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A SURVEY OF ORGANOCHLORINE PESTICIDE CONTAMINATION IN
COSTA RICAN WILDLIFE

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Abstract

Amphibians, turtles, rodents, and birds collected from a tropical conservation area in northwestern Costa Rica where pesticides are not directly applied were analyzed for organochlorine (OC) pesticide contamination. Six of thirty-nine amphibians (three of eight species), three of six turtles (two species), one of eight rodents (one species), and nine of twenty-five insectivorous birds (four species) contained OC compounds ranging from 2.77 ng/g to 277.70 ng/g. The most frequently detected compound (found in thirteen organisms) was p,p'-DDE. Heptachlor, delta-BHC, dieldrin, endosulfan II, and p,p'-DDD were each found in four or more organisms, while eight additional OC compounds were detected in at least one organism. The average body mass of contaminated amphibians was 156.40 g, compared to 56.89 g for uncontaminated amphibians, which suggests that biomagnification of OC compounds may occur in this taxon. The presence of OCs in wildlife from this conservation area indicates that long-distance transport of pesticides through the atmosphere is likely.

Keywords: organochlorine, pesticides, Costa Rica, turtles, amphibians, birds

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. No park exists in complete isolation, however, as interactions among organisms inside a conserved wildland will inevitably be impacted by conditions in the surrounding agricultural, industrial, and residential landscapes (Janzen, 1999). Global factors, such as increases in atmospheric carbon dioxide concentration, increasing ultraviolet radiation, and atmospheric transport of pesticides, will affect wildlands in ways that are dependent upon topography, climate, and the human use patterns of the site and surrounding area, rather than the size of the conserved area or the degree of protection from local threats. Although the impacts of these global scale phenomena have not been quantified, or even documented, in most conservation areas, they are often proffered as explanations for unexpected phenomena, such as the perceived population declines of species or groups of species. For example, Pounds and Crump (1995) quantified population declines in two amphibian species in a Costa Rican conservation area and suggested that pesticide contamination from regional agricultural sites may have been a contributing factor in those declines. However, no evidence was presented that suggested that these organisms had ever been exposed to pesticides.

Organochlorine compounds accumulate in the tissue of many biota (Gonzalez-Barros et al., 1998; Poole et al., 1998; Lunden and Noren, 1998; Harper et al., 1996; Klemens et al., in press; Standley and Sweeney, 1994), and have half-lives of up to 20 years, which results in long residence time in the environment (Cooke and Stringer, 1982). OCs can adversely affect a variety of physiological functions in vertebrates and invertebrates (Ibrahim et al., 1998; Kelce et al., 1995; Berrill et al., 1993; Guana et al.,

1991), but for most organisms, it is unknown to what extent community-level interactions may be impacted 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 structures, there are little data on the extent to which OCs have been and continue to be applied (Murray, 1994). Costa Rica is one of the few countries with reliable information regarding the history of its pesticide use. Application of OC pesticides is estimated to be 6 kg/ha higher than in most industrialized countries (Duszeln, 1991). In 1981, most OC pesticides in Costa Rica were restricted for agricultural purposes only, while DDT could only be used in efforts to eradicate malaria (Castillo et al., 1997). DDT and a series of other persistent and/or hazardous OC pesticides (e.g., aldrin, dieldrin, etc.) were then banned for use in 1988, while chlordane, heptachlor, endosulfan, and a series of other OC compounds were restricted in their use (Duszeln, 1991). Nevertheless, persistent OC pesticides in Costa Rica have been detected in water samples (Duszeln, 1988), crops (Cetinkaya, 1984), insect larvae (Standley and Sweeney, 1995), fish (Rodriguez, 1990), birds (Klemens et al., in review; Fyfe et al., 1990), beef (Rojas and Ruiz, 1989), animal milk (Ruiz and Rojas, 1988), bird eggs (Hidalgo, 1986), human fat (Barquero and Constenla, 1986), and human milk (Umana and Constenla, 1984; Barquero and Thiel, 1986; Lunden and Noren, 1998).

This study presents a survey of OC contamination in amphibians, reptiles, rodents, and birds from a tropical conservation area in northwestern Costa Rica to which pesticides have not been directly applied. Except for birds, no studies have been published on pesticide contamination levels in these taxa from this locality. These

baseline data are crucial to understanding the potential effects of OC chemicals on tropical wildlife population. Because the ecosystem examined in this study is a tropical conservation area, these data will provide important information for management of these land reserves.

2. Methods

2.1. Collection of animals

Thirty-nine amphibians, six reptiles, twenty-five birds, and eight mice were collected in kill traps, netting, or with a shotgun at the Area de Conservacion Guanacaste (ACG) in Costa Rica in June and July of 1998. The animals were frozen on dry ice upon collection and were later transferred to a -80°C freezer.

2.2. Site Description

Located in the extreme northwestern region of the province of Guanacaste, Costa Rica (see Fig. 1), the ACG encompasses 120,000 terrestrial ha and 43,000 marine ha, in which there exist approximately 230,000 species. Animals for this study were collected from the tropical dry forest habitat of the park at Santa Rosa.

2.3. Species Accounts

Information regarding size, habitat preferences and feeding habits of organisms from this study are presented in Table 1.

2.4. Residue analysis

The extraction of pesticides from bird tissues and the chemical analyses using gas chromatography were performed as described in Frick et al. (1998) and Harper et al. (1996). Modifications to this procedure for amphibians and reptiles involved the inclusion of skins in analyses, and the addition of greater amounts of sodium sulfate to the carcasses (approximately 100% of the weight of the carcass). Egg masses were removed from nine of the 39 amphibians and were analyzed separately from the carcasses. The procedure for rodents differed only in that their skins were included in the analysis. 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 and Martin, 1994). Detection limits were 0.01 µg for all pesticides except the following: heptachlor (0.02 µg), aldrin (0.03 µg), endosulfan I (0.03 µg), and endosulfan sulfate (0.10 µg). 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. Levels of OC pesticides in duplicate samples were within five percent of each other.

3. Results

3.1. Amphibians

Organochlorine residues were present in six of thirty-nine individuals (Table 2), representing three of eight species (*B. marinus*, *R. forreri*, and *R. dorsalis*). These were the three largest amphibian species in the study. The average body mass of the six contaminated amphibians was 156.40 g, compared to 56.89 g for the thirty-three uncontaminated amphibians. The relationship between mass and total OC contamination for all individual amphibians is depicted in Fig. 2.

Twelve OC compounds were detected in amphibians. The most frequently detected compounds were p,p'-DDE, delta-BHC, heptachlor, and dieldrin (Table 3). Levels of p,p'-DDE contamination ranged from 3.55 ng/g in a single *B. marinus* to 54.62 ng/g in a single *R. forreri*. Delta-BHC contamination levels ranged from 6.76 ng/g in a single *B. marinus* to 39.87 ng/g in a single *R. forreri*. The levels of heptachlor contamination ranged from 3.79 ng/g in a single *B. marinus* to 32.14 ng/g in a single *R. forreri*. Dieldrin contamination levels ranged from 2.92 ng/g to 4.79 ng/g, both in individual specimens of *B. marinus*. Heptachlor epoxide, beta-BHC, gamma-BHC, endosulfan I, endosulfan II, aldrin, alpha-BHC, and p,p'-DDT were detected in amphibians at levels lower than 10 ng/g.

No OCs were detected in egg masses collected from two *H. variolosus*, two *S. boudinii*, and five *R. forreri*. Their average mass was 4.70 g. One of the egg masses was from a contaminated *R. forreri*, and the other egg masses were collected from uncontaminated individuals.

3.2. Turtles

OCs were detected in two of three *K. leucostomum* and one of three *R. pulcherrima* analyzed (Table 2). The average body mass of all six of these animals (without the shell) was 202.59 g.

Of the ten OC compounds detected in turtles, p,p'-DDE, delta-BHC, and heptachlor were the most frequently detected (Table 3). Levels of p,p'-DDE ranged from 4.36 ng/g in an individual *R. pulcherrima* to 239.74 ng/g in an individual *K. leucostomum*. Delta-BHC contamination levels ranged from 6.10 ng/g in an individual *K. leucostomum* to 11.03 ng/g in an individual *R. pulcherrima*. Heptachlor contamination levels ranged from 6.73 ng/g in an individual *K. leucostomum* to 16.69 ng/g in an individual *R. pulcherrima*. Alpha-BHC, dieldrin, p,p'-DDD, heptachlor epoxide, gamma-BHC, endosulfan I, and aldrin were detected in turtles at levels lower than 10 ng/g.

3.3. Rodents

One of the eight *L. salvinii* was contaminated with endrin at a level of 12.96 ng/g (Table 1). The average body mass of all eight of the *L. salvinii* was 47.29 g.

3.4. Birds

OCs were detected in nine of twenty-five birds (Table 2), representing three of the four species (*N. albicollis*, *M. tyrannulus*, and *V. flavoviridis*). The average body mass of the contaminated birds was 32.0 g, compared to 20.9 g for the uncontaminated birds. The relationship between body mass and OC contamination for all individual birds is depicted in Fig. 2.

Six OC compounds were detected in the birds. The most frequently detected compounds were p,p'-DDE, endosulfan II, and p,p'-DDD. Levels of p,p'-DDE contamination ranged from 3.84 ng/g in one *N. albicollis* individual to 16.41 ng/g in another *N. albicollis* individual. Endosulfan II contamination levels ranged from 2.77 ng/g in an individual *N. albicollis* to 65.76 ng/g in an individual *M. tyrannulus*. Levels of p,p'-DDD ranged from 3.60 ng/g in an individual *N. albicollis* to 94.05 ng/g in an individual *V. flavoviridis*. Dieldrin, aldrin, and heptachlor epoxide were also detected in birds at levels lower than 20 ng/g.

4. Discussion

These findings indicate that OC compounds are present in amphibians, turtles, rodents, and birds from a Costa Rican conservation area where pesticides are not known to have been previously applied. Except for the birds, no studies from this region have documented OC contamination in the species examined in this study. Other studies of turtles and birds have shown OCs to be present in those organisms throughout the world at levels similar to those in this study. OCs have been found in freshwater turtles (Kannan et al., 2000) and sea turtles (Rybitski et al., 1995) at low to middle ng/g levels (10-200 ng/g range). Likewise, Harper et al. (1996) and Klemens et al. (in press) found OCs in neotropical migrant birds at low levels (2-30 ng/g range). In Costa Rica, Standley and Sweeney (1995) found OCs at similar levels in mayfly larvae.

The adverse effects of high levels of OCs on organisms from these taxa have been well documented. Developmentally, OCs have been shown to lead to egg shell thinning in snapping turtles (*Chelydra serpentina serpentina*) (Bishop et al., 1996) and birds

(Faber, 1973; Wiemeyer et al., 1984) at levels ranging from 50-5000 ng/g. In addition to physiological and developmental effects on individuals, OCs have been shown to be potential endocrine disruptors (Dickerson et al., 1999). They have been linked to feminization of bird embryos at 2000 ng/g levels (Fry and Toone, 1981), alteration of reproductive development in rodents (Gray et al., 1989), and abnormalities in turtle hatchlings (Bishop et al., 1998; de Solla et al., 1998). Most of these abnormalities have been linked to relatively high levels of OC contamination (high $\mu\text{g/g}$ concentrations). Organisms in this study were contaminated at low to high ng/g concentrations of OCs. It is difficult to assess the impact of OC pollutants at these levels. Few toxicological studies have been published regarding OCs at these levels. However, studies exist linking low $\mu\text{g/g}$ and high ng/g concentrations of OCs to reproductive and hormonal dysfunction and immune system failure in barn owls (*Tyto alba*) and marsh turtles (*Mauremys caspica*) (Goldman and Yawetz, 1991), catfish (*Heteropneustis fossilis*) (Saxena et al., 1992), and snapping turtle eggs (Bishop et al., 1996; Bishop et al., 1991). Furthermore, interactive effects of more than one OC may lead to abnormalities at low levels of contamination. In a recent study, Willingham and Crews (1999) suggested that the exposure of eggs of the red-eared slider turtle (*Trachemys scripta*) to OC pesticides and PCBs produced significant sex reversal compared to eggs that were incubated in the presence of a single OC compound.

While some members of all of the taxa examined were contaminated with OCs, birds, amphibians, and turtles exhibited more frequent contamination and higher levels of contamination than did rodents. Birds, unlike the other taxa examined, may visit more contaminated regions outside of the conservation area due to their mobility and

potentially broader range. Turtles and amphibians may be exposed to chemicals due to the long periods of time they may be in contact with contaminated water. However, it is interesting to note that none of the amphibian egg masses had detectable levels of OCs. This may be due to their small masses ($x = 4.70$ g), which may have rendered egg masses too small for detection of OCs, even though one of the egg masses was from a contaminated female. Conversely, rodents occupy entirely terrestrial habitats, and therefore may receive less exposure to OCs than the other taxa. In addition, the primarily granivorous diet of the *L. salvinii*, the only mammal examined in this study, may reduce the risks of OC contamination. Furthermore, larger terrestrial mammals outside of Costa Rica have been shown to accumulate OCs in their tissues (Gonzalez-Barros et al., 1998; Poole et al., 1998), suggesting that body mass may be the reason for the discrepancy among taxa.

In this study, amphibians and birds were the only taxa in which body masses were highly variable. Within the amphibians, the average body mass of contaminated animals (156.40 g) was far greater than that of uncontaminated animals (56.89 g). Likewise, the average body mass of contaminated birds (34.76g) was far greater than that of uncontaminated birds (20.35g). Turtles, which were contaminated at a high frequency (50%), had average body masses (202.59 g) comparable to those of the contaminated amphibians. Furthermore, the rodents in this study, which had a relatively low frequency of contamination (1 of 8 animals), had average body masses that were similar to the uncontaminated amphibians (47.29 g). This suggests that higher contamination levels may be associated with larger body mass. There is also the potential for magnification of OC compounds in predators that feed on organisms in these taxa. That is, animals from

higher trophic levels are more likely to be contaminated than animals from lower trophic levels. However, because of the relatively small sample sizes in this study, a definite relationship between trophic level (best represented by body mass) and OC contamination cannot be reached. Furthermore, small animals exposed to large quantities of chemicals will likely show higher levels of contamination than larger animals exposed to smaller quantities of these compounds. Nevertheless, these results suggest that larger organisms did, on average, concentrate pesticides to a greater degree than smaller organisms.

There are two possible explanations for the presence of OC compounds in organisms from the ACG, where pesticides are not known to have been previously applied. Klemens et al. (in review) suggested that there may be residual OC contamination on the western side of the ACG. Another explanation is the long-distance transport of pesticides through the atmosphere from surrounding agricultural areas and other external sources. This phenomenon has been documented by numerous studies (Bevenue et al., 1972; Bidleman et al., 1975; Rapaport et al., 1985; Kurtz, 1990). Furthermore, these compounds have been detected in remote regions such as the Antarctic and Arctic, where pesticides have never been applied (Gregor and Gummer, 1989; Gregor, 1990). Standley and Sweeney (1995) hypothesized that their findings of OC contamination in mayfly larvae and vegetation from mountain catchments in the ACG was due to atmospheric transport and deposition of pesticides from adjacent agricultural areas. Klemens et al. (in review) documented OC contamination in insectivorous birds from the ACG. They examined birds from three different sites in the ACG, and found that birds collected in the Santa Rosa region (the collection site for this

study) were the least contaminated. They suggested that this was due to decreased OC deposition on the lee side of the Cordillera de Guanacaste mountain range (Fig. 1). Therefore, the dry forest habitat of Santa Rosa was less susceptible to OC deposition from eastern agricultural areas than the eastern, wetter regions. However, OC deposition in Santa Rosa may possibly be explained by multi-directional winds that occur during the rainy season (May-December). These winds may carry with them OCs from surrounding agricultural areas and from other areas in Central America.

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Figures and tables

Figure 1. Map of the ACG

Figure 2. Levels of OC contamination in relation to body mass in amphibians and birds

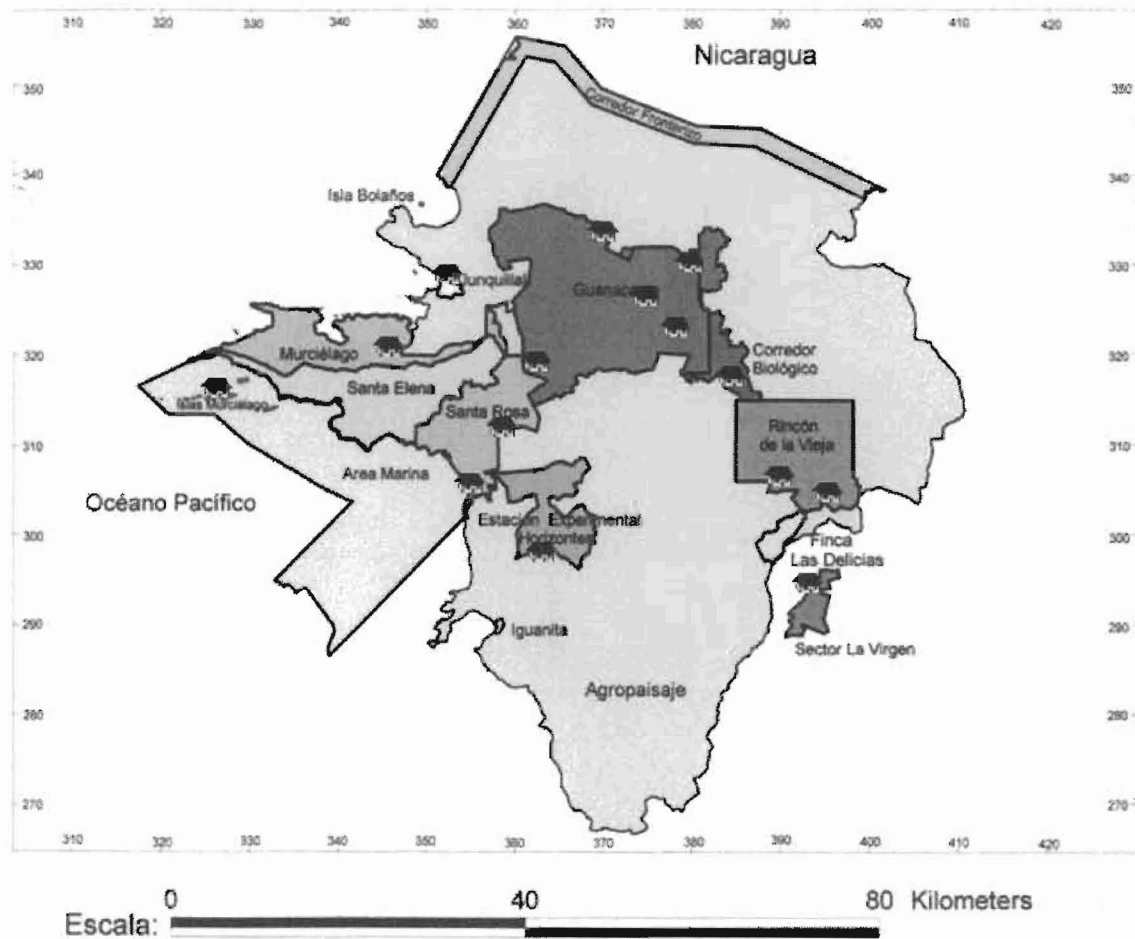
Table 1. Length, habitat preferences and feeding habits of species surveyed for Ocs

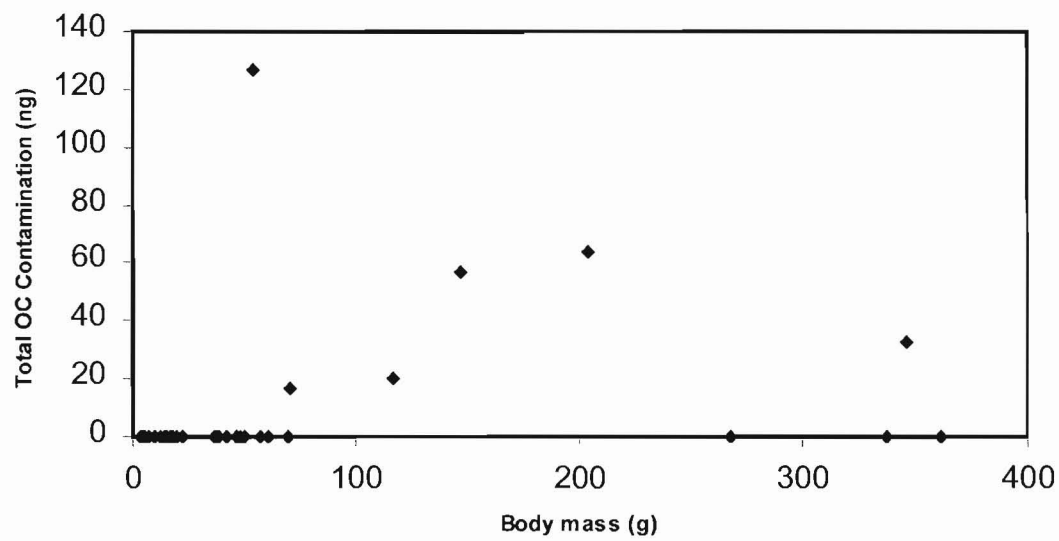
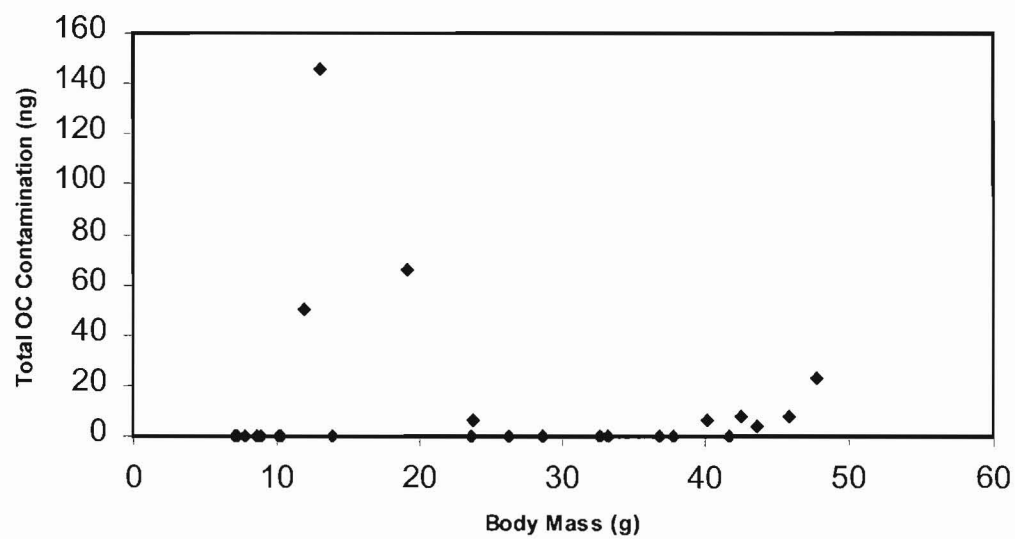
Table 2. Frequency of OC contamination of organisms surveyed

Table 3. Most frequently detected OC compounds in Costa Rican amphibians and turtles

Table 4. Most frequently detected OC compounds in Costa Rican birds

Area de Conservación Guanacaste



a. Amphibians**b. Birds**

Species	Length (mm) ^a	Habitat	Feeding Habits
Amphibians			
<i>Bufo marinus</i> (Marine Toad)	90-200	Savannah to open forest, now commensal with humans (Zug, 1983)	Active forager, feeds on amphibians, many insects, primarily ants and beetles (Zug, 1983; Evans and Lampo, 1996)
<i>Rhinophrynus dorsalis</i> (Mexican Burrowing Toad)	60-65	Fossorial	Ant and termite specialist, also feeds on other insects (Foster and McDiarmid, 1983)
<i>Rana forreri</i> (Leopard Frog)	120	Along rivers, permanent ponds and temporary pools (Norman, 1998)	Active forager day and night, probably feeds on juvenile fish and amphibians (Brooks and Klemens, pers. ob.)
<i>Phrynohyus venulosa</i> (Marbled Rubber Frog)	70-80	Arboreal	Opportunistic insectivore (Brooks and Klemens, pers. ob.)
<i>Smilisca baudinii</i> (Baudin's Smilisca)	65-75	Arboreal	Opportunistic insectivore (Brooks and Klemens, pers. ob.)
<i>Hypopachus variolosus</i> (Sheep Frog)	50	Fossorial, particularly in ant and Termite mounds	Ant and termite specialist, also feeds on other insects (Brooks and Klemens, pers. ob.)

<i>Scinax boulengeri</i> (Boulenger's Treefrog)	50	Arboreal	Opportunistic insectivore (Brooks and Klemens, pers. ob.)
<i>Leptodactylus melanonotus</i> (Black-backed Frog)	35-45	Leaf litter and other vegetation near water (Norman 1998)	Active forager, probably an arthropod generalist (Brooks and Klemens, pers. ob.)
Reptiles			
<i>Kinosternon leucostomum</i> (Yellow Turtle)	200	Semi-aquatic, found in swamps, slow Streams, and temporary ponds.	Opportunistic omnivore (Vogt and Guzman, 1988). Feeds heavily on aquatic snails (Scott and Limerick, 1983)
<i>Rhinoclemmys pulcherrima</i> (Red Turtle)	230	Common in cleared areas close to streams and in gallery forests	Feeds on a variety of meat and plants, with a marked preference for plants (Ernst, 1983)
Mammals			
<i>Liomys salvinii</i> (Spiny Pocket Mouse)		Forest floor in mature and successional tropical dry forest	Primarily seed predators, but also consume pupae of Lepidopterans and other insects (Fleming, 1983)

Table 1. (continued)

Species	Length (mm) ^a	Habitat	Feeding Habits
Birds			
<i>Nyctidromus albicollis</i> (Common Pauraque)	280	Roads, pastures, and riverbanks	Sits on open ground, captures flying insects, mostly moths and beetles (Stiles and Skutch, 1986; Willis, 1980; Klemens, pers. ob.)
<i>Myiarchus tyrannulus</i> (Brown-crested Flycatcher)	190	Large clearings or open areas in forest, along roads and trails	Forages from middle understory to low canopy and feeds on insects, small fruits and arillate seeds (Stiles and Skutch, 1986; Willis, 1980; Klemens, pers. ob.)
<i>Vireo flavoviridis</i> (Yellow-green Vireo)	145	Forest canopy	Forages throughout the canopy and feeds on insects, spiders, small fruits, and arillate seeds (Stiles and Skutch 1986; Willis, 1980; Klemens, pers. ob.)

<i>Basileuterus rufifrons</i> (Rufous-capped Warbler)	130	Forest edge and interior	Forages on ground level and throughout the understory, feeds on insects, spiders, larvae, and small fruits (Stiles and Skutch, 1986; Willis, 1980; Klemens pers. ob.)
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^aApproximate average adult length (literature value)

Taxa	Species	Average	Number	Number
		Mass (g) ^a	Contaminated ^b	Uncontaminated
Amphibians	<i>Bufo marinus</i>	254.58	4	3
	<i>Rana forerri</i>	54.31	1	6
	<i>Rhinophrynus dorsalis</i>	52.92	1	2
	<i>Phrynohyus venulosa</i>	37.36	0	5
	<i>Srinax bulengeri</i>	15.41	0	5
	<i>Smilisca boudinii</i>	14.61	0	5
	<i>Leptodactylus melanonotus</i>	12.36	0	1
	<i>Hypopachus variolosus</i>	5.29	<u>0</u>	<u>6</u>
Subtotal			6	33
Turtles	<i>Kinosternon leucostomum</i>	244.19 ^c	2	1
	<i>Rhinoclemmys pulcherrima</i>	200.43 ^c	<u>1</u>	<u>2</u>
Subtotal			3	3

Rodent	<i>Liomys salvinii</i>	27.87	1	7
Birds	<i>N. albicollis</i>	40.19	5	5
(from Klemens et al.)	<i>Myiarchus tyrannulus</i>	24.31	2	3
	<i>Vireo flavoviridis</i>	11.86	2	3
	<i>Basileuterus rufifrons</i>	7.89	<u>0</u>	<u>5</u>
Subtotal			9	16
Totals			19	59

^a Average mass of all animals collected.

^b Number contaminated with any OC compound.

^c Without shell

Taxa	Species	p,p'- DDE			Delta-BHC		Heptachlor		Dieldrin	
		Average	Prop.	Level ^c	Prop.	Level ^c	Prop.	Level ^c	Prop.	Level ^c
		Mass (g) ^a	Cont. ^b	(ng/g)	Cont. ^b	(ng/g)	Cont. ^b	(ng/g)	Cont. ^b	(ng/g)
Amphibians	<i>B. marinus</i>	203.67	4/7	12.8 ± 0.06	3/7	8.20 ± 0.04	3/7	6.40 ± 0.004	3/7	3.70 ± 0.008
	<i>R. forreri</i>	53.26	1/7	54.62	1/7	39.87	1/7	32.14	0/7	ND
	<i>R. dorsalis</i>	70.47	1/3	16.85	0/3	ND	0/3	ND	0/3	ND
Turtles	<i>K. leucostomum</i>	238.34	2/3	124.9 ± 18.5	2/3	7.60 ± 0.003	1/3	6.73	0/3	ND
	<i>R. pulcherrima</i>	175.65	1/3	4.36	1/3	11.03	1/3	16.69	1/3	4.64

^a average mass of contaminated animals (without the shells in turtles)

^b proportion contaminated

^c mean ± Standard Error

Species	p,p'-DDE			p,p'-DDD		Endosulfan II	
	Average	Prop.	Level ^c	Prop.	Level ^c	Prop.	Level ^c
	Mass (g) ^a	Cont. ^b	(ng/g)	Cont. ^b	(ng/g)	Cont. ^b	(ng/g)
<i>N. albicollis</i>	27.40	4/10	9.15 ± 2.29	1/10	3.60	1/10	2.77
<i>M. tyrannulosa</i>	18.40	1/5	6.75	0/5	ND ^d	1/5	65.76
<i>V. flavovoria</i>	12.44	1/5	13.18	1/5	94.05	1/5	51.45
<i>B. rufifrons</i>	10.49	0/5	ND ^d	0/5	ND ^d	0/5	ND ^d

^aaverage mass of contaminated animals

^bproportion contaminated

^cmean ± Standard Error

^dnot detected