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The Environmental Contaminant DDE Fails to Influence the Outcome of Sexual Differentiation in the Marine Turtle *Chelonia mydas*

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In many turtles, the temperature experienced during the middle of egg incubation determines the sex of the offspring. The implication of steroid sex hormones as the proximate trigger for sex determination opens the possibility that endocrine-disrupting contaminants may also influence the outcome of sexual differentiation. In this study we investigate the potential effects of DDE (a common DDT metabolite) on sexual differentiation of *Chelonia mydas* (green sea turtle). Four clutches of eggs collected from Heron Island, Queensland, Australia, were treated with DDE at the beginning of the thermosensitive period for sexual determination. An incubation temperature of 28°C or less produces male hatchlings in this species, whereas 30°C or more produces female hatchlings. Dosed eggs were consequently incubated at two temperatures (27.6°C and 30.4°C) on the upper and lower boundaries of the sex determination threshold for this species. DDE, ranging from 3.3 to 66.5 μg, was dissolved in 5, 10, and 25 μl ethanol and applied to eggshells above the embryo. Less than 2.5 ng/g DDE was present in eggs prior to dosing. Approximately 34% of the applied DDE was absorbed in the eggs, but only approximately 8% of applied DDE was found in embryos. Thus, treated eggs, corrected for background DDE, had up to 543 ng/g DDE. The sex ratio at these doses did not differ from what would be expected on consideration of temperature alone. Incubation time, hatching success, incidence of body deformities, hatching size, and weight were also within the limits of healthy developed hatchlings. This indicates that the eggs of *C. mydas* in the wild with concentrations of DDE less than 543 ng/g should produce hatchlings with relatively high hatching success, survival rate, and normally differentiated gonads. *Key words*: *Chelonia*, DDT, endocrine-disrupting contaminant, temperature-dependent sex determination. *Environ Health Perspect* 106:185–188 (1998). [Online 26 February 1998]

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In many turtles, the temperature experienced during the middle third of incubation determines the sex of the offspring (*1,2*). Recent evidence indicates that steroid sex hormones are the proximate trigger for sex determination (*3–5*), though the mechanism by which temperature influences hormonal balance or hormone receptors in the developing embryo is not yet fully understood. Offspring sex can be influenced in the laboratory by administration of compounds such as tamoxifen (*6*), 17β-estradiol (*7–9*), estradiol benzoate (*4*), and testosterone propionate (*4*), which opens the possibility that endocrine-disrupting contaminants will influence hatchling sex ratios in the wild. DDT, its metabolic product DDE (*10*), and polychlorinated biphenyl compounds (PCBs) (*11*) are likely candidates (*12*) and should be of special concern, as they are the most common contaminants accumulated in animal tissues (*13–16*).

Although banned in some countries, DDT is widely used in developing countries (*17,18*), and this insecticide and its derivatives are well established as environmental contaminants (*19,20*). DDT accumulates in lipid tissues and is progressively metabolized to DDE (*21,22*), which is more persistent than its parent compound.

An estimated rate of over 12,000 tons of DDE per year ended up in the oceans in the 1970s (*23,24*). While some is adsorbed to sediments of the ocean floor (*25*), most of it enters the marine food chain (*23,24*). Once in an organism, DDE can affect the endocrine system, resulting in effects on growth, development, and reproduction (*10,26,27*). The embryo stage appears to be the developmental stage most vulnerable to the effects of endocrine-disrupting contaminants (*12*). Abnormal development, embryonic deformities, increased mortality, and lower hatching success have been shown to occur because of DDE contamination in a range of organisms including reptiles (*28,29*).

In sea turtles, DDE accumulates in adipose tissue (*15*) and is eventually passed to eggs (*30,31*). DDE concentrations occurring in turtle and other reptile eggs in their natural environment have been measured in many studies (Table 1), but the effects of this pollutant on embryos are mentioned in only a few (*28,29,32,33*). In this paper we examine the effects of DDE contamination on offspring sex ratios and embryonic development in the marine turtle *Chelonia mydas*. Background DDE levels in eggs collected in the wild were also measured and compared to published DDE values for reptile eggs from other studies, and the effectiveness of topical administration of DDE in solution was assessed.

Materials and Methods

Source of eggs. Freshly laid eggs from the clutches of four female *Chelonia mydas* (Table 2) were collected from Heron Island, Queensland, Australia, on 12 January 1995, chilled to 12°C, and transported by air to the University of Canberra, Australian Capital Territory. All eggs were assigned to experimental incubators within 72 hr. Initially, all eggs were placed in incubators set at 27.5°C to await an assessment of their viability. They were placed in 5-liter circular plastic food containers and covered by moist vermiculite (three parts vermiculite to four parts water by weight). After 15 days of incubation, eggs were checked for shell whitening (*34*), which indicates that development had commenced. Extreme care was exercised when inspecting the eggs so as not to jolt or rotate them, as cases of movement-induced mortality are well documented (*35,36*). Eggs that were not turgid or that lacked the white patch were removed, and the remaining eggs were consolidated into the minimum number of containers in preparation for allocation to experiments.

DDE background levels. Fifteen eggs from the four clutches (Table 2) were used for determination of background levels of DDE. They were incubated at 27.5°C until stage 21 [day 15 of incubation (*34,37*)] and then dissected. Stage of the embryo was determined by comparison with a standard series (*34*). Albumin, clear fluids, yolk, and

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embryo were separated from shell and shell membranes and stored frozen.

DDE concentrations in albumin, fluids, yolk, and embryo were determined using methods modified from those recommended for chicken eggs (*38*). The samples were homogenized and duplicate subsamples of approximately 5 g were weighed and placed in glass Kimax tubes (Kimble Kontes, Vineland, NJ). Acetonitrile (nanograde, 25 ml; Mallinckrodt, Phillipsburg, NJ) was added and the sealed tubes were shaken manually for 2 min and centrifuged at 2,500 rpm for 5 min. Supernatant (25 ml) containing any DDE was decanted and diluted to 100 ml with distilled deionized water. This aqueous acetonitrile solution (50 ml) was purified using tandem C18 (Alltech Associates, Inc., Deerfield, IL) and Florisil SPE columns (Alltech Associates, Inc.). The columns were eluted with 100% ethyl acetate (omnisolv; EM Science, Gibbstown, NJ) and 100% hexane (nanograde; Mallinckrodt) at a flow rate of 1 ml/min. The collected eluate, evaporated to 2 ml (using a warm bath and a stream of high purity nitrogen), was analyzed for DDE using a gas chromatograph fitted with an electron capture detector (HP-5890 Series II GC fitted with HP-1 and BP-10 capillary columns, HP7673 auto-injector; Hewlett-Packard Corporation, North Ryde, Australia). Matching duplicate determinations for each sample were averaged to obtain estimates of DDE in the egg tissues. Recoveries of DDE from spiked samples were high (98 ± 23% for whole egg and 101 ± 6% for embryo).

DDE exposure. Eggs were dosed at the beginning of the sexual determination period [stage 21 (*34*)] with 2,2-bis (4-chlorophenyl)-1,1dichloroethylene (*p,p'*-DDE; purity 99%; Aldrich Chemical Co., Milwaukee, WI) dissolved in ethanol (Table 3). A stock solution of 0.8 g DDE in 300 ml 95% ethanol was used to prepare dilutions of 0.67, 1.33, 2.00, and 2.66 µg/µl, which were applied to eggs in 5, 10, and 25 µl aliquots. There were two levels of control: eggs that were not dosed at all and eggs that were dosed with one of each of the three quantities of ethanol only (Table 3), as there are no studies on the effects of ethanol on *Chelonia mydas* embryos. Approximately 20 eggs were used per experimental regime. DDE solution was pipetted directly onto the eggshell [topical application (*7*)] immediately overlying the embryo. This was preferred to DDE injection into the egg and avoided unacceptable mortality arising from penetration of the eggshell during injection (*7*).

A subsample of 12 eggs, 2/dose, was selected to assess the degree of penetration of DDE across a range of DDE concentrations and quantities of ethanol (Table 3), while the remaining eggs were incubated as part of the DDE effects on hatchlings experiment.

DDE rates of uptake. The eggs were incubated at 27.6°C until Stage 28 [49 days (*34,37*)], which is at the end of the thermosensitive period for *Chelonia mydas*. DDE concentrations were determined as described above, separately for the embryo and the remaining contents (minus shell and membranes), and corrected for background DDE levels. Rates of uptake following the topical application of DDE were used to estimate DDE dose in the sex determination experiments described below.

DDE effects on hatchlings. Eggs were dosed with DDE according to the experimental regime described above. Eggs at stage 21 (*34*) were placed in two incubators set at 30.5°C and two incubators set at 27.5°C. Hence, there were 8–12 eggs per experimental regime per incubating temperature. In the absence of contamination, the eggs incubated at the higher temperature would be expected to produce approximately 100% female hatchlings and the eggs incubated at lower temperature would be expected to yield approximately 100% males (*37,39*). Any observed shift of sex ratio in dosed eggs would therefore be explained by the effects of DDE, ethanol, or both on developing embryos between dosing time (stage 21) and the end of the sex determination period [stage 27 (*34,37*)].

Sixteen eggs were placed in each 5-liter circular plastic food container and covered by moist vermiculite (three parts vermiculite to four parts water by weight). Eggs were allocated to ensure that eggs from each of the 16 treatments, including controls, and eggs from each of the four clutches were represented in each container. The containers were fitted with lids that allowed gas exchange while minimizing water loss and placed in four incubators until hatching. Water trays were placed in the bottom of each incubator to maintain a high but unmeasured humidity in the incubation chamber. Container weights were monitored during incubation, but replenishment of water was not necessary. Actual temperatures in the incubators, monitored using mercury thermometers placed in close proximity to the eggs and calibrated against a certified National Association of Testing Authorities of Australia thermometer, were 30.4 ± 0.6°C and 27.6 ± 0.4°C, respectively.

Hatchlings were removed from the treatments at Stage 28 (*34,37*), killed by intracranial injection of sodium pentabarbitone, labeled, and stored in 10% formalin. They were weighed to the nearest 0.01 g, and head width and straight carapace length were measured to the nearest 0.1 mm.

Table 1. Range of values of DDE measured in eggs of reptiles with temperature-dependent sex determination compared to values obtained in the present study for dosed eggs and undosed eggs collected from Heron Island, Queensland, Australia

Species name	DDE (ng/g wet wt)	Locality	Reference
Alligator mississippiensis	890–29,000	Lake Apopka, Florida	(*32*)
Crocodylus acutus	370–2,900	Everglades National Park, Florida	(*31*)
Chelydra s. serpentina	877 ± 481[a]	Cootes Paradise, Lake Ontario	(*28*)
Chelonia mydas	Up to 543	Lab dosing, University of Canberra	Present study
Chelydra s. serpentina	150–430	Hamilton Harbour, Lake Ontario	(*16*)
Caretta caretta	56–150	Merritt Island, Florida	(*14*)
Chelonia mydas	ND–9	Ascension Island	(*33*)
Chelonia mydas	ND–5	Merritt Island, Florida	(*13*)
Chelonia mydas	1.3–2.4	Heron Island, Queensland, Australia	Present study

ND, level not detectable.
[a]Mean ± standard deviation.

Table 2. Details of four turtles and their clutches and the concentrations of DDE measured in a sample of eggs from each clutch

Turtle tag no.	Clutch no.	Clutch size	DDE concentration (ng/g)
G2-T12791	A	156	2.0 ± 0.3 (*n* = 5)
G1-T82886	B	88	1.5 ± 0.0 (*n* = 5)
G4-T6287	C	73	1.7 (*n* = 1)
G3-T81574	D	139	1.5 ± 0.1 (*n* = 4)

DDE concentrations are mean ± one standard deviation. Because only one egg was used from clutch C, no standard deviation can be reported.

Table 3. Quantities of DDE administered to eggs of *Chelonia mydas* in solutions of 5, 10, and 25 µl absolute ethanol (EtOH).

DDE (µg)/5 µl EtOH	DDE (µg)/10µl EtOH	DDE (µg)/25 µl EtOH
0	0	0
3.3	6.7[a]	16.6
6.7[a]	13.3[a]	33.3[a]
10.0	20.0	49.8
13.3[a]	26.6	66.5[a]

One set of control eggs received neither ethanol nor DDE, and there were 12 DDE treatments and three ethanol-only controls.
[a]Treatments chosen for analysis of the penetration rates of DDE; two eggs per dose were treated at stage 21 ± 0.4 and DDE was extracted at stage 28 ± 0.5.

Hatchling success and any hatchling deformities were recorded. The right gonad, kidney complex, and associated ducts of each hatchling were removed, embedded in wax, sectioned, and stained with hematoxylin and eosin. The sex of each gonad was assessed by examination under a light microscope according to criteria established by Miller and Limpus (*37*). Where an assessment was not possible, the second gonad was examined.

Results

DDE background levels. Untreated eggs had between 1.3 ng/g and 2.4 ng/g DDE (Table 2) (mean ± standard error, 1.7 ± 0.3, $n = 15$). There was a significant added variance component due to differences among clutches in DDE concentrations ($F = 5.96$; $df = 3,11$; $p<0.05$). Eggs in clutch A had significantly higher concentrations of DDE (2.0 ng/g) than eggs in clutch B (1.5 ng/g) or D (1.5 ng/g) (Tukey Multiple Comparison Test; $p<0.05$) (Table 2). There were no significant differences between clutches A and C (1.7 ng/g), or B, D, and C (Table 2).

DDE rates of uptake. The amount of DDE taken up by the whole egg contents (excluding shell and shell membranes) and the embryo alone was regressed against the amount of DDE applied and the volume of ethanol used to apply the DDE. Once the amount of DDE applied was included in the model, no further variation in DDE taken up could be explained by the volume of ethanol used. DDE uptake could be predicted from DDE applied using the following equations:

$$DDE_{\text{uptake by the whole egg}} = 0.3387 \times DDE_{applied} - 0.2339 \quad (1)$$

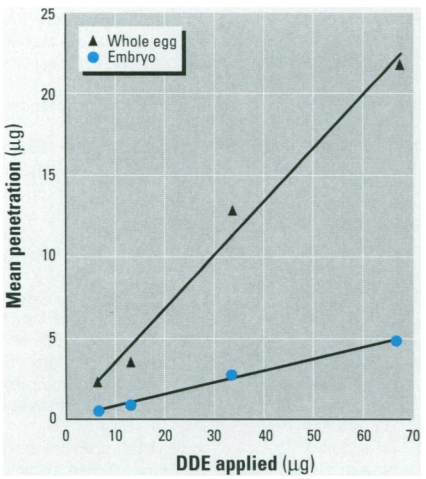

Figure 1. Relationship between the amount of DDE applied and the amount that penetrated the egg. The whole egg does not include shell and shell membranes. Typical standard deviations were 1.9 µg for whole eggs and 1.1 µg for embryos.

$$DDE_{\text{uptake by the embryo}} = 0.0757 \times DDE_{applied} - 0.0545 \quad (2)$$

where DDE is in micrograms and $DDE_{applied}$ ranged from 6.7 µg to 66.5 µg (Fig. 1). The slopes of both relationships were significant (Equation 1: $F = 206.6$; $df = 1,2$; $p<0.01$; $R^2 = 0.99$; and Equation 2: $F = 179.7$; $df = 1,2$; $p<0.01$; $R^2 = 0.99$), but the intercepts were not. Hence, about one-third of DDE applied penetrated the eggs (Fig. 1), and about 8% of DDE applied was absorbed by the embryo (Fig. 1); therefore, levels up to 543 ng/g DDE were predicted in eggs exposed to up to 66.5 µg DDE dissolved in 25 µl of ethanol.

DDE effects on hatchlings. Stepwise multiple logistic regression of hatchling sex against the predicted amount of DDE taken up by the whole egg, the amount of ethanol applied, and incubation temperature indicated that temperature alone exerted an influence on offspring sex ($x^2 = 204.0$; $df = 1$; $p<0.0005$). The probability of an egg yielding a female hatchling at 27.6°C was 9%, compared to 94% at 30.4°C. Predicted outcomes of sexual differentiation were consistent, with expectation based on the effect of temperature alone (males at low temperatures and females at high temperatures). Hence, we could not demonstrate that either DDE or ethanol affected hatchling sex at either temperature.

Multiple logistic regression was also used to explore any relationships between embryo survival and the amount of DDE taken up by the whole egg, the amount of ethanol applied, and incubation temperature. Only incubation temperature was found to have a significant effect on embryo survival ($x^2 = 21.7$; $df = 1$; $p<0.0005$). The probability of an egg hatching at 27.6°C was 94% compared to 72% at 30.4°C.

The incubation time to pipping, deformity rate, and hatchling size and weight were regressed against predicted total DDE in the egg and volume of ethanol applied for each temperature. Duration of incubation, hatchling weight, head width, or carapace length were not significantly related to the amount of DDE or ethanol applied. However, eggs incubated at the lower temperature of 27.6°C took longer to develop (62.6 vs. 52.3 days; $F = 2,437.2$; $df = 1,283$; $p< 0.0005$) and yielded hatchlings that were heavier (25.0 vs. 24.2 g; $F = 17.0$; $df = 1,272$; $p<0.0005$) and had significantly wider heads (12.5 vs. 12.3 mm; $F = 7.99$; $df = 1,282$; $p<0.01$) than hatchlings that emerged from the 30.4°C incubation. Carapace length was unaffected by all treatments (46.2 mm).

One female hatchling incubated at 30.4°C and exposed to 42.8 ng/g DDE in the egg (predicted by Equation 1) showed a saddleback condition of extreme celosomia (body deformity) (*34*). No other cases of body deformities were observed.

Discussion

DDE concentrations in *Chelonia mydas* eggs collected from Heron Island were much lower than the levels reported in other studies, i.e., in the eggs from Florida beaches in 1976 [up to about four times lower (*13*)] and from the Ascension Islands in 1972 [up to about 7 times lower (*33*)] (Table 1). Some DDE in *C. mydas* eggs from Heron Island was expected because of the global contamination of oceans with DDT (*23,24*). Concentrations of DDE are even higher in other reptile species with temperature-dependent sex determination from other areas (Table 1). The foraging grounds of the four turtles whose eggs were collected on Heron Island must therefore be relatively uncontaminated by DDE. Differences in DDE concentration between clutches were statistically significant but small (e.g., 2 vs. 1.5 ng/g; Table 2). These differences probably reflect variances in the background levels of DDE in the foraging area of the female turtles (*23*), differences in time since last nesting (*40*), or natural variation in DDE uptake and retention time (*41*) of each individual.

Topical administration of DDE dissolved in ethanol was found to be an effective method of contaminating eggs in the laboratory because the relationship between the concentration and amount applied and the amount taken up by the egg and embryo was statistically significant and suitable for prediction (Fig. 1). However, only 34% of the initial dose found its way into the egg, and only about 8% of applied DDE found its way into the stage 28 embryo (*34*) (Fig. 1). The remainder presumably remains on the surface and within the pores of the eggshell and shell membranes or is lost by volatilization. Guillette (*29*) reported an uptake of up to 62% when *Alligator mississippiensis* eggs were dosed with estradiol in oil. It is likely that the penetration of solutes varies from species to species depending on the eggshell structure (*42,43*) and on the solvents used. Nevertheless, there is a limit to the amount of ethanol that can be applied to an egg before risking its survival (thought to be about 25 µl); because about one-third passes into the egg of *Chelonia mydas* and only 8% is taken up by the embryo, there is a limit to the amount of DDE that can be administered to the developing embryo by topical administration using ethanol.

Within these limitations, we could demonstrate no effects of contamination on incubation time to pipping, hatching success, sexual differentiation, or hatchling deformities. While hatching success decreased and

hatchling mortality and incidence of body deformities increased in *A. mississippiensis* eggs exposed to up to 10 µg/g DDE (*29*), this was not observed for *C. mydas* exposed to an 18-times lower dose (543 ng/g DDE).

Hatchling size and weight of *C. mydas* varied only with temperature. Normal hatchling size and weight were also observed with *A. mississippiensis* (*29*). It appears that the concentrations of DDE applied to the eggs of *C. mydas* in the present study (up to 543 ng/g of whole egg administered in up to 25 µl of ethanol) were not sufficient to disrupt cellular processes governing embryonic growth, development, and sexual differentiation. We conclude that embryos of *C. mydas*, and possibly those of other species with environmental sex determination, will not be impacted in the wild by levels of DDE contamination in the eggs similar to or less than those measured in the dosed eggs of the present study (Table 1). This is in contrast to other studies, which have reported reduced hatchling success, increased deformities, and modified development when reptiles are exposed to DDT and its metabolites (*12,28*). However, the range of DDE levels that trigger these responses in embryos is still unknown. The use of less volatile solvents with greater DDE solubility coefficient, hence allowing increased levels of DDE to be applied, should be considered in future studies. The problem of low applicable DDE that generates no physiological effects in the present study could possibly be overcome by using a less degenerative solvent in greater quantities and the exposure of embryos to DDE at an earlier stage.

The exceptionally low levels of DDE in control eggs from Heron Island and the failure to demonstrate any effects of DDE contamination up to 543 ng/g of whole egg suggests that the populations of green turtles feeding in the same area as the four turtles whose eggs were collected on Heron Island are currently secure from the effects of DDE contamination. The same may be true for a number of populations of this and other reptile species that are subjected to moderate levels of contamination (Table 1). However, the possible synergistic effects between DDE and other endocrine-disrupting contaminants (*44–46*) on sex differentiation, a possible continuous exposure to a surrounding media contaminated with DDE during the incubation period, and transgenerational effects need to be considered.

References

1. Ewert MA, Nelson CE. Sex determination in turtles: diverse patterns and some possible adaptive values. Copeia 1991:50–69 (1991).
2. Janzen FJ, Paukstis GL. Environmental sex determination in reptiles: ecology, evolution, and experimental design. Q Rev Biol 66:149–179 (1991).
3. Crews D, Cantu AR, Bergeron JM. Temperature and non-aromatizable androgens—a common pathway in male sex determination in a turtle with temperature-dependent sex determination. J Endocrinol 149:457–463 (1996).
4. Gutzke WHN, Bull JJ. Steroid hormones reverse sex in turtles. Gen Comp Endocrinol 64:386–372 (1986).
5. Raynaud A, Pieau C. Embryonic development of the genital system. In: Biology of the Reptilia, Vol 15, Development B (Gans C, Billett F, eds). New York:John Wiley and Sons, 1985;149–300.
6. Lance VA, Bogart MH. Tamoxifen 'sex reverses' alligator embryos at male-producing temperatures, but is an antiestrogen in female hatchlings. Experientia 47:263–266 (1991).
7. Crews D, Bull JJ, Wibbels T. Estrogen and sex reversal in turtles: a dose dependent phenomenon. Gen Comp Endocrinol 81:357–364 (1991).
8. Crews D, Cantu AR, Rhen T, Vohra R. The relative effectiveness of estrone, estradiol-17β, and estriol in sex reversal in the red-eared slider (*Trachemys scripta*), a turtle with temperature-dependent sex determination. Gen Comp Endocrinol 102:317–326 (1996).
9. Jeyasuria P, Roosenburg WM, Place AR. Role of P-450 aromatase in sex determination of the diamondback terrapin, *Malaclemys terrapin*. J Exp Zool 269:1–17 (1994).
10. Guillette LJ Jr, Gross TS, Masson GR, Matter JM, Percival HF, Woodward AR. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ Health Perspect 102:680–688 (1994).
11. Bergeron JM, Crews D, McLachlan JA. PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:780–781 (1994).
12. Guillette LJ Jr, Crain DA, Rooney AA, Pickford DB. Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environ Health Perspect (suppl 7):103:157–164 (1995).
13. Clark DR Jr, Krynitsky AJ. Organochlorine residues in eggs of loggerhead and green sea turtles nesting at Merritt Island, Florida—July and August 1976. Pestic Monit J 14:7–10 (1980).
14. Clark DR Jr, Krynitsky AJ. DDE residues and artificial incubation of loggerhead sea turtle eggs. Bull Environ Contam Toxicol 34:121–125 (1985).
15. Rybitski MJ, Hale RC, Musick JA. Distribution of organochlorine pollutants in Atlantic sea turtles. Copeia 1995:379–390 (1995).
16. Struger J, Elliot JE, Bishop CA, Obbard ME, Norstrom RJ, Weseloh DVC, Simon M, Ng P. Environmental contaminants in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes–St. Lawrence River basin of Ontario, Canada (1981, 1984). J Gt Lakes Res 19:681–694 (1993).
17. Dinham B. The Pesticide Hazard, A Global Health and Environmental Audit. London:Zed Books, 1993.
18. Murti CRK, Dikshith TSS. Application of biodegradable pesticides in India. In: Biodegradation of Pesticides (Matsumura F, Murti CRK, eds). New York:Plenum Press, 1982;257–305.
19. Boul HL. DDT residues in the environment—a review with a New Zealand perspective. NZ J Agric Res 38:257–277 (1995).
20. Cooper K. Effects of pesticides on wildlife. In: Handbook of Pesticide Toxicology, Vol 1, General Principles (Hayes WJ Jr, Laws ER Jr, eds). San Diego:Academic Press, 1991;463–496.
21. Dorough HW, Ballard SK. Degradation of pesticides by animals. In: Biodegradation of Pesticides (Matsumura F, Murti CRK, eds). New York:Plenum Press, 1982;3–20.
22. Khan MAQ, Korte F, Payne JF. Metabolism of pesticides by aquatic animals. In: Pesticides and Aquatic Environments (Khan MAQ, ed). New York:Plenum Press, 1977;191–220.
23. Brown AWA. Ecology of Pesticides. New York:John Wiley and Sons Inc, 1978.
24. Edwards CA. Pesticide residues in soil and water. In: Environmental Pollution by Pesticides (Edwards CA, ed). London:Plenum Publishing Company Ltd, 1973;409–458.
25. Gillis CA, Bonnevie NL, Su SH, Ducey JG, Huntley SL, Wenning RJ. DDT, DDD, and DDE contamination of sediment in the Newark Bay estuary, New Jersey. Arch Environ Contam Toxicol 28:85–92 (1995).
26. Facemire CF, Gross TS, Guillette LJ Jr. Reproductive impairment in the Florida panther: nature or nurture? Environ Health Perspect 103(suppl 4):79–86 (1995).
27. Oestreicher MI, Shuman DH, Wurster CF. DDE reduces medullary bone formation in birds. Nature 229:571 (1971).
28. Bishop CA, Brooks RJ, Carey JH, Ng P, Norstrom RJ, Lean DRS. The case for a cause-effective linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s.serpentina*) from Ontario, Canada. J Toxicol Environ Health 33:521–547 (1991).
29. Guillette LJ Jr. Statement of Louis J. Guillette, Jr. In: Health Effects of Estrogenic Pesticides. Hearing before the Subcommittee on Health and the Environment of the Committee on Energy and Commerce House of Representatives. 103 Congress, 1st Session, 21 October 1993. Serial No. 103-87. Washington, DC:U.S. Government Printing Office, 1994;42–49.
30. Herbert CE, Glooschenko V, Haffner GD, Lazar R. Organic contaminants in snapping turtle (*Chelydra serpentina*) populations from southern Ontario, Canada. Arch Environ Contam Toxicol 24:35–43 (1993).
31. Hall RJ, Kaiser TE, Robertson WB Jr, Patty PC. Organochlorine residues in eggs of the endangered American crocodile (*Crocodylus acutus*). Bull Environ Contam Toxicol 23:87–90 (1979).
32. Heinz GH, Percival HF, Jennings ML. Contaminants in American alligator eggs from Lake Apopka, Lake Griffin, and Lake Okeechobee, Florida. Environ Monit Assess 16:277–285 (1991).
33. Thompson NP, Rankin PW, Johnston DW. Polychlorinated biphenyls and *p,p'* DDE in green turtle eggs from Ascension Island, South Atlantic Ocean. Bull Environ Contam Toxicol 11:399–406 (1974).
34. Miller JD. Embryology of marine turtles. In: Biology of the Reptilia, Vol 14 (Gans C, ed). New York:John Wiley & Sons, 1985;269–328.
35. Limpus CJ, Baker V, Miller JD. Movement induced mortality of loggerhead eggs. Herpetologica 35:335–338 (1979).
36. Parmenter CJ. Incubation of the eggs of the green sea turtle, *Chelonia mydas*, in Torres Strait, Australia: the effect of movement on hatchability. Aust Wildl Res 7:487–491 (1980).
37. Miller JD, Limpus CJ. Incubation period and sexual differentiation in the green turtle *Chelonia mydas*. In: Proceedings of the Melbourne Herpetological Symposium (Banks C, Martin A, eds). Melbourne:The Royal Melbourne Zoological Gardens, 1981;66–73.
38. Schenck FJ, Wagner R, Hennessy MK, Okrasinski JL Jr. Screening procedures for organochlorine and organophosphorus pesticide residues in eggs using a solid-phase extraction cleanup and gas chromatographic detection. J AOAC 77(4):1036–1040 (1994).
39. Morreale SJ, Ruiz GJ, Spotila JR, Standora EA. Temperature-dependent sex determination: current practices threaten conservation of sea turtles. Science 216:1245–1247 (1982).
40. Kwan D. Fat reserves and reproduction in the green turtle, *Chelonia mydas*. Wildl Res 21:257–266 (1994).
41. Matsumura F. Absorption, accumulation, and elimination of pesticides by aquatic organisms. In: Pesticides in Aquatic Environments (Khan MAQ, ed). New York:Plenum Press, 1977;77–105.
42. Ewert MA. Embryology of turtles. In: Biology of the Reptilia, Vol 14 (Gans C, ed). New York:John Wiley & Sons, 1985;75–267.
43. Ferguson MWJ. Reproductive biology and embryology of the Crocodilians. In: The Biology of the Reptilia, Vol 14 (Gans C, ed). New York:John Wiley & Sons, 1985;329–491.
44. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 103:582–587 (1995).
45. Matsumura F. Absorption, accumulation, and elimination of pesticides by aquatic organisms. In: Pesticides in Aquatic Environments (Khan MAQ, ed). New York:Plenum Press, 1977;77–105.
46. Sumpter JP, Jobling S. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect 103(suppl 7):173–178 (1995).