Endocrine-Disrupting Contaminants and Reproductive Abnormalities in Reptiles

Recent studies show that environmental contamination of reptiles is associated with population declines due to lethal and reproductive effects of the contaminants in embryos, juveniles, or adults, developmental abnormalities of embryos, including major teratogenic effects in turtles and more subtle effects on the development of the reproductive system of alligators, and abnormalities of the endocrine system. We examine the data available on abnormalities of the reproductive system in reptiles induced by endocrine-disrupting xenobiotics. We discuss the role of these xenobiotics in light of experimental evidence showing that estrogenic steroids are capable of stimulating sex reversal—male to female—in developing reptilian embryos exhibiting environmental sex determination. A hypothesis is presented suggesting that any compound that disrupts the normal steroid milieu of the developing embryo will have significant, life-long consequences on sex determination and the organization and function of the reproductive system. These endocrine-disrupting compounds may directly influence embryonic development by altering the steroid dynamics that facilitate sex determination.

Key Words: *alligator, turtle, sex determination, gonadal steroidogenesis, estradiol-17b, testosterone, androgen, estrogen, gonadal morphology, egg viability*

Many environmental contaminants kill wildlife: the species affected are as diverse as water fleas, hermit crabs, bass, bald eagles, and alligators. It is not surprising that this is so, as many xenobiotic compounds, for example, insecticides, herbicides, and fungicides,—are designed to be lethal. Although most of these compounds are developed for specific applications in agriculture or industry, their affect on the environment is not specific, for some have had and continue to have wide-ranging and long-term influences on the environment. The lethal effects of pesticides and their

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most obvious mechanisms of actions were presented to the public in Rachel Carson's classic book *Silent Spring*. She clearly reviewed the mutagenic and carcinogenic qualities of many of the common chemicals in use in the United States and throughout much of the developed world during the middle part of this century. Although the chemicals Carson identified as extremely detrimental to wildlife (such as dichlorodiphenyl trichloroethane [DDT], dieldrin, aldrin) are today banned or controlled in the United States, many are still synthesized, exported, and used in other countries.[1]

Although the use of DDT was controlled almost 20 years ago, DDT and its major breakdown products, DDE and DDD, are still commonly obtained in chemical analyses of wildlife and human tissue.[2] The concentration of these compounds in wildlife and human tissue has decreased markedly since the 1960s so that today many regulatory agencies believe that the current concentrations pose no health hazard. This assumption has persisted because regulatory agencies continue to focus principally on the lethal, carcinogenic, or extreme teratogenic actions of these compounds. Evidence from a number of sources suggests that we must now consider another mechanism of action for xenobiotic environmental chemicals, that of endocrine disruption.

CASE STUDIES—FIELD STUDIES

Lake Apopka's Alligators

Specific problems associated with the eggs and the reproductive system of alligators (*Alligator mississippiensis*) obtained from Lake Apopka, Florida, have been documented recently.[3-7] Lake Apopka is adjacent to an Environmental Protection Agency (EPA)-designated Superfund site, the former Tower Chemical Company. Lake Apopka is the fourth largest body of freshwater (12,500 ha) in Florida and is highly polluted[8,9] with contaminants historically derived from agricultural activities, a sewage treatment facility, and a major pesticide spill. The Tower Chemical Company was the site of a major spill of dicofol (contaminated with DDT and its metabolites DDD, DDE, and chloro-DDT), and sulfuric acid in 1980 (EPA: Unpublished report).

The alligator population on Lake Apopka has been studied for many years by a team of collaborating researchers.[3-6] Studies of the American alligator on Lake Apopka from 1979 to 1981, characterized the population as com-

posed of healthy and reproductively viable individuals (Woodward A: Personal communication). In fact, this lake was chosen for studies of alligator harvest-management because of the large numbers of adults and nests inhabiting the lake. The alligator population changed dramatically during the early and mid 1980s. A significant decline in the juvenile alligator population occurred following the Tower Chemical spill into Lake Apopka in 1980.[3,5] The population declined by 90% over the years 1980–1984 and remains at this low density today. In contrast to Lake Apopka, other study populations showed stable or increasing population densities during the same period. In addition to a severe decline in the population density of juvenile and hatchling alligators, clutch viability on this lake decreased significantly during the mid-1980s and remains depressed today when compared to clutch viability on other comparable lakes in central and southern Florida.[4-6] Poor clutch viability contributes to the reduced density of hatchlings and juvenile alligators seen today. Interestingly, a rapid decline in clutch viability was not observed until 1984, 4 years after the spill and 3 years after a significant decline in juvenile population density had occurred. We suggest that the initial decline in the population was a direct consequence of the lethal effects of high dicofol/DDT (or its metabolites) contamination of hatchlings and juveniles living in the lake, whereas the current reduced population density of these age groups is a result of poor clutch viability.

Why is clutch viability poor today, almost 15 years after the spill? An analysis of embryonic mortality in alligator eggs has been performed and suggests that greater than 80% of all embryonic mortality takes place during the first month after fertilization of the egg.[10] Numerous contaminants are lethal to wildlife embryos. Specifically, dicofol, DDT, or DDE kill embryos of fish and birds.[11-13] Bald eagle eggs containing concentrations greater than 3.5 ppm DDE produce no viable neonates.[13] A study has identified elevated levels of the contaminant *p,p'*-DDE in alligator eggs from Lake Apopka (collected during 1984 and 1985) when compared to eggs from two other lakes,[14] but this study could not directly correlate elevated organochlorine compounds with poor egg viability. The mean levels observed, 5.8 ppm wet weight (1984: n = 3 eggs; range, 3.4 to 7.6 ppm) and 3.5 ppm wet weight (1985: n = 23 eggs; range, 0.89 to 29 ppm) are above the concentrations known to influence adversely avian eggs and embryos.[13] In addition to *p,p'*-DDE, 23 alligator eggs collected in 1985 from Lake Apopka had detectable levels of *p,p'*-DDD (ND 1.8 ppm), dieldrin (0.02 to 1.0 ppm), and cis-chlordane (ND, 0.25 ppm).[14] These concentrations are elevated compared to eggs collected on several other lakes. Each of these compounds has been identified as an endocrine disruptor.[15]

Laboratory studies have shown that exposure to subthreshold concentrations of many contaminants are additive and even synergistic, leading to biological actions even though the concentrations of the individual compounds never exceed known threshold levels.[16,17]

The lethal effects of contaminants are well documented but what is surprising is that juvenile alligators hatched from eggs collected on Lake Apopka exhibit abnormal gonadal morphology and plasma sex steroid concentrations.[6] Male juvenile alligators from Lake Apopka, siblings of the females described later had poorly organized testes with unique, aberrant structures within the seminiferous tubules.[6] A number of the germ cells have clear mitotic figures, suggesting that premature spermatogenesis has been induced as no mitotic or meiotic activity is seen in the testes obtained from the control males. Ovaries from alligators 6 months of age, hatched from eggs collected on Lake Apopka, had prominent polyovular follicles and many of the oocytes were multinucleated.[6] A normal ovarian follicle contains a single oocyte that has a single nucleus. Polyovular follicles contain more than one oocyte and in the case of the alligator as many as six discrete oocytes can be counted in a single follicle. All of the females hatched from eggs collected on Lake Apopka exhibited this condition, whereas it was not observed in control females.[6]

The abnormal morphology of these ovarian follicles and oocytes is similar to that observed in mice treated with the estrogenic chemical diethylstilbestrol (DES). Rodent polyovular follicles can be stimulated to ovulate and are capable of fertilization in vitro and in vivo, although the number of eggs fertilized is significantly lower than for uniovular follicles.[18,19] Fertilized ova from DES-induced polyovular follicles develop to implantation stage embryos, but the frequency of embryos reaching this stage is significantly reduced compared to embryos derived from uniovular follicles.[20] Thus, the reported reduction in the viability of embryos in those species exposed to xenobiotic estrogens could be in part due to their release from polyovular follicles. This suggests one method by which clutch viability is reduced in alligators and this phenomenon could contribute to the low hatching rates reported for various wildlife species exposed to estrogenic xenobiotics.[15]

In addition to morphological abnormalities of the gonad, Lake Apopka male and female juvenile (6 months of age)[6] and hatchling (Guillette and Gross: Unpublished data) alligators exhibited abnormal plasma sex steroid concentrations. Males from Lake Apopka have greatly reduced plasma testosterone concentrations; concentrations are one fourth of those observed

in juvenile males from the control lake, Lake Woodruff. Additionally, the males from Lake Apopka exhibit elevated plasma estradiol-17β concentrations compared to control males. The females from Lake Apopka have elevated plasma estradiol-17β concentrations compared to the females from the control lake. These data concerning plasma sex steroid concentrations suggest several hypotheses: (1) steroidogenesis is abnormal due to developmental abnormalities of the gonad, (2) gonadal steroidogenesis is normal but degradation of the sex steroids is modified, thus resulting in abnormal plasma concentrations, (3) the ratio of free to bound hormone has been modified due to a modification in the concentrations of the various plasma proteins bound to steroid hormones, or (4) abnormal secretion of gonadotropin (luteinizing hormone, follicle stimulating hormone) or gonadotropin-releasing hormone from the pituitary and hypothalamus, respectively, could result in altered stimulation of the gonad and, thus, abnormal plasma sex steroid concentrations. These hypotheses are not exclusive and the observations reported may be due, to various degrees, to all of these factors.

The first hypothesis is supported by experiments in which in vitro incubation of the gonads was used to determine endogenous and gonadotropin-stimulated rates of steroid synthesis.[7] In vitro synthesis of estradiol-17β was significantly different, with ovaries from females obtained from Lake Apopka synthesizing more estradiol-17β than ovaries from control females.[7] Additionally, testes from males obtained from the contaminated lake, Lake Apopka, synthesized significantly higher concentrations of estradiol-17β when compared to testes obtained from control males. In contrast, testosterone synthesis from the testes displayed a normal pattern with no difference noted between lakes. As expected, testosterone production from the testes was greater than that observed from ovaries from females obtained from either lake. Interestingly, the pattern of in vitro gonadal steroidogenesis[7] differs from plasma concentrations of these hormones obtained from the same individuals.[6]

Taken together, these data suggest that the differences in plasma steroid concentrations observed between alligators from contaminated and control lakes are due in part to modifications of gonadal steroidogenesis with additional changes in degradation pathways and gonadotropin release from the hypothalamo-hypophysial system. Although no data on the activity of various enzymes associated with gonadal steroidogenesis nor data on degradation pathways in alligators exposed to various contaminants are available at this time, it is clear that changes in these systems have occurred and future studies must examine these systems.

Do these changes in plasma sex steroid concentrations persist in animals from Lake Apopka? Juveniles alligators (2 to 5 years of age: 30 to 122 cm) from three lakes in central Florida (Apopka, Woodruff, Orange) have been examined.[21] Females exhibited similar plasma estradiol-17β concentrations among all three lakes, whereas plasma testosterone concentrations were significantly increased on Apopka animals compared with those from the other lakes. Morphologically, females exhibited normal secondary sex characteristics except for a few females on Lake Apopka, which had a hypertrophied clitoris. Wild juvenile males from Lake Apopka showed plasma estradiol concentrations significantly elevated compared with the males collected on the other lakes. Plasma testosterone concentrations were significantly lower in males from Lake Apopka compared with Lake Woodruff but not Orange Lake. These data suggest that endocrine functioning of the wild juvenile males from Lake Apopka was modified and further implies that males from other lakes (such as Orange Lake) may also be affected to some degree.

A significant modification in androgen functioning in male alligators from Lake Apopka is further supported by morphological observations that many of the males from Lake Apopka had a significantly reduced phallus size when compared to males from the control lake, Lake Woodruff (Figure 1 from Guillette, Percival, Abercrombie, Pickford, Rooney, and Rice: Unpublished data). Interestingly, the greatest reduction in phallus size is observed in juvenile males living in the vicinity of Gourd Neck Spring (Apopka-GNS), located in the southwest corner of Lake Apopka and immediately adjacent to the stream draining the marsh that connects the Tower Chemical Superfund site and Lake Apopka. Although experimental studies have not been performed on Lake Apopka males to determine if they would exhibit phallic growth in response to exogenous testosterone treatment, previous studies have demonstrated that phallus development and growth is androgen dependent in alligators[22] and crocodilians,[23] as observed in other reptiles[24] and in mammals.[25] Interestingly, Ramaswani and Jacob[23] observed that the penis of juvenile Indian Mugger crocodiles (*Crocodylus palustris*) exhibited the greatest androgen responsiveness of any male reproductive organ they examined, which included the secondary sexual ducts, the Wolffian duct, and renal sex segment of the kidney. Thus, phallus size apparently represents an obvious marker of abnormal androgen concentrations in the alligators of Lake Apopka.

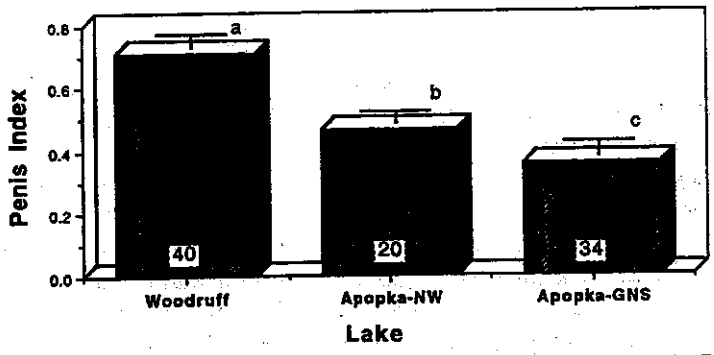

FIGURE 1 Mean phallus size in alligators from two regions of a contaminated lake (Lake Apopka) and a control lake (Lake Woodruff). Lake Apopka samples are separated into two localities, the Gourd Neck Spring area (Apopka-GNS) where the contaminants from the Tower Chemical Spill entered the lake and the northwestern part of the lake (Apopka-NW), away from the spill. Penis size is presented as an index (penis tip length × penis base width / snout vent length). Values from the different lake regions are significantly different ($F = 23.99$; d.f. = 2, 91; $p < 0.0001$). (Data are from Guillette, Percival, Abercrombie, Pickford and Rice; Unpublished findings.)

Although the effects of contaminants on Lake Apopka alligators are obvious, two fundamental questions remain: (1) Which contaminants are responsible for the developmental abnormalities described? (2) What is the source of the contaminants in alligator eggs? Although *p,p'*-DDE is present in the greatest concentration in eggs and alligator tissue, many other contaminants detected in alligator eggs are known endocrine disruptors, such as *p,p'*-DDD, dieldrin, and cis-chlordane. The effects described are likely to be the result of the combined effects of all the contaminants present. Two possible sources for the contaminants found in the eggs exist: maternal transfer of contaminants, via the blood, into the oocyte during growth, or transfer through the shell once the egg is laid in a nest. We believe that the major source of egg contamination is via transfer directly from the female as previously reported in other reptiles.[26-28] However, as will be described, experimental evidence is available demonstrating that contaminants applied to the outside of the egg shell of turtles and alligators are transported internally and affect embryonic development.[21] Further research is needed to elucidate the individual, additive, and synergistic effects of particular contaminants and to clarify the modes of contaminant exposure in the alligators of Lake Apopka.

Snapping Turtles of the North American Great Lakes

Snapping turtles (*Chelydra serpentina*), like the American alligator already discussed, feed near the top of the food chain. Thus, they bioaccumulate and biomagnify environmental contaminants found in their habitats. The snapping turtles of the Laurentian Great Lakes region of North America have been examined to determine the relationship between contaminant body burdens, egg contaminant loads, and reproductive abnormalities.[29] Snapping turtle eggs collected from various localities on Lake Ontario, Lake Erie, and the upper St. Lawrence River exhibit elevated levels of polychlorinated biphenyls (PCBs), dibenso-*p*-dioxins, dibenzofurans, and various organochlorine pesticides or their metabolites (*p,p'*-DDE, dieldrin).[27-32] Using the practical causal inference techniques of ecoepidemiologists,[33] Bishop and colleagues[29] examined the cause-effect linkage between environmental contamination and the development of snapping turtle eggs. They observed that eggs containing the highest contaminant levels exhibited significantly higher rates of embryonic mortality and embryonic deformities when compared to control sites where egg contamination was lower. Although they could not show that contamination of eggs preceded the occurrence of elevated levels of embryonic mortality, low embryonic mortality and deformity is the norm for eggs from control sites in the Great Lakes[29] and elsewhere.[34,35] Interestingly, as described for the alligator eggs collected from Lake Apopka, the majority of embryonic mortality in the highly contaminated snapping turtle eggs occurred early in development.[29]

Although gross anatomical deformities were noted in many hatchlings obtained from the eggs collected at the sites with the highest contaminant levels, a histological examination of these hatchlings was not performed. Thus, at this time it is unknown whether the reproductive organs of these animals are normal or abnormal. Likewise, no data are available on the hormonal milieu present in these hatchlings. It would be extremely interesting to know the sex ratio of the offspring produced at these sites. Snapping turtles exhibit environmental sex determination[36] and estrogenic chemicals stimulate sex reversal of male embryos to females (see later). Examining the sex ratio of eggs incubated at a temperature expected to produce a 1:1 sex ratio could provide a simple bioassay of the presence of estrogenic compounds in the eggs of turtles. A recent study has demonstrated that various PCB congeners can induce sex reversal in turtles when applied to the outside of the egg shell, as has been repeatedly demonstrated following estradiol-17β treatment (see discussion on

Mechanisms). Bergeron et al[37] observed that 2′,4′,5′-trichloro-4-biphenylol induced 100% sex reversal (based on histological examination of gonads and internal ducts) in the red-eared turtle (*Trachemys scripta*), whereas treatment with 2′,3′,4′,5′-tetrachloro-4-biphenylol stimulated total sex reversal in 50% of the embryos and partial sex reversal (intersex) in 21% of the embryos. Additionally, treatment of eggs with both of these estrogenic PCBs together (given at a dosage 10 to 20 times below the effective dose of the individual compounds) produced a synergistic effect.[37] Interestingly, turtle neonates from Lake Apopka have either apparently normal ovaries or ovotestes (Gross and Guillette: Unpublished data). Moreover, chorioallantoic fluid concentrations of estradiol-17β and testosterone indicate that few hatchlings from Lake Apopka produce a normal androgen synthesis pattern (Gross and Guillette: Unpublished data). Reptiles represent excellent models to determine the extent of estrogenic xenobiotic contamination in an ecosystem due to the apparent lability of sex determination in response to the presence of estrogen or estrogen-like compounds.

POSSIBLE MECHANISMS OF ACTION

Lability of Gonadal Differentiation in Temperature-Dependent Sex Determination Reptiles

Sex determination in many reptiles is dependent on the temperature of embryonic development, and the specific sex-determining regimen varies among species.[36] For example, warm temperatures produce males and cool temperatures produce females in the American alligator,[38,39] whereas the opposite is true in the freshwater turtle *Trachemys scripta elegans*.[40] Over the last decade much research has been dedicated to understanding temperature-dependent sex determination (TSD) in reptiles, and *T. scripta elegans* has been used as a model species for elucidating the complexities of TSD. In this species, temperature-shift experiments have revealed that both duration and magnitude of incubation temperature affect gonadal development during roughly the second trimester of egg incubation.[41,42] These experiments indicate that sex determination is extremely labile during the temperature-sensitive window. Could factors other than temperature alter sex determination in animals with TSD? An answer to this question requires an understanding of the events surrounding and mechanisms controlling TSD.

The exact mechanisms by which temperature affects gonadal development are still unknown, but several lines of evidence suggest that the gonad is influenced indirectly by temperature. In cultured gonads excised from the sea turtle *Lepidochelys olivacia* (a species with TSD), culture temperature has no effect on the differentiation of the gonad.[43] Additionally, cultured gonads of embryonic *T. scripta elegans* secrete no detectable progesterone, testosterone, or estradiol-17β during embryonic development.[44] Cultured kidney-adrenal complexes, however, secrete progesterone, corticosterone, and testosterone, but these steroid secretions do not exhibit sexually dimorphic patterns.[44] If gonadal development is facilitated by the presence of sex-specific steroids (testosterone in males and estradiol-17β in females) as traditionally thought,[45,46] then two alternative hypotheses exist: (1) differential steroid production occurs in organs other than the gonad, adrenal, or mesonephros, or (2) differential activity of enzymes with steroidogenic action is present in or around the developing gonad. The second hypothesis is supported by studies on the fetal adrenal of mammals showing that estrogen can directly regulate steroidogenesis by altering steroidogenic enzyme activity.[47]

It is widely acknowledged that exogenous estradiol-17β can cause gonadal feminization of *T. scripta elegans* embryos that are incubated at male-producing temperatures. Higher doses of estradiol-17β result in the production of significantly more female turtles and, thus, the effects of estradiol are said to be dose dependent.[48] Exogenous estradiol-17β alters gonadal differentiation during the same developmental period as temperature sensitivity.[49] These results suggest that temperature and estradiol act in a common pathway in TSD, but the direct effects of estradiol are not completely understood. Autoradiography reveals that radiolabeled estradiol-17β is localized in the mesonephros prior to gonadal differentiation, the mesonephros and oviduct during gonadal differentiation, and the mesonephros, oviduct, and interrenal gland after gonadal differentiation.[50] The lack of estradiol-17β incorporation in the gonad and the significant incorporation in the mesonephric area support the hypothesis that sex determination in animals with TSD is not a direct influence on the gonad. It has recently been suggested that both estradiol-17β and female producing temperatures ensure female sex determination by facilitating medullary cord regression.[51]

Experimental treatments have been used to discern the effects of various hormones or endocrine-disrupting compounds on gonadal development of turtles[52] and alligators[53] (see Table I). Exogenous estradiol-17β alters sex determination in both turtle (*T. scripta elegans*) and American

TABLE I

Affects on Sex Determination Following Treatment of Alligator or Turtle Eggs with Various Compounds.*

Treatment	Action	Red Eared Turtle†		American Alligator	
		% Male 26°C ♂♀○	% Female 31°C ♂♀○	% Male 33°C ♂♀○	% Female 30°C ♂♀○
Control	Control	100/0/0	0/100/0	100/0/0	0/100/0
Vehicle control	Control	0/100/0	0/100/0	100/0/0	0/100/0
Estradiol-17β	Steroid (estrogenic)	53/47/0		0/100/0	0/100/0
Testosterone	Steroid (androgenic)	100/0/0	0/100/0	100/0/0	0/100/0
Dihydrotestosterone	Steroid (androgenic)	100/0/0	9/91/0	100/0/0	0/85/15
4-OH-androstenedione	Steroid-aromatase blocker	100/0/0	0/100/0		
ATD‡	Steroid-aromatase blocker			100/0/0	0/71/29
Aminoglutethimide	Aromatase blocker			100/0/0	0/0/100
CGS 16949‡	Aromatase blocker	100/0/0	0/100/0	0/100/0	0/100/0
Tamoxifen	Antiestrogen estrogenic			100/0/0	0/100/0
ICI M154384§	Antiestrogen			100/0/0	0/100/0
Cyproterone acetate	Antiandrogen				
OH-flutamide	Antiandrogen	94/6/0			

* Data for alligator eggs are obtained from Lance and Bogert[32] (eggs injected) whereas the turtle data are derived from Wibbels and Crews [33] (high dose, topical administration).

† ♂ = male; ♀ = female; ○ = ambiguous sex.

‡ 4-(5,6,7,8-Tetrahydroimidazo [1,5α]pyridin-5yl) benzonitrile monohydrochloride (Ciba-Geigy, Summit, NJ).

§ 11*N*-n-butyl-11-(3, 17β-dihydroxyoestra-1,3,5(10)-trien-7α-yl)-*N*-methylundecanamide), (ICI, United Kingdom).

alligator (*A. mississippiensis*) embryos; thus, environmental contaminants that alter the dynamics of endogenous estradiol and possibly other sex steroids could also affect gonadal development. A review of both studies suggests that estrogenic chemicals can induce embryonic feminization, some androgens can induce feminization, and alterations in aromatase (the enzyme responsible for conversion of androgens to estrogens) activity can cause abnormal gonadal differentiation. All of these effects may be species or dose specific. The finding that testosterone can induce feminization seems odd, but can be explained by the fact that aromatase converts this androgen readily to an estrogen. Any contaminant that alters aromatase dynamics is expected to cause abnormal estrogen to androgen ratios which, in turn, can alter reproductive tract development and function. Wibbels and Crews[54] use fadrozole (a potent inhibitor of aromatase activity) to induce male sex determination in the embryos of the parthenogenic lizard *Cnemidophorus uniparens*, and in freshwater turtles (*T. scripta elegans*). Taken together, these studies indicate that masculinization and feminization of developing reptilian embryos can be induced by exogenous agents. Based on the relative doses and potencies of the administered agents, it appears that feminization is more easily induced. This can be attributed to the fact that ovarian differentiation appears dependent on estrogens, but that testicular differentiation may be independent of steroid hormones.[53]

Thus, xenobiotics that alter estrogen dynamics could have greater developmental effects than those that alter androgen dynamics. That is, if a compound acts as an estrogen or antiandrogen, it will change the hormonal mileau so that the developing gonad experiences an estrogenic environment. It is again important to emphasize that it is not the absence or presence of sex steroids that is important to the developing embryo but the relative ratio of these steroids that influences sex determination and the organizational development of the reproductive system. The relationship between the steroidal milieu and the development of the reproductive system is not specific to reptiles with TSD, but appears to be almost universal among vertebrates.[55] Reptiles with TSD, however, make excellent model systems due to the responsiveness of the reproductive system to exogenous estrogenic compounds.

Mechanisms of Endocrine Disruption

Three variables regulate the potency with which a contaminant disrupts the endocrine system: functional similarity of the contaminant to a hormone, magnitude of exposure, and timing of exposure. The action of many

endocrine disruptors appears to be defined more by function than structure[56] and, thus, the functional similarity of the contaminant to an endocrine regulator is of importance. Magnitude of exposure is of obvious importance (higher exposures elicit greater effects), but the timing of an exposure is perhaps the most overlooked and important variable in dictating the potency of an endocrine-disrupting contaminant. For a developing embryo, development and growth are accelerated by hormones, and exposure to endocrine-disrupting contaminants are especially harmful during this critical period. This has been shown in humans,[57] wildlife in general,[15] and reptiles in particular.[29,52] With these three variables in mind, we shall explore specific mechanisms by which contaminants may disrupt normal endocrine control of reproduction. Contaminants can act by altering the expression of hormone-induced genes, the expression of genes whose product can modify hormone-induced genes, or the genetic expression of steroidogenic enzymes (Fig. 2).

In the simplest scenario, the environmental contaminant binds directly to a hormone receptor, stimulating or blocking the expression of hormone-induced genes. A number of synthetic estrogens (such as diethylstilbesterol) and xenobiotic contaminants (such as *o,p'*-DDT, some PCBs, nonylphenol) are known to elicit effects through estrogen receptors,[16,58,59,61] but the majority of environmental contaminants have not been tested for their ability to bind estrogen, or any steroid, receptors.[56] Detergent components in sewage effluent stimulate in vivo[60] and in vitro[61] production of vitellogenin (a major yolk protein found in female plasma after estrogen stimulation of the liver) from rainbow trout (*Oncorhyncus mykiss*), a phenomenon experimentally demonstrated to be mediated via the estrogen receptor.[61] If contaminants mimic the actions of an estrogen, one would predict that sex ratios would be skewed in wildlife populations where estrogens are capable of stimulating sex reversal of males to females—populations of reptiles exhibiting TSD.

Contaminants may also bind to hormone receptors, blocking normal hormonal functions. The fungicide vinclozolin is an example of a contaminant that is an androgen antagonist. Two metabolites of vinclozolin bind to and block the action of androgen receptors, causing demasculinization (but not complete feminization) of male rats exposed to this contaminant's breakdown products in utero.[62] The increased frequency of developmental abnormalities of male phallic structures, as reported in humans,[63] fish,[64] and alligators,[6] is most easily explained by hypothesizing that circulating androgen concentrations have been reduced during embryonic develop-

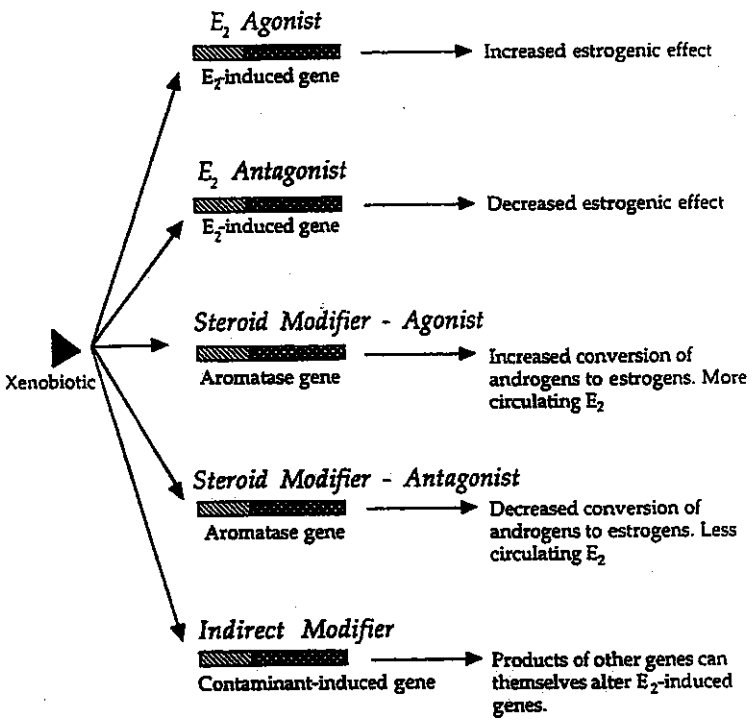

FIGURE 2 Mechanisms of action of possible endocrine-disrupting contaminants. The binding of a contaminant to a hormone-induced gene (in this case the estrogen-responsive element of an estrogen-induced gene) can result in agonistic and antagonistic effects. Similarly, the production of steroid modifiers such as aromatase can be induced or inhibited. Contaminants also may alter endocrine functioning through indirect mechanisms. E_2 = estradiol.

ment and early life. If xenobiotic contaminants can act as antiandrogens, the developing male reproductive tract would see a cellular environment deficient in androgens but relatively rich in estrogens. This internal environment would create the abnormalities observed in the alligators of Lake Apopka—a reduced or structurally abnormal phallus. To test for endocrine-disrupting effects of specific contaminants, McLachlan[56] has suggested screening contaminants for their ability to bind and activate or inactivate various steroid receptors. It is important to note that inactivation of a receptor-hormone complex required for normal development is as critical to the embryo as its inappropriately timed activation.

Many contaminants elicit a broad spectrum of responses. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) exerts partial demasculinization and feminization of male rats exposed in utero without altering estrogen receptor binding.[65] The exact mechanisms have not been discerned, but the antiestrogenic effects of TCDD appear to be mediated indirectly through the aryl hydrocarbon (AH) receptor.[66] Many PCBs may also exert effects via the AH receptor,[67] and AH receptor induction should be studied to elucidate any associated endocrine disruptions.

Environmental contaminants may also alter hormonal conditions by expression of genes whose product can modify the synthesis or functioning of steroidogenic enzymes. The cytochrome P450 enzyme system is the predominant biotransformation pathway in the initial detoxification of toxicants, and eight distinct gene families of cytochrome P450 enzymes have been characterized. The gene families can be separated into two groups differing in function: families I, II, III, and IV are involved in xenobiotic biotransformation, whereas families XI, XVII, XIX, and XXI are involved in steroid hormone biosynthesis. Cytochrome P450 enzymes add a hydroxyl moiety to a compound that, in the case of xenobiotics, facilitates excretion of the substance and, in the case of steroid biotransformation, converts androgens to estrogens. On chemical insult from inducing agents (such as halogenated pesticides, PCBs, steroids, and dioxins), the synthesis of cytochrome P450 enzymes is increased.[68] This enzyme induction is thought to function in xenobiotic transformation for enhanced excretion but, if steroid hormone biosynthesis is also stimulated, may be responsible for the altered hormonal conditions associated with exposure to endocrine-disrupting contaminants. For example, juvenile male and female alligators from Lake Apopka have elevated plasma estrogens and males have greatly reduced plasma androgen concentrations compared to control juveniles. Although it has been hypothesized that modified gonadal steroidogenesis is responsible for this phenomenon,[6] in vitro testing of gonadal steroidogenesis suggests that other organs may be involved, such as a modification in liver P450 degradation of steroids.[7] Other enzymes that facilitate hormone degradation may be up-regulated directly or indirectly by contaminants. The hypothesis that contaminant-induced activation of liver or gonadal aromatase activity stimulates the degradation or conversion of androgens to estrogens, and thus modifies the steroid milieu of developing embryos, has rarely been considered but deserves much further examination. This research is of particular importance given the data demonstrating that many chemicals

have apparent endocrine-disrupting actions but are structurally unrelated to the hormones they mimic or block.

FINAL PERSPECTIVES

This article reviews some of the data on the lethal and reproductive effects of environmental contaminants on reptiles. Among the reproductive effects are sex reversal as well as other, less conspicuous changes such as altered sex hormone dynamics and increased morphological abnormalities of the reproductive system (such as polyovular follicles, decreased phallus size). It is important for researchers to recognize the continuum of effects that can be caused by environmental contaminants—from death to a subtle change in hormonal regulation. The "subtle" changes can have a tremendous impact on populations of animals, evidence of which is obtained by consideration of the Lake Apopka alligator population. Abnormalities of reproductive function may be sublethal to individuals, but such alterations are lethal to a population of animals. As stewards of our environment, scientists and conservationists must monitor and attempt to rectify any abnormalities induced by our own actions.

Reptiles with TSD are particularly susceptible to disruption of the endocrine system and, thus, provide a good model for monitoring the endocrine perturbations caused by environmental contaminants. Both acute (such as sex reversal) and chronic (such as increased frequency of polyovular follicles; decreased secretion of hormones) reproductive effects have been recognized in reptiles, and such parameters should be considered when monitoring the effects of contaminants. The effects of contaminants on reproduction of reptiles are important in themselves, but may also elucidate a problem of broad spectrum and consequence. In humans, elevations in the rates of breast cancer, cervical cancer, testicular cancer, and infertility are all attributed to abnormal endocrine function, and it has been hypothesized that endocrine-disrupting contaminants are involved.[63,69] Intense effort should be made to establish a clear cause-effect relationship between environmental contaminants and reproductive dysfunction in as many model systems as are relevant and economically (time and funds) realistic, eliminate problematic contaminants, and establish screening tests for detecting chemicals that are endocrine disruptors. Studies of wildlife clearly demonstrate that environmental contamination continues adversely to affect them and their offspring.

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References

1. C.W. Bason and T. Colborn. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing, Princeton, NJ, 1992), pp. 335–346.
2. K.B. Thomas and T. Colborn. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing, Princeton, NJ 1992), pp. 365–394.
3. M.L. Jennings, H.F. Percival, and A.R. Woodward. Proc. Ann. Conf. Southeast Assoc. Fish. Wildl. Agencies **42**:283 (1988).
4. H.F. Percival, G.R. Masson, and K.G. Rice. Florida Cooperative Fish and Wildlife Research Unit, University of Florida (1992).
5. A.R. Woodward, M.L. Jennings, H.F. Percival, and C.T. Moore. Fl. Sci. **56**:52 (1993).
6. L.J. Guillette Jr., T.S. Gross, G.R. Masson, J.M. Matter, H.F. Percival, and A.R. Woodward. Environ. Health Perspect. **102**:680 (1994).
7. L.J. Guillette Jr., T.S. Gross, D. Gross, A. Rooney, and H.F. Percival. Environ. Health Perspect. **103** (Suppl 4): (1995).
8. U.S. Environmental Protection Agency. U.S. EPA, Region 4, Atlanta, GA (1979).
9. C. Schelske and P. Brezonik. *Restoration of Aquatic Ecosystems: Science, Technology and Public Policy.* (National Academy Sciences, Washington, DC, 1992), pp. 393–398.
10. G.R. Masson. Ph.D. Dissertation. University of Florida, (1995).
11. D.R. Clark. US Fish Wildl. Tech. Report **29**:1 (1990).
12. T.E. Colborn, A. Davidson, S.N. Green, R.A. Hodge, C.I. Jackson, and R.A. Liroff. *Great Lakes Great Legacy?* (The Conservation Foundation, Washington, DC, 1990).

13. K. Cooper. In Hayes W.J., E.R. Laws, Jr., eds., *Handbook of Pesticide Toxicology*. Vol. 1 (Academic Press, New York, 1991), pp. 463–495.
14. G.H. Heinz, H.F. Percival, and M.L. Jennings. Environ. Monit. Assess. **16**: 277 (1991).
15. T. Colborn, F.S. vom Saal, and A.M. Soto. Environ. Health Perspect. **101**: 378 (1993).
16. A.M. Soto, T.-M. Lin, H. Justicia, R.M. Silvia, and C. Sonnenschein. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing, Princeton, NJ, 1992), pp. 295–309.
17. A.M. Soto, K.L. Chung, and C. Sonnenschein. Environ. Health. Perspect. **102**: 380 (1994).
18. T. Iguchi, Y. Fukazawa, Y. Uesugi, and N. Takasugi. Biol. Reprod. **43**: 478 (1990).
19. T. Iguchi, K. Kamiya, Y. Uesugi, K. Sayama, and N. Takasugi. In vivo **5**: 359 (1991).
20. A. Halling, J.-G. Forsberg. Biol. Reprod. **45**: 157 (1991).
21. L.J. Guillette, Jr. Hearing on: "Health effects of estrogenic pesticides." Subcommittee on Health and the Environment, Congressman Henry A. Waxman, Chairman 103-87; U.S. Govern. Print. Off.: Washington, DC, (1994).
22. T.R. Forbes. Anat. Rec. **75**: 51 (1939).
23. L.S. Ramaswani and D. Jacob. Experientia **21**: 206 (1965).
24. A. Raynaud and C. Pieau. In C. Gans and F. Billett, eds., *Biology of the Reptilia*: Vol. 15: Development B (John Wiley & Sons, New York, 1985), pp. 149–300.
25. A. van Teinhoven. *Reproductive Physiology of Vertebrates* (Cornell University Press, Ithaca., NY, 1983).
26. D.R. Clark and A.J. Krynitsky. Bull. Environ. Contam. Toxicol. **34**: 121 (1985).
27. A.M. Bryan, W.B. Stone, and P.G. Olafsson. Bull. Environ. Contam. Toxicol. **39**: 791 (1987).
28. C.E. Hebert, V. Glooschenko, G.D. Haffner, and R. Lazar. Arch. Environ. Contam. Toxicol. **24**: 35 (1993).
29. C.A. Bishop, R.J. Brooks, J.H. Carey, P. Ng, R.J. Norstrom, and D.R.S. Lean. J. Toxicol. Environ. Health **33**: 521 (1991).
30. W.B. Stone, E. Kiviat, and S.A. Butkas. NY Fish Game J. **27**: 39 (1980).
31. J.J. Ryan, B.P.-Y. Lau, J.A. Hardy, W.B. Stone, P. O'Keefe, and J.F. Gierthy. Chemosphere **15**: 537 (1986).
32. J. Struger, J.E. Elliott, C.A. Bishop, M.E. Obbard, R.J. Norstrom, D.V. Weseloh, M. Simon, and P.J. Ng. Great Lakes Res. **19**: 681 (1993).
33. G.A. Fox. J. Toxicol. Environ. Health **33**: 359 (1991).
34. G.C. Packard, G.L. Paukstis, T.J. Boardman, and W.H.N. Gutzke. Can. J. Zool. **63**: 2422 (1985).
35. G.C. Packard, M.J. Packard, K. Miller, and T.J. Boardman. Ecology **68**: 983 (1987).
36. J.J. Bull. Quart. Rev. Biol. **55**: 3 (1980).
37. J.M. Bergeron, D. Crews, and J.A. McLachlan. Environ. Health Perspect. **102**: 780 (1994).
38. M.W.J. Ferguson and T. Joanen. Nature **296**: 850 (1982).
39. J.M.P. Joss. J. Zool. **21**: 679 (1989).
40. J.J. Bull, R.C. Vogt, and C.J. McCoy. Evolution **36**: 326 (1982).
41. J.J. Bull, T. Wibbels, and D. Crews. J. Exp. Zool. **256**: 339 (1990).
42. T. Wibbels, J.J. Bull, and D. Crews. J. Exp. Zool. **260**: 371 (1991).
43. H. Merchant-Larios and I. Villalpando. J. Exp. Zool. **254**: 327 (1990).
44. R.B. White and P. Thomas. Gen. Comp. Endocrinol. **88**: 10 (1992).
45. M.H. Bogart. J. Theor. Biol. **128**: 349 (1987).
46. F.D.A. Uchima, A.K. Vallerga, G.L. Firestone, and H.A. Bern. Proc. Soc. Exp. Biol. Med. **195**: 218 (1990).
47. G.J. Pepe and E.D. Albrecht. Endocrinol. Rev. **11**: 151 (1990).
48. D. Crews, J.J. Bull, and T. Wibbels. Gen. Comp. Endocrinol. **81**: 357 (1991).

49. T. Wibbels, J.J. Bull, and D. Crews. J. Exp. Zool. **260**: 130 (1991).
50. M. Gahr, T. Wibbels, and D. Crews. Biol. Reprod. **46**: 458 (1992).
51. T. Wibbels, P. Gideon, J.J. Bull, and D. Crews. Differentiation **53**: 149 (1993).
52. T. Wibbels and D. Crews. J. Endocrinol. **133**: 121 (1992).
53. V.A. Lance and M.H. Bogar. Gen. Comp. Endocrinol. **86**: 59 (1992).
54. T. Wibbels and D. Crews. J. Endocrinol. **141**: 295 (1994).
55. F.S. vom Saal, M.M. Montano, and M.H. Wang. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing Princeton, NJ, 1992), pp. 17–83.
56. J.A. McLachlan. Environ. Health Perspect. **101**: 386 (1993).
57. T. Mori and N. Nagasawa, eds. *Toxicity of Hormones in Perinatal Life*. (CRC Press, Boca Raton, FL, 1988).
58. K.S. Korach, P. Sarver, K. Chae, J.A. McLachlan, and J.D. McKinney. Mol. Pharmacol. **33**: 120 (1987).
59. L.E. Gray. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing, Princeton, NJ, 1992), pp. 203–230.
60. C.E. Purdom, P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler, and J.P. Sumpter. Chemis. Ecol. **8**: 275 (1994).
61. S. Jobling and J.P. Sumpter. Aqua. Toxicol. **27**: 361 (1993).
62. W.R. Kelce, E. Monosson, M.P. Gamcsik, S.C. Laws, and L.E.J. Gray. Toxicol. Appl. Pharmacol. **126**: 276 (1994).
63. R.M. Sharpe and N.E. Skakkebaek. Lancet **341**: 1392 (1993).
64. W.P. Davis and S.A. Bortone. In T. Colborn and C. Clement, eds., *Chemically-induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection* (Princeton Scientific Publishing, Princeton, NJ, 1992), pp. 113–127.
65. D.L. Bjerke, T.J. Brown, N.J. MacLusk, R.B. Hochberg, and R.E. Peterson. Toxicol. Appl. Pharmacol. **127**: 258 (1994).
66. S. Safe, B. Astroff, M. Harris, T. Zacharwski, R. Dickerson, M. Romkes, and L. Biegel. Pharmacol. Toxicol. **69**: 400 (1991).
67. A. Brouwer. Biochem. Soc. Trans. **19**: 731 (1991).
68. I.G. Sipes and A.J. Gandolfi. In M. Amdur, J. Doull, and C. Klaassen, eds., *Casarrett and Doull's Toxicology: The Basic Science of Poisons* (McGraw-Hill, New York, 1993), pp. 88–126.
69. D.L. Davis, H.L. Bradlow, M. Wolff, T. Woodruff, D.G. Hoel, and H. Anton-Culver. Environ. Health Perspect. **101**: 372 (1993).