile hormones are of importance in many groups especially insects and crustaceans (for review see Pinder and Pottinger, 1998). Both of these systems are potentially susceptible to interference by environmental contaminants. A range of vertebrate steroids (androgens, estrogens, progestins, corticosteroids) have been detected in insects and crustaceans, but their functional role remains to be established. Mollusks utilize a wide range of peptide hormones to control and coordinate physiological processes, but ecdysteroids do not appear to play an important role in this group. The presence of vertebrate-type steroids has been reported for a number of

TABLE 5
Reproductive and Nonreproductive Effects in Invertebrates

Effect/disorder	Location	Species	Associated contaminants	Ref.
Imposex	World-wide different geographic regions	150 different species of prosobranchs snails	TBT	Fioroni et al., 1991; Oehlmann et al., 1998; Matthiessen et al., 1999
Imposex	UK and French coastal waters	Dogwhelk (<i>Nucella lapillus</i>)	TBT	Gibbs et al., 1987; Bryan et al., 1988; Huet et al., 1996 Strand, 1998
Imposex	Danish waters	Common whelk (<i>Buccinum undatum</i>)	TBT	Barreiro et al., 1998; Coelho et al., 1998; El Hamdani et al., 1998
Imposex	Iberian Atlantic Sea	Several species of gastropods	TBT	Mensink et al., 1997; Hallers-Tjabbes et al., 1994
Imposex and reduced growth	Eastern Scheldt, North Sea	Common whelk (<i>Buccinum undatum</i>)	TBT	Terlizzi et al., 1998; Morcillo and Porte, 1988; Sole et al., 1988 Bright and Ellis, 1990
Imposex	Mediterranean Sea	<i>Hexaplex trunculus</i> , <i>Bolinus brandaris</i>	TBT	
Masculinization (imposex) loss of reproductive potential	Northeast Pacific	Several neogastropod species	TBT	
Imposex	Japanese waters	Rock shell (<i>Thais clavigera</i>)	TPHT	Horiguchi et al., 1994
Intersex	North Sea	Periwinkle (<i>Littorina littorea</i>)	TBT	Bauer et al., 1995
Abnormally developed shells; declined spatfall	France, Arcachon Bay; UK and several other countries, estuaries	Pacific oyster (<i>Crassostrea gigas</i>)	TBT	Alzieu et al., 1986, 1991; Waldock and Thain, 1983; Stephenson, 1991
Intersex	Scotland	Harpacticoid copepods	Sewage	Moore and Stevenson, 1994

TABLE 5 (continued)
Reproductive and Nonreproductive Effects in Invertebrates

Effect/disorder	Location	Species	Associated contaminants	Ref.
Previtellogenic oocytes; altered steroid profiles in testes and hemolymph	Atlantic Canada, Halifax Harbor	Lobster (<i>Homarus americanus</i>)	Contaminants	Sangalang and Jones, 1997
Effects on CYP450 monooxygenase; no effect on oocyte maturation	North Sea	Sea star (<i>Asteria rubens</i>)	Pollutants unknown	Postma and De Valk, 1996, 1997

molluskan species. In some cases, especially in the prosobranch gastropods, there is strong evidence that these steroids play a functional role. Evidence for a significant role of vertebrate-type steroids is strongest within the echinoderms where they may play a role in the control of growth and reproduction. For other invertebrate taxa there is only limited information on endocrine-type processes (Pinder and Pottinger, 1998). Given the physiological diversity it is likely that an endocrine-disrupting chemical would have a different effect in different invertebrates.

1. Tributyltin-Induced Imposex in Marine Snail

To date, the tributyltin (TBT)-induced masculinization (imposex/intersex) in female mollusks, particularly prosobranch snails belonging to the orders Mesogastropoda and Neogastropoda, is the best example of endocrine disruption in invertebrates that is causally linked to an environmental pollutant (for reviews see Fioroni et al., 1991; Matthiessen and Gibbs, 1998). It is also a key example of population-level impact resulting from reproductive abnormalities. Widespread TBT contamination derives from the antifouling paint active ingredient TBT applied below the waterline on ship hulls. Imposex comprises the growth of a penis and occlusion of the oviduct due to the development of a superficial vas deferens. Whether imposex results in sterilization depends in part on the normal morphological configuration of the reproductive apparatus. Imposex-affected populations have been observed in the wild in 150 different species of marine prosobranch snails (Matthiessen et al., 1999) from across the world. Imposex-affected populations have been seen in Europe, in many estuaries (Gibbs et al., 1987; Huet et al., 1996; Fioroni et al., 1991; Oehlmann et al., 1996; 1998; Mensink et al., 1997; Strand, 1998), the Iberian Atlantic and the Mediterranean Sea (Terlizzi et al., 1998; Morcillo and Porte, 1988; Sole et

al., 1988; Coelho et al., 1998). All populations of the dogwhelk in the coastal areas of the North Sea are more or less affected by imposex leading in some areas to complete population loss (North Sea Task Force, 1993). Signs of imposex were observed in the majority of neogastropod taxa in waters of British Columbia (Bright and Ellis, 1990). Recently, imposex has been reported in the common whelk *Buccinum undatum* from the open North Sea where 20 years ago no signs of imposex were observed, and this is apparently related to the intensity of commercial shipping traffic in the area resulting in release of the anti-fouling ingredient TBT (Hallers-Tjabbes et al., 1994) (Figure 6). Laboratory studies show that imposex development occurs at very low exposure concentrations to TBT (1 ng/l) in the dogwhelk (Gibbs et al., 1987) and 7 ng/l in common whelk (Mensink et al., 1997). The most sensitive species currently seems to be *Ocenebrina aciculata* with a threshold concentration of 0.1 ng TBT-Sn/l (see Oehlmann et al., 1996). Based on these laboratory studies with the wildlife species, it is concluded that the concentrations of TBT measured in areas with imposex are sufficiently high to cause this condition.

How TBT induces imposex is not precisely known, but a considerable weight of evidence suggests an inhibition of aromatase enzyme metabolism of testosterone to 17 β -estradiol (Bettin et al., 1996). This can lead to increased testosterone levels and masculinization. In the dogwhelk (*Nucella lapillis*, Neogastropoda) the degree of masculinization is a graded response dependent on the level of TBT exposure (Bettin et al., 1996). Notably, exposure to testosterone may also lead to imposex. Simultaneous exposure to TBT (50 ngSn.l) and an anti-androgen (1.5 mg cyproterone acetate) did not result in imposex in contrast to the same concentration of TBT alone (Oehlmann et al., 1996). Exposure to the aromatase inhibitor SH-489 also resulted in imposex. These results can all be explained by the hypothesis that TBT interferes with the synthesis of steroid hor-



FIGURE 6. A female whelk from the open North Sea showing the development of a penis (imposex). (Photo courtesy of H. Kralt.)

mones by inhibiting the conversion of testosterone to estradiol by the enzyme aromatase (CYP19). In addition to aromatase-inhibition, another hypothesis has been postulated recently, namely, that TBT inhibits the formation of sulfate conjugates of testosterone, thus resulting in reduced elimination of testosterone from the body (Ronis and Mason, 1996). Furthermore, because TBT is reported to accumulate specifically in neural ganglia in the dogwhelk (Bryan et al., 1993) and common whelk (Mensink et al., 1997), it is possible that interference with the neuroendocrine system by TBT could result in the development of imposex, mediated by alteration of steroid metabolism. In England, Norway, and in the Pacific Ocean recovery of imposex-affected populations of dogwhelks and whelks have occurred after measures were taken to cease the use of TBT on ships smaller than 25 meters (Tester et al., 1996; Evans et al., 1994; Smith, 1996). Despite these reports that TBT concentrations in coastal waters and imposex occurrences in the U.K. and elsewhere are generally in decline, evidence put forward by other authors

indicate that organotin concentrations are still high enough to endanger sensitive species (see Oehlmann et al., 1998).

Another condition termed intersex is observed exclusively in the periwinkle (*Littorina littorea*; Mesogastropoda). Intersex is defined as a disturbance of the phenotypic sex determination between gonad and genital tract and is characterized by supplantation of the distal part of the pallial oviduct by a prostate gland, a sperm groove and ultimately also a penis, whereas the proximal part of the pallial oviduct retains its female structure (Bauer et al., 1995, 1997). Intersex in the periwinkle occurs in populations in the German Wadden Sea; the fertility of female animals was affected in the direct vicinity of harbors and marinas. Intersex development was found at concentrations of 10 to 15 ng/liter. Juvenile animals seem more sensitive for these effects than adult animals. For example, adult periwinkle are much less sensitive for TBT, although high concentrations show a significant decrease in egg production. In periwinkle, adult females lose their sensitivity to TBT once the whole pallial oviduct is established (Bauer

et al., 1997). However, exposure to concentrations > 100 ng TBT/l reduces the egg production significantly in females of this species (Matthiessen et al., 1995). Laboratory experiments showed that exposure to concentrations higher than 10 ng TBT acetate/l induced the development of male sexual characteristics in a dose-dependent manner when whelks were exposed *in ovo* or just after hatching. Growth rates were affected at an even lower dose. However, adult female whelks were not affected (Mensink et al., 1996).

In addition to TBT, antifouling paints may contain triphenyltin (TPhT), a chemical that is heavily used in agriculture as a fungicide, but has also been added to technical formulations of antifouling paints (up to 10% of the TBT concentration). TPhT has been found in sea stars and (imposex-affected populations of) whelks in the North Sea and Eastern Scheldt (NL) (Mensink et al., 1997). Imposex was also found in Japanese waters in areas with a low shipping traffic intensity, but which are likely to receive TPhT compounds from neighboring agricultural areas (Horiguchi et al., 1994). Experimental evidence with regard to the ability to develop imposex due to TPhT exposure is at present contradictory, because both negative and positive experimental studies have been reported (Bryan et al., 1988; Horiguchi et al., 1997). From the positive study it can be concluded that TPhT enhances imposex development in the rock shell (*Thais clavigera*). Thus, a role of TPhT with respect to the development of imposex seems quite likely in this species. Hawkins and Hutchinson (1990) also reported that monophenyltin (MPhT) induces imposex in *Ocenebra erinacea*.

In several species of bivalve mollusks, increased shell thickness resulting from TBT exposure has been reported. In bivalves, TBT is highly toxic to larvae and gives rise to malformations of the shells of adult animals by inhibition of calcification, but the exact underlying mechanism is presently unknown. Changes in morphology of the sexual organs

have not been found in these species (Alzieu et al., 1991).

Deleterious effects of organotin compounds were first noted in Pacific oyster (*Crassostrea gigas*) populations in Arcachon Bay, France, in the late 1970s: from 1977 to 1983 oyster production was severely affected (Alzieu et al., 1986, 1991). Shells developed abnormally and spatfall declined dramatically. Abnormally developed shells have been observed also in the U.K. and in estuaries of several other countries (Waldock and Thain, 1983; Stephenson, 1991). Shell abnormalities included chambering and the formation of a protein-containing jelly and effects on calcification occurred at concentrations of TBT as low as 2 ng/l, although the sensitivity varied between oyster species. Larvae survived only a few days in Arcachon Bay water and retarded growth and high mortality occurred in larvae exposed to 50 ng/l of TBT in laboratory tests. At a concentration of 20 ng/l growth of *C. gigas* was inhibited, but the related species *C. virginica* was less sensitive. Effects of TBT on reproduction have also been reported for several other bivalve mollusks, including the native oyster *Ostrea edulis* (Thain et al., 1986) and the deposit feeding clam *Scrobicularia plana* (Ruiz et al., 1994, 1995a,b). In the latter field and laboratory studies it was found that the toxic action of TBT has a negative influence on embryonic development and could have contributed in northern Europe to population declines or disappearances from certain estuarine sites. Remarkably, there are no reports in the literature on effects of TBT and related compounds in fresh water mollusks. From a population point of view the effects on reproduction are obviously more important than shell deformities (see above).

There is evidence that the endocrine-disrupting effects of TBT have led to changes in marine communities. Detailed temporal trend studies of benthic and epibenthic community structure (Waldock et al., 1999; Rees et al., 1999) have been conducted in the Crouch

estuary, U.K., since 1987 when the use of TBT for small vessels was banned. The estuary is rather unique because of its primary TBT inputs; other polluting inputs to the estuary have been negligible historically. The findings show over a period of 5 to 10 years a considerable increase in species diversity in the upper- and mid-estuarine areas where TBT concentrations declined to relatively low levels. However, there were no significant changes in the originally less-contaminated areas of the outer estuary. Therefore, it is likely that the effects were at least partly due to the decline in TBT input. Recovery was seen not only in several mollusk species (including the oyster *Ostrea edulis*), but also in crustaceans and ascidians, although it is not known whether these latter groups were originally affected directly or indirectly.

Another observation in invertebrates concerns the very high proportion of intersex in harpacticoid copepods in the Firth of Forth in Scotland (Moore and Stevenson, 1994). This observation has been linked but not confirmed to a sewage effluent with elevated levels of alkylphenoxy-ethalates from the local textile industry. Furthermore, the lobster *Homarus americanus* has been observed with ovotestis in Nova Scotia, but it was not clear whether this was a natural or site-related phenomenon (Sangalang and Jones, 1997).

Semifield studies with the echinoderm *Asterias rubens* have shown that rates of P450-dependent steroid metabolism can be decreased as a result of exposure to contaminants. Short-term cadmium exposure of this animal affected steroid synthesis and tissue levels of progesterone and testosterone (Voogt et al., 1987). Den Besten (1991a,b) found that both cadmium and PCBs significantly reduced levels of progesterone and testosterone in the pyloric caeca of male and female *Asterias rubens*. No effect of cadmium was found in the gonads, while PCB exposure resulted in increased testosterone levels in testes and ovaries. Although the physiological

role of progesterone and testosterone in sea stars is still unknown, it has been suggested that changes in their levels represent a hormonal trigger for the pyloric caeca, being the onset of vitellogenesis and gametogenesis (Voogt et al., 1987). Results from field studies are inconclusive. In a number of surveys in the North Sea, *Asteria rubens* were collected at different sites in order to investigate the quality of their offspring and to perform biomarker measurements. No pollution gradient-related effects were observed on oocyte maturation or on the early development of sea star embryos. However, slight effects were observed on the P450 system in the pyloric caeca of female sea stars collected from polluted coastal sites under the influence of rivers like Humber, Elbe, Rhine, and Western Scheldt (Postma and De Valk, 1996 and 1997). Given the experimental findings, these decreased P450 activities found in the North Sea study may signal adverse effects.

A range of pesticides that act on insect endocrine systems have been deliberately introduced in the environment and have shown to have also impacts on non-target species. There are no documented examples of non-pesticidal EDC-related effects on freshwater and terrestrial invertebrates. There are, however, a substantial number of laboratory based studies that identify the effects of potential EDCs on freshwater and terrestrial invertebrate species (for literature overview see Pinder and Pottinger, 1998; Janssen et al., 1998). Woin and Bronmark (1992) showed that exposure to non-lethal concentrations of DDT cause a substantial reduction in the fecundity of the freshwater snail (*Lymnaea stagnalis*). More than 100 phytosteroids are structurally similar to ecdysteroids and have been shown to exert hormonal activity in insects (Adler and Grebenok, 1995). For example, moulting was arrested in dargonfly larvae (*Macromia cingulata*) when exposed to paper and pulp mill effluent containing phytoecdysteroids (Subramanian and Varadaraj, 1993). Alkylphenol (4-NP) apparently reduces the rate of

elimination of testosterone in freshwater crustacean *Daphnia magna* leading to accumulation of androgenic products and reduced fecundity of females (Baldwin et al., 1997). The alkylphenol *p*-tert-pentylphenol (PTP) may induce irreversible changes in morphology and reproduction of *Daphnia magna*, including masculinization of females (Gerritsen, 1997).

VII. DISCUSSION WEIGHT OF EVIDENCE ON WILDLIFE HEALTH EFFECTS

The precise endocrine mechanisms regulating the reproductive process of a species are diverse and may be influenced by a range of biological and physicochemical factors. These environmental factors include xenoestrogens and other chemicals observed to affect the reproductive physiology of fish and other wildlife species (Campbell and Hutchinson, 1998). It is also recognized that environmental stress can have an effect on the survival and immunocompetence of fish populations and that such stress can reduce the reproductive success of aquatic and terrestrial species. In addition, nutritional deficiencies may have profound effects on the survival, development, and reproductive health of organisms. Therefore, there is no doubt that a variety of factors impinge on the reproductive output and fitness of natural populations. Many feedback mechanisms and redundancies at various levels of organization have to be overruled before populations are affected.

The ultimate goal of ecological risk assessment is to protect communities and whole ecosystems from detrimental effects caused by chemical pollutants (Landis et al., 1995). To date, however, apart from the community effects of TBT there seems to be no good example to demonstrate that a defined toxic effect resulting from exposure to an endocrine disrupter has caused an adverse effect

that becomes visible at the ecosystem or community level. Moreover, as is evident from the overview of data given above, there are only a few cases where an observed effect on the population level can be firmly linked to a chemical acting on the endocrine system. Some of these cases have been highlighted above. This situation is not unique for endocrine disrupter research but is rather a situation characteristic for (eco)epidemiology in general. While the ecoepidemiological approach aims at establishing relationships between reproductive and health disturbances on the one hand and exposure to environmental pollutants in wild populations on the other hand, it will generally provide little if any information about the underlying mechanisms of action of the chemicals involved. Therefore, it is evident that an evaluation of the ecological significance of endocrine-disrupting chemicals needs to be based on a combination of ecoepidemiological field studies and controlled experimental studies. The strengths and weaknesses of these approaches, depicted in Figure 7, indicate that the so-called semi-field studies would represent a useful approach to bridge the gap between the controlled conditions of the laboratory experiments and the uncontrolled exposure conditions in the field. The use of sentinel species to examine markers for exposure and biological effects, and the application of defined test organisms to determine effects of exposure at contaminated sites, represent promising approaches of semi-field type (*in situ* experiments).

While it seems difficult to reach definite conclusions about cause-effect relationships in the field, there are numerous examples of ecotoxicological effects where a mechanism of endocrine disruption would seem possible or even likely. Several of these cases refer to persistent organic pollutants, many of which are known to exert experimental toxicity by different mechanisms of action relating to endocrine disruption. Compounds of this type include chlorinated hydrocarbons such as PCBs, PCDFs, and PCDDs, as well as hydroxy-

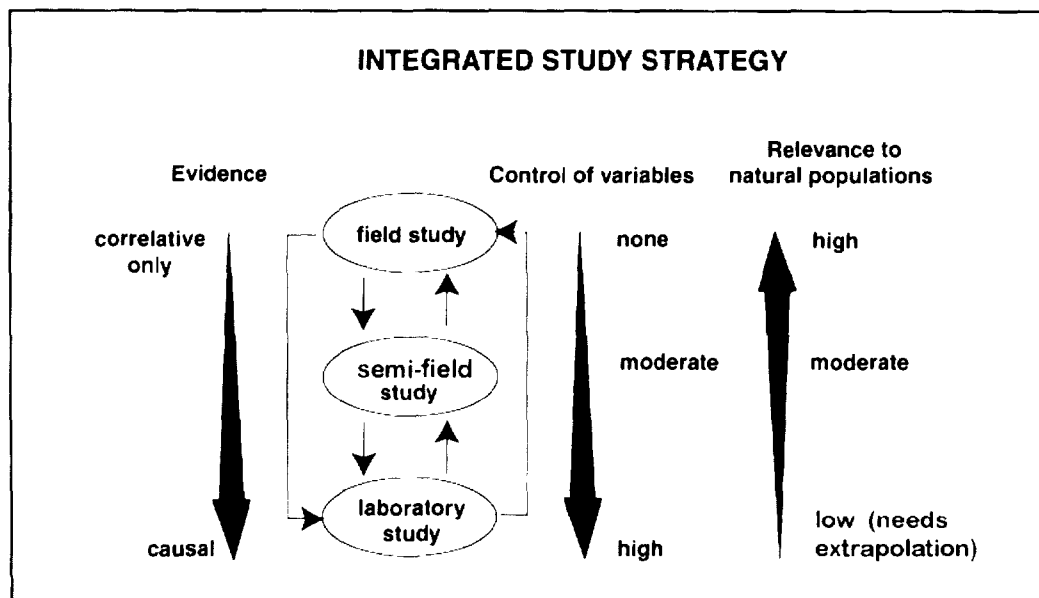


FIGURE 7. Integrated approach to study the causes of wildlife diseases. (After Vethaak, 1993.)

lated and methylsulfonylated metabolites of PCBs and DDT.

The best documented cases of reproductive disturbances and population declines due to exposure by EDCs in Europe and abroad include: TBT-induced imposex in marine snails, DDE-induced eggshell thinning in birds, and reproductive and immunosuppressive effects in seals by PCBs and metabolites. It is remarkable that in the discussed cases of EDC exposure, notably in roach, despite widespread vitellogenin induction and ovotestis formation, no effect has been reported at the population level. For most studies included in this overview, however, the evidence for a causal link to endocrine disruption is weak or nonexisting. Specific remarks for the various animal groups are given below.

A. Mammals and Birds

Most studies on endocrine-modulating effects in mammals refer to aquatic mammalian species, only few to terrestrial species. In most

cases the causal evidence for effects due to EDCs in terrestrial and aquatic mammals remains circumstantial. Apart from the PCB-induced decline of the gray seal population in the Baltic Sea (see above), probably the best evidence for cause-effect relationships comes from Dutch semifield studies on harbor seals, in which both reproduction and immune function impairments have been reported, and PCBs have been implicated. In some cases good correlations are found between, on the one hand, concentrations of certain organochlorines and their metabolites and, on the other hand, hormone concentrations and observed disorders, but the mode of action is not well understood. In many other cases no clear-cut correlations between organochlorine-residue levels and observed effects could be established. This is mainly due to the incomplete nature of the data, for example, lack of data on residue levels, on dose-response relationships, and on ecological background such as normal population parameter values. The complexity of a causal relationship is illustrated by the observation that some of the af-

affected populations to date are growing in number despite clear localized evidence of effects caused by exposure to EDCs. An example is the recently increased population of the Baltic gray seals that occurred despite the fact that this population locally still suffers from lesions induced by persistent organochlorines.

B. Reptiles/Amphibians

There is increasing evidence from non-European countries that some chlorinated aromatic compounds are capable of disrupting reproductive, developmental, and behavioral functions in reptiles and amphibians. Experimental data indicate that larval stages of amphibians may be particularly sensitive to endocrine disruption. So far no effects on amphibian and reptile populations in Europe have been demonstrated, possibly because such investigations have, as yet, not been conducted. Therefore, field and semifield studies are needed throughout Europe to examine the population status of amphibians and reptiles and the possible impact of EDCs on these populations.

C. Fish

There is convincing evidence that the endocrine system of a variety of fish species has been adversely affected by endocrine-disrupting chemicals. The early data indicate that effects occur at geographically defined contaminated sites and sources (e.g., effluents of water treatment plants, wood- and paper-processing industries, textile industry). This exposure appears to be associated with endocrine disruption and reduced reproductive success, which could result in decreased fish populations. However, there are recent indications for a more widespread occurrence of estrogenic effects in certain fish populations in the U.K. Significant estrogenic exposure predominantly due to natural and synthetic

steroids but also to other compounds is not restricted to freshwater systems, but is also demonstrated in estuaries, fjords, and sheltered coastal areas. Endocrine-disrupting compounds associated with physiological and reproductive effects in fish to date are natural and synthetic as well as plant-derived steroids. However, estrogenic effects observed in fish in industrialized estuaries in the North Sea and elsewhere indicate that industrial effluents including alkyl phenols, and conventional problem substances such as DDT, PCBs/PCDFs/PCDDs, heavy metals as well as yet unidentified chemicals may play a more important role. It is unclear to what extent endocrine-disrupting chemicals are implicated in the decline of fresh water/coastal fish populations, although natural factors such as habitat destruction and fisheries impact may override the contaminant effects in most instances. Effects on important offshore fish stocks have not been demonstrated so far, although certain North Sea flatfish appear to be maturing earlier than 30 years ago. However, this effect could be explained by a natural cause. In most cases the biological and ecological significance of observed changes such as precocious female maturation, intersexuality, ovotestis formation, vitellogenin induction for reproductive success is unclear and merits further examination.

Little is known about nonreproductive endocrine effects of contaminants in fish, although an endocrine-disrupting component could play a role in embryo malformation, immunosuppression, decreased vitamin A levels, thiamin deficiency, infectious disease, and epizootic liver tumors in wild fish populations. Chemically induced alterations in immune function occurring in early life-stages or in adult fish can translate into an altered host resistance to infectious diseases (IPCS, 1996). Clearly, more research is required to understand the role of contaminants in susceptibility of fish species to infectious diseases. In particular, more emphasis should be placed on immunosuppressive effects of TBT.

D. Shellfish and Other Invertebrates

Perhaps the best example of an endocrine disruption effect is that of TBT causing imposex worldwide in many species of marine snails. This phenomenon is held responsible for global declines in populations of several species. There is now an understanding of the effects at all levels of biological organization from molecular to population levels. TBT is considered to inhibit the cytochrome P450-dependent aromatase (CYP 19) activity responsible for conversion of testosterone to estradiol. Apart from the example of imposex in marine snails, EDC-induced effects in other invertebrates are poorly documented. Not surprisingly, effects of pesticidal endocrine disruptors on development of single organisms or populations of non-target species have been identified. However, it is likely that endocrine disruption is also occurring in other invertebrates (e.g., crustaceans). Appropriate field studies are therefore needed to investigate the effects of EDCs on invertebrates, especially in the fresh water environment.

VIII. CONCLUSIONS AND RECOMMENDATIONS

Many wildlife species may be exposed to biologically active concentrations of endocrine-disrupting chemicals, including a large number of persistent organochlorines, natural and synthetic steroid hormones, organotins, alkylphenols. There is strong evidence obtained from laboratory studies showing the potential of several environmental chemicals to cause endocrine disruption at environmentally realistic exposure levels. In wildlife populations, associations have been reported between reproductive and developmental effects and endocrine-disrupting chemicals. Effects have been observed in a broad number of species representing many major taxa, including mammals, birds,

reptiles, fish, and mollusks from Europe, North America, and other continents. Most of these studies refer to aquatic food chain organisms, only few to terrestrial systems. The observed abnormalities in various wildlife species vary from subtle changes to overt and permanent structural and functional alterations, including disturbed sex differentiation with malformed (feminized or masculinized) sex organs, changed sexual behavior, and altered immune function.

Impaired reproduction and development causally linked to endocrine-disrupting chemicals are well documented in a number of species and have caused local or regional population changes. These include the following.

A. Marine Snails

Imposex occurs in many species of gastropods and correlates with harbors and shipping lanes. The proven causative agent is tributyltin, a biocide used in antifouling paints. The dogwhelk appears particularly sensitive, and the development of imposex has resulted in decline or extinction of local populations worldwide, including coastal areas all over Europe and the open North Sea.

B. Birds

DDE-induced egg-shell thinning caused severe population declines in a number of raptor species in Europe and North America. Developmental exposure to the DDT complex has been firmly linked to the induction of ovotestis in male Western gulls.

C. Fish

Endocrine-disrupting chemicals have adversely affected a variety of fish species. Effects on reproductive organs are causally linked

to exposure mostly in the vicinity of certain sources (e.g., effluents of water treatment plants, wood- and paper-processing industries, textile industry) and in the most contaminated areas. However, there are recent indications for significant estrogenic exposure of fish not only in freshwater systems, but also in estuaries and coastal areas in the U.K.

D. Mammals

Field studies on Baltic gray and ringed seals, and semifield studies on Wadden Sea harbor seals, have shown that both reproduction and immune functions are impaired by PCBs in the food chain. Reproduction effects resulted in population declines, whereas in impairment of immune function has likely contributed to the mass mortalities due to morbillivirus infections.

E. Reptiles

Distorted sex organ development and function in alligators has been related to a major pesticide spill into a lake in Florida, U.S.A. In experimental studies with alligator eggs, the observed estrogenic/antiandrogenic effects have been causally linked to the DDT complex present in the formulation.

For most other reported effects in wildlife, however, the evidence for a causal link with endocrine disruption is weak or nonexisting. This is mainly due to the complexity of contaminant mixtures, the lack of data on both exposure and sensitivity of the species concerned, along with a poor knowledge of the mechanisms of action. Crucial in establishing causal evidence for chemical-induced wildlife effects are semifield or laboratory studies using the wildlife species of concern.

Although most observed effects currently reported concern heavily polluted areas, there is a potential global problem. This is exemplified by the widespread occurrence of imposex in marine snails and the recent findings of high levels of persistent potential

endocrine-disrupting chemicals in several marine mammalian species inhabiting oceanic waters. Therefore, it is recommended:

- To assess the full environmental significance of endocrine disruption, including studies on effects in terrestrial systems and in amphibian and reptile populations in Europe.
- To conduct further field, semifield, and laboratory studies in order to establish cause-and-effect relationships. Because it is recognized that this relationship is multifactorially defined, including many other environmental or stock-related factors such as habitat destruction and fisheries impact, it is needed to establish baseline data in nonexposed reference populations.
- To encourage the establishment of coordinated biomonitoring programs by international monitoring organizations (e.g., OSPAR, HELCOM, and MEDPOL), the European Environment Agency and national environmental agencies to identify the hazards of endocrine disruption to aquatic and terrestrial wildlife. In doing so, consideration should be given to the strategies and guidelines provided by two international Expert Workshops EMWAT and EDIETA.
- To establish the long-term consequences at the population and community levels of the observed effects on reproductive organ structure and function (such as vitellogenin induction, precocious female maturation, intersexuality, ovotestis formation, and altered sexual behavior), as well as on thyroid status and immune function.

REFERENCES

- Addison RF (1989) Organochlorines and marine mammal reproduction. *Can J Fish Aquat Sci* **46**:360–8.
- Adkins EK (1975) Hormonal basis of sexual differentiation in the Japanese quail. *J Comparative Physiol Psychol* **89**:61–71.

- Adler JH and Grebenok RJ (1995) Biosynthesis and distribution of insect-molting hormones in plants — a review. *Lipids* **30**:257–62.
- AFS (1996) Early Mortality Syndrome: Reproductive Disruptions in Fish in the Great Lakes, New York Finger Lakes, and the Baltic Region. Symposium as part of the 126th Annual Meeting of the American Fisheries Society, August 26–29, 1996, Dearborn, Michigan, U.S.. Abstract volume, 172 pp.
- Aguilar A and Borrell A (1994) Abnormally high polychlorinated biphenyls levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. *Sci. Total Environ* **154**:237–47.
- Allen Y, Matthiessen P, Haworth S, Thain JE, and Feist S (1999) Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*. *Environ Toxicol Chem* **18**:1791–800.
- Alzieu C, Sanjuan J, Deltreil JP, and Borel M (1986) Tin contamination in Arcachon Bay: Effects on oyster shell anomalies. *Mar Pollut Bull* **17**:494–8.
- Alzieu C, Michel P, Tolosa I, Bacci E, Mee LD, and Readman JW (1991) Organotin compounds in the Mediterranean: a continuing cause of concern. *Mar Environ Res* **32**:261–70.
- Amcoff P, Börjeson H, Eriksson R, and Norrgren L (1998) Effects of thiamine treatments on survival of M74-affected ferchi baltic salmon. Early Life Stage Morality Syndrome in Fishes of the Great Lakes and Baltic Sea, *Am Fish Soc.*, **21**:31–40.
- Amcoff P, Börjeson H, Landergren P, Vallin L, and Norrgren L (1999) Thiamine (vitamin B₁) concentrations in salmon (*Salmo salar*), brown trout (*Salmo trutta*) and cod (*Gadus morhua*) from the Baltic sea. *Ambio* **28**:48–54.
- Anderson RS (1993) Modulation of nonspecific immunity by environmental stressors. In: Fournie J, Couch J (Eds.) *Pathobiology of Marine and Estuarine Organisms*. CRC Press Inc., Boca Raton, FL, pp. 483–510.
- Andersson T, Förlin L, Härdig J, and Larsson Å (1988) Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. *Can J Fish Aquat Sci* **45**:1525–36.
- Ankley G, Mihaich E, Stahl R, Tillitt D, Colborn T, McMaster S, Miller R, Bantle J, Campbell P, Denslow N, Dickerson R, Folmar L, Fry M, Giesy J, Gray LE, Guiney P, Hutchinson T, Kennedy S, Kramer V, LeBlanc G, Mayes M, Nimrod A, Patino R, Peterson R, Purdy R, Ringer R, Thomas P, Touart L, Van Der Kraak G, and Zacharewski T (1998) Overview of a workshop on screening methods for detecting potential (anti-)estrogenic/androgenic chemicals in wildlife. *Environ Toxicol Chem* **17**:68–87.
- Ashfield LA, Pottinger TG, and Sumpter JP (1998) Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. *Environ Toxicol Chem* **17**(3):679–86.
- Asplund L, Athanasiadou A, Bergman A, and Börjesson H (1999) Organohalogen substances in muscle, egg, and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produced offspring with the M74 syndrome. *Ambio* **28**:66–76.
- Aulerich RJ, Bursian SJ, Breslin WJ, Olson BA, and Ringer RK (1985) Toxicological manifestations of 2,4,5,2',4',5'—,2,3,6,2',3',6'— and 3,4,5,3',4',5',—hexachlorobiphenyl and Aroclor 1254 in mink. *J Toxicol Environ Health* **15**:63–79.
- Baker Mata M, Cairncross C, and Kocan RM (1998) Possible effects of polychlorinated biphenyls on sex determination in rainbow trout. *Environ Toxicol Chem* **17**:26–9.
- Baldwin WS, Graham SE, Shea D, and LeBlanc GA (1997) Metabolic androgenization of female *Daphnia magna* by the xenoestrogen 4-nonylphenol. *Environ Toxicol Chem* **16**:1905–11.
- Barreiro R, Ruiz JM, and Quintela M (1998) Respuesta a la contaminacion por TBT en individuos trasplantados de *Nucella lapillus* (L.) desde zonas poco contaminadas a los puertos de Ferrol y A Coruna. *Cuad Invest Biol* **20**:279–82.
- Bauer B, Fioroni P, Ide I, Liebe S, Oehlmann J, Stroben E, and Watermann B (1995) TBT effects on the female genital system of *Littorina littorea*: a possible indicator of tributyltin pollution. *Hydrobiologia* **309**:15–27.
- Bauer B, Fioroni P, Schulte-Oehlmann U, Oehlmann J, and Kalbfus W (1997) The use of *Littorina littorea* for tributyltin (TBT) effect monitoring results from the German TBT survey 1994/

- 1995 and laboratory experiments. *Environ Pollut* **96**:299–309.
- Beland PR, Michaud R, and Martneau D (1987) Recensements de la population de belugas du Saint °-Laurent en 1985 parembarcations. *Rapp Techn Can Sci Halieut Aquat No* 1545.
- Bengtsson B-E, Hill C, Bergman Å, Brandt I, Johansson N, Magnhagen C, Södergren A, and Thulin J (1999) Reproductive disturbances in Baltic fish: a synopsis of the FIRE project. *Ambio* **28**:2–8.
- Berg C, Halldin K, Brunström B, and Brandt I (1998) Methods for studying xenoestrogenic effects in birds. *Toxicol Lett* **103**:671–6.
- Bergeron JM, Crews D, and McLachlan JA (1994) PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* **102**:780–1.
- Bergman A and Olsson M (1985) Pathology of Baltic ringed seal and gray seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? *Finn Game Res* **44**:47–62.
- Bergman A, Olsson M, and Reiland S (1992) Skull-bone lesions in the Baltic gray seal (*Halichoerus grypus*). *Ambio* **21**:517–9.
- Bergman Å, Klasson-Wehler E, and Kuroki H (1994) Selective retention of hydroxylated PCB metabolites in blood. *Environ Health Perspect* **102**:464–9.
- Bergman A (1999) Health condition of the Baltic gray seal (*Halichoerus grypus*) during two decades. Gynaecological health improvement but increased prevalence of colonic ulcers. *Acta Pathol Microbiol Immunol Scand* **107**:270–82.
- Bern H (1992) The fragile fetus. In: Colborn T, Clement C (Eds.) *Chemically induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton Science Publications, Princeton, NJ. XXI, pp. 9–15.
- Bernhoft A, Wiig Ø, and Skaare JU (1997) Organochlorines in polar bears (*Ursus maritimus*) at Svalbard. *Env Pollut* **95**:159–75.
- Besselink HT, Flipsen EMTE, Eggens ML, Vethaak AD, Koeman JH, and Brouwer A (1998) Alterations in plasma and hepatic retinoid levels in flounder (*Platichthys flesus*) after chronic exposure to contaminated harbor sludge in a mesocosm study. *Aquat Toxicol* **42**:271–85.
- Bettin C, Oehlmann J, and Stroben E (1996) TBT induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgoländer Meeresunters* **50**:299–317.
- Bignert A, Litzén K, Odsjö T, Olsson M, Persson W, and Reutergårdh L (1995) Time-related factors influence the concentrations of sDDT, PCBs and shell parameters in eggs of Baltic guillemot (*Uria aalge*). *Environ Pollut* **89**:27–36.
- Bishop CA, Brooks RJ, and Carey JH (1991) The case for a cause-effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. *J Toxicol Environ Health* **33**:521–48.
- Bitman J, Cecil HC, and Fries GF (1970) DDT-induced inhibition of avian shell gland carbonic anhydrase: a mechanism for thin eggshells. *Science* **168**:594–6.
- Blaustein AR and Wake DB (1990) Declining amphibian populations: a global phenomenon? *Trends Ecol Evol* **5**:203–4.
- Boon JP, van Arnhem E, Jansen S, Kannan N, Petrick G, Duinker JC, Reijnders PJH, and Goksyr A (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. In: Walker CH, Livingstone DR (Eds.) *Persistent Pollutants in Marine Ecosystems*. Pergamon Press, Oxford, pp. 119–59.
- Borg K (1958) Inverkan av betat utsade pa viltfaunan. VIII Nord Veterinarmote, Helsingfors, 394–400.
- Bosveld ATC and Van den Berg M (1994) Effects of polychlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans on fish-eating birds. *Environ Rev* **2**:147–66.
- Bosveld ATC, Gradener J, Murk A, Brouwer A, van Kampen M, Evers EHG, and den Berg M (1995) Effects of PCDDs, PCDFs and PCBs in common tern (*Sterna Hirundo*) breeding in estuarine and coastal colonies in the Netherlands and Belgium. *Environ Toxicol Chem* **1**:99–115.
- Boudewijn TJ, Dirksen S, Mes RG, and Teunissen WA (1988) Aalscholvers in de Dordtse Biesbosch: broedsucces en fourageerplaatskeuze in

- een vervuild ecosysteem. Bureau Ecoland, Utrecht, The Netherlands Report 88-6, 115 pp.
- Brandt I, Darnerud PO, Bergman Å, and Larsson Y (1982) Metabolism of 2,4',5-trichlorobiphenyl: enrichment of hydroxylated and methyl sulphone metabolites in the uterine luminal fluid of pregnant mice. *Chem.-Biol Interactions* **40**:45–56.
- Brandt I, Jönsson C-J, and Lund B-O (1992) Comparative studies on adrenocorticolytic DDT-metabolites. *Ambio* **21**:602–5.
- Brandt I, Lund J, Bergman Å, Klasson-Wehler E, Poellinger L, and Gustafsson J-Å (1995) Target cells for the PCB metabolite 4,4'-bis(methylsulphonyl)-2,2',5,5'-tetrachlorobiphenyl in lung and kidney. *Drug Metabolism Disp* **13**:490–6.
- Brandt I, Berg C, Halldin K, and Brunström B (1997) Developmental and reproductive toxicity of persistent environmental pollutants. *Arch Toxicol Suppl* **20**:111–9.
- Bright DA and Ellis DV (1990) A comparative survey of imposex in northeast Pacific neogastropods (Prosobranchia) related to tributyltin contamination and choice of suitable bioindicator. *Can J Zool* **68**:1915–24.
- Brouwer A, Reijnders PJH, and Koeman JH (1989) Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal *Phoca vitulina*. *Aquat Toxicol* **15**:99–106.
- Brouwer A, Morse DC, Lans MC, Schuur G, Murk AJ, Klasson-Wehler E, Bergman A, and Visser TJ (1998) Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol Industr Health* **14**(1/2):59–84.
- Brunström B, Bergman A, Backlin BM, Lund BO, and Orberg J (1997) Effects of long-term exposure to PCB and PCB methylsulphones on reproduction in the mink. *Organohalogen Comp* **20**:471–80.
- Bryan GW, Gibbs PE, and Burt GR (1988) A comparison of the effectiveness of tri-n-butyltin chloride and five other organotin compounds in promoting the development of imposex in the dog-whelk, *Nucella lapillus*. *J Mar Biol Assoc UK* **68**:733–44.
- Bryan TE, Gildersleeve RP, and Wiard RP (1989) Exposure of Japanese quail embryos to o,p'-DDT has long-term effects on reproductive behaviors, hematology and feather morphology. *Teratology* **39**:525–36.
- Bryan GW, Bright DA, Hummerstone LG, and Burt GR (1993) Uptake, tissue distribution and metabolism of ¹⁴C-labelled tributyltin (TBT) in the dog-whelk, *Nucella lapillus*. *J Mar Biol Assoc UK* **73**:889–912.
- Bulkley RV (1972) Diethylstilbestrol in catfish feed. *Trans Am Fisheries Soc* **101**:537–99.
- Burkhart JG, Helgen JC, Fort DJ, Gallagher K, Bowers D, Propst TL, Gernes M, Magner J, Shelby MD, and Lucier G (1998) Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. *Environ Health Perspect* **106**:841–8.
- Cameron P, Berg J, Dethlefsen V, and Von Westernhagen H (1992) Developmental defects in pelagic embryos of several flatfish species in the southern North sea. *Neth J Sea Res* **29**(1–3):239–56.
- Campbell PM and Hutchinson TH (1998) Wildlife and endocrine disrupters: requirements for hazard identification. *Environ Contam Toxicol* **17**:127–35.
- Carey C and Bryant CJ (1995) Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environ Health Perspect* **103**:13–7.
- Cattet M (1988) Abnormal sexual differentiation in black bears (*Ursus americanus*) and brown bears (*Ursus arctos*). *J Mamm* **69**:849–52.
- Coelho MR, Bebianno MJ, and Gibbs PE (1998) Imposex in neogastropod snails from southern Portugal. *Cuad Inv Biol (Bilbao)* **20**:273.
- Colborn T, Vom Saal F, and Soto A (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* **101**:378–84.
- Colborn T and Smolen MJ (1996) Epidemiological analysis of persistent organochlorine contaminants in cetaceans. *Rev Environ Contam Toxicol* **146**:91–171.
- Conover MR and Hunt GL Jr. (1984) Experimental evidence that female-female pairs in gulls result from a shortage of breeding males. *Condor* **86**:472–6.

- Cooke AS (1973) Shell thinning in avian eggs by environmental pollutants. *Environ Pollut* **4**:85–152.
- Cooke JB and Hinton DE (1999) Promotion of 17 β -estradiol and β -hexachlorocyclohexane of hepatocellular tumors in medaka, *Oryzias latipes*. *Aquat Toxicol* **45**:127–145.
- Cowan DF (1967) Pathology of the pilot whale. *Arch Pathol* **82**:178–99.
- Crain DA and Guillette LJ (1997) Endocrine-disrupting contaminants and reproduction in vertebrate wildlife. *Rev Toxicol* **1**:207–31.
- Crain DA, Guillette LJ, Rooney AA, and Pickford DB (1997) Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmentally contaminants. *Environ Health Perspect* **105**:528–33.
- Crain DA and Guillette LJ (1998) Reptiles as models of contaminant-induced endocrine disruption. *Animal Reproduct Sci* **53**:77–86.
- Crain DA, Guillette LJ, Pickford DB, Percival HF, and Woodward AR (1998) Sex-steroid and thyroid hormone concentrations in juvenile alligators (*Alligator mississippiensis*) from contaminated and references lakes in Florida, U.S. *Environ Toxicol Chem* **17**:446–52.
- Crews D (1994) Temperature, steroids and sex determination. *J Endocrinology* **142**:1–8.
- Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, Hoffmann JL, Morrow MS, Rodier DJ, Schaeffer JE, Touart LW, Zeeman MG, and Patel YM (1998) Environmental endocrine disruption: an effects assessment and analysis. *Environ Health Perspect* **106**, Suppl. 1:11–56.
- Darnerud PO, Brandt I, Klasson-Wehler E, Bergman Å, d'Argy R, Dencker L, and Sperber GO (1986) 3,3',4,4'-tetrachloro-[¹⁴C]biphenyl in pregnant mice: enrichment of phenol and methyl sulfone metabolites in late gestational fetuses. *Xenobiotica* **16**:295–306.
- Davis DL and Bortone SA (1992) Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning? In: Colborn T, Clement C (Eds.) *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton Scientific Publishing, Inc, Princeton, NJ, pp. 113–27.
- De Boer J, Wester PG, Klammer HJC, Lewis WE, and Boon JP (1998) Do flame retardants threaten ocean life. *Nature* **394**:28–9.
- De Guise S, Lagace A, and Béland P (1994a) True hermaphroditism in a St. Lawrence beluga whale (*Delphinapterus leucas*). *J Wildl Dis* **30**:287–90.
- De Guise S, Lagace A, and Béland P (1994b) Tumors in the St. Lawrence beluga whales (*Delphinapterus leucas*). *Veter Pathol* **31**:444–9.
- De Maagd PGJ and Vethaak AD (1997) Biotransformation of PAHs and their carcinogenic effects in fish. In: Nelson A (Ed.) *Handbook of Environmental Chemistry*, Chap. 15, pp. 265–309.
- De Solla SR, Bishop CA, Van Der Kraak G, and Brooks RJ (1998) Impact of organochlorine contamination on levels of sex hormones and external morphology of common snapping turtles (*Chelydra serpentina serpentina*) in Ontario, Canada. *Environ Health Perspect* **106**:253–60.
- De Swart RL, Ross PS, Vedder LJ, Timmerman HH, Heisterkamp SH, Loveren H van, Vos JG, Reijnders PJH, and Osterhaus ADME (1994) Impairment of immune function in harbor seals (*Phoca vitulina*) feeding on fish from polluted waters. *Ambio* **23**:155–9.
- De Swart RL, Ross PS, Timmerman HH, Vos HW, Reijnders PJH, Vos JG, and Osterhaus ADME (1995) Impaired cellular immune response in harbor seals (*Phoca vitulina*) feeding on environmentally contaminated herring. *Clin Exp Immunol* **101**:480–6.
- De Waal EJ, Schuurman HJ, Van Loveren H, and Vos JG (1997) Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, bis(tri-*n*-butyltin)oxide and cyclosporine on thymus histophysiology. *Crit Rev Toxicol* **27**:381–430.
- Den Besten PJ, Van Donselaar EG, Herwig HJ, Zandee D, and Voogt PA (1991a) Effects of cadmium on gametogenesis in the sea star *Asterias rubens* L. *Aquat Toxicol* **20**:83–94.
- Den Besten PJ, Elenbaas JML, Maas JR, Dieleman SJ, Herwig HJ, and Voogt PA (1991b) Effects of cadmium and polychlorinated biphenyls (Clophen A50) on steroid metabolism and cytochrome P-450 monooxygenase system in the sea star *Asterias rubens* L. *Aquat Toxicol* **20**:95–110.
- Delong RL, Gilmartin WG, and Simpson JG (1973) Premature births in California sea lions: associa-

- tion with high organochlorine pollutant residue levels. *Science* **181**:1168–70.
- Dent JN (1985) Hormonal interaction in the regulation of migratory movements in urodele amphibians. In: Follett BK, Ishii S, and Chandola A (Eds.) *The Endocrine System and the Environment*. Japan Scientific Society Press, Tokyo/Springer-Verlag, Berlin, pp.79–84.
- Desbrow C, Routledge E, Brighty GC, Sumpter JP, and Waldock M (1998) Identification of estrogenic chemicals in STW effluent. I. Chemical fractionation and *in vitro* biological screening. *Environ Sci Technol* **32**:1549–58.
- Dethlefsen V, von Westerhagen H, and Cameron P (1996) Malformations in North Sea pelagic fish embryos during the period 1984–1995. *ICES J Marine Science* **53**:1024–35.
- DFG (Deutsche Forschungsgemeinschaft), Eisenbrand G (1996) Symposium: Hormonally active agents in food, organised by the DFG Senate Commission of Food Safety, Kaiserslautern, October 6 to 9.
- Dirksen S, Boudewijn LK, Slager IK, Mes RG, Van Schalck MJM, and Voogt P (1995) Reduced breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. *Environ Pollut* **88**:119–32.
- Donohoe RM and Curtis LR (1996) Estrogenic activity of chlordecone, *o,p'*-DDT and *o,p'*-DDE in juvenile rainbow trout: induction of vitellogenesis and interaction with hepatic estrogen binding sites. *Aquat Toxicol* **36**:31–52.
- Drysdale DT and Bortone SA (1989) Laboratory induction of intersexuality in the mosquitofish *Gambusia affinis*, using paper mill effluent. *Bull Environ Contam Toxicol* **43**:611–7.
- ECETOC (1996) Environmental estrogens: a compendium of test methods. ECETOC Document No. 33, Brussels.
- Elbrecht A and Smith RB (1992) Aromatase enzyme activity and sex determination in chickens. *Science* **255**:467–70.
- El Hamdani A, Ferrer JM, and Garcia Carrascosa AM (1998) Imposex in prosobranch molluscs: an indicator of TBT pollution in the Valencian Coast (Spain, Western Mediterranean). *Cuad Inv Biol (Bilbao)* **20**:275–8.
- Elliott JM (1990) Mechanisms responsible for population regulation in young migratory trout, *Salmo trutta*. II. Fish growth and size variation. *J Anim Ecol* **59**:171–85.
- EPA (1997) Special report on Environmental Endocrine Disruption: An Effects Assessment and Analysis. United States Environmental Protection Agency. Office of Research and Development Washington DC 20460. EPA/630/R—96/012, February 1997, 116 pp.
- Evans SM, Hawkins ST, Porter J, and Samosir AM (1994) Recovery of dogwhelk populations on the Isle of Cumbrae, Scotland following legislation limiting the use of TBT as an antifoulant. *Mar Pollut Bull* **28**:15–7.
- Facemire CF, Gross TS, and Guillette LJJ (1995) Reproductive impairment in the Florida panther: nature or nurture? *Environ Health Perspect* **103**:79–86.
- Falandysz J, Yamashita N, Tanabe S, Tutsukawa R, Rucinska L, Mizera T, and Jakuczum B (1994) Congener-specific analysis of polychlorinated biphenyls in white-tailed sea eagles *Haliaeetus albicilla* collected in Poland. *Arch Environm Contam Toxicol* **26**:13–22.
- Fioramonti E, Semlitsch RD, Reyer HU, and Fent K (1997) Effects of triphenyltin and pH on the growth and development of *Rana lessonae* and *Rana esculenta* tadpoles. *Environ Toxicol Chem* **16**:1940–7.
- Fioroni P, Oehlmann J, and Stroben E (1991) The pseudohermaphroditism of prosobranchs; morphological aspects. *Zool Anz* **226**:1–26.
- Firestone D (1973) Etiology of chick edema disease. *Environ Health Perspect* **5**:59–66.
- Fitzsimons JD, Brown SB, Honeyfield DC, and Hnath JG (1999) A review of early mortality syndrome (EMS) in Great Lakes salmonids: relationship with thiamine deficiency. *Ambio* **28**:9–15.
- Folmar LC, Denslow ND, Rao V, Chow M, Crain DA, Enblom J, Marcino J, and Guillette LJ (1996) Vitellogenin induction and reduced serum testosterone concentrations on feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. *Environ Health Perspect* **104**:1096–1101.
- Förlin L, Larsson DGJ, Parkonen J, Nilsson M, Hellman H, Olsson P-E, and Adolfsson M (1999). Endo-

- crine disruption responses in fish near sewage treatment works and pulp mills in Sweden. Abstract 9th Annual Meeting of SETAC Europe May 25 to 29, 1999, Leipzig, Germany
- Fry DM and Toone CK (1981) DDT-induced feminization of gull embryos. *Science* **213**:922–4.
- Fry DM, Toone CK, Speich SM, and Peard RJ (1987) Sex ratio skew and breeding patterns of gulls: demographic and toxicological considerations. *Stud Avian Biol* **10**:26–43.
- Fry DM (1995) Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ Health Perspect* **103** (Suppl. 7):165–71.
- Gabrielsen GV, Skaare JU, Polder A, and Bakken V (1995) Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. *Sci Total Sci* **160/161**:337–46.
- Gagnon MM, Bussieres D, Dodson JJ, and Hodson PV (1995) White sucker (*Catostomus commersoni*) growth and sexual maturation in pulp mill-contaminated and reference rivers. *Environ Toxicol Chem* **14**:317–27.
- Galton VA (1992) The role of thyroid hormone in amphibian metamorphosis. *TEM* **3**:96–100.
- Geraci JR, Palmer NC, and St. Aubin DJ (1987) Tumors in cetaceans: analysis and new findings. *Can J Fish Aquat Sci* **44**:1289–1300.
- Gerritsen A (1997) The influence of body size, life stage and sex on toxicity of alkylphenols to *Daphnia magna*. PhD thesis, University of Utrecht, The Netherlands, 109 pp.
- Gibbs PE, Bryan GW, Pascoe PL, and Burt GR (1987) The use of the dog-whelk, *Nucella lapillus*, as an indicator of tributyltin (TBT) contamination. *J Mar Biol Assoc UK* **67**: 507–23.
- Giesy JP, Verbrugge DA, Othout RA, Bowerman WW, Mora MA, Jones PD, Newsted JL, Vandervoort C, Heaton SN, Aulerich RJ, Bursian SJ, Ludwig JP, Dawson GA, Kubiak TJ, Best DA, and Tillitt DE (1994a) Contaminants in fishes from Great Lakes—influenced sections and above dams of 3 Michigan rivers. II. Implications for health of mink. *Arch Environ Contam Toxicol* **27**:213–23.
- Giesy JP, Ludwig J, and Tillitt D (1994b) Deformities in birds of the Great Lakes region. Assigning causality. *Environ Sci Technol* **28**:128–35.
- Gilbertson M, Kubiak T, Ludwig J, and Fox G (1991) Great Lake embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. *J Toxicol Environ Health* **33**:455–520.
- Gimeno S, Gerritsen A, Bowmer T, and Komen H (1996) Feminization of male carp. *Nature* **384**:221–2.
- Grachev MA, Kumarev VP, Mamaev LV, Zorin VL, Baranova LV, Denikja NN, Belikov SI, Petrov EA, Kolesnik VS, Dorofeev VM, Beim AM, Kudelin VN, Nagieva FG, and Sidorov VN (1989) Distemper virus in Baikal seals. *Nature* **338**:209.
- Gray MA and Metcalfe CD (1997) Induction of testis—ova in Japanese medaka (*Oryzias latipes*) exposed to p—nonylphenol. *Environ Toxicol Chem* **16**(5):1082–6.
- Guillette LJJ, Gross TS, Masson GR, Matter JM, Percival HF, and Woodward AR (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* **102**:680–8.
- Guillette LJJ, Gross TS, Rooney AA, et al. (1995) Gonadal steroidogenesis in vitro from juvenile alligators obtained from contaminated or control lakes. *Environ Health Perspect* **103**:1–5.
- Guillette LJJ, Pickford DB, Crain DA, Rooney AA, and Percival HF (1996) Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. *Gen Comp Endocrinol* **101**:32–42.
- Gutleb AC, Appelman J, Bronkhorst M, Van den Berg JHJ, and Murk AJ (1998) Delayed teratogenic effects of Aroclor 1254 and PCB 126 in frog embryos in a newly developed prolonged-FETAX assay. *Organohalogen Compounds* **37**: 301–4.
- Hall RJ and Henry PFP (1992) Assessing effects of pesticides on amphibians and reptiles: status and needs. *Herpetol J* **2**:65–71.
- Hallers-Tjabbes CCT, Kemp JF, and Boon JP (1994) Imposex in whelks (*Buccinum undatum*) from the open North Sea: relation to shipping traffic intensities. *Mar Poll Bull* **28**:311–13.
- Hansen PD, von Westernhagen H, and Rosenthal H (1985) Chlorinated hydrocarbons and hatching

- success in Baltic Herring spring spawners. *Mar Environ Res* **15**:59–76.
- Haraguchi K, Athanasiadou M, Bergman Å, Hovander L, and Jensen S (1992) PCB and PCB methyl sulfones in selected groups of seals from Swedish waters. *Ambio* **21**:546–9.
- Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Routledge E, Rycroft R, Sumpter JP, and Taylor T (1996) A survey of estrogenic activity in U.K. inland waters. *Environ Toxicol Chem* **15**:1993–2002.
- Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Sumpter JP, Taylor T, and Zaman N (1997) Estrogenic activity in five U.K. rivers detected by measurement of vitellogenesis in caged male trout. *Environ Toxicol Chem* **16**:534–42.
- Harrison PTC, Humfrey CDN, Litchfield M, Peakall D, and Shuker LK (1995) IEH Assessment on Environmental Estrogens: Consequences to Human Health and Wildlife. Medical Research Council, Institute for Environment and Health, Page Bros, Norwich, 107 pp.
- Hart LR, Cheng KM, Whitehead PE, Shah RM, Lewis RJ, Ruschkowski RE, Blair RW, Bennett DC, Bandiera SM, and Norstrom RJ (1991) Dioxin contamination and growth and development in great blue heron embryos. *J Toxicol Environ Health* **32**:331–44.
- Hawkins LE and Hutchinson S (1990) Physiological and morphogenetic effects of monophenyltin trichloride on *Ocenebra erinacea* (L.). *Funct Ecol* **4**:449–54.
- Heinz GH, Percival HF, and Jennings ML (1991) Contaminants in American alligator eggs from lake Apopka, lake Griffin, and lake Okeechobee, Florida. *Environ Monitor Assess* **16**:277–85.
- Helander B, Olsson M, and Reutergård L (1982) Residue levels of organochlorine and mercury compounds in unhatched eggs and the relationships to breeding success in white-tailed sea eagles *Haliaeetus albicilla* in Sweden. *Holarctic Ecol* **5**:349–66.
- Helle E, Olsson M, and Jensen S (1976a) DDT and PCB levels and reproduction in Ringed seal from the Bothnian Bay. *Ambio* **5**:188–9.
- Helle E, Olsson M, and Jensen S (1976b) PCB levels correlated with pathological changes in seal uteri. *Ambio* **5**:261–3.
- 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 Zool Fenn* **17**:147–58.
- Henriksen EO, Brunström B, Skaare JU, and Gabrielsen GW (1998) Bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents and mono-*ortho* polychlorinated biphenyl concentrations in liver of glaucous gulls (*Larus hyperboreus*) from Svalbard. *Organohalogen Compounds* **39**:415–8.
- Herman RL and Kincaid HL (1988) Pathological effects of orally administered estradiol to rainbow trout. *Aquaculture* **72**:165–72.
- Hirsch KS, Weaver DE, Black LJ, Falcone JF, and MacLusky NJ (1987) Inhibition of central nervous system aromatase activity: a mechanism for fenarimol-induced infertility in the male rat. *Toxicol Appl Pharmacol* **91**:235–45.
- Hoffman DJ (1990) Embryotoxicity and teratogenicity of environmental contaminants to bird eggs. *Rev Environ Contam Toxicol* **115**:39.
- Hoffman DJ, Rice CP, and Kubiak TJ (1996) PCBs and dioxins in birds. In: Beyer WN, Heinz GH, Redmon-Norwood AW (Eds.) *Environmental Contaminants in Wildlife — Interpreting Tissue Concentrations*. SETAC Special Publications Series, Lewis Publishers, CRC Press, Boca Raton, FL, pp. 165–207.
- Horiguchi T, Shiraishi H, Shimizu M, and Morita M (1994) Imposex and organotin compounds in *Thais clavigera* and *T. bronni* in Japan. *J Mar Biol Assoc UK* **74**:651–69.
- Horiguchi T, Shiraishi H, Shimizu M, and Morita M (1997) Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell *Thais clavigera*. *Environ Pollut* **95**:85–91.
- Howell WM and Denton TE (1989) Gonopodial morphogenesis in female mosquitofish, *Gambusia affinis affinis*, masculinized by exposure to degraded products from plant sterols. *Environ Biol Fish* **24**:43–51.
- Huet M, Paulet YM, and Glémarec M (1996) Tributyltin (TBT) pollution in the coastal waters of west Brittany as indicated by imposex in *Nucella lapillus*. *Mar Environ Res* **41**:157–67.
- Hunt GL Jr and Hunt MW (1973) Clutch size, hatching success, and eggshell-thinning in Western Gulls. *Condor* **75**:483–6.

- Hunt GL Jr and Hunt MW (1977) Female-female pairing in Western Gulls (*Larus occidentalis*) in southern California. *Science* **196**:1466–7.
- Hyvärinen H, Sipilä T, Kunnasranta M, and Koskela JT (1998) Mercury pollution and the Saimaa ringed seal (*Phoca hispida saimensis*). *Mar Pollut Bull* **36**:76–81.
- ICES (1998) Report of the working group on biological effects of contaminants. Mont-Joli, Quebec, Canada, 30 March–3 April 1998 Marine habitat committee ICES CM 1998/E:3 Ref: ACME.
- IPCS (1996) Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. International Programme on Chemical Safety Environmental Criteria 180, World Health Organization, Geneva.
- Janssen PAH, Lambert JGD, Vethaak AD, and Goos HJT (1997) Environmental pollution causes elevated concentrations of estrogens and vitellogenin in the female flounder, *Platichthys flesus* (L.). *Aquat Toxicol* **39**:195–214.
- Janssen PAH, Faber JH, and Bosveld ATC (1998) (Fe)male? How contaminants can disrupt the balance of sex hormones and affect reproduction in terrestrial wildlife species. DLO Institute for Forestry and nature research (IBN-DLO), Wageningen, The Netherlands. IBN Scientific Contributions 13.
- Jensen S (1966) Report of a new chemical hazard. *New Scientist* **32**:612.
- Jensen S (1972) The PCB story. *Ambio* **1**:123–31.
- Jensen S and Jansson B (1976) Anthropogenic substances in seal from the Baltic: methylsulfone metabolites of PCB and DDE. *Ambio* **5**:257–60.
- Jensen S, Kihlström JE, Olsson M, Lundberg C, and Oerberg J (1977) Effects of PCB [polychlorinated biphenyls] and DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] on mink (*Mustela vison*) during the reproductive season [laboratory experiments]. *Ambio* **6**:239.
- Jobling S, Sheahan DA, Osborne JA, Matthiessen P, and Sumpter JP (1996) Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environ Toxicol Chem* **15**:194–202.
- Jobling S, Tyler CR, Nolan M, and Sumpter JP (1998) The identification of estrogenic effects in wild fish. R&D Technical Report W119, Environment Agency, Bristol, UK.
- Johansson M, Larsson C, Bergman Å, and Lund B-O (1998a) Structure-activity relationship for inhibition of CYP11B1-dependent glucocorticoid synthesis in Y1 cells by aryl methyl sulfones. *Pharmacol Toxicol* **83**:225–30.
- Johansson M, Nilsson S, and Lund B-O (1998b) Interactions between methylsulfonyl PCBs and the glucocorticoid receptor. *Environ Health Perspect* **106**:769–72.
- Johnson LL, Casillas E, Collier TK, McCain BB, and Varanasi U (1988) Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puget Sound, Washington. *Can J Fish Aquat Sci* **45**:2133–46.
- Johnson LL, Sol SY, Ylitalo GM, Hom T, French B, Olson OP, and Collier TK (1997a) Precocious sexual maturation and other reproductive anomalies in English sole from an urban waterway. ICES CM 1997/U:07.
- Johnson LL, Sol SY, Lomax DP, Nelson GM, Sloan CA, and Casillas E (1997b) Fecundity and egg weight in English sole, *Pleuronectes vetulus*, from Puget Sound, Washington: influence of nutritional status and chemical contaminants. FB 95:231–49.
- Johnstone R, Simpson TH, and Youngson AF (1978) Sex reversal in salmonid cultures. *Aquaculture* **13**:115–34.
- Jönsson C-J, Rodríguez-Martínez H, Lund B-O, Bergman Å, and Brandt I (1991) Adrenocortical toxicity of 3-methylsulfonyl-DDE in mice (II): Mitochondrial changes following ecologically relevant doses. *Fundam Appl Toxicol* **16**:365–74.
- Jönsson C-J, Lund B-O, Bergman Å, and Brandt I (1992) Adrenocortical toxicity of 3-methylsulfonyl-DDE (III): studies in fetal and suckling mice. *Reproductive Toxicol* **6**:233–40.
- Jönsson C-J, Lund B-O, Bergman Å, and Brandt I (1993) Toxicity of *o,p'*-DDD and *p,p'*-DDD in the adrenal cortex of mink (*Mustela vison*). *Eco-toxicology* **2**:41–53.
- Jönsson C-J, Lund B-O, Brunström B, and Brandt I (1994) Toxicity and irreversible binding of two DDT metabolites — 3-methylsulfonyl-DDE and *o,p'*-DDD — in adrenal interrenal cells in birds. *Environ Toxicol Chem* **13**:1303–10.
- Kannan K and Falandysz J (1997) Butyltin residues in sediment, fish, fish-eating birds, harbor porpoise and human tissues from the Polish coast

- of the Baltic Sea. *Mar Pollut Bull* **34**: 203–7.
- Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, and Wilson EM (1995). Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist. *Nature* **375**:581–5.
- Kelce WR, Lambright CR, Gray LE Jr, and Roberts KP (1997) Vinclozolin and *p,p'*-DDE alter androgen-dependent gene expression: in vivo confirmation of an androgen receptor-mediated mechanism. *Toxicol Appl Pharmacol* **142**:192–200.
- Keymer LF, Wells GAF, Mason CF, and Macdonald SM (1988) Pathologicval changes and organochlorine residues in tissues of wild otters (*Lutra lutra*). *Veterinary Records* **122**:153–5.
- Kihlström J-E, Olsson M, Jensen S, Johansson Å, Ahlbom J, and Bergman Å (1992) Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). *Ambio* **21**:563–9.
- Kime DE (1995) The effects of pollution on reproduction in fish. *Rev Fish Biol Fish* **5**:52–96.
- Klasson-Wehler E, Bergman Å, Athanasiadou M, Ludwig JP, Auman HJ, Kannan K, Van den Berg M, Murk AJ, Feyk LA, and Giesy JP (1998) Hydroxylated and methylsulfonyl polychlorinated biphenyl metabolites in albatrosses from Midway Atoll, North Pacific Ocean. *Environ Toxicol Chem* **17**:1620–5.
- Koeman JH, Van Beusekom S, and Goeij JJM (1972) Eggshell and population changes in the sparrowhawk (*Accipiter nisus*). *TNO-nieuws* **27**:542–50.
- Kovacs TG, Gibbons JS, Tremblay LA, O'Connor BI, Martel PH, and Voss RH (1995) The effects of a secondary treated bleached kraft mill effluent on aquatic organisms as assessed by short-term and long-term laboratory test. *Ecotoxicol Environ Saf* **31**:7–22.
- Krajnc EI et al. (1984) Toxicity of bis(tri-*n*-butyltin)oxide in the rat. I. Short-term effects on general parameters and on the endocrine and lymphoid systems. *Toxicol Appl Pharamacol* **75**:363–86.
- Kruuk H and Conroy JWH (1996) Concentrations of some organochlorines in otters (*Lutra lutra* L) in Scotland: Implications for populations. *Environ Pollut* **2**:165–71.
- Kubiak TJ, Harris HJ, Smith LM, Schwartz TR, Stalling DL, Trick JA, Sileo L, Docherty DE, and Erdman TC (1989) Microcontaminants and reproductive impairment of the Foster's tern on Green Bay, Lake Michigan-1983. *Arch Environm Contam Toxicol* **18**: 706–27.
- Laenge R, Schweinfurth H, Croudace CP, and Panter GH (1997) Growth and reproduction of fathead minnow (*Pimephales promelas*) exposed to the synthetic steroid hormone ethynylestradiol in a life—cycle test Abstract, 7th Annual Meeting, Society of Environmental Toxicology and Chemistry— Europe, Amsterdam, The Netherlands, April 7 to 10, p. 43.
- Lahvis GP, Wells RS, Kuehl DW, Stewart JL, and Rhinehart HL, Via CS (1995) Decreased lymphocyte responses in free—ranging bottlenose dolphins (*Turiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ Health Perspect* **103** (Supplement 4):67–72.
- Lair S, Béland P, De Guise S, and Martineau D (1997) Adrenal hyperplastic and degenerative changes in beluga whales. *J Wildlife Diseases* **33**:430–7.
- Landis WG, Matthews RA, and Matthews GB (1995) A contrast of human health risk and ecological risk assessment: risk assessment for an organism versus a complex non-organismal structure. *Human Ecolog Risk Assess* **1**:485–8.
- Lang T, Damm U, and Dethlefsen V (1995) Changes in the sex ratio of North Sea dab (*Limanda limanda*) in the period 1981–1995. ICES CM 1995/G:25 Ref E.
- Larsson DGJ, Adolfsson-Erici M, Parkkonen J, Pettersson M, Berg AH, Olsson P-E, and Förlin L (1999) Ethinylestradiol — an undesired fish contraceptive? *Aquatic Toxicol* **45**:91–97.
- Laws SC, Carey SA, and Kelce WR (1995) Differential effects of environmental toxicants on steroid receptor binding [Abstract]. *Toxicologist* **15**:294.
- Leatherland JF (1992) Endocrine and reproductive function in Great Lakes salmon. In: Colborn T, Clement C (Eds.) *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Advances in Modern Environmental Toxicology* (Vol. 21). Princeton Scientific Publishing Co., Inc., Princeton, NJ, pp. 129–45.
- Leblanc J, Couillard CM, and Brethes JCF (1997) Modifications of the reproductive period in mum-

- michog (*Fundulus heteroclitus*) living downstream from a bleached kraft pulp mill in the Miramichi estuary, New Brunswick, Canada. *Can J Fish Aquat Sci* **54**:2564–73.
- Leonards PEG (1997) PCBs in mustelids: Analysis, food chain transfer and critical levels. Dissertation. Free University of Amsterdam
- Leonards PEG, Elmeros M, Cofino W, Conroy J, Gutleb AC, Mason CF, Murk AJ, Smit M, Van Hattum B, and Madsen AB (1996) Toxic PCBs in European otter populations in relation to biological factors and health status. In: Smit MD, Leonards PEG, Murk AJ, De Jongh AJJ, Van Hattum B (Eds.) *Development of Otter-Based Quality Objectives for PCBs*. Institute for Environmental Studies, Amsterdam
- Lincer JL (1975) DDE-induced eggshell thinning in the American kestrel: a comparison of the field situation and laboratory results. *J Appl Ecol* **12**: 781–93.
- Lund B-O, Bergman Å, and Brandt I (1988) Metabolic activation and toxicity of a DDT-metabolite, 3-methylsulfonyl-DDE, in the adrenal zona fasciculata in mice. *Chem-Biol Interactions* **65**: 25–40.
- Lund B-O (1994) In vitro adrenal bioactivation and effects on steroid metabolism of DDT, PCBs and their metabolites in the gray seal (*Halichoerus grypus*). *Environ Toxicol Chem* **13**:911–17.
- Lund J, Brandt I, Poellinger L, Bergman Å, Klasson-Wehler E, and Gustafsson J-Å (1995) Target cells for the polychlorinated biphenyl metabolite 4,4'-bis(methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl. Characterization of high affinity binding in rat and mouse lung cytosol. *Molecular Pharmacol* **27**:314–23.
- Lundholm C-E (1997) DDE-induced eggshell thinning in birds: Effects of *p,p'*-DDE on the calcium and prostaglandin metabolism of the eggshell gland. *Comp Biochem Physiol-C-Pharmacol Toxicol Endocrinol* **118**:113–28.
- Lye CM, Frid CLJ, Gill ME, and McCormick D (1997) Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. *Mar Pollut Bull* **34**:34–41.
- Mac MJ and Edsall CC (1991) Environmental contaminants and the reproductive success of lake trout in the Great lakes: an epidemiological approach. *J Toxicol Environ Health* **33**:375–94.
- Marquenie JM, Roele P, and Hoornsman G (1986) Onderzoek naar de effecten van contaminanten op duikeenden. TNO-report 86/0686, Delft, The Netherlands.
- Martineau D, Lagace A, Béland P, Higgins R, Armstrong D, and Shugart LR (1988) Pathology of stranded beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Quebec, Canada. *J Compar Pathol* **98**:287–311.
- Martineau D, De Guise S, Fournier M, Shugart L, Girard C, Lagace A, and Béland P (1994) Pathology and toxicology of beluga whales from the St. Lawrence Estuary, Quebec, Canada. Past, present and future. *Sci Total Environ* **154**:201–15.
- Matthiessen P, Waldock R, Thain JE, and Waite ME (1995) Chances in periwinkle (*Littorina littorea*) populations following the ban on TBT-based antifouling on small boats in the United Kingdom. *Ecotoxicol Environ Saf* **30**:180–94.
- Matthiessen P, Allen Y, Allchin CR, Feist SW, Kirby MF, Law RJ, Scott AP, Thain JE, and Thomas KV (1998) Estrogenic endocrine disruption in flounder (*Platichthys flesus* L.) from U.K. estuarine and marine waters. Sci Ser Tech Rep, CEFAS, Lowestoft (107), 48 pp.
- Matthiessen P and Gibbs PE (1998) Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. *Environ Toxicol Chem* **17**:37–43.
- Matthiessen P, Reynoldson T, Billinghamstz, Brassard DW, Cameron P, Chandler GT, Davies IM, Horinguchi T, Mount DR, Dehlmann J, Pottinger TG, Sibley PK, Thompson H, and Vethaak AD (1999). Field assessment for endocrine disruption in invertebrates. In: DeFur PI, Crane M, Ingersall CG, Tattersfield L (Eds.) *Endocrine Disruption in Invertebrates: Endocrinology, Testing, and Assessment*. Society of Environmental Toxicology and Chemistry (SETAC), pp. 199–257.
- McMaster ME et al. (1991) Changes in hepatic mixed-function oxidase (MFO) activity, plasma steroid levels and age of maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft mill effluent. *Aquat Toxicol* **21**:199–218.
- Mensink BP, Van Hattum BGM, Ten Hallers-Tjabbes CC, Everaarts JM, Kralt H, Vethaak AD, and Boon JP (1996) Tributyltin causes imposex in the common whelk, *Buccinum undatum*. Mecha-

- nism and occurrence. Netherlands Institute for Sea Research. NIOZ Report 1997-6: 56 pp.
- Mensink BP, Van Hattum BGM, Vethaak AD, and Boon JP (1997) The development of imposex in relation to organotin contamination in the common whelk, *Buccinum undatum*. Field observations in the Eastern Scheldt and experimental exposure in a laboratory study. Netherlands Institute for Sea Research. NIOZ-Report 1996-3.
- Moccia R, Fox G, and Britton AJ (1986) A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environmental contaminants. *J Wildl Dis* **22**:60–70.
- Moore CG and Stevenson JM (1994) Intersexuality in benthic harpacticoid copepods in the Firth of Forth, Scotland. *J Nat History* **28**:1213–30.
- Morcillo Y and Porte C (1988) Monitoring of organotin compounds and their effects in molluscs from the Catalan Coast (NW Mediterranean). *Trends Anal Chem* **17**:109–16.
- Mortensen P, Bergman A, Bignert A, Hansen H-J, Härkönen T, and Olsson M (1992) Prevalence of skull lesions in harbor seals (*Phoca vitulina*) in Swedish and Danish museum collections: 1835–1988. *Ambio* **21**:520–4.
- Mueller GC and Kim UH (1978) Displacement of estradiol from estrogen receptors by simple alkylphenols. *Endocrinology* **102**:1429–35.
- Murk AJ, Bosveld ATC, Van den Berg M, and Brouwer A (1994a) Effects of polyhalogenated aromatic hydrocarbons (PHAHs) on biochemical parameters in chicks of the common tern (*Sterna hirundo*). *Aquat Toxicol* **30**:91–115.
- Murk AJ, Morse DC, Boon J, and Brouwer A (1994b) *In vitro* metabolism of 3,3',4,4'-tetrachlorobiphenyl in relation to ethoxyresorufin-O-deethylase activity in liver microsomes of some wildlife species and rat. *Eur J Pharmacol* **270**:253–61.
- Murk AJ, Boudewijn TJ, Meininger PL, Bosveld ATC, Rossaert G, Ysebaert T, Meire P, and Dirksen S (1996) Effects of polyhalogenated aromatic hydrocarbons and related contaminants on common tern reproduction: integration of biological, biochemical and chemical data. *Arch Environ Contam Toxicol* **31**:128–40.
- Myers MS, Landahl JT, Krahn MM, Johnson LL, and McCain BB (1990) Overview of studies on liver carcinogenesis in English sole from Puget Sound: Evidence for a xenobiotic chemical etiology I. Pathology and Epizootiology. TSTE 94: 33–50.
- Nagler JJ and Cyr DG (1997) Exposure of male american plaice (*Hippoglossoides platessoides*) to contaminated marine sediments decrease the hatching success of their progeny. *Environ Toxicol Chem* **16**(8):1733–8.
- Norris DO (1997) *Vertebrate Endocrinology*. Academic Press, San Diego, U.S.
- North Sea Task Force (1993) Quality Status Report 1993. Oslo and Paris Commissions, London. Frederiksborg, Denmark: Olsen & Olsen, 132 + vi pp.
- O'Shea TJ, Brownell RL (1998) California Sea lion (*Zalophus californianus*) populations and sumDDT contamination. *Mar Pollut Bull* **36**:159–64.
- Odsjö T and Sondell J (1982) Eggshell thinning and DDT, PCB and mercury in eggs of osprey (*Pandion haliaetus* (L.)) in Sweden and their relations to breeding success. In: Odsjö T (Ed.) Eggshell thickness and levels of DDT, PCB and mercury in eggs of osprey (*Pandion haliaetus* (L.)) and marsh harrier (*Circus aeruginosus* (L.)) in relation to their breeding success and population status in Sweden. (Ph. D thesis). Stockholm.
- Oehlmann J, Fioroni P, Stroben E, and Markert B (1996) Tributyltin (TBT) effects on *Ocenebrina aciculata* (Gastropoda: Muricidae): imposex development, sterilization, sex change and population decline. *Sci Total Environ* **188**:205–23.
- Oehlmann J, Stroben E, Schulte-Oehlmann U, and Bauer B (1998) Imposex development in response to TBT pollution in *Hinia incrassata* (Strom, 1768) (Prosobranchia, Stenoglossa). *Aquat Toxicol* **43**: 239–60.
- Olsson M, Karlsson B, and Ahnland E (1994) Diseases and environmental contaminants in seals from the Baltic and the Swedish west coast. *Sci Tot Environ* **154**:217–27.
- Olsson PE, Borg B, Brunström B, Håkansson H, and Klasson-Wehler E (1998) Endocrine-disrupting substances — Impairment of reproduction. Swedish Environmental Protection Agency Customer Services, Stockholm.
- Osterhaus ADME and Vedder EJ (1988) Identification of virus causing recent seal deaths. *Nature* **335**:20.

- Osterhaus ADME, Groen J, UytdeHaag FGCM, Visser IKG, Van de Bildt MWG, Bergman A, and Klingeborn B (1989) Distemper virus in Baikal seals. *Nature* **338**:209–10.
- Palmer BD and Palmer SK (1995) Vitellogenine induction by xenobiotic estrogens in the red-eared turtle and the African clawed frog. *Environ Health Perspect* **103**:19–25.
- Palmer BD, Hutch LK, Pioto DL, and Selcer KW (1997) Vitellogenine as a biomarker for xenobiotic estrogens in an amphibian model system. *Environ Toxicol Chem* **17**:30–6.
- Parnell JF, Shields MA, and Frierson D Jr (1984) Hatching success of brown pelican eggs after contamination with oil. *Colon. Waterbirds* **7**:22.
- Peakall DB (1970) *p,p'*-DDT: effect on calcium metabolism and concentration of estradiol in blood. *Science* **168**:592–4.
- Peakall DB, Lincer JL, Risebrough RW, Pritchard JB, and Kinter WB (1973) DDE-induced eggshell thinning: structural and physiological effects. *Comp Gen Pharmac* **4**:305–13.
- Peakall DB (1992) *Animal Biomarkers as Pollution Indicators*. London: Chapman and Hall.
- Pereira JJ, Ziskowski J, and Mercaldo-Allen R, et al. (1992) Vitellogenin in winter flounder (*Pleuronectes americanus*) from Long Island Sound and Boston harbor. *Estuaries* **15**:289–97.
- Petersen GI, Gerup J, Nilsson L, Larsen JR, and Schneider R (1997) Body burdens of lipophilic xenobiotics and reproductive success in Baltic cod (*Gadus morhua* L.). ICES CM 1997/U:10.
- Piferrer F, Zanuy S, Carillo M, Solar II, Devlin RH, and Donaldson EM (1994) Brief treatment with an aromatase inhibitor during sex differentiation causes chromosomally female salmon to develop as normal, functional males. *J Exp Biol* **270**:255–62.
- Pinder LCV and Pottinger TG (1998) Endocrine function in aquatic invertebrates and evidence for disruption by environmental pollutants. Draft report to the United Kingdom Environment Agency and the CEFIC Endocrine Modulators Steering Group, 178 pp.
- Postma JF and De Valk S (1996) Ecotoxicologische monitoring op Loswal Noord en Noord-West met behulp van zeesterren. Technical Report no. 96.0409-2, Amsterdam: AquaSense Laboratory.
- Postma JF and De Valk S (1997) Biomar-II project: biomarkers voor reproductie toxiciteit in mariene invertebraten. Technical Report no.97.0409, Amsterdam: AquaSense Laboratory
- Purdum CE, Hardiman PA, Bye VJ, et al. (1994) Estrogenic effects of effluents from sewage treatment works. *Chem Ecol* **8**:275–85.
- Ratcliffe DA (1967) Decrease in eggshell weight in certain birds of prey. *Nature* **215**:208–10.
- Ratcliffe DA (1970) Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *J Appl Ecol* **7**:67–115.
- Rees HL, Waldock R, Matthiessen P, and Pendle MA (1999) Surveys of the epibenthos of the Crouch estuary in relation to TBT contamination. *J Mar Biol Assoc UK*, in press.
- Reijnders PJH (1986) Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **324**:456–7.
- Reijnders PJH and Brasseur SMJM (1992) Xenobiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans. In: Colborn T, Clement C, Eds. *Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection*. Princeton, Princeton Sci. Publ., pp. 131–46.
- Reijnders PJH and De Ruiter-Dijkman EM (1995) Toxicological and ecological significance of pollutants in marine mammals. In: Blix, AS, Walloe, L, and Ulltang O, Eds. *Whales, Seals, Fish and Man*. Elsevier Science BV, Amsterdam, pp. 575–87.
- Reijnders PJH (1996) Organohalogen and heavy metal contamination in cetaceans: observed effects, potential impact and future prospects. In: Simmonds MP, Hutchinson JD (Eds.) *The Conservation of Whales and Dolphins*. John Wiley & Sons Ltd, Chichester, UK, pp. 205–17.
- Reijnders PJH (1999) Reproductive and developmental effects of endocrine-disrupting chemicals on marine mammals. In: O'Shea TJ, Reeves RR, and Kirk Long A, Eds. *Marine Mammals and Persistent Ocean Contaminants*. Proc Marine Mammal Commission Workshop, Keystone, Colorado, 12–15 October, 1998. Marine Mammal Commission, Bethesda Md, pp. 93–100.

- Reinke EE and Chadwick CS (1939) Inducing land stage of *Triturus viridescens* to assume water habitat. *Proc Soc Exp Biol Med* **40**:691–3.
- Reis-Henriques MA, Cruz MM, and Pereira JO (1997) The modulating effect of vitellogenin on the synthesis of 17-beta-estradiol by rainbow trout (*Oncorhynchus mykiss*) ovary. *Fish Physiol Biochem* **16**:181–6.
- Rijnsdorp AD and Vethaak AD (1997) Changes in reproductive parameters of North Sea plaice and sole between 1960 and 1995. ICES C.M. 1997/U:14.
- Ronis MJJ and Mason AZ (1996) The metabolism of testosterone by the periwinkle (*Littorina littorea*) in vitro and in vivo effects of tributyltin. *Mar Environ Res* **42**:161–66.
- Roos A, Bergman A, Grayerz E, and Olsson M (1998) Time trend studies on SDDT and PCB in juvenile gray seals (*Halichoerus grypus*), fish and guillemot eggs from the Baltic Sea. *Organohalogen Compounds* **39**:109–12.
- Roos A, Grayerz E, Olsson M, and Sandegren F. The otter (*Lutra lutra*) and mink (*Mustela vison*) in Sweden — Population trends in relation to SDDT and total PCB concentrations during 1968–1998. Submitted for publication.
- Rose FL and Harshbarger JC (1977) Neoplastic and possibly related skin lesions in neotenic tiger salamanders from a sewage lagoon. *Science* **196**:315–7.
- Ross PS, De Swart RL, Reijnders PJH, Van Loveren H, Vos JG, and Osterhaus ADME (1995) Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ Health Perspect* **103**:162.
- Ross PS, De Swart RL, Van Loveren H, Osterhaus ADME, and Vos JG (1996a) The immunotoxicity of environmental contaminants to marine wildlife: a review. *Ann Rev Fish Dis* **6**:151–65.
- Ross PS, De Swart RL, Addison R, Van Loveren H, Vos JG, and Osterhaus ADME (1996b) Contaminant-induced immunotoxicity in harbor seals: wildlife at risk? *Toxicology* **112**:157–69.
- Routledge EJ, Sheanan D, Desbrow C, Brighty GC, Waldoock M, and Sumpter J (1998) Identification of estrogenic chemicals in STW Effluent 2. In vivo responses in trout and roach. *Environ Sci Technol* **32**:1559–65.
- Ruiz JM, Bryan GW, and Gibbs PE (1994) Chronic toxicity of water tributyltin (TBT) — polluted sediment to spat of the bivalve *Scrobicularia plana*. *Marine Ecol Prog Series* **113**:105–17.
- Ruiz JM, Bryan GW, Wigham GD, and Gibbs PE (1995a) Effects of tributyltin (TBT) exposure on the reproduction and embryonic development of the bivalve *Scrobicularia plana*. *Mar Environ Res* **40**:363–79.
- Ruiz JM, Bryan GW, and Gibbs PE (1995b) Acute and chronic toxicity of tributyltin (TBT) to pediveliger larvae of the bivalve *Scrobicularia plana*. *Mar Biol* **124**:119–26.
- Sandegren F, Olsson M, and Reutergård L (1980) Der ryckgang der Fischotterpopulation in Schweden. In: Reuter C & Festetics A (Eds.) 1980: *Der Fischotter in Europa — Verbreitung, Bedrohung, Erhaltung. Selbstverlag, Oderhaus & Göttingen*, pp. 107–13.
- Sandström O, Förlin L, Grahn O, Lander L, Larsson Å, and Lindesjö E (1997) Environmental impact of individual organism responses in fish populations exposed to pulp mill effluents. In: Proceedings of 3rd International Conference on Environmental Fate and Effects of Pulp and Paper Mill Effluents. November 1997, Rotorua, New Zealand.
- Sangalang G and Jones G (1997) Oocytes in testis and intersex in lobsters (*Homarus americanus*) from Nova Scotian sites: natural or site related phenomenon? Canadian Technical Report on Fisheries and Aquatic Science **2163**:46.
- Schäfer WR, Zahradnik HP, Fijus-Plesner N, and Schneider K (1996) Anthropogene Substanzen mit unerwünschter Östrogenwirkung. *Umwelt-med* **1**:35–42.
- Schumacher M, Hendrick JC, and Balthazart J (1989) Sexual differentiation in quail: critical period and hormonal specificity. *Horm Behav* **23**:130–49.
- Scott GP, Burn DM, and Hansen IJ (1988) The dolphin dieoff: long-term effects and recovery of the population. In: Proceedings of the Ocean 88 Conference, 31 October–2 November 1988, Baltimore, MD. Vol 3, pp 819–23.
- SEPA (1995) Second workshop on reproduction disturbances in fish. Swedish Environmental Protection Agency, Stockholm, Sweden, November 20 to 23.

- Sharpe RM and Skakkebaek NE (1993) Are estrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **341**:1392–5.
- Sharpe RM, Fisher JS, Millar M, Jobling S, and Sumpter JF (1995) Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. *Environ Health Perspect* **103**:1136–4.
- Sheahan DA, Bucke D, Matthiessen P, et al. (1994). The effects of low levels of 17 α -ethinylestradiol upon plasma vitellogenin levels in male and female rainbow trout, *Oncorhynchus mykiss*, held at two acclimation temperatures. In: Miller R, Lloyd R (Eds.) *Sublethal and Chronic Effects of Pollutants on Freshwater Fish*. Oxford Fishing News Books, Oxford. pp. 99–112.
- Shi YB (1994) Molecular biology of amphibian metamorphosis. A new approach to an old problem. *TEM* **5**:14–20.
- Sindermann CJ (1993) Interactions of pollutants and disease in marine fish and shellfish. In: Couch JA, Fournie JW (Eds.) *Pathology of Marine and Estuarine Organisms*. CRC Press, Boca Raton. pp. 451–82.
- Smit MD Leonard PEG, Murk AJ, Jongh AWJJ de, and Hattum B van (1994) PCBs in European otter (*Lutra lutra*). Stichting Otterstation Nederland/Instituut voor Milieuvraagstukken, Amsterdam, The Netherlands R94/7, 77 pp.
- Smith PJ (1996) Selective decline in imposex levels in the dogwhelk *Lepsiella scobina* following a ban on the use of TBT antifoulants in New Zealand. *Mar Pollut Bull* **32**:362–5.
- Smits JEG, Haines DM, Blakley BR, and Wobeser GA (1996) Enhanced antibody responses in mink (*Mustela vison*) exposed to dietary bleached—kraft pulp mill effluent. *Environ Toxicol Chem* **15**:1166–70.
- Sole M, Morcillo Y, and Porte C (1988) Imposex in the Commercial Snail *Bolinus brandaris* from the Northwestern Mediterranean. *Environ Pollut* **99**:241–6.
- Spies RB and Rice DW (1988) Effects of organic contaminants on reproduction of starry flounder (*Platichthys stellatus*) in San Francisco Bay. *Mar Biol* **98**:191–200.
- St. Aubin DJ and Geraci JR (1989) Seasonal variations in thyroid morphology and secretion in the white whale, *Delphinapterus leucas*. *Can J Zoology* **67**:263–7.
- Stede G and Stede M (1990) Orientierende Untersuchungen von Seehundschädeln auf pathologische Knochenveränderungen. In: Zoologische und Ethologische Untersuchungen zum Robbensterben. Inst. f. Haustierkunde, Kiel, Germany, pp.31–53.
- Stephenson MA (1991) A field bioassay approach to determining tributyltin toxicity to oysters in California. *Mar Environ Res* **32**:51–9.
- Stott GG, Haensly WE, Neff JM, and Sharp JR (1983) Histopathologic survey of ovaries of plaice, *Pleuronectes platess* L., from Aber Wrac'h and Aber benoit, Brittany, France: long-term effects of the Amoco Cadix crude oil spill. *J Fish Dis* **6**:429–37.
- Strand J (1998) Widespread occurrence of imposex in the sublittoral whelks *Buccinum undatum* and *Neptunea antiqua* in the Danish waters. Abstract 3C/P060. SETAC-Europe 8th Annual Meeting. 14-18 April 1998, Bordeaux, France p 229.
- Subramanian AN, Tanabe S, Tatsukawa R, Saito S, and Myazaki N (1987) Reductions in the testosterone levels by PCBs and DDE in Dall's porpoises of Northwestern North Pacific. *Mar Pollut Bull* **18**:643–6.
- Subramanian MA and Varadaraj G (1993) The effect of industrial effluents on moulting in *Macromia cingulata* (Rambur) (Anisoptera: Corduliidae). *Odonatologica* **22**:229–32.
- Sumpter JP and Jobling S (1995) Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ Health Perspect* **103** (Suppl.7):173–8.
- Tanabe S, Watanabe S, Kan H, and Tatsukawa R (1988) Capacity and mode of PCB metabolism in small cetaceans. *Mar Mamm Sci* **4**:103–24.
- Terlizzi A, Gerachi S, and Minganti V (1998) Tributyltin (TBT) pollution in the coastal waters of Italy as indicated by imposex in *Hexaplex trunculus* (Gastropoda, Muricidae). *Mar Pollut Bull* **36**:749–52.
- Tester M, Ellis DV, and Thompson JAJ (1996) Neogastropod imposex for monitoring recovery from marine TBT contamination. *Environ Toxicol Chem* **15**:560–7.

- Thain JE, Waldock MJ, and Helm M (1986) The effect of tributyltin on the reproduction of the oyster *Ostrea edulis*. *Int Council Explor Sea*, CM 1986/E:14, 14 pp.
- Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Müller E, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, and Skakkebaek N (1995) Male reproductive health and environmental chemicals with estrogenic effects. Danish Environmental Protection Agency, Copenhagen, 166 pp.
- Ulfstrand S, Södergren A, and Raböl J (1971) Effect of PCB on nocturnal activity in caged robins (*Erithacus rubecula* L.) *Nature* **231**:467–8.
- Van den Berg M, Craane BLHJ, Sinnige T, Van Mourik S, Dirksen S, Boudewijn T, Van der Gaag M, Lutke-Schipholt IJ, Spenkelink B, and Brouwer A (1994) Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environ Toxicol Chem* **13**:803–16.
- Van der Kraak GJ, Munkittrick KR, McMaster ME, Portt CB, and Chang JP (1992) Exposure to bleached kraft pulp mill effluent disrupts the pituitary-gonadal axis of white sucker at multiple sites. *Toxicol Appl Pharmacol* **115**:224–33.
- Vethaak AD and Rheinallt T ap (1992) Fish disease as a monitor of marine pollution: the case of the North Sea. *Rev Fish Biol Fish* **2**:1–32.
- Vethaak AD (1993) Fish disease and marine pollution. The case study of the flounder (*Platichthys flesus*) in Dutch coastal and estuarine waters. PhD thesis, University of Amsterdam.
- Vial JL and Saylor L (1993) The status of amphibian populations. Working Document No 1. Corvallis OR: Declining Amphibian Populations Task Force.
- Vonier PM, Crain DA, McLachlan JA, Guillette LJ, and Arnold SF (1996) Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. *Environ Health Perspect* **104**:1318–22.
- Von Westernhagen H, Dethlefsen V, Cameron P, Berg J, and Furstenberg G (1988) Developmental defects in pelagic fish embryos from the western Baltic. *Helg. Meeresunters* **42**:13–36.
- Voogt PA, Den Besten PJ, Kusters GCM, and Messing MWJ (1987) Effects of cadmium and zinc on steroid metabolism and steroid level in the sea star *Asterias rubens* L. *Comp Biochem Physiol* **86C**:83–9.
- Vos JG, De Klerk A, Kranjc EI, Kruizinga W, van Ommen B, and Rozing J (1984) Toxicity of bis(tri-*n*-butyltin)oxide in the rat. II. Suppression of thymus-dependent immune response and parameters of non-specific resistance after short term exposure. *Toxicol Appl Pharmacol* **75**:387–408.
- Vos JG and Luster MI (1989) Immune alterations. In: Kimbrough RD, Jensen S (Eds.) *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products*. Elsevier Science Publishers, pp 295–322.
- Vos JG, De Klerk A, Krajnc EI, Van Loveren H, and Rozing J (1990) Immunotoxicity of bis(tri-*n*-butyltin)oxide in the rat: effects on thymus-dependent-immunity and non-specific resistance following long-term exposure in young versus aged rats. *Toxicol Appl Pharmacol* **105**:144–55.
- Vos JG, Van Loveren H, and Schuurman HJ (1991) Immunotoxicity of dioxin: immune function and host resistance in laboratory animals and humans. In: *Banbury Report 35: Biological Basis for Risk Assessment of Dioxins and Related Compounds*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor. pp. 79–93.
- Vos JG, Van Loveren H, Osterhaus ADME, Vaal M, Vethaak AD, and Wester P (1996) Immunotoxicity of environmental pollutants: adverse effects on resistance to infectious diseases. Proceedings OECD Workshop “Ecotoxicology: Responses, Biomarkers and Risk Assessment”, Maui, April 10–13, 1996. SOS Publications, Fair Haven, NJ, pp. 127–36.
- Vos JG, De Heer C, Van and Loveren H (1997/98) Immunotoxic effects of TCDD and toxic equivalency factors. *Teratog Carcinog Mutagen* **17**:275–84.
- Vuorinen PJ, Paasivirta J, Keinänen M, Koistinen J, Rantio T, Hyötyläinen T, and Welling L (1997a) The M74 syndrome of Baltic salmon (*Salmo salar*) and organochlorine concentrations in the muscle of female salmon. *Chemosphere* **34**(5–7):1151–66.
- Vuorinen PJ, Keinänen M, Brouwer A, Vartiainen T, Spenkelink A, and Schipholt I (1997b) Retinoids

- and thyroid hormones and toxicant loads with the occurrence of the M74 syndrome in Baltic salmon. In: Proceedings "Third Finnish Conference of Environmental Sciences, Jyväskylä 1997. Ambiotica 1997, paginanr.
- Wagemann R and Muir DGC (1984) Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. *Can Tech Rep Fish Aquat Sci* **1297**:1–97.
- Waldock R, Rees HL, Matthiessen P, and Pendle MA (1999) Surveys of the benthic infauna of the Crouch estuary in relation to TBT contamination. *J Mar Biol Assoc UK*, **79**:225–32.
- Waldock MJ and Thain JE (1983) Shell thickening in *Crassostrea gigas*: Organotin antifouling or sediment induced? *Mar Pollut Bull* **14**: 411–5.
- Waring CP, Stagg RM, Fretwell K, McLay HA, and Costella MJ (1996) The impact of sludge exposure on the reproduction of the sandy goby, *Pomatoschistus minutus*. *Environ Pollut* **93**:17–25.
- Wester PW, Canton JH, and Bisschop A (1985) Histopathological study of *Poecilia reticulata* (guppy) after long-term b-hex-achlorocyclohexane exposure. *Aquat Toxicol* **6**:271–96.
- Wester PW and Canton JH (1986) Histopathological study of *Oryzias latipes* (medaka) after long term b-hexachlorocyclohexane exposure. *Aquat Toxicol* **9**:21–45.
- Wester PW, Vethaak AD, and Van Muiswinkel W (1994) Fish as biomarkers in immunotoxicology: a review. *Toxicol* **86**(3):213–32.
- Weybridge (1996) Proceedings European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife, 2-4 December 1996, Weybridge, U.K.
- White R, Jobling S, Hoare SA, Sumpter JP, and Parker MG (1994) Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* **135**:175–82.
- Wiig Ø, Derocher AE, Cronin MM, and Skaare JU (1998) Female pseudohermaphrodite polar bears at Svalbard. *J Wildlife Dis* **34**:792–6.
- Woin P and Bronmark C (1992) Effect of DDT and MCPA (4-chloro-2-methylphenoxyacetic acid) on reproduction of the pond snail *Lymnaea stagnalis* L. *Bull Environ Contam Toxicol* **48**(1):7–13.
- Wolff MS, Toniolo PG, Lee EW, Rivera M, and Dubin N (1993) Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst* **85**:648–52.
- Woodward AR, Jennings ML, Percival HF, and Moore CT (1993) Low clutch viability of American alligators on Lake Apopka. *Florida Sci* **56**:52–63.
- Wren CD (1991) Cause-effect linkages between chemicals and populations of mink (*Mustela vison*) and otter (*Lutra canadensis*) in the Great Lakes. *J Toxicol Environm Health* **33**:549–85.
- Zacharenski-Timothy R, Berhane K, Gillesby BE, and Burmison BK (1995). Detection of estrogens and dioxin-like activity in pulp and paper mill black liquor and effluent using *in vitro* recombinant receptor/reporter gene assays. *Environ Sci Tech* **29**:2140–6.