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# Contaminant-induced feminization and demasculinization of nonmammalian vertebrate males in aquatic environments

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**Abstract**

Many chemicals introduced into the environment by humans adversely affect embryonic development and the functioning of the male reproductive system. It has been hypothesized that these developmental alterations are due to the endocrine-disruptive effects of various environmental contaminants. The endocrine system exhibits an organizational effect on the developing embryo. Thus, a disruption of the normal hormonal signals can permanently modify the organization and future function of the male reproductive system. A wide range of studies examining wildlife either in laboratories or in natural settings have documented alterations in the development of males. These studies have begun to provide the causal relationships between embryonic contaminant exposure and reproductive abnormalities that have been lacking in pure field studies of wild populations. An understanding of the developmental consequences of endocrine disruption in wildlife can lead to new indicators of exposure and a better understanding of the most sensitive life stages as well as the consequences of exposure during these periods.


*Keywords:* Endocrine-disrupting contaminants; Feminization; Development; Reproduction; Endocrinology; Sexual dimorphism; Steroids; Fertility

## 1. Introduction

Many chemicals introduced into the environment by humans adversely affect embryonic development and the functioning of the vertebrate reproductive system. It has been hypothesized that ubiquitous environmental contaminants can induce developmental alterations through disruption of the endocrine system. The endocrine system exhibits an organizational effect on the developing embryo, altering gene expression and dosing. Thus, a disruption of the normal hormonal signals can permanently modify the organization and future functioning of the reproductive system. The development of the male reproductive systems is a common target of endocrine-disrupting contaminants (EDCs) (see Fig. 1). Studies in wild, free-ranging male mammals, reptiles, amphibians, and fish have documented depressed plasma androgen profiles, altered spermatogenesis, altered penis/gonopodium development, and altered male behavior. Laboratory-based exposure studies have provided powerful documentation that similar alterations can be induced in developing male embryos exposed to ecologically relevant concentrations of various estrogenic and antiandrogenic EDCs. Further, although the mechanism of sex determination can vary among species, endocrine control of the testis and the role of androgens in male secondary sex development and functioning is highly conserved among vertebrates, indicating that wildlife are effective and important sentinels of human public health.

## 2. Reproductive tract—form and function

The effects of EDCs on testicular and reproductive tract development and function have been investigated

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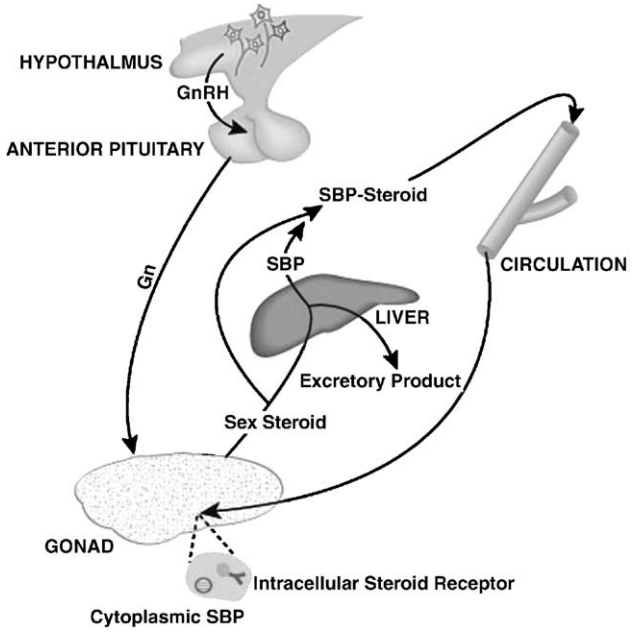


Fig. 1. Endocrine-disrupting contaminants can alter the biology of males at many levels of the endocrine system, besides the interaction with cellular-level receptors. Alterations of neuroendocrine function (e.g., gonadotropin-releasing hormone (GnRH) or gonadotropins (Gn)), serum-binding protein concentrations (SBP), and modification of liver biotransformation of hormones would alter the endocrine biology of males, leading to feminization or demasculinization.

in many fish species, but rarely in amphibians or reptiles. Most studies have focused on the effects of estrogens such as estradiol-17$\beta$ ($E_2$) or ethynylestradiol on sexual development. There are several studies on the effects of individual estrogenic and antiandrogenic contaminants, but noticeably few that look at the effects of chemical mixtures on the male reproductive tract. Environmental contaminants are known to affect sexual differentiation of the gonad, timing of sexual maturation, gonadosomatic index (GSI), and reproductive tract and testis morphology. Here, we present examples of each of these in fish and then present examples from the amphibian and reptilian literature.

### 2.1. Primary sex determination

When sex ratios are skewed in fish populations exposed to environmental estrogens or antiandrogens, the ratio is typically biased in favor of females. Sex ratios can be affected in the developing embryos of exposed parents or in juvenile fish exposed during the period of sex determination and differentiation. Exposure to $E_2$ during sexual differentiation increased the frequency of ovarian differentiation in *Cyclopterus lumpus* (Martinrobichaud et al., 1994), *Odontesthes bonariensis* (Strussmann et al., 1996), and *Silurus asotus* (Kim et al., 2001). Similarly, populations of Japanese medaka (*Oryzias latipes*) exposed during development to the estrogenic contaminants octylphenol (Knorr and Braunbeck, 2002) and bisphenol A (Yokota et al., 2000) exhibited female-biased sex ratios. More females than expected were also observed in juvenile guppies (*Poecilia reticulata*) exposed during development to the antiandrogens vinclozolin and *p,p′*-DDE (Bayley et al., 2002). Heavy metals such as mercury have also been shown to cause feminization of the gonads in fish (Matta et al., 2001).

Intersex is a condition that results from disrupted gonadal differentiation and typically describes the simultaneous presence of ovarian and testicular tissue in the same gonad. In feminized males, oocytes may be scattered throughout the testis or occur within distinct regions of ovarian tissue that are well delineated from testicular tissue (Nolan et al., 2001). Transgenic zebrafish assays in which transactivation of the estrogen receptor was linked to expression of the luciferase reporter gene have shown that estrogen receptor activity is very high during sexual development and could be one reason that the period of gonadal differentiation may be especially susceptible to disruption by estrogenic compounds (Legler et al., 2000). Experimental research has shown that estrogens administered during the period of gonadal differentiation can induce intersex in a variety of fish species such as Japanese medaka (Knorr and Braunbeck, 2002; Metcalfe et al., 2001), carp (*Cyprinus carpio*) (Gimeno et al., 1998a, b), and sheepshead minnow (*Cyprinodon variegatus*) (Zillioux et al., 2001). Several xenoestrogens have also been shown to induce development of intersex gonads. Examples include 4-*tert*-pentylphenol in carp (Gimeno et al., 1998a, b) and medaka (Gronen et al., 1999), and bisphenol A in medaka (Yokota et al., 2000).

Examples of wild fish populations exhibiting intersex gonads include flounder taken from Tokyo Bay (Hashimoto et al., 2000); rainbow trout (Tyler and Routledge, 1998) and gudgeon (*Gobio gobio*) (van Aerle et al., 2001) collected from rivers in the UK polluted with estrogenic sewage treatment effluent; wild barbel from polluted portions of the Po River in Italy (Vigano et al., 2001), and lake whitefish (*Coregonus clupeaformis*) collected from the St. Lawrence River in Quebec, Canada (Mikaelian et al., 2002). The lake whitefish had elevated tissue levels of polychlorinated biphenyls (PCBs), chlorobenzenes, pesticides, and trace metals. Similar effects were observed in shovelnose sturgeon from the Mississippi River below Saint Louis, where fish tissues and roe were found to be contaminated with chlordane, PCBs, and *p,p′*-DDE (Harshbarger et al., 2000).

### 2.2. Sexual maturation

Whether exposure to EDCs causes gross gonadal changes such as intersex or more subtle changes such as