DDE in Eggs of Two Crocodile Species from Belize

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Organochlorine (OC) residues were recently detected in nonviable Morelet's crocodile (*Crocodylus moreletii*) eggs from northern Belize. To further the assessment of contaminant exposure in Belizean crocodiles, nonviable Morelet's crocodile eggs ($n = 11$) from southern Belize and American crocodile *(Crocodylus acutus)* eggs ($n = 12$) from the coastal zones of Belize were screened for 20 OCs. Results indicated *p*,*p*-DDE to be the most prevalent OC (96% occurrence) in eggs examined, with concentrations ranging from 5 to 372 ng/g. These concentrations are similar to those observed in crocodile eggs (10−180 ng/g) from northern Belize. A general trend toward higher DDE concentrations in Morelet's crocodile eggs (mean = 103 ppb) compared with American crocodile eggs (mean = 31 ppb) was observed. However, this trend may be due to site-specific contamination rather than differences in interspecific susceptibility to chemical exposure. Other OCs detected in crocodile eggs included the parent compound, *p*,*p*-DDT, and its metabolite, *p*,*p*-DDD.

Keywords: *DDE; crocodile eggs; Belize; organochlorine residues*

INTRODUCTION

Widespread use of organochlorine (OC) pesticides in agriculture, industry, and vector control has resulted in worldwide distribution of OCs in the environment (Snedaker et al., 1999). This is particularly true in developing countries where regulations governing the production, distribution, application, and disposal of these chemicals are either not legislated or poorly enforced (Murray, 1994). In industrialized countries, the persistence of OC pesticides in the environment has led to their discontinuation in favor of less persistent alternatives (Waliszewski et al., 1999). However, OC residues are still detected worldwide in abiotic and biotic samples, particularly in samples with high lipid content. For example, OCs have been recently detected in human adipose tissue from residents in the United States (Stellman et al., 1998), foodstuffs from Nigeria (Osibanjo and Adeyeye, 1997), snapping turtle (*Chelydra serpentina serpentina*) eggs from Canada (Bishop et al., 1998), and pelican (*Pelecanus crispus* and *Pelecanus onocrotalus*) eggs from Greece (Crivelli et al., 1999).

A persistent OC that is frequently encountered in soils and biological systems is DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], a degradation product of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene] (Helling et al., 1971). DDE can be formed by photochemical processes (Maugh, 1973) and by bacterial (Subba-Rao and Alexander, 1985) or abiotic (Boul, 1995) dehydrochlorination. Studies have shown that DDE is extremely recalcitrant to further biological degradation under aerobic or anaerobic conditions (Strompl and Thiele, 1997), resulting in its long-term contamination of the environment. For example, in New Zealand, a pasture contained substantial levels of DDE in the soil 27 years after treatment with DDT (Boul et al., 1994).

OC contaminants have a tendency to bioaccumulate in the food chain at high concentrations, placing top-level predators in a position of high toxicological risk (Fossi et al., 1999). Crocodilians would be in this position because of their high trophic status and long life span. To our knowledge, OCs have been detected in 7 of the 23 species of crocodilians. A frequently occurring OC detected in crocodilian eggs is DDE (Ogden et al., 1974; Wessels et al., 1980; Phelps et al., 1986; Heinz et al., 1991; Skaare et al., 1991; Wu et al., 2000). OC exposure at these early developmental stages can have toxicological implications. Current studies indicate that certain OCs have the potential to disrupt hormones in the endocrine system necessary for reproductive development. For example, Willingham and Crews (1999) recently reported significant (40%) sex reversal in red-eared sliders (*Trachemys scripta*) following administration of DDE to eggs incubated at male-producing temperatures. In addition, high DDE levels may have contributed to abnormally developed testes and reduced phalli in juvenile alligators from Lake Apopka, Florida, following a spill of DDT and dicofol (Guillette et al., 1994; Heinz et al., 1991). The data from Lake Apopka suggest that young crocodilians are sensitive to OC exposure. However, very little is known about the variability of chemical residues in crocodilian eggs, such as variability within a clutch, between clutches, or among different crocodile species.

OC residues in crocodilian eggs are likely the result of maternal transfer from exposed females. The lipophilic nature of OCs facilitates their mobilization from fat depots in the female into developing follicles during vitellogenesis (Ferguson, 1985). Exposure to OCs in contaminated nest material and soil may also add to chemical burdens in eggs following oviposition (Wu et

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al., 2000). Large (diameters = 6.0−7.3 μm) and small (diameters averaging 0.51 ± 0.18 μm) pores necessary for gas exchange exist in the eggshell and shell membrane (Kern and Ferguson, 1997). Gas exchange through eggshell pores may facilitate the transfer of contaminants from the external environment into eggs.

We recently reported the detection of several OCs in eggs from Morelet's crocodile (*Crocodylus moreletii*) and associated sediments and nest media from northern Belize (Wu et al., 2000). To further our assessment of OC exposure to crocodiles in Belize, we included eggs from southern Belize as well as another crocodile species. In Belize, the American crocodile (*Crocodylus acutus*) lives on offshore cays and atolls, with a few individuals inhabiting the coastal mainland (Platt and Thorbjarnarson, 1997). In contrast, Morelet's crocodiles tend to inhabit freshwater wetlands (Platt, 1996; Rainwater et al., 1998).

MATERIALS AND METHODS

Reagents. A certified organochlorine pesticide mixture consisting of tetrachloro-*m*-xylene (TCMX), α-BHC, heptachlor, γ-BHC (lindane), endosulfan I, dieldrin, endrin, *p,p*-DDD, *p,p*-DDT, methoxychlor, aldrin, β-BHC, δ-BHC, heptachlor epoxide, γ-chlordane, α-chlordane, *p,p*-DDE, endosulfan II, endrin aldehyde, endosulfan sulfate, endrin ketone, and decachlorobiphenyl (DCBP) was obtained for use as a standard. Organic solvents (acetone, hexane, and methylene chloride) were of pesticide or GC-MS grade.

Egg Collection and Handling. Eleven nonviable eggs from nests of Morelet's crocodiles and 12 nonviable eggs from nests of American crocodiles were collected in April and May 1997. Morelet's crocodile eggs were collected from four nests along various rivers and creeks in southern Belize, whereas American crocodile eggs were collected from five nests found along the coastal mainland on offshore cays (islands) and the Turneffe Atoll (Figure 1). Each egg was marked for identification, placed in a sterile Whirl Pak (NASCO, Fort Wilkinson, WI) bag, and stored at −25 °C at Lamanai Field Research Center until shipment on ice to Texas Tech University. Eggs were stored at −20 °C in the laboratory until extraction.

Sample Extraction. Egg contents were removed and weighed in acetone-rinsed glass beakers. Samples were thoroughly mixed with a hand-held blender (3−5 min). A portion of each egg (3−5 g) was weighed and mixed with 10 g of anhydrous sodium sulfate (EM Science, Gibbstown, NJ; baked at 150 °C for 24 h prior to use). Samples were transferred into cellulose extraction thimbles and fortified with an internal standard (TCMX and DCBP). Samples were Soxhlet-extracted with 100 mL of acetone/hexane (1:1, v/v) for 20−24 h. Extracts were reduced to near dryness using rotary evaporation and solvent-exchanged with methylene chloride. Concentrated extracts were transferred to a 2-mL volumetric flask, brought to volume with methylene chloride, and filtered through a 0.45-μm Acrodisc filter into a 2-mL amber autosample vial.

Extract Cleanup. Lipids were removed from egg extracts using gel permeation chromatography similar to EPA Method 3640. A Hewlett-Packard 1100 liquid chromatograph equipped with a UV detector and a Plgel column (pore size = 50 A°) was used to separate and collect the appropriate fraction. Methylene chloride was the mobile phase. The collected fraction was concentrated to near dryness and then reconstituted in 1 mL of hexane.

Chemical Analysis. A Hewlett-Packard 6890 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and a 30 m × 0.32 mm DB-5 column was used for separation and quantification of OC residues in extracts. Inlet and detector temperatures were 200 and 300 °C, respectively. The temperature program was as follows: initial temperature = 80 °C; increased to 180 °C at 25 °C/min; increased from 180 to 205 °C at 2.5 °C/min with a 2-min hold; increased from 205 to 250 °C at 15 °C/min with a 1-min hold; and increased from 250 to 300 °C at 20 °C/min. Chemical conformation was conducted on selected extracts using a Hewlett-Packard 6890/5937 GC-MS in selected ion mode.

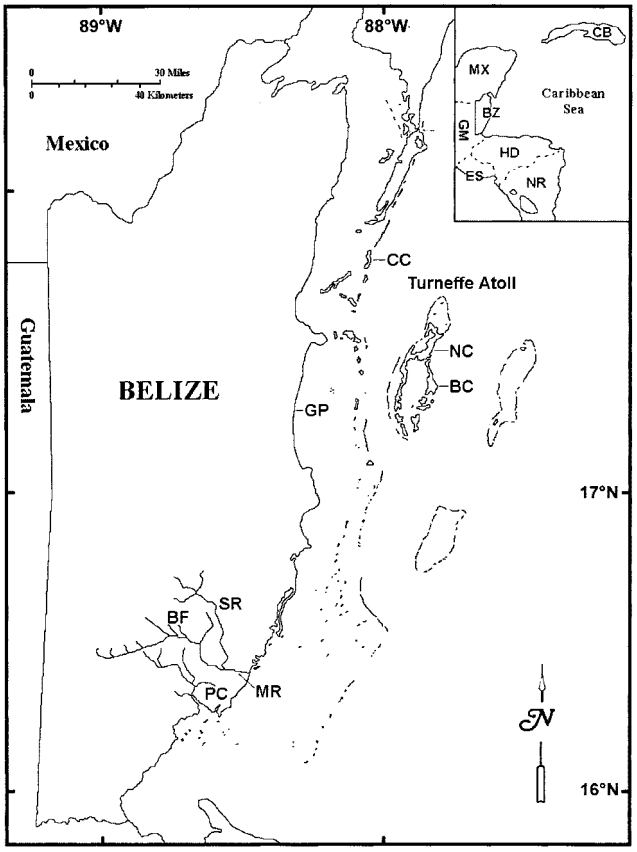

Figure 1. Map of Belize showing locations from where crocodile eggs were collected for contaminant analysis. American crocodile eggs were collected from BC, CC, GP, and NC. Morelet's crocodile eggs were collected from BF, MR, PC, and SR. [BC, Blackbird Cay; BF, BFREE (Belize Foundation for Research and Environmental Education); CC, Cay Caulker; GP, Gales Point; NC, Northern Cay; MR, Monkey River; PC, Paynes Creek; SR, Swasey River.]

Statistical Analysis. Residue data from eggs within nests were analyzed using ANOVA. Because nesting areas of the two crocodile species did not overlap, no species comparisons were made. Rather, the influence of nest location on DDE levels in the eggs was evaluated.

RESULTS

A total of 23 eggs, 11 from four Morelet's crocodile nests and 12 from five American crocodile nests, were analyzed for OCs. Organochlorine residues were detected in 22 of the 23 eggs (96%); DDE was the major OC detected (Table 1). DDT was detected in 9 eggs (39%) and DDD in 12 eggs (52%). Concentrations of DDE ranged from 5 ppb (ng of chemical/g of egg) to >370 ppb (ng of chemical/g of egg). Most eggs contained DDE at concentrations between 30 and 70 ppb.

There were some species differences with respect to the OC residues contained in the eggs. Although DDE and DDD concentrations in eggs from both species were fairly similar, concentrations of DDT in eggs from the two species varied. Five of the 12 eggs (42%) from nests of American crocodiles contained DDE, whereas all of the Morelet's crocodile eggs contained DDE. Interestingly, none of the eggs from American crocodile nests contained DDT. In contrast, 9 of 11 Morelet's crocodile

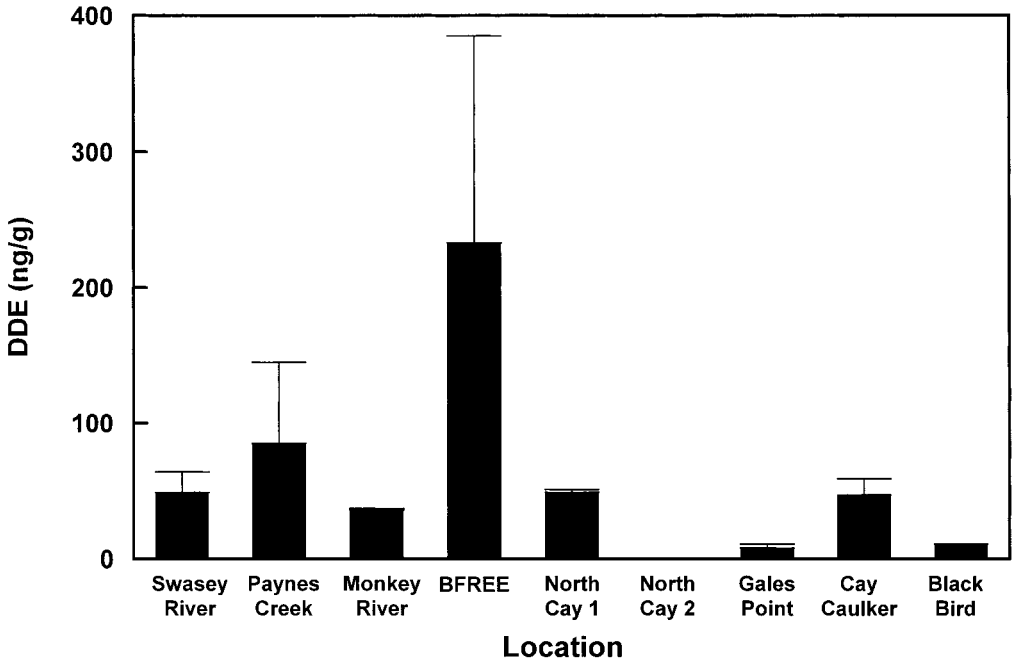

Figure 2. Mean residue concentrations of DDE (ng/g) in nonviable Morelet's and American crocodile eggs from various locations in Belize. Morelet's crocodile eggs were collected from nests at BFREE, Paynes Creek, Monkey River, and Swasey River. American crocodile eggs were collected from Blackbird Cay, Cay Caulker, Gales Point, and North Cay.

Table 1. Detection of *p,p*-DDT and *p,p*-DDD and Concentrations of *p,p*-DDE in Morelet's and American Crocodile Eggs from Belize

species, sample location, and egg no.[b]	contaminant[a]		
	p,p-DDT	*p,p*-DDD	*p,p*-DDE (ppb)
Morelet's crocodile			
Swasey River			
egg 1	X	X	49
egg 2	X	X	47
egg 3			31
egg 4	X	X	68
Paynes Creek			
egg 1	X		42
egg 2	X	X	127
Monkey River			
egg 1			35
egg 2	X		36
BFREE			
egg 1	X	X	372
egg 2	X	X	70
egg 3	X	X	256
American crocodile			
North Cay 1			
egg 1			47
egg 2			50
North Cay 2			
egg 1			ND
Gales Point			
egg 1			11
egg 2			5
egg 3			9
Cay Caulker			
egg 2		X	64
egg 3		X	50
egg 4		X	48
egg 5		X	31
egg 6		X	42
Blackbird Cay			
egg 3			11

[a] Concentrations were determined only for the eggs containing DDE. [b] Egg number does not refer to the order of oviposition. [c] ND, not detected by our analytical procedure.

eggs (82%) contained DDT. Detection of DDD was similar in both species, with 7 of 11 (64%) eggs from Morelet's nests and 5 of 12 (42%) eggs from American crocodile nests containing this metabolite.

A general trend toward higher DDE concentrations in eggs from Morelet's crocodile nests compared to eggs from American crocodile nests was observed. However, this trend may be simply a function of nest location rather than a true species difference. An analysis of nest versus DDE concentration in eggs revealed a significant location effect ($p = 0.017$, $n = 9$ nests). Morelet's crocodile eggs from a site associated with the Belize Foundation for Research and Environmental Education (BFREE) had the highest levels [mean = 233 ppb (Figure 2)]. Eggs from other locations had mean levels ranging from 8 ppb (Gales Point) to 85 ppb (Paynes Creek).

DISCUSSION

The primary route of DDE contamination in crocodile eggs is likely through maternal transfer. Female crocodiles could ingest DDT-contaminated prey, and the DDT may then be metabolized to DDE and retained in fat. Females may also directly ingest DDE-contaminated prey. In either scenario, DDE could be transferred from fat to developing follicles during vitellogenesis and yolk production.

High tropical temperatures in Belize may also facilitate uptake of volatile DDE from surrounding matrices (e.g., nest media), resulting in potential chemical diffusion through eggshell pores and the shell membrane. However, this mechanism has not been well studied. In addition to habitat differences, the nesting ecology of the two crocodile species we examined also differs slightly. American crocodiles build sand nests, whereas Morelet's crocodiles build mound-type nests utilizing plant material and sediment. Such differences may have also contributed to the egg residue variability observed in this study.

The variation in DDE residues from eggs of Morelet's and American crocodiles observed in the present study

may also represent differences in site-specific contamination rather than interspecific differences in susceptibility to OC exposure. The fact that Morelet's crocodile inhabits mainland Belize and is potentially closer to anthropogenic sources of contaminants may account for the contamination levels detected in the eggs. Lower contamination levels would be expected in more secluded areas such as the cays and atolls inhabited by American crocodiles. Eggs from the BFREE site contained the highest DDE levels detected. Interestingly, this site is considered to be the most pristine (remote) of those sampled in southern Belize.

In the present study, DDT was detected in a majority of Morelet's crocodile eggs but was not detected in American crocodile eggs by our analytical procedure. This suggests a more recent exposure of female Morelet's crocodiles to DDT, as opposed to American crocodiles in which DDT from past exposure may have already been metabolized to DDE.

Organochlorine residues (including DDE) have been identified in a variety of abiotic and biotic samples from several other Central American countries including Guatemala (Lopez Garcia and Alvarado-Chavez, 1990), El Salvador (Dominguez-Pantoja and Paz-Quevedo, 1988), Costa Rica (Standley and Sweeney, 1995), Nicaragua (Carvalho et al., 1999), and Mexico (Norena-Barroso et al., 1998). Due to the paucity of information on past or present chemical use in Belize, the sources of contaminants detected in this study are unknown. However, we speculate that the majority of OC contamination in these areas is the result of nonpoint sources including atmospheric deposition.

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