

# A cross-taxa survey of organochlorine pesticide contamination in a Costa Rican wildland

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**“Capsule”:** *Amphibians, turtles, mice and birds from a protected Costa Rican wildland were contaminated with organochlorine pesticides and metabolites.*

## Abstract

Amphibians, turtles, birds (mostly passerines) and mice collected from a conservation area in northwestern Costa Rica were analyzed for organochlorine (OC) pesticide contamination. Six of 39 amphibians (three of eight species), three of six turtles (two species), one of eight mice (one species) and 19 of 55 birds (five of seven species) contained OCs at levels up to 580 ng/g. The most frequently detected compound in 23 of 108 organisms was p,p'DDE. Dieldrin, delta-BHC, heptachlor, p,p'DDD, and endosulfan II were each found in at least four organisms, while eight other OCs were found in at least one organism. The presence of OCs in taxa from the conservation area indicates the likelihood of long-distance transport of such compounds through the atmosphere.

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## 1. Introduction

Tropical conservation areas have been set aside not only to protect a suite of organisms, but also to preserve the interactions among them. However, no park is completely isolated, as interactions between organisms inside a conserved wildland will inevitably be impacted by conditions in the surrounding agricultural, industrial, and residential landscapes. Many of these impacts decrease in magnitude as park size increases (Janzen, 1999), and are often lumped into the category of “edge effects”. Impacts that operate on a global scale, such as increases in atmospheric carbon dioxide, increased ultraviolet radiation, and long-distance atmospheric transport of pesticides (LeNoir et al., 1999) may affect wildlands in ways that are unpredictable from their size or degree of protection from other external threats. The

magnitude of such impacts may instead depend on topography, climate, latitude, and geologic history of the site and surrounding area. The degree of influence of these types of factors on a conservation area will be unique for each park, and will probably be unique for the different habitats protected within a large conservation area. Although very few of these global level impacts have been quantified (or even documented) in most conservation areas, they are often offered as explanations for unexpected phenomena, such as the perceived population declines of species or groups of species. For example, Pounds and Crump (1994) quantified population declines in two amphibian species in a Costa Rican conservation area, and suggested that pesticide contamination from regional agricultural sites may be a contributing factor in these declines. However, there was no direct evidence that these organisms had ever been exposed to pesticides. For most organisms it is unknown to what extent population dynamics may be affected by pesticide contamination.

Organochlorine pesticide use is thought to be widespread in Central and South American countries (Castillo et al., 1997), but because of underdeveloped regulatory

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structures, there are little data on the extent to which they are used (Murray, 1994). Costa Rica is one of the few countries with reliable information regarding the history of its pesticide use. About 10% of Costa Rica's total land area is used for crop production, and pesticide imports have been reported to be up to 9000 metric tons annually (von Duzeln, 1991). Although exact statistics on the quantity of OC pesticides applied to crops is not available, it is estimated to be 6 kg/ha higher than in most industrialized countries (von Duzeln, 1991). In 1981, most OC pesticides in Costa Rica were restricted for agricultural use only, while DDT could only be used in efforts to eradicate malaria (Castillo et al., 1997). DDT and a series of other OC pesticides (e.g. aldrin, dieldrin) were then banned for use in 1988, while chlordane, heptachlor, endosulfan, and a series of other OC compounds were restricted in their use (von Duzeln, 1991). All of these laws restricted pesticides to these uses from a former wide range of permitted uses. Persistent OC residues in Costa Rica have been detected in water samples (von Duzeln, 1988), coffee (Cetinkaya et al., 1984), insect larvae (Standley and Sweeney, 1995), bovine meat (Rojas and Ruiz, 1989), bovine milk (Ruiz and Rojas, 1988), human fat (Barquero and Constenla, 1986), and human milk (Umana and Constenla, 1984).

The objective of this study was to document OC contamination in amphibians, reptiles, birds, and mammals from a tropical conservation area in Northwest Costa Rica. Although no studies have been published on pesticide contamination levels in vertebrates from this locality, Standley and Sweeney (1995) documented OC contamination in mayfly larvae and vegetation collected from sites on the eastern and western slopes of the continental divide within the conservation area. They found an east to west gradient in OC contamination, which they attributed to local weather patterns and the location of agricultural areas in relation to the conservation area. We collected birds from locations near the two sites used by Standley and Sweeney (1995) and from a site further west. In addition, we examined OC contamination in tissues of amphibians, reptiles and mice from our westernmost site. These became available from a parasite inventory conducted at the same time the birds were collected. To our knowledge, no data exist on OC contamination in vertebrates from this conservation area. In fact, very few data exist on OC contamination of Neotropical wildlife.

## 2. Methods

### 2.1. Collection of animals

After securing the appropriate permits, thirty-nine amphibians, six turtles, and 55 birds were collected in June and July, 1998. Once collected, gut contents and all

endoparasites and ectoparasites were removed and the animals were frozen. Eight mice that had been collected for the parasite inventory one year earlier and stored in a freezer were also included in this study. The viscera and associated fat deposits of the mice had been discarded. All organisms were later transferred to a  $-80^{\circ}\text{C}$  freezer.

### 2.2. Site description

Birds, reptiles, amphibians and mice were collected in Sector Santa Rosa of the Area de Conservación Guanacaste (ACG) ( $10^{\circ} 50' \text{ N}$ ,  $85^{\circ} 37' \text{ W}$ ). Additionally, birds were collected from forests and pastures near the Pitilla and Maritza Biological Stations of the ACG. These sites correspond to the eastern and western catchments, respectively, of Standley and Sweeney (1995). The ACG is a conserved wildland located approximately 40 km north of the city of Liberia in northwestern Costa Rica (<http://www.acguanacaste.ac.cr>), and includes 120,000 ha of terrestrial habitat and 43,000 ha of marine habitat. The vegetation of sector Santa Rosa is remnant, old-growth tropical dry forest embedded in a matrix of successional tropical dry forest of varying ages regenerating on cattle pasture. Santa Rosa averages 1500 mm rain/year and is located at an elevation of about 300 m. Station Maritza ( $10^{\circ} 57' \text{ N}$ ,  $85^{\circ} 29' \text{ W}$ ) is located at the transition between successional tropical dry forest that is regenerating on old cattle pastures, and moister montane forest. Maritza averages 2000 mm of rainfall/year, and is located at an elevation of 650 m. The vegetation at Pitilla ( $10^{\circ} 59' \text{ N}$ ,  $85^{\circ} 25' \text{ W}$ ) is regenerating montane rainforest. Pitilla averages between 3000 and 4000 mm of rainfall/year and is located at an elevation of 600 m.

### 2.3. Species accounts

Information regarding habitat preferences, feeding habits and sizes of organisms from this study are presented in Tables 1 and 2. For birds, one species of flycatcher (Tyrannidae), vireo (Vireonidae), warbler (Parulidae) and nightjar (Caprimulgidae) were collected at each of the three sites. The flycatcher *Myiarchus tyrannulus* was collected at Santa Rosa and Maritza, while *Elaenia flavogaster* was collected at Pitilla. The vireo *Vireo flavorividis* was collected at Santa Rosa, while *Hylophilus decurtatus* was collected at Maritza and Pitilla. The warbler *Basileuterus rufifrons* was collected at Santa Rosa and Maritza, while *Geothlypis poliocephala* was collected at Pitilla. The nightjar *Nyctidromus albigollis* was collected at all three sites.

### 2.4. Residue analysis

The extraction of pesticides from bird tissues and subsequent chemical analyses using gas chromatography were performed in a similar manner as described

Table 1  
Most frequently detected OC compounds in amphibians, reptiles and mice

Taxa	Species	N	Mean ± SE levels (ng/g, wet weight)			
			p,p'-DDE	Delta-BHC	Heptachlor	Dieldrin
<i>Amphibians</i>						
Family Bufonidae:	<i>Bufo marinus</i> <sup>a</sup>	7	12.8 ± 0.06 (4) <sup>b</sup>	8.20 ± 0.04 (3)	6.40 ± 0.004 (3)	3.70 ± 0.008 (3)
Family Ranidae:	<i>Rana. forreri</i> <sup>c</sup>	7	54.62 (1)	39.87 (1)	32.14 (1)	ND <sup>d</sup>
Family Rhinophrynidae:	<i>Rhinophrynus dorsalis</i> <sup>e</sup>	3	16.85 (1)	ND	ND	ND
Family Microhylidae:	<i>Hypopachus variolosus</i> <sup>f</sup>	6	ND	ND	ND	ND
Family Hylidae:	<i>Smilisca baudini</i> <sup>g</sup>	4	ND	ND	ND	ND
	<i>Scinax boulengeri</i> <sup>h</sup>	4	ND	ND	ND	ND
Family Leptodactylidae:	<i>Phrynohyus venulosus</i> <sup>i</sup>	5	ND	ND	ND	ND
	<i>Leptodactylus melanonotus</i> <sup>j</sup>	1	ND	ND	ND	ND
<i>Turtles</i>						
Family Kinosternidae:	<i>Kinosternon scorpioides</i> <sup>k</sup>	3	124.9 ± 18.5 (2)	7.60 ± 0.003 (2)	6.73 (1)	ND
Family Emydidae:	<i>Rhinoclemmys pulcherrima</i> <sup>l</sup>	3	4.36 (1)	11.03 (1)	16.69 (1)	4.64(1)
<i>Mice</i>						
Family Heteromyidae:	<i>Liomys salvini</i> <sup>m</sup>	8	ND	ND	ND	ND

<sup>a</sup> Inhabits savannah and open forest (Zug, 1983); insectivorous but feeds primarily on ants and beetles (Zug, 1983; Evans and Lampo, 1996); mean mass was 254 g.

<sup>b</sup> Number contaminated.

<sup>c</sup> Inhabits standing water and streams; likely feeds on juvenile fish and amphibians; mean mass was 54 g.

<sup>d</sup> Not detected.

<sup>e</sup> Fossorial; feeds on ants and termites (Foster and McDiarmid, 1983); mean mass was 53 g.

<sup>f</sup> Fossorial; feeds on ants and termites; mean mass was 5 g.

<sup>g</sup> Arboreal; insectivorous; mean mass was 15 g.

<sup>h</sup> Arboreal; insectivorous; mean mass was 18 g.

<sup>i</sup> Inhabits vegetation near water (Norman, 1998); likely insectivorous; mean mass was 12 g.

<sup>j</sup> Arboreal; insectivorous; mean mass was 37 g.

<sup>k</sup> Semi-aquatic; feeds on aquatic snails and invertebrates (Acuña-Mésén, 1990); mean mass was 244 g.

<sup>l</sup> Inhabits cleared areas near streams and in gallery forests; omnivorous, but prefers plants (Ernst, 1983); mean mass was 200 g.

<sup>m</sup> Inhabits forest floor in mature/regenerating tropical dry forest; granivorous/consume larvae of Lepidopterans and other insects (Fleming, 1983); mean mass (without viscera) was 28 g.

in Frick et al. (1998) and Harper et al. (1996). However, carcasses were plucked and all parts (including the skin and fat) except the feathers, feet and claws were included in the analyses. Just prior to analysis each carcass was thawed and the digestive tract was rinsed with water to remove the contents, and then replaced with the carcass. Sodium sulfate (not exceeding 50% of the weight of the carcass) was added to the carcass, which was then ground into a paste with a tissue homogenizer. The mixture was then transferred to a soxhlet thimble and extracted with hexane (approximately 150 ml, pesticide-grade, Fisher) for 15–24 h. The extract was concentrated under 5 ml and then transferred to a chromatography column containing Florisil<sup>®</sup> (20 g, 60–100 mesh, activated at 130 °C for 16 h) and sodium sulfate (1–2 cm).

The column, which had been washed with hexane (~40 ml), was eluted with 200 ml portions of 6% diethyl ether in hexane (fraction 1), 15% diethyl ether in hexane (fraction 2), and 50% diethyl ether in hexane (fraction 3). These elutions were collected, concentrated to about 5 ml using a rotary evaporator and rediluted to 10.0 ml in a volumetric flask.

Each fraction was analyzed with a Hewlett Packard (HP) 6890 series gas chromatograph equipped with two Ni<sup>63</sup> electron capture detectors operated at 300 °C. One microliter injections were made with an autosampler (HP 18596-C) into a split/splitless injector operated at 230 °C. The analyte was separated on two different fused silica capillary gas chromatograph columns using helium as the carrier gas. A 30-m DB-35 (0.32-mm

Table 2  
Most frequently detected OC compounds in birds × site combinations

Species	Site	N	Mean ± SE levels (ng/g, wet weight)			
			p,p'-DDE	p,p'-DDD	Endosulfan II	Dieldrin
Family Caprimulgidae:						
<i>Nyctidromus albicollis</i> <sup>a</sup>	Santa Rosa	10	3.7 ± 1.8 (4) <sup>b</sup>	3.6 (1)	2.8 (1)	0.7 (1)
<i>Nyctidromus albicollis</i>	Maritza	4	145 (1)	88 (1)	ND <sup>c</sup>	ND
<i>Nyctidromus albicollis</i>	Pitilla	5	13 ± 7.7 (3)	ND	ND	ND
Family Tyrannidae:						
<i>Myiarchus tyrannulus</i> <sup>d</sup>	Santa Rosa	5	1.4 (1)	ND	66 (1)	ND
<i>Myiarchus tyrannulus</i>	Maritza	5	ND	ND	ND	ND
<i>Elaenia flavogaster</i> <sup>e</sup>	Pitilla	4	16 ± 0.6 (4)	37 (1)	ND	11 ± 4.3 (3)
Family Vireonidae:						
<i>Vireo flavoviridis</i> <sup>f</sup>	Santa Rosa	5	2.7 (1)	94 (1)	51 (1)	3 (1)
Family Parulidae:						
<i>Hylophilus decurtatus</i> <sup>g</sup>	Maritza	3	ND	ND	ND	ND
<i>Hylophilus decurtatus</i>	Pitilla	5	ND	ND	ND	ND
<i>Basileuterus rufifrons</i> <sup>h</sup>	Santa Rosa	5	ND	ND	ND	ND
<i>Basileuterus rufifrons</i>	Maritza	3	ND	ND	ND	ND
<i>Geothlypis poliocephala</i> <sup>i</sup>	Pitilla	1	ND	29 (1)	19 (1)	ND

<sup>a</sup> Inhabits roadsides, pastures, riverbanks; insectivorous, primarily feeds on moths and beetles; mass = 55 g (Stiles and Skutch, 1989; Willis, 1980). Masses for all species are from Stiles and Skutch (1989).

<sup>b</sup> Number contaminated.

<sup>c</sup> Not detected.

<sup>d</sup> Inhabits forest clearings and roadsides; insectivorous/granivorous; mass = 34 g (Stiles and Skutch, 1989; Willis, 1980).

<sup>e</sup> Inhabits forest clearings, edge and pastures; insectivorous/frugivorous/granivorous; mass = 25 g (Stiles and Skutch, 1989).

<sup>f</sup> Inhabits forest canopy; insectivorous/frugivorous/granivorous; mass = 18.5 g (Stiles and Skutch, 1989; Willis, 1980).

<sup>g</sup> Inhabits forest canopy and edge; insectivorous/frugivorous/granivorous; mass = 9 g (Stiles and Skutch, 1989; Willis, 1980).

<sup>h</sup> Inhabits forest interior; insectivorous/frugivorous; mass = 11.5 g (Stiles and Skutch, 1989; Willis, 1980).

<sup>i</sup> Inhabits pastures, forest edge; insectivorous/frugivorous/granivorous; mass = 15.5 g (Stiles and Skutch, 1989).

inside diameter) served as the primary column for pesticide quantification, while a 30-m DB-1701 (0.32-mm inside diameter) was used for pesticide confirmation. The oven temperature was raised from an initial temperature of 150–200 °C at a rate of 8 °C/min, then from 200–290 °C at 4 °C/min, and maintained at 290 °C for 7 min. Data were collected and analyzed with HP environmental analysis software. Peak areas from eight calibration standards were used to calculate response factors. Curve fit was performed by linear regression, and linearity of each calibration curve was verified by determining the coefficient of determination of the line formed by the eight response factors for each pesticide. The coefficient of determination was always greater the 95%. The average response factor from the calibration curve was used to quantify pesticide levels. In all cases the retention times of the peaks closely matched the standards.

All other organisms were analyzed in the same manner, with the following changes in procedure. Compared to birds, more sodium sulfate was added to the carcasses of turtles and amphibians (approximately 100% of the weight of the carcass). Gut contents and turtle shells were excluded from analyses. Egg masses were removed from nine of the 39 amphibians, and were analyzed separately from the carcasses. The six *Hypo-*

*pachus variolosus* were too small to analyze individually; they were instead randomly assigned to one of two groups, and each group was homogenized and processed as a single sample. Hair was removed from mice before processing. Skins and rinsed viscera of all organisms were included in the analysis, except for mice, which did not include viscera. The volume of some samples exceeded the capacity of the soxhlet extraction thimbles, and in those cases the carcass was homogenized and a subsample of the homogenate was analyzed for OC contamination.

The chemicals assayed for were aldrin, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (p,p'-DDD), 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE), 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (p,p'-DDT), dieldrin, endosulfan I, endosulfan II, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, alpha-hexachlorocyclohexane, beta-hexachlorocyclohexane, gamma-hexachlorocyclohexane, lindane, and methoxychlor. Most of these compounds have been detected in studies of other organisms (DeWeese et al., 1986; Fyfe et al., 1990; Elliott et al., 1994). Detection limits ranged from 4–6.5 µg/kg for all pesticides except the following: heptachlor (8–13 µg/kg), aldrin (12–20 µg/kg), endosulfan I (12–20 µg/kg), and endosulfan sulfate (40–65 µg/kg). Positive identification of pesticides was made

when sample retention times were within 0.05 min of the average retention time of the calibration standards on both columns. Values were converted to ng/g (wet weight) of tissue sample.

### 3. Results

#### 3.1. Amphibians

Organochlorine residues were present in six of 39 individuals (three of eight species). Four of seven *Bufo marinus*, one of seven *Rana forreri*, and one of three *Rhinophrynus dorsalis* contained OC compounds. These species were the three largest amphibian species in the study (Table 1). Twelve OC compounds were detected in amphibians. The most frequently detected compounds were p,p'-DDE, delta-BHC, heptachlor, and dieldrin (Table 1). Heptachlor epoxide, beta-BHC, gamma-BHC, endosulfan II, alpha-BHC, and p,p'-DDT were detected in amphibians at levels above detection limits but lower than 10 ng/g.

No OCs were detected in *H. variolus* ( $n=2$  samples from six individuals), *S. baudinii*, *S. boulengeri*, *L. melanotus*, and *P. venulosa*. In addition, no OCs were detected in egg masses collected from two *H. variolus*, two *S. baudinii*, and five *R. forreri*. One of the egg masses was from the contaminated *R. forreri*.

#### 3.2. Turtles

OCs were detected in two of three *Kinosternon scorpioides*, and one of three *Rhinoclemmys pulcherrima* (Table 1). Of the 10 OC compounds detected in turtles, p,p'-DDE, delta-BHC, and heptachlor were the most frequently detected. Alpha-BHC, dieldrin, p,p'-DDD, heptachlor epoxide and gamma-BHC were detected in turtles at levels above detection limits but lower than 10 ng/g.

#### 3.3. Mice

Endrin was the only OC compound detected in one of eight *Liomys salvinii* at a level of 13 ng/g. This was the largest individual (44.7 g) analyzed in the study.

#### 3.4. Birds

Nineteen of 55 birds (five of seven species) contained detectable levels of 13 of the 17 OC compounds. The most commonly detected compound was p,p'-DDE, occurring in 14 of the 19 contaminated birds. The frequency of occurrence and the range of contamination levels for each compound are presented in Table 2. The most highly contaminated individual was a *Nyctidromus albicollis* from Maritza, which contained 580 ng/g p,p'-DDE, 164 ng/g p,p'-DDT and 88 ng/g p,p'-DDD.

### 4. Discussion

OC compounds were present in amphibians, turtles, mice, and birds of the ACG. These data suggest that larger amphibian species were more contaminated than the smaller species. Larger predators may be expected to feed higher in the food chain than smaller animals, and may accumulate higher concentrations of OC compounds. In addition, larger species may be longer-lived than smaller species, and thus any given individual may be more likely to have had more time to accumulate OC compounds. Turtles showed the highest frequency of contamination of any group (50%) but were also from the taxon with the smallest sample size ( $n=6$ ). Turtles may live longer than the amphibians in this study, which would give them more time to accumulate higher concentrations of OC compounds. Both the turtles and the more aquatic amphibian species (*R. forreri* and *L. melanotus*) may absorb OC compounds directly from water, and they may also consume aquatic insects and other prey that have concentrated OC compounds (Standley and Sweeney, 1995; Bishop et al., 1996). *B. marinus*, which is mostly terrestrial, had the highest frequency of contamination among the amphibians.

Rodents were the least contaminated organisms in the study. This is not unexpected as *L. salvinii* is mostly granivorous. In addition, samples of *L. salvinii* did not include the viscera and associated fatty deposits, so contamination in this group of organisms was probably underestimated.

Birds were more frequently contaminated than were amphibians or mice. This could possibly be explained by the larger home ranges of birds compared to those of the other taxa, if one assumes that there was a patchy distribution of OC compounds within sites. Although all birds used in this study were year-round residents, there is likely some variability in home range size among species. Due to small sample sizes and low frequencies of contamination, we were unable to analyze differences in contamination levels between species and sites.

The pattern of OC contamination in this area detected by Standley and Sweeney (1995) was dominated by endosulfan compounds, whereas the metabolites of DDT (p,p'-DDE and p,p'-DDD) were the most commonly detected compounds in organisms from this study. Because endosulfans break down more quickly and are more water soluble than DDT compounds (Kamrin, 1997), it is not surprising that the more persistent DDT compounds dominated the OC signature of vertebrates. Castillo et al. (1997) reported that endosulfans were imported into Costa Rica as recently as 1993, but most OCs, including DDT, were restricted to anti-malarial uses in the early 1980s and were banned by 1990. Although DDT and other OCs are apparently no longer imported into Costa Rica, it is unknown what reserves remain in the country and to what degree these pesticides may still be used.

Atmospheric transport of pesticides from surrounding agricultural areas is likely the major source of OC contamination within the ACG. Atmospheric transport of organochlorines has been documented in numerous studies (e.g. Simonich and Hites, 1995; Blais et al., 1998). Based on the spatial pattern of OC contamination in vegetation samples, Standley and Sweeney (1995) hypothesized that OC compounds were entering the ACG from agricultural areas to the east of the conservation area. However, there are few data regarding the origin and distribution of pesticides in Central America (Castillo et al., 1997). It is also possible that some OC compounds may have been applied to the study area before it was declared a national park in the mid-1960s (Janzen, 1986).

In summary, OC contamination in vertebrates of the ACG is widespread both geographically and across taxonomic boundaries. Levels of OCs detected in passerine birds in the ACG are below those known to have deleterious reproductive effects in other passerine species (e.g. Custer et al., 1998; Bishop et al. 1999). Likewise, developmental and other abnormalities in three species of frog tadpoles have been linked to higher levels (in the 100 ng/g or ug/g ranges) of OCs than those found in this study (Savage et al., 2002; Rosenshield et al., 1999). However, developmental abnormalities in common snapping turtles (*Chelydra serpentina*) have been linked to OCs at levels in the same range as those observed in this study (Bishop et al., 1998). Most of the organisms surveyed in this study do not occupy the highest trophic levels in their respective food chains. The presence of OCs in these organisms indicates the possibility for biomagnification in organisms that feed at higher trophic levels.

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## References

Acuña-Mésén, R.A., 1990. The impact of fire and drought on the population structure of *Kinosternon scorpioides* (Testudines, Kinosternidae) in Palo Verde, Guanacaste, Costa Rica. *Brenesia* 20, 85–98.

- Barquero, M., Constenla, M., 1986. Residuos de plaguicidas organoclorados en tejido adiposo humano en Costa Rica. *Revista de Biología Tropical* 34, 7–12.
- Bishop, C.A., Mahony, N.A., Trudeau, S., Pettit, K.E., 1999. Reproductive and biochemical effects in tree swallows (*Tachycineta bicolor*) exposed to chlorinated hydrocarbon contaminants in wetlands of the Great Lakes and St. Lawrence River Basin, USA and Canada. *Environmental Toxicology and Chemistry* 18, 263–271.
- Bishop, C.A., Ng, P., Norstrom, R.J., Brooks, R.J., Pettit, K.E., 1996. Temporal and geographic variation of organochlorine residues in eggs of the common snapping turtle (*Chelydra serpentina serpentina*) (1981–91) and comparisons to trends in the herring gull (*Larus argentatus*) in the Great Lakes Basin in Ontario, Canada. *Archives of Environmental Contamination and Toxicology* 31, 512–524.
- Bishop, C.A., Ng, P., Pettit, K.E., Kennedy, S.W., Stegeman, J.J., Norstrom, R.J., Brooks, R.J., 1998. Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St Lawrence River basin (1989–1991). *Environmental Pollution* 101, 143–156.
- Blais, J.M., Schindler, D.W., Muir, D.C.G., Kimpe, L.E., Donald, D.B., Rosenberg, B., 1998. Accumulation of persistent organochlorine compounds in mountains of western Canada. *Nature* 395, 585–588.
- Castillo, L.E., de la Cruz, E., Ruepert, C., 1997. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environmental Toxicology and Chemistry* 16, 41–51.
- Cetinkaya, M., von Duzeln, J., Thiemann, W., Silwar, R., 1984. Untersuchung von organochlor-pesticidrückständen in roh- und röstkaffee und deren abbauverhalten beim röstprozeb. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 179, 5–8.
- Custer, C.M., Custer, T.W., Allen, P.D., Stromborg, K.L., Melancon, M.J., 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River drainage and Green Bay, Wisconsin, USA. *Environmental Toxicology and Chemistry* 17, 1786–1798.
- DeWeese, L.R., McEwen, L.C., Hensler, G.L., Petersen, B.E., 1986. Organochlorine contaminants in passeriformes and other avian prey of the peregrine falcon in the western United States. *Environmental Toxicology and Chemistry* 5, 675–693.
- Elliott, J.E., Martin, P.A., Arnold, T.W., Sinclair, P.H., 1994. Organochlorines and reproductive success of birds in orchard and non-orchard areas of central British Columbia, Canada, 1990–1991. *Archives of Environmental Contamination and Toxicology* 26, 435–443.
- Ernst, C.H., 1983. *Rhinoclemmys pulcherrima*. In: Janzen, D.H. (Ed.), *Costa Rican Natural History*. University of Chicago Press, Chicago, pp. 418–419.
- Evans, M., Lampo, M., 1996. Diet of *Bufo marinus* in Venezuela. *Journal of Herpetology* 30, 73–76.
- Fleming, T.H., 1983. *Liomys salvini*. In: Janzen, D.H. (Ed.), *Costa Rican Natural History*. University of Chicago Press, Chicago, pp. 475–477.
- Foster, M.S., McDiarmid, R.W., 1983. *Rhinophrynus dorsalis*. In: Janzen, D.H. (Ed.), *Costa Rican Natural History*. University of Chicago Press, Chicago, pp. 419–421.
- Frick, J.A., Klemens, J.A., Harper, R.G., Capparella, A.P., 1998. Effect of skin removal on estimated levels of organochlorine pesticide contamination in passerine birds. *Bulletin of Environmental Contamination and Toxicology* 61, 658–663.
- Fyfe, R.W., Banasch, U., Benavides, V., De Benavides, N.H., Luscombe, A., Sanchez, J., 1990. Organochlorine residues in potential prey of peregrine falcons, *Falco peregrinus*, in Latin America. *Canadian Field-Naturalist* 104, 285–292.
- Harper, R.G., Frick, J.A., Capparella, A.P., Borup, B., Nowak, M., Biesinger, D., Thompson, C.F., 1996. Organochlorine pesticide contamination in Neotropical migrant passerines. *Archives of Environmental Contamination and Toxicology* 31, 386–390.

- Janzen, D.H., 1986. Guanacaste National Park: tropical ecological and cultural restoration. Editorial Universidad Estatal a Distancia, San Jose, Costa Rica.
- Janzen, D., 1999. Gardenification of tropical conserved wildlands: multitasking, multicropping, and multiusers. *Proceedings of the National Academy of Science* 96, 5987–5994.
- Kamrin, M.A., 1997. Endosulfan. In: Kamrin, M.A. (Ed.), *Pesticide Profiles, Toxicity, Environmental Impact and Fate*. Lewis Publishers, Boca Raton, FL, USA.
- LeNoir, J.S., McConnell, L.L., Fellers, G.M., Cahill, T.M., Seiber, J.N., 1999. Summertime transport of current-use pesticides from California's Central Valley to the Sierra Nevada Mountain Range, USA. *Environmental Toxicology and Chemistry* 18, 2715–2722.
- Murray, D., 1994. *Cultivating Crisis, the Human Cost of Pesticides in Latin America*. University of Texas Press, Austin, TX, USA.
- Norman, D., 1998. *Common Amphibians of Costa Rica*. US Fish and Wildlife Service.
- Pounds, J.A., Crump, M.L., 1994. Amphibian declines and climate disturbances: the case of the golden toad and the harlequin frog. *Conservation Biology* 8, 72–85.
- Rojas, J.L., Ruiz, O., 1989. Residuos quimicos en carne bovina en Costa Rica. *Ciencias Veterinaria* 11, 23–28.
- Rosenshield, M.L., Jofré, M.B., Karasov, W.H., 1999. Effects of polychlorinated biphenyl 126 on green frog (*Rana clamitans*) and leopard frog (*Rana pipiens*) hatching success, development, and metamorphosis. *Environmental Toxicology and Chemistry* 18, 2478–2486.
- Ruiz, O., Rojas, J.L., 1988. Residuos de plaguicidas organoclorados en leche bovina. *Ciencias Veterinaria* 10, 19–23.
- Savage, W.K., Quimby, F.W., DeCaprio, A.P., 2002. Lethal and sub-lethal effects of polychlorinated biphenyls on *Rana sylvatica* tadpoles. *Environmental Toxicology and Chemistry* 21, 68–174.
- Simonich, S.L., Hites, R.A., 1995. Global distribution of persistent organochlorine compounds. *Science* 269, 1851–1854.
- Standley, L.J., Sweeney, B.W., 1995. Organochlorine pesticides in stream mayflies and terrestrial vegetation of undisturbed tropical catchments exposed to long-range atmospheric transport. *Journal of the North American Benthological Society* 14, 38–49.
- Stiles, F.G., Skutch, A.F., 1989. *A Guide to the Birds of Costa Rica*. Comstock Publishing Associates, Ithaca, NY, USA.
- Umana, V., Constenla, M., 1984. Determinacion de plaguicidas organoclorados en leche materna en Costa Rica. *Revista de Biología Tropical* 32, 233–239.
- von Duzeln, J., 1988. Analysis of Pesticides in Costa Rica with Special Emphasis on Water and Fish Samples. Technical report (GTZ Project No. 85.2039.7/Aquaculture). Bremen, FRG.
- von Duzeln, J., 1991. Pesticide contamination and pesticide control in developing countries: Costa Rica, Central America. In: Richardson, M.L. (Ed.), *Chemistry, Agriculture and the Environment*. Royal Society of Chemistry Press, Cambridge, pp. 410–428.
- Willis, E., 1980. Ecological roles of migratory and resident birds on Barro Colorado Island, Panama. In: Keast, A., Morton, E.S. (Eds.), *Migrant Birds in the Neotropics: Ecology, Behavior, Distribution, and Conservation*. Smithsonian Institution Press, Washington, DC, pp. 205–225.
- Zug, G., 1983. *Bufo marinus*. In: Janzen, D.H. (Ed.), *Costa Rican Natural History*. University of Chicago Press, Chicago, pp. 386–387.