SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 32, No. 11, pp. 2576–2583, 2013

PESTICIDES IN BLOOD FROM SPECTACLED CAIMAN (*CAIMAN CROCODILUS*) DOWNSTREAM OF BANANA PLANTATIONS IN COSTA RICA

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(*Submitted 7 May 2013; Returned for Revision 9 July 2013; Accepted 28 July 2013*)

Abstract: Spectacled caiman (*Caiman crocodilus*) are fish-eating crocodilians that inhabit freshwater habitat in tropical regions of the Americas. To assess the exposure of caiman to pesticides from banana plantations, the authors collected whole blood samples (30 mL) from 14 adult caiman that were captured in the North Atlantic region of Costa Rica. Blood samples were analyzed for 70 legacy- and current-use pesticides and breakdown products using newly developed ultra-trace, high-resolution mass spectrometry (HRMS). Caiman accumulated pesticides ranked by concentration as dieldrin > permethrin > mirex > 4,4′-DDE > alpha-endosulfan > heptachlor epoxide > oxychlordane > heptachlor > cypermethrin. Caiman within the high-intensity banana crop watershed of Rio Suerte had higher pesticide burdens relative to other more remote locations ($F = 12.79$; $p = 0.00$). Pesticide concentration decreased with distance from upstream banana plantations in this river system ($F = 20.76$; $p = 0.00$). Caiman body condition was negatively correlated with total pesticide concentrations ($F = 6.23$; $p = 0.02$) and with proximity to banana plantations ($F = 5.05$; $p = 0.04$). This suggests that either pesticides elicited toxic effects in caiman, resulting in diminished overall health, or that the quantity or quality of their prey was reduced by pesticides downstream of plantation waterways. The authors' results indicate that pesticide use in banana plantations is impacting a high trophic level species inhabiting one of the most important wilderness areas in Costa Rica (Tortuguero National Park). *Environ Toxicol Chem* 2013;32:2576–2583. © 2013 SETAC

Keywords: Pesticides Persistent organic pollutants Aquatic toxicology Caiman Crocodilian

INTRODUCTION

The North Atlantic region of Costa Rica is a prime banana-producing area, characterized by warm temperatures, high rainfall, and soils with extensive drainage systems where surplus water flows into local streams and rivers. The headwaters of the Rio Suerte flow through this major banana-producing area and drain into the Tortuguero Conservation Area, one of the most important wilderness areas found in the country. The Tortuguero Conservation Area comprises the Tortuguero National Park (19 000 ha) and the Barra del Colorado National Wildlife Refuge (92 000 ha). This species-rich area (Figure 1) filled with black water rivers and canals and lowland tropical wet forest provides critical habitat for many International Union for Conservation of Nature red-listed species, including 2 crocodilian species, the American crocodile (*Crocodylus acutus*—vulnerable) and the spectacled caiman (*Caiman crocodilus*—lower risk) [1].

Intensive pesticide use on banana plantations can contaminate downstream aquatic ecosystems and the taxa they support [2]. In the North Atlantic region of Costa Rica, this is compounded by frequent heavy rains, which regularly wash pesticides from target areas and necessitate constant reapplication to crops [2]. In addition to the application of large quantities of pesticides, certain practices, such as overuse, aerial spraying in close proximity to streams and rivers, washing application equipment in rivers and streams, and inappropriate disposal of excess pesticides, can increase pesticide contamination of downstream areas [3].

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Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2358

Lack of developed regulatory infrastructure and adequate enforcement have contributed to environmental contamination related to high pesticide use [3,4]. Although current use of pesticides in Costa Rica has shifted to less persistent but still toxic pesticides [3], total pesticide use has doubled over the last 20 yr throughout Central America in response to agricultural demands [5]. Costa Rica alone ranks second in the world for intensity of pesticide use, with applications averaging 52 kg of active ingredient per hectare [5].

Exposure to elevated levels of pesticides have been associated with adverse health outcomes in humans and wildlife, including increased disease susceptibility, reproductive failure, developmental abnormalities, and mortality [6–8]. Within Costa Rica, a number of human and environmental poisonings have been documented in commercial banana plantations [2,3,9–12], and high levels of persistent organic pollutants (POPs), including dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene, and heptachlor, have been detected in human tissue samples [13]. Within the Rio Suerte in the North Atlantic region of Costa Rica, and elsewhere in the country, significant fish kills have been recorded as a direct result of exposure to pesticides [2,3]. Concentrations from multiple pesticides also may act additively, resulting in a total concentration that might be biologically relevant, even within less contaminated habitats [14]. Detrimental effects in high-trophic-level crocodilians are all the more troubling because they may signal consequences for the entire ecosystem [15,16].

Within tropical aquatic environments, heavy rains cause rapid fluctuations in pesticide concentrations, causing inconsistencies in pesticide detection within abiotic samples such as sediments [17–19], air [20,21], and water [3,12,22]. This emphasizes the importance of using long-lived, high-trophic-level organisms

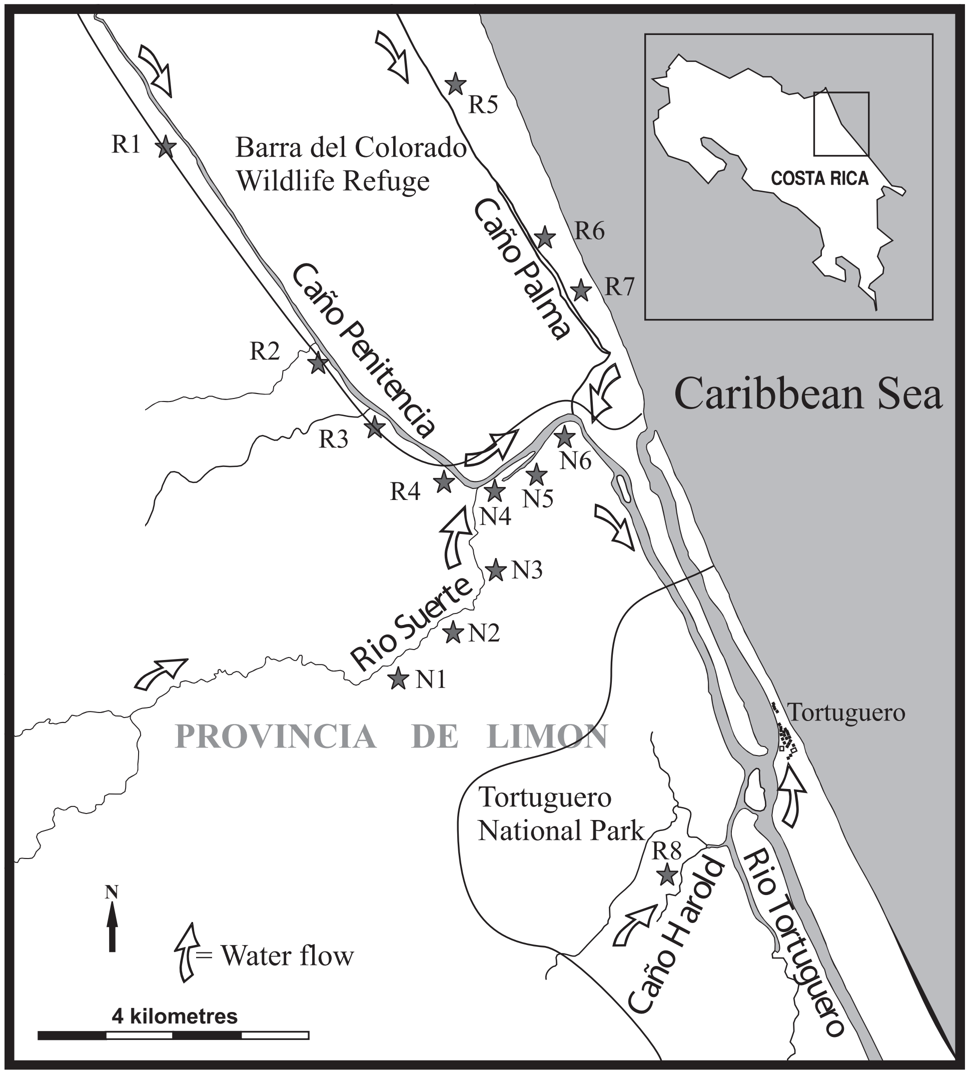

Figure 1. Caiman captured in various waterways of the North Atlantic region of Costa Rica. Caiman (N1–N6) were classified as near-field, under the direct influence of banana plantations in upstream Rio Suerte. Caiman (R1–R8) were classified as reference, captured in areas outside the direct influence of banana plantations.

such as crocodilians, to provide a more integrated assessment of the fate of pesticides used in such areas [23]. Despite the obvious advantages of using crocodilians as environmental sentinels, these species remain underused in tropical regions [24]. The spectacled caiman (*Caiman crocodilus*) is a predatory reptile that lives in freshwater habitats in the Americas. Within the North Atlantic region of Costa Rica, adult spectacled caiman are relatively abundant, territorial, and tend to have high site fidelity [16,25]. Although spectacled caiman are generalist predators, most of the adult diet consists of fish [26].

The objective of the present study was to use a new ultra-trace, high-resolution mass spectrometry (HRMS) analytical procedure to characterize the concentrations and patterns of 70 current-use and legacy pesticides in caiman that were live-captured in Costa Rica.

MATERIALS AND METHODS

Sampling

Fourteen spectacled caiman were live-captured between March 2006 and June 2006 in 4 connected waterways in the North Atlantic region of Costa Rica (Figure 1). Six individuals were captured in near-field sites, within the immediate influence of banana plantations. Of those, 3 individuals were captured within the Rio Suerte, which is directly downstream of approximately 5730-ha banana plantations [12], and 3 individuals were captured in the Caño Penitencia downstream of where the Rio Suerte enters. In addition, 8 individuals were captured within reference areas that were more remote and removed from the direct influence of banana plantations. These included 4 individuals captured in the Caño Penitencia upstream of where the Rio Suerte enters, 3 individuals in Caño Palma, which flows into Caño Penitencia, and 1 individual in Caño Harold, which flows into the Rio Tortuguero before flowing into the Caribbean Sea.

Caiman were located by nocturnal spotlight surveys by boat, because crocodilians have a red eye shine that is seen up to 150 m away [27]. Adults have a total length of approximately 1.25 to 2.50 m [28], although diet can affect growth and condition (A.N. Rice, 2004, Master’s thesis, University of Florida, Gainesville, FL, USA) [29]. Efforts were made to capture adults of a similar size range to minimize potentially confounding influences of age on pesticide accumulation. Individuals were hand- or noose-captured under permit from the Costa Rican Ministry of Environment and Energy, which manages the national system of conservation areas.

For each animal, sex was determined by cloacal examination of the genitalia [30]. Measurements, in meters, of total body length or snout–tail length (measured ventrally from tip of the snout to tip of the tail), snout–vent length (measured ventrally from the tip of the snout to the anterior margin of the cloaca), tail circumference (widest measurement at the base of the tail), and neck circumference (widest neck measurement) were taken with a measuring tape. Head width (maximum head width measurement), head depth (largest vertical measurement), and head length measurements (measured from the tip of the snout to the posterior edge of the supra-occipital bone) were taken using vernier calipers. Mass was determined using Pesola spring scales (20 kg and 10 kg; Pesola) to the nearest 0.1 kg. Each animal was permanently marked for future identification by removing a unique series of caudal scutes that correspond to a numerical code, thus providing an individual number to each caiman [31].

Whole blood (30 mL) was obtained from the postcranial sinus using a vacutainer blood collection system into nonadditive tubes (Becton-Dickenson). Samples were stored on ice in the field and then transferred to certified pesticide-grade glass vials with Teflon-lined caps, and stored at −20 °C. Samples were then shipped to the Institute of Ocean Sciences (Sidney, British Columbia, Canada) and stored at −80 °C until pesticide analysis at AXYS Analytical Services (Sidney, British Columbia, Canada). Blood has been used in previous contaminant studies of crocodilians [6,24,32,33]. All caiman were treated in accordance with the principles of the Canadian Council on Animal Care.

After sample collection, which took approximately 30 min per individual, caiman were released at their site of capture. Distance of caiman from pesticide source was calculated using the closest sample point to banana plantations in upstream Rio Suerte (N1) and measuring the distance (km) from N1 to each site along the course of the river. All discharge areas from banana plantations enter into Rio Suerte, upstream of N1.

Pesticide Analysis

Whole blood samples were analyzed by AXYS Analytical Services using a newly developed ultra-trace HRMS analytical procedure for the detection of 70 current-use and legacy pesticides and breakdown products (Table 1). The samples included a procedural blank, an in-house reference spiked blood sample (human blood), and a spiked matrix sample prepared using blood from 1 of the caiman. The mean recoveries were $79.9 \pm 23\%$ for the human blood sample and $93 \pm 17\%$ for the spiked matrix caiman blood. Twenty-nine isotope-labeled standards were spiked into each sample, and their recovery values were used as general indicators of method performance for the individual samples. Accurately weighed (10 g wet wt) whole-blood samples were placed into 250-mL round-bottom flasks. The samples were extracted with a mixture of 10 mL ethanol, 50 mL hexane, and 10 mL saturated ammonium sulfate using a shaker table. The hexane layer was decanted into a 500-mL separatory funnel. The aqueous residue was further extracted with 50 mL fresh hexane, and the hexane layer combined with that in the separatory funnel. The combined hexane extract was backwashed with reagent-grade water. The resulting extract was cleaned and analyzed as described by Woudneh et al. [34]. Analytes were identified once the HRMS data satisfied all reported criteria. Lipid content was determined gravimetrically. When analytes were detected in more than 70% of the samples, any nondetects were replaced with the detection limit [35]. Data were lipid normalized to correct for the variability in accumulation of lipophilic contaminants caused by differences in lipid content [36]. All reagents were pesticide grade quality obtained from Mallinckrodt. Analytical labeled standards were all obtained from Cambridge Isotope Laboratories. All analysis was conducted using an AutoSpec Ultima HRMS (Micromass) equipped with an HP 6890 gas chromatograph (Agilent Technologies), a CTC auto-sampler (CTC Analytics), and an Alpha data system running on Micromass software.

Data Analysis

Condition analyses provide a measure of the basic health of an individual. To determine body condition of spectacled caiman, Fulton's Condition Factor K was used, providing a numerical score based on skeletal length and volumetric measurement (C. Zweig, 2003, Master's thesis, University of Florida, Gainesville, FL, USA).

$$K = \left(\frac{W}{L^3}\right) \times 10^n$$

Where W is the mass of the caiman in kg, L is the length (snout–vent length in cm), and n is chosen so that the mean of K is larger than 1; in this case, $n = 5$.

Statistical analyses were performed with Statistica 10 [37]. Regression analyses were used to investigate relationships between caiman morphometric data, body condition, distance from source, and pesticide concentration. One-way analyses of variance were used to investigate differences between near-field and reference sites. Principal components analysis (PCA) were performed following normalization of pesticide data to the pesticide concentration total to remove artifacts related to concentration differences among samples. Centered log ratio transformation was then applied to this compositional data set to produce a data set that was unaffected by negative bias or closure [35] and where the average concentration and concentration total were identical for every sample. Data were then autoscaled to give every variable equal weight.

RESULTS AND DISCUSSION

The 14 caiman captured were all of good health, and near-field and reference caiman did not vary by sex ($F = 0.10$; $p = 0.76$), mass ($F = 0.05$; $p = 0.81$), percentage of lipid in blood ($F = 3.06$; $p = 0.11$), snout–tail length ($F = 0.15$; $p = 0.70$), snout–vent length ($F = 0.07$; $p = 0.80$), tail circumference ($F = 0.58$; $p = 0.46$), neck circumference ($F = 0.64$; $p = 0.70$), head width ($F = 0.30$; $p = 0.59$), head depth ($F = 0.03$; $p = 0.86$), or head length ($F = 0.02$; $p = 0.86$). These factors were thereby eliminated as potentially confounding influences (Table 2). Because adult female crocodilians reduce their body burdens of lipophilic contaminants through maternal transfer to eggs [38], all data within the present study were analyzed with and without the 4 female caiman (2 near-field, 2 reference) to ensure that sex of individuals did not alter results. Their exclusion did not significantly alter any of the analyses, and no significant differences between males and females in terms of morphometrics, pesticides concentrations, or body conditions existed, allowing both male and female caiman to be analyzed together to increase sample size.

Both legacy- and current-use pesticides were detected in blood samples from all 14 caiman, irrespective of whether individuals were caught in near-field waterways within the immediate influence of banana plantations or in more remote locations. Of the 70 current-use and legacy pesticides and breakdown products analyzed, the 9 detected in caiman blood were insecticides (Table 3). The remaining 61 pesticides were

Table 1. Whole blood from spectacled caiman (*Caiman crocodilus*) analyzed to detect 70 current-use and legacy pesticides and breakdown products[a]

2,4′-DDD	I	1	Dimethenamid	H	9	Methoxychlor	I	1
2,4′-DDE	I	1	Disulfoton	I	2	Metolachlor	H	4
2,4′-DDT	I	1	Disulfoton sulfone	I	2	Metribuzin	H	5
4,4′-DDD	I	1	Endosulfan, alpha-	I	1	Mirex	I	1
4,4′-DDE	I	1	Endosulfan, beta-	I	1	Nonachlor, cis-	I	1
4,4′-DDT	I	1	Endosulfan sulfate	I	1	Nonachlor, trans-	I	1
Alachlor	H	4	Endrin	I	1	Octachlorostyrene	I	
Aldrin	I	1	Endrin ketone	I	1	Parathion-ethyl	I	2
Ametryn	H	5	Ethalfluralin	H	6	Parathion-methyl	I	2
Atrazine	H	5	Ethion	I	2	Pendimethalin	H	6
Azinphos-methyl	I	2	Fenitrothion	I	2	Permethrin	I	3
Butralin	H	6	Flufenacet	H	9	Perthane	I	1
Butylate	H	7	Fonofos	I	2	Phorate	I N	2
Chlordane, alpha	I	1	HCH, alpha	I	1	Phosmet	I	2
Chlordane, gamma	I	1	HCH, beta	I	1	Pirimiphos-methyl	I	2
Chlordane, oxy-	I	1	HCH, delta	I	1	Quintozene	N	9
Chlorpyrifos	I	2	HCH, gamma	I	1	Simazine	H	5
Chlorpyrifos-methyl	I	2	Heptachlor	I	1	Tebuconazol	F	9
Chlorpyrifos-oxon	I	2	Heptachlor epoxide	I	1	Tecnazene	F	9
Cypermethrin	I	3	Hexachlorobenzene	F I	1	Terbufos	N	2
Dacthal	H	8	Linuron	H	9	Triallate	H	7
Diazinon	I	2	Malathion (carbofos)	I	2	Trifluralin	H	6
Diazinon-oxon	I	2	Methamidophos	I	2			
Dieldrin	I	1	Methoprene	1	9			

[a]Chemical classes: 1 = organochlorine; 2 = organophosphorus; 3 = pyrethroid; 4 = chloroacetanilide; 5 = triazine; 6 = 2,6-dinitroaniline; 7 = thiocarbamate; 8 = alkyl Phthalate; 9 = other.
I = insecticide; H = herbicide; F = fungicide; N = nematicide.

not detected. Of these, 7 were legacy organochlorines, listed as POPs under the Stockholm Convention; together, these accounted for an average of 73.6% of the total pesticides detected in caiman. These consisted of dieldrin (36.6%), mirex (18.1%), 4,4′-DDE (8.1%), alpha-endosulfan (8%), heptachlor epoxide (1.2%), oxy-chlordane (0.8%), and heptachlor (0.8%). Dieldrin, DDE, alpha-endosulfan, heptachlor epoxide, and oxy-chlordane are all major metabolites of aldrin, DDT, endosulfan, heptachlor, and chlordane, respectively. Each of these parent compounds and its metabolites are believed to have endocrine-disrupting properties [7,39–41], which have detrimental effects on reproduction and development [33,40]. Dieldrin and DDT were banned in Costa Rica in 1988, followed by heptachlor and chlordane in 1990, and no importation of mirex occurred after 1993. A recent global ban on the manufacture and use of endosulfan was also negotiated under the Stockholm Convention in 2011. However, at the time of the present study endosulfan was only being voluntarily phased out.

Breakdown products (DDE, heptachlor epoxide, oxy-chlordane, and alpha-endosulfan), within caiman blood highlight the long half-lives legacy POP parent compounds have within aquatic systems. This is of concern, as breakdown products are often as mobile and as toxic as the parent compound [3]. High temperatures, common in tropical environments, can also enhance toxicity of POPs [3,42,43]. Dominance of these POPs in environmental matrices demonstrates their persistence, despite decades-old regulation. Of the 9 pesticides detected, 2 were in current use; these pyrethroid insecticides accounted for an average of 26.4% of the total pesticides detected in caiman. These were permethrin (25.7%), the second most prevalent pesticide detected, and cypermethrin (0.7%). Detection of these current-use pesticides confirms their presence in the caiman food web. Pseudopersistence as a consequence of extensive current use in the region may also explain part of this phenomenon.

Across all waterways, caiman had a mean total pesticide concentration of 3.33 ± 3.26 ng/g lipid (mean $\pm$ standard deviation). However, caiman (N1–N6) exposed to the direct influence of banana plantations within the Rio Suerte and downstream Caño Penitencia had higher total pesticide concentrations ($F = 12.79$; $p = 0.00$) of 6.03 ± 3.43 ng/g lipid compared with 1.30 ± 0.41 ng/g lipid, than in caiman outside the direct influence of banana plantations within the upstream areas of Caño Penitencia, Caño Palma, and Caño Harold (Figure 2).

Pesticide profiles of caiman within the direct influence of banana plantations (N1–N6) had higher concentrations of dieldrin ($F = 5.46$; $p = 0.03$), permethrin ($F = 18.62$; $p = 0.00$), mirex ($F = 6.23$; $p = 0.02$), 4,4′-DDE ($F = 5.47$; $p = 0.03$), heptachlor epoxide ($F = 5.69$; $p = 0.03$), and oxy-chlordane ($F = 5.23$; $p = 0.04$). In contrast, alpha-endosulfan ($F = 3.21$; $p = 0.09$), heptachlor ($F = 1.37$; $p = 0.26$), and cypermethrin ($F = 0.734$; $p = 0.40$) were more evenly distributed and did not vary among near-field or reference sites. In addition, no relationship was found between pesticide concentrations in caiman and log K_{OW} (log of the octanol–water partition coefficient) of the pesticides ($F = 0.32$; $p = 0.59$) within caiman. The influence of and log K_{OW} was likely masked by bioavailability of pesticides. Therefore, pesticide profiles appear to be driven by differences in bioavailability between near-field and reference areas, reflecting the degree to which their prey have been contaminated by current and past use of pesticides.

A PCA was applied to the data set to further explore the spatial variability of pesticide distribution within the region (Figure 3). Data processing within these PCA models eliminates the effect of concentration to interpret pattern of pesticide distribution. Reference caiman R7 was removed as an outlier, because it was the only individual in which cypermethrin was detected and therefore contained a very different pattern of accumulation relative to other caiman. Cypermethrin is a current-use pyrethroid insecticide used for vector control and sold locally for domestic use to control termite populations. The location of caiman R7 in Caño Palma is unique, because it is adjacent to human habitations (the Caño Palma Biological

Table 2. Morphometric details of spectacled caiman (*Caiman crocodilus*) sampled in the present study[a]

Number	Location	Sex	Scute ID	Mass	STL	SVL	TC	NC	HW	HD	HL	CI
N1	Rio Suerte	F	23	7.2	1.35	0.73	0.26	0.28	0.11	0.07	0.19	1.87
N2	Rio Suerte	M	21	10.0	1.50	0.80	0.30	0.30	0.12	0.09	0.22	1.97
N3	Rio Suerte	M	20	6.0	1.17	0.63	0.27	0.25	0.10	0.07	0.17	2.40
N4	Penitencia (downstream)	M	5	8.1	1.41	0.73	0.31	0.32	0.11	0.07	0.19	2.06
N5	Penitencia (downstream)	M	18	17.5	1.70	0.92	0.37	0.39	0.14	0.10	0.24	2.25
N6	Penitencia (downstream)	F	16	8.2	1.30	0.68	0.31	0.28	0.07	0.07	0.20	2.61
R1	Penitencia (upstream)	F	12	6.2	1.60	0.65	0.27	0.35	0.10	0.06	0.18	2.26
R2	Penitencia (upstream)	M	17	8.0	1.34	0.70	0.31	0.30	0.11	0.08	0.18	2.38
R3	Penitencia (upstream)	M	15	15.0	1.62	0.90	0.37	0.35	0.13	0.09	0.25	2.04
R4	Penitencia (upstream)	M	19	7.9	1.34	0.69	0.30	0.30	0.10	0.07	0.19	2.40
R5	Caño Palma	M	2	6.0	1.20	0.58	0.25	0.26	0.09	0.06	0.17	3.08
R6	Caño Palma	F	13	11.0	1.51	0.79	0.35	0.35	0.12	0.08	0.21	2.23
R7	Caño Palma	M	14	17.0	1.55	0.84	0.36	0.40	0.15	0.09	0.24	2.87
R8	Caño Harold	M	30	9.2	1.36	0.72	0.34	0.32	0.10	0.07	0.20	2.43

[a]Other than mass (kg), all measurements are in meters.
STL = snout–tail length; SVL = snout–vent length; TC = tail circumference; NC = neck circumference; HW = head width; HD = head depth; HL = head length; CI = condition index.

Station, and lodge Vista al Mar), which use cypermethrin. Because runoff after rainfall events has been identified as a major source of pesticides in water bodies [12,22,44], the frequent heavy rains in this region likely wash this frequently used pesticide into Caño Palma.

Within the sample projection plot (a), caiman cluster into 2 groups. Most near-field caiman (N1–N4) fall into group I. All reference caiman (R1–R8) fall into group II, along with the 2 near-field caiman that were sampled furthest downstream of the banana plantations (N5–N6). Thus, the PCA clearly distinguishes between those caiman that are exposed to and directly influenced by applications at the banana plantations.

Conversely, that these 2 groups are close together suggests some commonalities in terms of overall pattern of pesticide distribution in these waterways. The variable projection plot (b) shows that most of the pesticides cluster together on the right-hand side of the plot, being associated with caiman from reference sites. This indicates that these pesticides are more ubiquitously distributed in the regional environment, something that is consistent with atmospheric transport and deposition on a larger scale. Within the Northern Atlantic region of Costa Rica, periodic flooding caused by high annual rainfall (~5000 mm) likely accelerates the dispersion of pesticides over time and space.

The variable projection plot (b) shows that dieldrin and mirex do not cluster with the other pesticides, but are found toward the left-hand side of the plot. These 2 pesticides explain the variation in caiman in group I. This can only be explained by local upstream use of these 2 pesticides, which most likely is taking place at banana plantations.

When dieldrin and mirex were removed from the PCA (not shown), it did not reveal any significant difference between principal component scores from *x*- and *y*-axis (PC1 and PC2), within the variable projection plot (b). Within the sample projection plot (a), it resulted in caiman within groups I and II merging closer together. This again indicates a background pattern of pesticides through all caiman and that dieldrin and mirex are factors that influence the pattern of pesticide accumulation within group I caiman. In addition, no relationship existed between log K_{OW} values [45] and PC1 ($F = 1.6$; $p = 0.23$) or PC2 ($F = 1.8$; $p = 0.21$), eliminating log K_{OW} as the dominant influence on the accumulation profiles of pesticides in caiman.

To explore spatial variation in pesticide concentration, we also examined the distance that caiman were sampled from the upstream Rio Suerte. Although various pesticides may have

Table 3. Concentrations in ng/g lipid weight of the pesticides detected in blood collected from spectacled caiman (*Caiman crocodilus*) in Costa Rica[a]

	Near-field sites[b]								Reference sites[c]									
	N1	N3	N2	N4	N5	N6	Mean ± SD	∑ %[d]	R3	R6	R2	R7	R8	R4	R1	R5	Mean ± SD	∑ %[d]
% Lipid	0.18	0.11	0.14	0.16	0.12	0.27	0.16 ± 0.05	30.91	0.19	0.17	0.37	0.59	0.21	0.26	0.15	0.25	0.27 ± 0.14	69.09
Dieldrin	7.94	3.87	2.59	1.08	0.08	0.12	2.61 ± 2.74	33.65	0.10	0.13	0.32	0.03	0.25	0.30	0.17	0.04	0.17 ± 0.11	2.90
Permethrin	1.06	1.53	0.87	1.76	0.89	2.19	1.38 ± 0.49	17.79	0.97	0.25	0.46	0.35	0.70	0.35	0.40	0.24	0.46 ± 0.23	7.96
Mirex	2.21	1.01	2.07	0.82	0.22	0.07	1.07 ± 0.82	13.72	0.66	0.40	0.29	0.27	0.06	0.12	0.13	0.08	0.25 ± 0.19	4.34
4,4′-DDE	0.89	0.26	0.25	0.51	0.86	ND	0.55 ± 0.28	5.94	0.16	0.47	0.21	0.18	ND	ND	ND	ND	0.26 ± 0.13	2.19
Alpha-endosulfan	0.26	0.44	0.40	0.37	0.49	0.06	0.33 ± 0.14	4.30	0.14	0.34	0.15	0.10	0.24	0.22	0.32	0.24	0.22 ± 0.08	3.74
Heptachlor epoxide	0.21	0.11	ND	0.07	0.10	ND	0.12 ± 0.05	1.04	ND	0.06	ND	0.03	ND	ND	ND	ND	0.05 ± 0.02	0.20
Oxy-chlordane	0.09	ND	ND	0.12	0.12	ND	0.11 ± 0.02	0.70	ND	ND	ND	0.04	ND	ND	ND	ND	0.04 ± 0.00	0.08
Heptachlor	0.06	ND	ND	0.07	0.09	ND	0.07 ± 0.01	0.48	ND	0.06	0.03	ND	ND	0.04	ND	ND	0.04 ± 0.01	0.28
Cypermethrin	ND	ND	ND	ND	ND	ND	–	–	ND	ND	ND	0.31	ND	ND	ND	ND	0.31	0.67
Total	12.71	7.21	6.18	4.80	2.85	2.44	6.03 ± 3.43	77.63	2.02	1.73	1.46	1.31	1.25	1.03	1.02	0.60	1.30 ± 0.41	22.37

[a]Samples are arranged from highest to lowest total concentration left to right, and pesticides are arranged from highest to lowest prevalence across all samples.
[b]Individuals (N1–N6) are near-field samples within the direct influence of banana plantations.
[c]Individuals (R1–R8) are reference samples outside the direct influence of banana plantations.
[d]Percentage of total (∑ %) is percentage of all 14 near-field and reference samples.
ND = not detected.

different effects on caiman and their food webs, combined they can act additively [14]. Therefore, total pesticide concentration serves as a proxy for habitat quality, pesticide use, and food web contamination. Total pesticide concentration (ng/g lipid) in

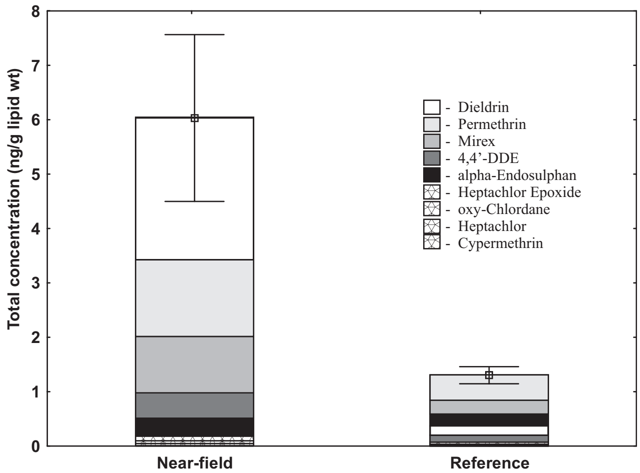

Figure 2. Mean ± standard error of pesticide concentration in spectacled caiman (*Caiman crocodilus*; ng/g lipid) in near-field and reference caiman ($F = 12.79$; $p = 0.00$).

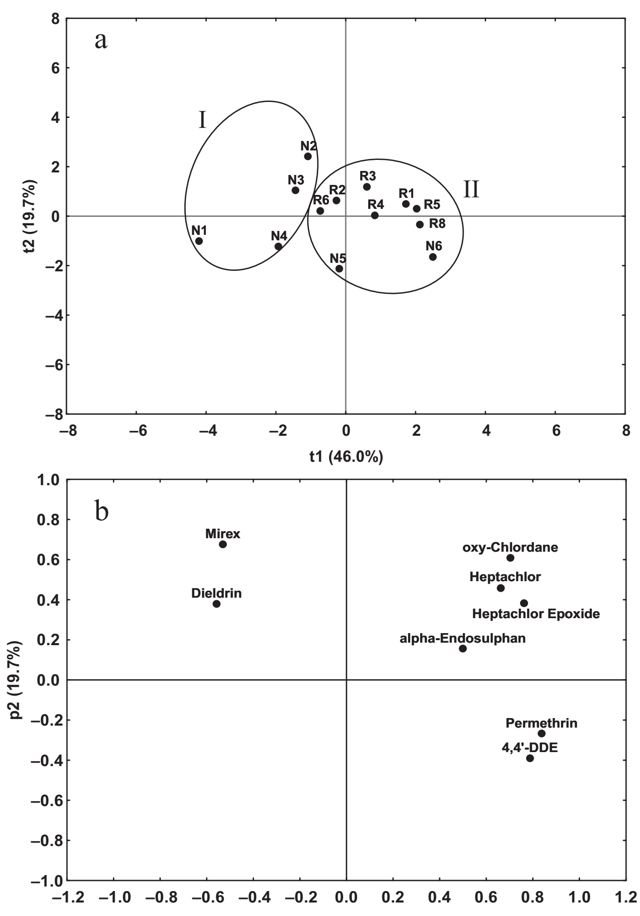

Figure 3. Principal component analysis (PCA) of pesticides in blood from spectacled caiman (*Caiman crocodilus*) captured in Costa Rica. (**A**) Sample projection and (**B**) variable projection. Circles in sample projection serve as a visual aid to identify caiman in groups I and II. Data processing eliminates the effect of concentration so that PCA interprets pattern. Caiman R7 was removed as an outlier from the pattern, as the only individual that contained cypermethrin.

caiman blood decreased with distance (km; $F = 20.76$; $p = 0.00$; Figure 4).

We also explored relationships between body condition and pesticide concentrations as a crude measure of health. Near-field and reference caiman had Fulton's condition factor K ranging from 1.87 to 3.08. Although no difference was seen in condition between near-field and reference groups of caiman ($F = 2.49$; $p = 0.14$), a negative correlation was found between body condition of caiman and proximity to the plantation-influenced Rio Suerte ($F = 5.06$; $p = 0.04$). Body condition also decreased with increasing pesticide concentrations ($F = 6.2$; $p = 0.02$; Figure 4).

Poor body condition was also related to lower blood lipid content in caiman ($F = 6.57$; $p = 0.02$). Condition is most likely attributable either to direct pesticide toxicity in caiman or to an indirect effect on the quality or abundance of prey for the caiman. If prey species within caiman food webs have been altered, essential nutrients are possibly lacking in their diets [23]. Low blood lipid content also may result in reduced egg viability [46]; however, we were unable to evaluate this in the present study. Although we have no data to substantiate a direct toxic injury to caiman, fish kills resulting from pesticide exposure observed within Rio Suerte in the past [2,3,12] provide tangible evidence that pesticides at a minimum contribute to an erosion of caiman habitat.

Research into the level at which multiple pesticides become biologically relevant and trigger health effects in crocodilians are limited [23,24]. However, exposure to pesticides has been implicated with detrimental effects on crocodilian reproduction and development in other studies [6,33,40,47]. Pesticides may disrupt endocrine systems by acting as anti-hormones or alter enzymes responsible for hormone synthesis and degradation [41]. As long-lived, high-trophic-level predators, crocodilians are important sentinels of ecosystem health [6,23]. Compared with other crocodilians, caiman within the present study generally had relatively low pesticide concentrations. Within blood samples from American crocodiles in the Tarcoles river, Costa Rica, 3 organochlorines were detected: aldrin (10 ng/g), 4,4′DDT (52 ng/g), and 4,4′-DDE (4.2 ng/g) [44]. The high amount of parent compounds (aldrin and DDT) indicates a more recent, heavy use of pesticide in the Tarcoles River than within the Rio Suerte. In Florida, blood samples from American alligators in Lake Woodruff had mean concentrations of 4,4′-DDE (0.92 ng/mL), dieldrin (0.24 ng/mL), and trace values for mirex and oxy-chlordane [6]. American alligators in nearby Lake Apopka had mean concentrations of 4,4′-DDE (7.35 ng/mL), dieldrin (1.68 ng/mL), mirex (0.28 ng/mL), and oxy-chlordane (0.39 ng/mL) [6]. Results in nanograms per milliliter are roughly comparable to ng/g, indicating that caiman in the present study had much lower DDE contaminant burdens than alligators in Lake Apopka, which were exposed to a spill containing high levels of dicofol, consisting largely of DDT/DDE. However, caiman had higher levels of dieldrin and mirex than Lake Apopka alligators. Differences in pesticide burdens reflect local pesticide bioavailability. The notable reproductive abnormalities in American alligators from Lake Apopka sheds more light on the potential for population-level effects in caiman inhabiting waterways downstream of banana plantations in Costa Rica and elsewhere.

The present study suggests that spectacled caiman and other aquatic taxa within the Tortuguero Conservation Area are at risk from exposure to pesticides that are used in upstream banana plantations. Although these plantations are economically important to Costa Rica, the ready detection of a range of legacy and currently used pesticides in caiman in a national

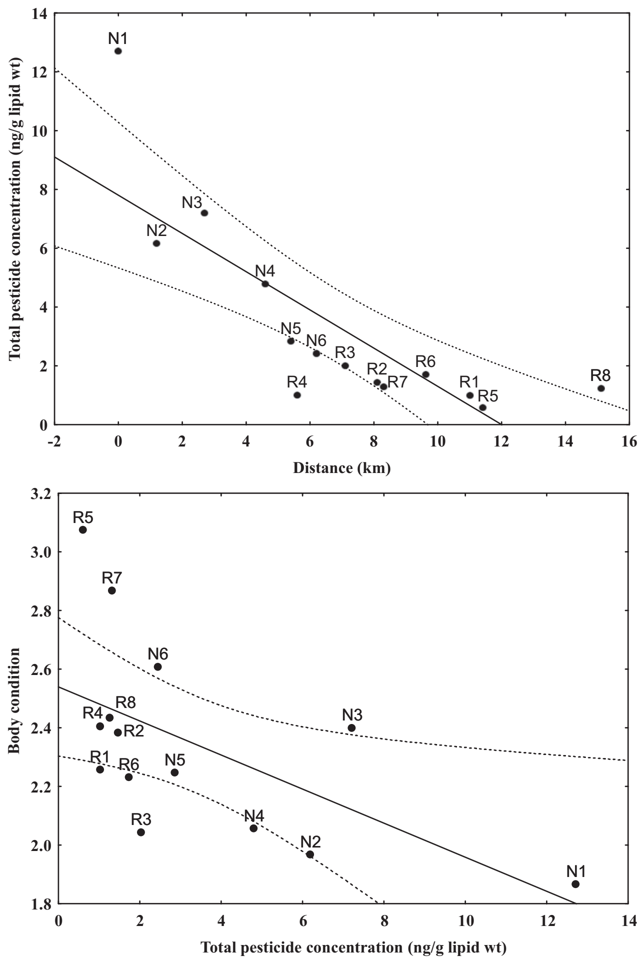

Figure 4. Total pesticide concentration (ng/g lipid) in spectacled caiman (*Caiman crocodilus*) blood significantly decreased with distance (km) from banana plantations located upstream in the Rio Suerte ($F = 20.76$; $p = 0.00$). Caiman body condition decreased with increasing total pesticide concentration (ng/g lipid; body condition details in Table 2; $F = 6.23$; $p = 0.02$). Dotted lines are 95% confidence intervals.

wilderness area raises important questions about the moral sustainability of banana agricultural production.

Acknowledgment—The authors gratefully acknowledge the assistance of AXYS Analytical Services including R. Grace, J. Cosgrove, B. Warning, and K. Pillay. We also wish to thank the Canadian Organization for Tropical Education and Rainforest Conservation, Ministro de Ambiente y Energía for providing research permit ACTo-GASP-PIN-006-06, the Earthwatch Institute, L. Castillo, M. Garcia, D. Retana, N. Dangerfield, Carlos at Vista al Mar, R. Ballard, T. Lewis, and D. Loth.

REFERENCES

1. International Union for Conservation of Nature. 2012. International Union for Conservation of Nature Red List of Threatened Species. Version 2012. 2. [cited 2012 December 11]. Available from: http://www.iucnredlist.org.
2. Henriques W, Jeffers RD, Lacher TE, Kendall RJ. 1997. Agrochemical use on banana plantations in Latin America: Perspectives on ecological risk. *Environ Toxicol Chem* 16:91–99.
3. Castillo LE, de la Cruz E, Ruepert C. 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environ Toxicol Chem* 16:41–51.
4. Murray DL. 1994. *Cultivating Crisis: The Human Cost of Pesticides in Latin America*. University of Texas Press, Austin, TX, USA.
5. Polidoro B, Dahlquist RM, Castillo LE, Morra MJ, Somarriba E, Bosque-Pérez N. 2008. Pesticide application practices, pest knowledge, and cost-benefits of plantain production in the Bribri-Cabécar Indigenous Territories, Costa Rica. *Environ Res* 108:98–106.
6. Guillette LJ, Brock JW, Rooney AA, Woodward AR. 1999. Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallus size in juvenile American alligators. *Arch Environ Contam Toxicol* 36:447–455.
7. Guillette LJ. 2006. Endocrine disrupting contaminants: Beyond the dogma. *Environ Health Perspect* 114:9–12.
8. Gilbertson M, Kubiak T, Ludwig J, Fox G. 1991. Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to chick edema disease. *J Toxicol Environ Health* 33:455–520.
9. Wesseling C, Ahlbom A, Antich D, Rodriguez AC, Castro R. 1996. Cancer in banana plantation workers in Costa Rica. *Int J Epidemiol* 25:1125–1131.
10. Wesseling C, Castillo L, Elinder CG. 1993. Pesticide poisonings in Costa Rica. *Scand J Work Environ Health* 19:227–235.
11. Sass R. 2000. Agricultural “killing fields”: The poisoning of Costa Rican banana workers. *Int J Health Serv* 30:491–514.
12. Castillo LE, Ruepert C, Solis E. 2000. Pesticide residues in the aquatic environment of banana plantation areas in the North Atlantic zone of Costa Rica. *Environ Toxicol Chem* 19:1942–1950.
13. Von Duszeln J. 1991. Pesticide contamination and pesticide control in developing countries: Costa Rica, Central America. In Richardson ML, ed, *Chemistry, Agriculture, and the Environment*. Royal Society of Chemistry, Cambridge, UK, pp. 410–428.
14. Guillette LJ, Vonier PM, McLachlan J. 2002. Affinity of the alligator estrogen receptor for serum pesticide contaminants. *Toxicology* 181:151–154.
15. Guillette LJ, Guillette EA. 1996. Environmental contaminants and reproductive abnormalities in wildlife: Implications for public health? *Toxicol Ind Health* 12:537–550.
16. Poletta GL, Larriera A, Kleinsorge E, Mudry MD. 2008. *Caiman latirostris* (broad-snouted caiman) as a sentinel organism for genotoxic monitoring: Basal values determination of micronucleus and comet assay. *Mutat Res* 650:202–209.
17. Gibbs RJ, Guerra C. 1997. Metals of the bottom muds in Belize City Harbor, Belize. *Environ Pollut* 98:135–138.
18. Carvalho FP, Villeneuve JP, Cattini C, Tolosa I, Montenegro-Guillen S, Lacayo M., et al. 2002. Ecological risk assessment of pesticide residues in coastal lagoons of Nicaragua. *J Environ Monit* 4:778–787.
19. Defew LH, Mair JM, Guzman HM. 2005. An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. *Mar Pollut Bull* 50:547–552.
20. Daly GL, Lei YD, Teixeira C, Muir DCG, Castillo LE, Wania F. 2007. Accumulation of current-use pesticides in neotropical montane forests. *Environ Sci Technol* 41:1118–1123.
21. Alegria HA, Bidleman TF, Shaw TJ. 2000. Organochlorine pesticides in ambient air of Belize, Central America. *Environ Sci Technol* 34:1953–1958.
22. Castillo LE, Martínez E, Ruepert C, Savage C, Gilek M, Pinnock M, Solis E. 2006. Water quality and macroinvertebrate community response following pesticide applications in a banana plantation, Limon, Costa Rica. *Sci Total Environ* 367:418–432.
23. Milnes MR, Guillette LJ. 2013. Alligator Tales: New lessons about environmental contaminants from a sentinel species. *BioScience* 58:1027–1036.
24. Campbell KR. 2003. Ecotoxicology of crocodilians. *Appl Herpetol* 1:45–163.
25. Grant PBC, Lewis TR. 2010. High-speed boat traffic: A risk to crocodilian populations. *Herpetol Conserv Biol* 5:456–460.
26. Da Silveira R, Magnusson WE. 1999. Diets of spectacled and black caiman in the Anavilhanas Archipelago, Central Amazonia, Brazil. *J Herpetol* 33:181–192.
27. Platt S, Thorbjarnarson JB. 2000. Population status and conservation of Morelet’s crocodile, *Crocodylus moreletii*, in northern Belize. *Biol Conserv* 96:21–29.
28. Leenders T. 2001. *A Guide to Amphibians and Reptiles of Costa Rica*. Zona Tropical, Miami, FL, USA.
29. Thorbjarnarson JB. 1994. Reproductive ecology of the spectacled caiman (*Caiman crocodilus*) in the Venezuelan Llanos. *Copeia* 4:907–919.
30. Allsteadt J, Lang JW. 1995. Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis*. *Herpetologica* 51:314–25.
31. Leslie AJ. 1997. *The Ecology and Physiology of the Nile Crocodile (Crocodylus niloticus) in Lake St. Lucia, KwaZulu-Natal, South Africa*. Drexel University, Philadelphia, PA, USA.

32. Rainwater TR, Millichamp NJ, Barrantes LDB, Barr BR, Montero JRB, Platt SG, Abel ST, Cobb GP, Anderson TA. 2011. Ocular disease in American crocodiles (*Crocodylus acutus*) in Costa Rica. *J Wildl Dis* 47:415–426.
33. Rainwater TR, Selcer KW, Nespoli LM, Finger AG, Ray D, Platt SG, Smith PN, Densmore LD, Anderson TA, McMurry ST. 2008. Plasma vitellogenin in Morelet's crocodiles from contaminated habitats in northern Belize. *Environ Pollut* 153:101–109.
34. Woudneh MB, Ziqing O, Sekela M, Tuominen T, Gledhill M. 2009. Pesticide multiresidues in waters of the lower Fraser Valley, British Columbia, Canada. Part I. Surface Water. *J Environ Qual* 38:940–947.
35. Ross PS, Jeffries SJ, Yunker MB, Addison RF, Ikonomou MG, Calambokidis J. 2004. Harbor seals (*Phoca vitulina*) in British Columbia, Canada, and Washington, USA, reveal a combination of local and global polychlorinated biphenyl, dioxin, and furan signals. *Environ Toxicol Chem* 23:157–165.
36. Glasius M, Christensen JH, Platz J, Vorkamp K. 2005. Halogenated organic contaminants in marine fish and mussels from southern Greenland: Pilot study on relations to trophic levels and local sources. *J Environ Monit* 7:127–131.
37. StatSoft Inc. 2011. STATISTICA (data analysis software system), version 10. [cited 09 February 2013]. Available from: www.statsoft.com.
38. Wu TH, Rainwater TR, Platt SG, Mcmurry ST, Anderson TA. 2000. Organochlorine contaminants in Morelet's crocodile (*Crocodylus moreletii*) eggs from Belize. *Chemosphere* 40:671–678.
39. Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101:378–384.
40. Guillette LJ, Crain DA, Gunderson MP, Kools SAE, Milnes MR, Orlando EF, Rooney AA, Woodward AR. 2000. Alligators and endocrine disrupting contaminants: A current perspective. *Am Zool* 40:438–452.
41. Guillette LJ, Gunderson MP. 2001. Alternations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. *Reproduction* 122:857–864.
42. Howe GE, Marking LL, Bills TD, Rach JJ, Mayer FL. 1994. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol and 2,4-dinitrophenol to the amphipod (*Gammarus pseudolimnaeus*) and rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 13:51–66.
43. Sprague JB. 1985. Factors that modify toxicity. In Rand GM, Petrocelli SR, eds, *Fundamentals of Aquatic Toxicology*. Taylor & Francis, Bristol, PA, USA, pp. 124–163.
44. Mortensen SR, Johnson K, Weisskop CP, Hooper MJ, Lacher TE, Kendall RJ. 1998. Avian exposure to pesticides in Costa Rican banana plantations. *Bull Environ Contam Toxicol* 60:562–568.
45. Sangster J. 2012. LOGKOW: A databank of evaluated octanol-water partition coefficients (Log P). Sangster Research Laboratories. Updated 2012-08-17. [cited 07 March 2013]. Available from: www.http://logkow.cisti.nrc.ca/logkow/index.jsp.
46. Noble RC, McCartney R, Ferguson MWJ. 1993. Lipid and fatty acid compositional differences between eggs of wild and captive-breeding alligators (*Alligator mississippiensis*): An association with reduced hatch-ability? *J Zoology Lond* 230:639–649.
47. Matter JM, McMurry CS, Anthony AB, Dickerson RL. 1998. Development and implementation of endocrine biomarkers of exposure and effects in American alligators (*Alligator mississippiensis*). *Chemosphere* 37:1905–1914.