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Reptiles as models of contaminant-induced endocrine disruption

D.A. Crain [a,*], L.J. Guillette Jr. [b]

[a] *Division of Natural Sciences, Maryville College, Maryville, TN 37804, USA*
[b] *Department of Zoology, University of Florida, Gainesville, FL 32611, USA*

Abstract

Historically, reptiles have been used as bioindicators of environmental contaminants and, currently, reptiles have the potential to elucidate the mechanisms of a newly described group of environmental contaminants—endocrine disrupters. Reptiles are particularly good models for studying endocrine altering compounds due to the fact that different species of reptiles have varying modes of gender determination (genotypic sex determination or temperature-dependent sex determination) and parity modes (oviparity or viviparity). This review focuses both on laboratory and field studies of contaminant-induced endocrine alterations in reptiles. Laboratory studies of oviparous reptiles with temperature-dependent sex determination reveal that embryonic exposure to natural hormones and many man-made chemicals (including the ubiquitous PCBs and common herbicides) can permanently alter the functioning of the reproductive system. It is hypothesized that similar permanent, organizational changes occur in wild reptiles exposed to endocrine-disrupting contaminants.

Keywords: Reptiles; Alligators; Endocrine-disrupters; Reproduction

1. Introduction

For years, reptiles have served as biomonitors of heavy metal (Overmann and Krajicek, 1995) and radionuclide (Meyers-Schone et al., 1993) contamination. Reptiles are particularly suitable as contaminant biomonitors due to their persistence in a variety of habitats, wide geographic distribution, longevity and, in many cases, site fidelity. Additionally, reptiles exhibit a sensitivity to contaminants similar to that reported for

* Corresponding author. Tel.: +1 423 981 8238; fax: +1 423 981 8010; e-mail: crain@maryvillecollege.edu

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birds and mammals (Hall and Clark, 1982) and they bioaccumulate and biomagnify contaminants to levels equal to or greater than that reported for birds and mammals (Olafsson et al., 1983; Bryan et al., 1987; Hall and Henry, 1992).

Recently, reptiles have been used as biomonitors of another class of environmental contaminants—endocrine disrupters. Many natural and synthetic compounds in the environment change the normal functioning of the endocrine system. These compounds are related only by effect (not structure) and are collectively termed endocrine-disrupting contaminants (EDCs) (Guillette et al., 1995a). Reptiles are excellent models for the study of contaminant-induced endocrine disruption because different species exhibit varying modes of gender determination (genotypic sex determination—GSD, or temperature-dependent sex determination—TSD) and parity (oviparity or viviparity) (Table 1). Comparisons among reptiles with GSD and TSD can clarify the effects of EDCs on the mechanisms and organization of the reproductive system, whereas comparisons among closely related oviparous and viviparous species can elucidate complexities surrounding maternal transfer of contaminants by comparing chronic placental transfer to acute oviductal transfer.

The many effects of EDCs on reptiles have been discussed elsewhere (Guillette et al., 1995a; Crain and Guillette, 1997) and are beyond the scope of this paper. This review focuses on the utility of reptilian models for the study of contaminant-induced endocrine-disruption. First, we present a case study of a population of American alligators (an oviparous reptile with TSD) exposed to EDCs. Second, we examine the advances gained from experimental studies of oviparous reptiles with TSD. Finally, we apply this knowledge to our understanding of the EDC-exposed alligators. It is hoped that such an approach will help focus future EDC research endeavors into the context of vertebrate gender-determining and reproductive strategies.

2. A case study of endocrine disruption

In the late 1970s, the Florida Game and Freshwater Fish Commission initiated studies to examine the status of Florida's alligators. More than two decades of research indicates that the alligator population on one particular lake, Lake Apopka, exhibits low clutch viability, decreased juvenile population densities and unexplained adult mortality (Woodward et al., 1993). There are many working hypotheses for the cause of these problems (Masson, 1995), but the most likely explanation is endocrine-disruption (Guillette and Crain, 1995).

Juvenile alligators from Lake Apopka express a number of characteristics that are consistent with endocrine-disruption, including having abnormal circulating hormone concentrations. When compared to alligators from a reference lake, Apopka females have elevated oestradiol-17β (Guillette et al., 1994) and males have depressed testosterone concentrations (Guillette et al., 1994, 1996, 1997; Crain et al., 1998a) These abnormal hormone concentrations are associated with several structural abnormalities of the juvenile alligators. Ovaries from Apopka females have numerous polyovular follicles and polynuclear oocytes (Guillette et al., 1994). Although the effect of these aberrations on adult reproduction is unknown, polyovular follicles typically yield eggs with low

fertilization success (Iguchi et al., 1990, 1991). Testes from Apopka males have poorly organized seminiferous tubules and aberrant bar-shaped nuclei (Guillette et al., 1994), similar to abnormalities seen in alligators treated with *p,p′*-DDD, an environmental metabolite of DDT (Crain and Guillette, unpubl.). Lake Apopka juvenile alligators also have abnormal sexual characteristics. Penis size is significantly reduced in animals of Lake Apopka compared to animals from a reference lake (Guillette et al., 1996). Collectively, these data suggest that the alligators of Lake Apopka experience endocrine disruption.

If the Lake Apopka alligators are so affected, what are the causative agents and how are they affecting the endocrine system? One possible explanation is that the effects seen today can be traced back to a pesticide spill in Lake Apopka in 1980. The spill consisted primarily of dicofol, but also had significant amounts of DDT, DDE and DDD (U.S. EPA, 1994). Analysis of alligator eggs taken from Lake Apopka in 1984 and 1985 revealed significant residues of toxaphene, dieldrin, *p,p′*-DDE, *p,p′*-DDD, *trans*-nonachlor, and PCBs (Heinz et al., 1991). Many of these compounds have been shown to bind to the alligator oestrogen receptor (Vonier et al., 1996). However, unlike oestradiol-17β, many of these EDCs do not bind to alligator serum and cytosolic steroid binding proteins (Arnold et al., 1996; Crain et al., 1998b) and, thus, the EDCs may be more bioavailable to cells and nuclear receptors when compared to endogenous oestradiol-17β. Although an interaction with the oestrogen receptor is only one of many means of causing endocrine disruption (Crain and Guillette, 1997), this mechanism is of particular interest because of the complex interactions of many compounds with the oestrogen receptor. For example, although many compounds actively compete for the oestrogen receptor (such as *p*-nonylphenol and *o,p′*-DDT), others such as butyl benzyl phthalate appear to displace endogenous oestradiol from the receptor through non-competitive mechanisms (Gaido et al., 1997). It is hypothesized that some of the endocrine-disrupting effects of the Lake Apopka alligators (such as polyovular follicles) are caused by the interaction of multiple EDCs with the alligator oestrogen receptor. This is consistent with data showing that activity of the gonadal steroidogenic enzyme aromatase is decreased in Lake Apopka alligators (Crain et al., 1997), perhaps through negative feedback circuits initiated at the oestrogen receptor. However, it is probable that mechanisms independent of the oestrogen receptor are involved because the decreased aromatase activity is inconsistent with the elevated oestradiol and depressed testosterone seen in the juvenile Apopka alligators. It has recently been proposed that hepatic degradation and the excretion of steroid hormones may also be altered in the Apopka alligators (Crain and Guillette, 1997), but this remains to be tested.

3. Oviparous reptiles with temperature-dependent sex determination

The temperature at which the eggs are incubated determines gender in many reptile species (Fig. 1). Research to date has shown that all crocodiles, many turtles and some lizards lack distinct sex chromosomes, and that the sex not organized until well after fertilization, during organogenesis. This pattern of gender determination differs greatly from that of animals with genotypic sex determination (GSD). In the latter, gender is

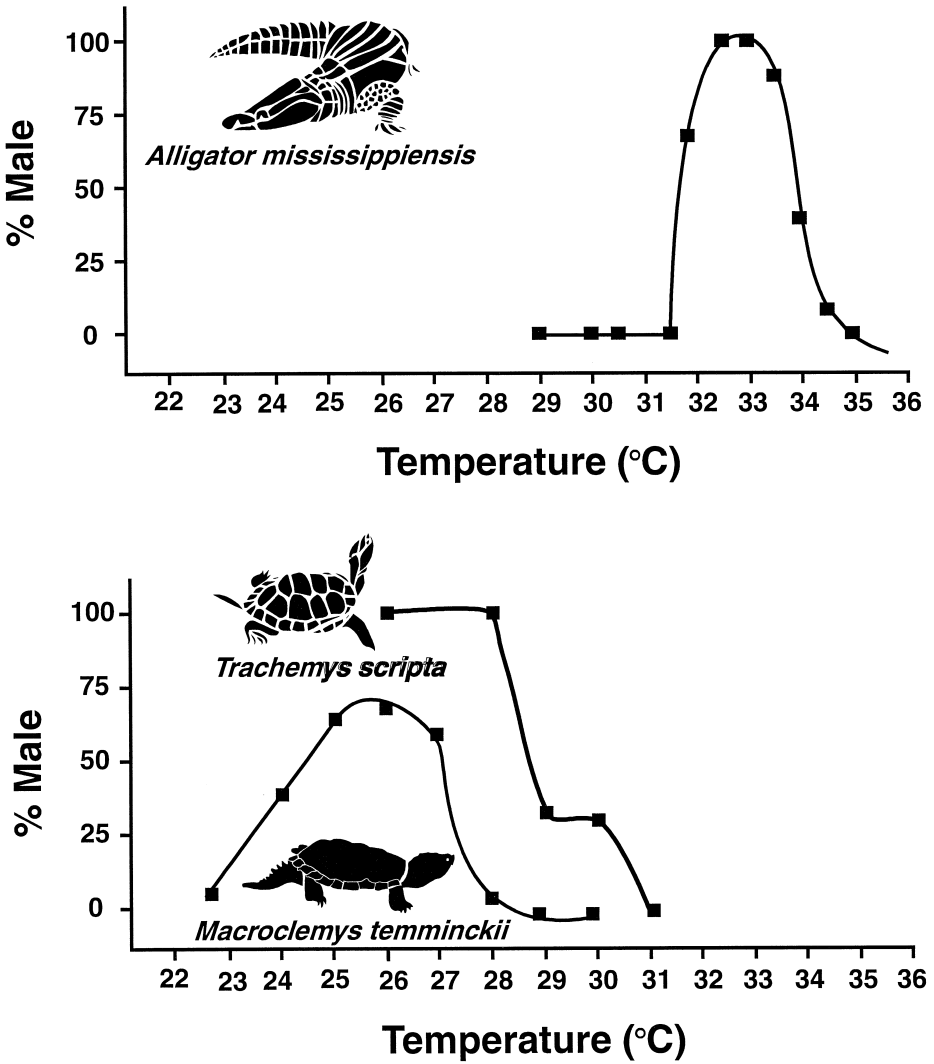

Fig. 1. Temperature-dependent sex determination in the American alligator (*Alligator mississippiensis*), the red-eared slider turtle (*Trachemys scripta elegans*), and the alligator snapping turtle (*Macroclemys temminckii*). Data from Ewert et al. (1994); Lang and Andrews (1994); Wibbels et al. (1991a,b).

determined at fertilization by sex chromosomes inherited from the parents that allow development of a default sex or override the development of this default sex (Crews, 1993). For example, in mammals females are the default gender and males are produced only after activation of genes on the Y chromosome (Norris, 1997). In animals with TSD, gender is determined during organogenesis by incubation temperature. The concept of default sex in animals with TSD has not been fully resolved, but is possible that temperature acts through similar mechanisms as sex chromosomes to override a

default gender. In animals with TSD, gender determination is sensitive to both duration and magnitude of incubation temperature, with temperature exerting an 'all or none' effect on ovarian or testicular differentiation (Wibbels et al., 1991a). In general, the period of temperature sensitivity occurs during the middle of embryonic development and is complete by the time the gonads begin to sexually differentiate (Wibbels et al., 1991a).

Interestingly, temperature is not the only factor that can influence gender determination and reproductive function in reptiles with TSD. When oestradiol-17β is administered to eggs during the temperature sensitive period, phenotypic females are produced at male-producing temperatures in turtles (Gutzke and Bull, 1986; Crews et al., 1991), lizards (Tousignant and Crews, 1994) and alligators (Lance and Bogart, 1991; Crain et al., 1997). Therefore, it has been suggested that temperature and oestradiol-17β share a common pathway in sex determination of animals with TSD (Wibbels et al., 1991b). Further, exposure to other oestrogens, oestrone and oestriol (Crews et al., 1996), and aromatizable androgens, androstenedione and testosterone (Crews et al., 1995b), produces females at a male-producing incubation temperature. In contrast, application of androgens to eggs incubated at a female-producing temperature fails to induce masculinization (Wibbels and Crews, 1992). It is possible, however, to induce the formation of males at a female temperature after exposure to aromatase inhibitors (Richard-Mercier et al., 1995). Collectively, these results indicate that the mechanisms leading to the production of males can more easily be overridden than those leading to the production of females. Regardless, animals with TSD appear to be particularly sensitive to developmental exposure to exogenous hormones, and temperature-dependent gender can be altered by hormone exposure.

In addition to natural endogenous oestrogens, exposure to man-made chemicals can also alter the normal pathway of gender determination in TSD animals. For instance, exposure of red-eared turtle (*Trachemys scripta elegans*) eggs to some polychlorinated biphenyls (PCB) causes feminization, even though the eggs are incubated at a male-producing temperature (Bergeron et al., 1994; Crews et al., 1995a). These results are identical to those of oestradiol-treated eggs, and suggest that these PCBs are having an 'oestrogenic' effect on the embryonic gonad. The effects of PCBs and oestradiol illustrate that exogenous chemicals can change the organization of the reproductive system. In general, contaminant effects on the endocrine system can be categorized as either organizational or activational (Guillette et al., 1995a). Organizational effects are those that cause permanent structural or functional change in the endocrine system, whereas activational effects are those that cause a temporary change in a normally organized system (Guillette et al., 1995a). Many studies have shown that PCBs (Biessmann, 1982; Gray et al., 1993) and *o,p′*-DDT (Palmer and Palmer, 1995) can change the reproductive system in an activational manner, but the study by Bergeron et al. (1994) illustrates that PCBs can also change reproductive function in an organizational fashion.

The gender-reversing effects of PCBs in embryonic red-eared turtles exemplify the extent to which contaminants can alter the endocrine system, but other endocrine-disrupting effects are much more subtle. Just as some PCBs can cause an organizational change in gender, other compounds may cause an organizational change in reproductive

function. For example, consider gender-specific enzyme activity. Animals have sexually dimorphic patterns of enzyme activity in the gonad and liver, and research indicates that this dimorphism is established by embryonic exposure to endogenous steroids (Lucier et al., 1982, 1985). Moreover, this normal dimorphism can be altered by embryonic exposure to exogenous steroids; androgens can masculinize a female liver and oestrogens can feminize a male liver (Gustafsson, 1994). Similarly, embryonic exposure to EDCs could also change the organization of normal sexually dimorphic enzyme patterns. For example, exposure of embryonic alligators to high dosages of the herbicide atrazine (14 ppm) causes hatchling male alligators to exhibit elevated gonadal aromatase activity (Fig. 2) (Crain et al., 1997). Aromatase is the enzyme that converts androgens to oestrogens by binding the C19 androgen substrate and catalyzing several reactions leading to a phenolic ring characteristic of oestrogens (Simpson et al., 1994). In reptiles with TSD, aromatase activity is associated with the production and function of females (Crews and Bergeron, 1994; Jeyasuria et al., 1994; Smith et al., 1995) and, therefore, although the atrazine-exposed alligators appear to have morphologically normal testes, these testes have steroidogenic enzyme activity similar to that of normal ovaries.

A change in the pattern of steroidogenic enzyme activity is only one example of how EDCs can organizationally alter the function of the reproductive system. Recent evidence suggests that changes in endogenous oestradiol concentrations during embryonic development can permanently alter the morphology and function of the adult rat prostate (vom Saal et al., 1992). Also, DDT exposure is thought to feminize female gulls in much the same way that PCBs feminize the hatchling turtles (Fry and Toone, 1981). Therefore, the organizational effects of EDCs appear to affect animals with GSD as well

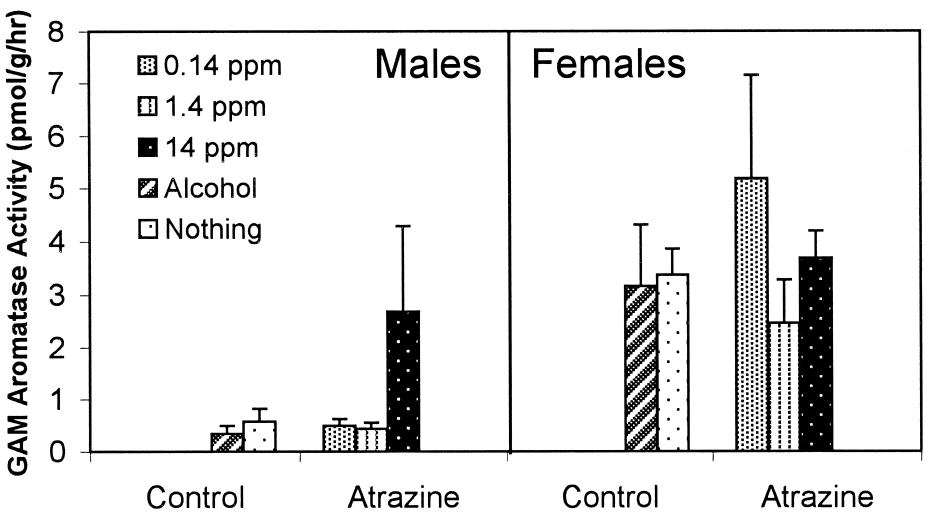

Fig. 2. Aromatase activity of the Gonad/Adrenal/Mesonephros complex from hatchling alligators that were exposed in ovo to varying concentrations of the common herbicide atrazine. Alligators incubated at a male-incubation temperature had testes that appeared structurally normal, however, these testes had enzymatic activity similar to that of ovaries. Data from Crain et al. (1997) with permission.

as those with TSD, but animals with TSD are particularly good bioindicators of organizational endocrine disruption due to the lability and oestrogen-dependence of gender determination and, ultimately, reproductive function.

4. EDC-exposed alligators—revisited

Several concepts gleaned from the experimental research on reptiles with TSD can help elucidate the endocrine disruption in the Lake Apopka alligators. First, it is apparent that an adequate assessment of endocrine disruption can only be obtained when multiple levels of study are considered. A single molecular endpoint (i.e. oestrogen receptor binding) is not, alone, an adequate indicator of endocrine disruption. Likewise, a single whole-organism endpoint may not detect endocrine disruption. For instance, while some EDCs cause feminization in embryonic reptiles (Bergeron et al., 1994), others elicit more subtle changes such as alteration of steroidogenic enzyme activity (Crain et al., 1997). Therefore, a multi-scale approach to studying endocrine disruption is needed. Fig. 3 presents an example of such a multi-scale approach by summarizing studies on the alligators of Lake Apopka. Only when such an approach is taken can an accurate assessment of endocrine disruption be made.

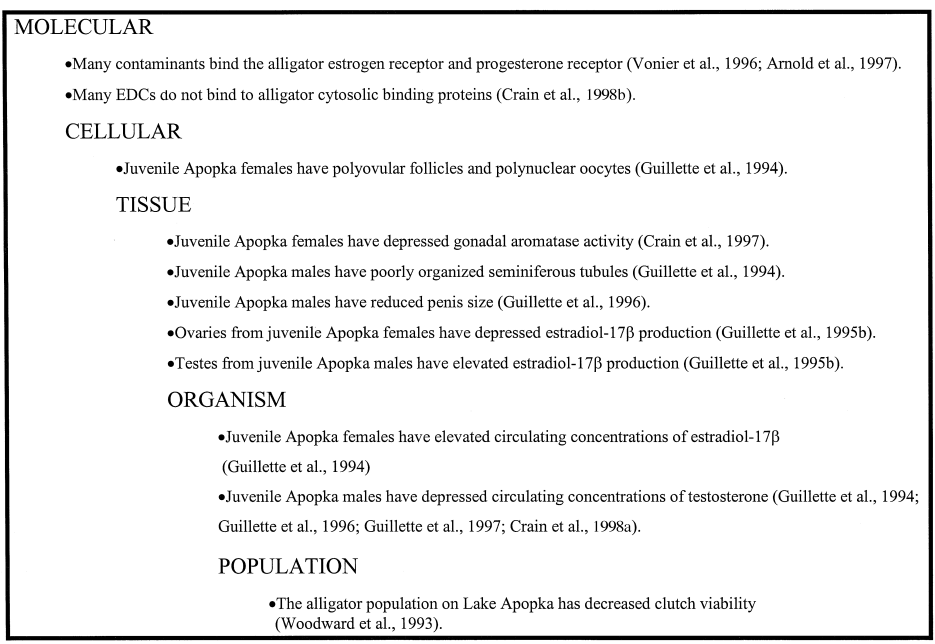

Fig. 3. A multi-scale approach is presented to better understand endocrine disruption in the Lake Apopka alligators. The five levels shown are: (1) molecular (Vonier et al., 1996; Arnold et al., 1997; Crain et al., 1998b); (2) cellular (Guillette et al., 1994); (3) tissue (Crain et al., 1997; Guillette et al., 1994, 1996, 1995b); (4) organism (Guillette et al., 1994, 1996, 1997; Crain et al., 1998a); (5) population (Woodward et al., 1993).

Second, it is clear that the reproductive function of adult reptiles is determined, at least in part, during an extremely labile embryonic period. Therefore, exposure of alligator embryos to EDCs could permanently alter the structure and function of the reproductive system. Indeed, the alligator eggs taken from Lake Apopka have high concentrations of many compounds known to be endocrine disrupters (Heinz et al., 1991), and the reproductive abnormalities appear at or before hatching (Guillette and Crain, unpubl.).

Third, natural and synthetic oestrogens are capable of reversing the effects of a male-producing temperature, but androgens are not able to override the effects of a female-producing temperature. Therefore, feminization of species with TSD is one of the best endpoints available for testing in vivo endocrine disruption of oestrogen agonists. This model may not be suitable for testing EDCs that act as oestrogen antagonists, androgen antagonists or androgen agonists.

Studies of oviparous reptiles with TSD have contributed greatly to our current understanding of contaminant-induced endocrine disruption. Reptiles, in general, are excellent models for endocrine disruption research, as different species of reptiles have various forms of gender determination (TSD and GSD) and have different parity modes (oviparity and viviparity). The complex nature of contaminant-induced endocrine disruption requires that scientists incorporate non-traditional endpoints and species into EDC research.

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