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Organochlorine pesticides in stream mayflies and terrestrial vegetation of undisturbed tropical catchments exposed to long-range atmospheric transport

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Abstract. We investigated whether biota and sediments in river catchments consisting of primary forest in northwestern Costa Rica are contaminated by atmospheric transport of organochlorine pesticides from nearby regions where their use is intense. We measured organochlorine residues in stream mayflies, as well as in the bark and leaves of trees in catchments of an undisturbed dry tropical forest west of Volcán Orosí and Cerro Cacao (i.e., western catchments), and in tree leaves and bark in partially disturbed rain forest catchments east of Cerro Orosí (i.e., eastern catchments) in a region where agricultural activity is intense. Samples were solvent extracted, purified by silica gel clean-up, and concentrated before analysis by gas chromatography/mass spectrometry. Twelve organochlorine pesticides were detected in samples, including hexachlorocyclohexanes, heptachlor epoxide, endosulfans, DDT, DDE, dieldrin, endrin, endrin aldehyde, and aldrin. Endosulfans dominated the organochlorine signature of mayflies collected in the western catchments and of leaves and bark collected from eastern catchments. Leaves and bark collected in the undisturbed western catchments contained ten-fold lower concentrations of the endosulfans than those collected from the eastern catchments. Hexachlorocyclohexane isomers were dominated by the gamma isomer and were present at comparable levels in samples of leaves and bark collected from both sides of the volcanic ridge. An exception was high residues in bark collected from one eastern catchment, suggesting a local source. Stream mayflies and terrestrial vegetation in the undisturbed dry tropical forest contained substantial quantities of organochlorine pesticides, the most likely source being long- and short-range atmospheric transport.

Key words: mayfly, macroinvertebrate, atmospheric transport, indicator organism, organochlorine pesticides, catchment, leaves, bark, endosulfan.

Few regions on earth are physically undisturbed by the activities of humans. Even fewer regions, and possibly none, are free from contamination by airborne chemicals of anthropogenic origin. Organic and inorganic contaminants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides, and heavy metals, have been measured in regions as remote as the Arctic and Antarctic (Kawano et al. 1984, 1986, Addison et al. 1986, Muir et al. 1988, Calamari et al. 1991) and have been documented in biota, water, and sediments throughout the world (e.g., Kawano et al. 1988, Calamari et al. 1991). Organochlorine pesticides, in particular, have atmospheric residence times on the order of weeks to months, allowing them to be transported great distances within a hemisphere but not appreciably across the atmospheric boundary between the two hemispheres. Long-range atmospheric transport of organochlorine pesticides is of special concern because of the well-documented impairment of reproductive and neurological systems in aquatic organisms and other wildlife (Hileman 1993, 1994). Recent evidence also links organochlorine compounds to human health effects, such as reduced fertility, fetotoxicity, and various cancers (Fein et al. 1984, Carlsen et al. 1992, Sharpe and Skakkebaek 1993).

The use of organochlorine pesticides has recently decreased in developed countries, with a corresponding reduction of some organochlorine residues in biomass (Moilanen et al. 1982, Addison et al. 1986). However, organochlorine use has actually increased in developing countries, especially those in the tropics (e.g., Rapaport et al. 1985, Standley and Hites 1991, Bidleman et al. 1992, PAHO 1992). For example, Canadian raptors have higher body burdens of organochlorine pesticides following their migration south to overwinter in South and Central America (Fyfe et al. 1991, Elliot and Shutt 1993), reflecting relatively high organochlorine pesticide use (including DDT) in countries in Central America, the Caribbean islands, the northern coast of South America, and Mexico.

Furthermore, Mexico imported almost 1000 tons of DDT in 1992 and Costa Rica imported approximately 11 tons of endosulfan in 1992 for use on coffee, cotton, sugarcane, and vegetables (Instituto Nacional de Biodiversidad, Costa Rica, personal communication).

Atmospheric transport of contaminants, such as pesticides from regions of intense agricultural activity, results in deposition to otherwise undisturbed river catchments. Impact on stream biota will depend on several factors: (a) proximity to the source region; (b) regional topography controlling the extent of atmospheric contaminant transport (i.e., barriers such as mountain ranges); and (c) the tendency for contaminants to be mobilized through the river catchment via surface runoff or groundwater transport.

In this study, we assessed the extent of organochlorine pesticide contamination in biota and sediments collected from forested catchments in northern Costa Rica where pesticides have not been used. Two key factors control long-range atmospheric transport of pesticides and other contaminants to this region. First, wind direction and intensity vary seasonally and are affected by the location of the Intertropical Convergence Zone (ITCZ), which defines the boundary between northern and southern hemispheric zones of atmospheric circulation. During the rainy season (approximately June through November), the ITCZ is directly over Costa Rica; thus, winds are mild and not unidirectional (i.e., the "doldrums", although storms can and do move from the east to the west during this period). However, during the dry season, the ITCZ shifts southward, and wind patterns over the region are dominated by strong northeasterlies.

The second factor is the physical barrier presented by the volcanic ridge dividing eastern and western Costa Rica. The ridge impedes atmospheric transport of pesticides from the eastern region to the west. As an air mass rises to pass the volcanic barrier, it cools, resulting in the condensation of moisture and thus rainfall. When the ITCZ shifts southward, substantially more rain falls on the eastern slopes of the volcanic ridge than on the western slopes. The strong northeasterly winds associated with the ITCZ shift originate from the Carribean, Nicaragua, and regions of northwestern Costa Rica located east of the volcanic ridge. Contaminants adsorbed on particles and dissolved in rain droplets are washed out of the atmosphere during rainfall onto eastern catchments.

Our study focused largely on contaminant levels in the tissues of stream mayflies and deciduous trees of the region. Mayflies are especially useful as indicators of aquatic contamination (Kovats and Ciborowski 1989, Cain et al. 1992) because larvae bioconcentrate the more lipophilic compounds, such as organochlorine pesticides, which often are too dilute to be detected in stream water. Thus, contaminants in larval mayfly tissues represent a fingerprint of material deposited in and transferred through the catchment.

This organochlorine fingerprint in mayflies, however, will not reflect total deposition to the catchment because the contaminants have been filtered through the catchment (in contrast to surface dwelling marine organisms, for example, that are exposed directly). Instead, the fingerprint will reflect processes occurring throughout the catchment interface, which include: physico-chemical properties of the pesticides, such as water solubility, Henry's Law constant, and the octanol-water partitioning coefficient (Kow, a measure of the lipophilicity); loss mechanisms such as microbial decomposition; and processing by aquatic organisms, such as bioaccumulation (direct uptake from food and water), metabolism, and biomagnification (enhanced uptake of contaminants over concentrations in prey) through ingestion of contaminated water and food supplies.

In contrast to mayfly tissues, tree tissue uptake is more straightforward, and previous studies have shown these tissues to be useful indicators of atmospheric deposition to a region (Calamari et al. 1991, Hermanson and Hites 1990, Simonich and Hites 1994a, 1994b). Uptake by surficial plant tissues such as leaves and bark is controlled by: physico-chemical properties of the pesticides (as above) (e.g., Hermanson and Hites 1990, Calamari et al. 1991, Paterson et al. 1991, Simonich and Hites 1994a); the distribution of pesticides in the atmosphere between gas and particle phases (Simonich and Hites 1994a); and wax (lipid) content of the plant tissues (Paterson et al. 1991, Simonich and Hites 1994a).

In this study, pesticide residues were measured in terrestrial vegetation and mayflies (note—also several sediment samples from one

western river) from catchments containing virgin (primary) tropical dry forest and, for comparative purposes, in plant tissues from eastern catchments near intensely cultivated regions. All catchments west of the mountain range were sparsely populated and unperturbed by direct agricultural activities. To our knowledge, organochlorine pesticides are not used within these catchments and never have been. Thus, we hypothesize that the presence of pesticide residues in samples from these catchments has to represent atmospheric transport from other regions.

Methods

Collection of samples

Samples were collected from streams and riparian vegetation in catchments on the eastern and western slopes of Volcán Orosí and Cerro Cacao, which are in the Parque Nationale de Guanacaste in northern Costa Rica (Fig. 1). A volcanic ridge separates the primary forest on the western slopes from the forest near intensely cultivated regions on the eastern slopes. Samples from the western slopes included larvae of the aquatic mayfly *Euthyplocia hecuba* (Ephemeroptera:Polymitarcyidae), stream sediments, and leaves and bark from deciduous trees. Mayfly larvae were collected from eight streams: Río Tempisquito, R. Tempisquito Sur, Quebrada Marilin, Q. el Jobo, Q. Zompopa, Q. Kathia, Q. Florcita, and R. San Josecito. Plant and stream-sediment samples were collected only from R. Tempisquito and its catchment. Samples from the eastern slope catchments R. Orosí and Q. Senderohi (represented as site 9, Fig. 1) included only plants.

Vegetation samples were collected using solvent-rinsed metal forceps and spatulas and wrapped in clean foil before being stored in plastic bags. Plant species were not identified; however, leaves and bark were sampled from the same tree. Mayflies were collected by removing larvae from the bottom surface of cobbles excavated from the stream (see Sweeney et al. 1995 for details). Mayfly larvae were stored in organically cleaned (muffled at 500°C for 4 h) vials. All samples were kept cool or frozen before transport to the Stroud Center and then kept frozen until extraction and analysis, which occurred within a year of collection. Stream sediments were collected by scooping the top few cm. Sediments were stored cool before transport to the Stroud Center and frozen until extraction, which occurred within a year of collection.

Extraction of samples

Mayfly larvae were weighed wet and were ground using a tissue homogenizer with a 1:1 mixture of methylene chloride : methanol, a solvent mixture appropriate for extracting contaminants from fatty tissues. Homogenized tissue was then sonicated (sonic bath) with two aliquots of 1:1 methylene chloride : methanol for 10 min per aliquot. Wet weights of samples containing either individuals or composites ranged from 0.004 to 2.1 g.

Plant leaves and bark were freeze-dried and ground using a mill (#20 mesh). Sediment samples were also freeze-dried before extraction. Dry mass ranges were: leaves, 0.4–1.6 g; bark, 0.8–3.0 g; and sediments, 1.8–3.3 g. Plant and sediment samples were weighed and then extracted for 24 h with 1:1 hexane : acetone using a Soxhlet apparatus. All extracts were filtered and exchanged to hexane by rotary evaporation. Extract mass (i.e., lipid content) was determined by dry weighing a small portion of the hexane-dissolved extract on a small pre-weighed aluminum foil pan. Pesticide components were separated from lipids and other chemical interferences using microcolumns of 1 g of 1% (w/w) water-deactivated silica gel for small samples (e.g., extracts of mayflies) and 10-g columns for larger samples (e.g., extracts of vegetation and sediments). Organochlorine pesticides were eluted from the column using an aliquot of 50% methylene chloride in hexane. More polar waxes and extractable insect tissues remained on the column, reducing our chemical interferences. Extracts were concentrated to ~1 mL using rotary evaporation and to 20–100 μL using a stream of dry nitrogen.

Mass spectral analysis

Concentrated extracts (20–100 μL total volume) were analyzed by gas chromatography/mass spectrometry for the following pesticides: α- and γ-hexachlorocyclohexane (abbreviated aHCH and gHCH, respectively), heptachlor (HEP), α- and β-endosulfan (aESN and bESN, respectively), 1,1-bis(4-chlorophenyl)-2,2,2-

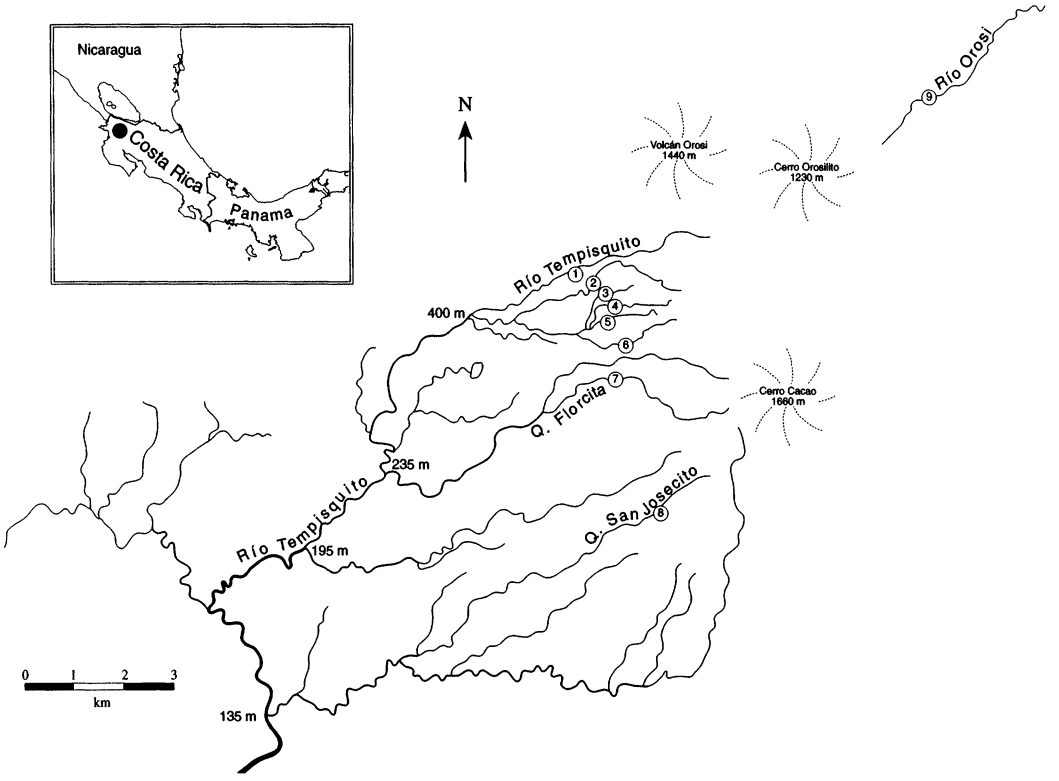

FIG. 1. Location of sampling sites, with approximate elevations, in Parque Nationale de Guanacaste (inset is regional map showing sampling region as black dot). Western river catchments included the following sites: R. Tempisquito (site 1), R. Tempisquito Sur (site 2), Q. Marilin (site 3), Q. el Jobo (site 4), Q. Zompopa (site 5), Q. Kathia (site 6), Q. Florcita (site 7), and R. San Josecito (site 8). Eastern catchments included R. Orosí (site 9) and the nearby Q. Senderohi (which is not shown because we could not find the exact location on regional maps).

trichloroethane (DDT), dieldrin (DLD), aldrin (ALD), endrin (END), and the metabolites or oxidation products heptachlor epoxide (HPX), 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (DDD), 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (DDE), endrin aldehyde (EA), and endosulfan sulfate (EST). Small volumes (1 μL) of extracts were co-injected with octafluoronaphthalene as an internal standard onto a 0.25-μm coating thickness, 0.25-mm i.d., 30-m DB-5 (J & W Scientific) fused-silica capillary column and separated using a temperature program of 60°C (held 2 min), increased to 200°C at 20°C/min, increased to 230°C at 2°C/min, increased to 280°C at 20°C/min, and held at 280°C for 3 min. Components were identified and quantified using a Hewlett Packard 5988, RTE-A, quadrupole mass spectrometer, with selected ion monitoring (SIM) and electron capture negative ionization (ECNI, 0.45 torr CH_4) to improve sensitivity and selectivity. Several standards were processed before each suite of samples, with a check standard following each set of ten samples. The instrumental detection limits were as follows: 1 pg for aESN, bESN, EST, and EA; 5 pg for aHCH, gHCH, HPX, DDT, DDE, ALD; 10 pg for HEP, DLD, and END; and 50 pg for DDD.

Data analysis

Means and medians were calculated for replicates by substituting half-detection-limit values for zeros when some (but not all) values were below the detection limits. For results where all replicates of a given sample type were below detection limits, only the range of detection limits was presented in Table 1; how-

TABLE 1. Mean ($\bar{x}$), standard error (SE), and median (M) of organochlorine pesticide levels (ng/g) in mayfly, leaf, and bark lipids collected from northern Costa Rica. Parenthetical data ( ) show the range of instrument detection limits for those samples where concentrations were below detection limits.

Catchment	n		aHCH	gHCH	HPX	aESN
Mayflies						
Western river[a]	24	$\bar{x}$, SE	<(6-250)	<(6-250)	37, 9	51, 24
		M	n.c.	n.c.	20	5
Leaves						
R. Tempisquito	4	$\bar{x}$, SE	19, 8	37, 20	60, 44	17, 10
		M	19	23	21	8
R. Orosi	4	$\bar{x}$, SE	<(5-51)	51, 37	<(5-51)	65, 47
		M	n.c.	21	n.c.	27
R. Senderohi	6	$\bar{x}$, SE	<(8-17)	17, 11	<(8-17)	31, 22
		M	n.c.	6	n.c.	7
Bark						
R. Tempisquito	5	$\bar{x}$, SE	<(2-43)	75, 66	<(2-43)	9, 6
		M	n.c.	11	n.c.	3
R. Senderohi	3	$\bar{x}$, SE	<(26-53)	590, 85	<(26-53)	140, 120
		M	n.c.	670	n.c.	31

[a] Includes larvae collected from R. Tempisquito, R. Tempisquito Sur, Q. Marilin, Q. el Jobo, Q. Zompopa, Q. Kathia, Q. Florcita, and R. San Josecito. Abbreviations: n = number of samples; $\bar{x}$ = mean; M = median; SE = standard error; n.c. = not calculated for sets of data entirely below the detection limit; aHCH = α-hexachlorocyclohexane; gHCH = γ-hexachlorocyclohexane; HPX = heptachlor epoxide; aESN = α-endosulfan; bESN = β-endosulfan; EST = endosulfan sulfate; DDE = 1,1-dichloro-2,2-bis-(4-chlorophenyl) ethylene; DDT = 1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane; ALD = aldrin; DLD = dieldrin; END = endrin; EA = endrin aldehyde. Means and medians were calculated with one half detection limits for values below detection.

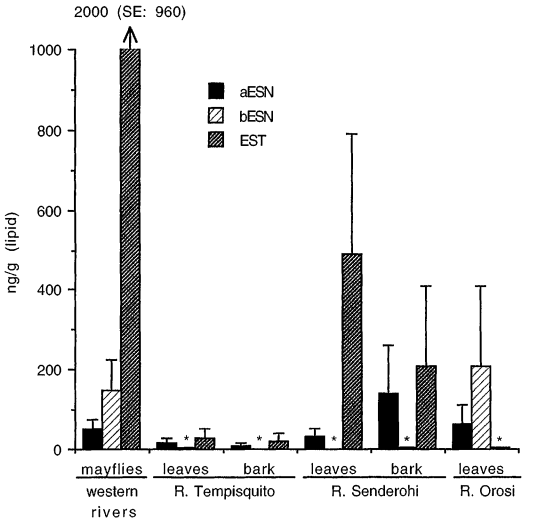

FIG. 2. Concentration (ng/g, lipid) and standard error of endosulfan pesticide (as α-endosulfan [aESN] and β-endosulfan [bESN]) and the metabolite endosulfan sulfate (EST) in samples of mayfly larvae (data composited for insects from eight western streams) and tree bark and leaves collected from catchments associated with the Volcán Orosí and Cerro Cacao on either the western, dry forest slopes (Río Tempisquito) or on the eastern, rain forest slopes (Río Senderohi and Río Orosí). Asterisks denote data below detection and were calculated as means of one half the detection limit.

ever, the mean of half-detection limits is shown in Figures 2 and 3 for replicates to facilitate comparisons with other data (denoted by asterisks). Recovery of individual compounds was not determined.

Organochlorine pesticide residues were normalized to lipid content (i.e., ng of pesticide per g of lipid) for mayfly and plant tissues to facilitate comparison between samples of varying lipid content. The correlation between lipid content and bioaccumulation of nonpolar contaminants such as organochlorine pesticides has been well documented for aquatic fauna (e.g., Chiou 1985, Bierman 1990, Viganò et al. 1992, Larsson et al. 1993). For plant samples, it is preferable to normalize pesticide residues to lipid content rather than to bulk organic matter content because leaves contain >90% of their dry

TABLE 1. Extended.

bESN	EST	DDE	DDT	ALD	DLD	END	EA
150, 75	2000, 960	67, 16	<(6-250)	54, 21	100, 25	<(12-500)	150, 90
7	9	60	n.c.	20	48	n.c.	4
<(2-13)	28, 23	<(12-66)	<(12-66)	<(12-66)	89, 58	54, 25	50, 46
n.c.	6	n.c.	n.c.	n.c.	41	41	4
210, 200	<(1-10)	<(5-51)	<(5-51)	<(5-51)	<(10-100)	<(10-100)	<(1-10)
3	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
<(2-3)	490, 300	51, 38	<(8-17)	<(8-17)	<(15-34)	46, 33	<(2-3)
n.c.	36	7	n.c.	n.c.	n.c.	13	n.c.
<(0.3-9)	22, 20	<(2-43)	<(2-43)	<(2-43)	<(3-86)	61, 50	<(0.3-9)
n.c.	2	n.c.	n.c.	n.c.	n.c.	17	n.c.
<(5-11)	210, 200	<(26-53)	11,000, 10,000	<(26-53)	<(52-110)	<(52-110)	<(5-11)
n.c.	6	n.c.	2100	n.c.	n.c.	n.c.	n.c.

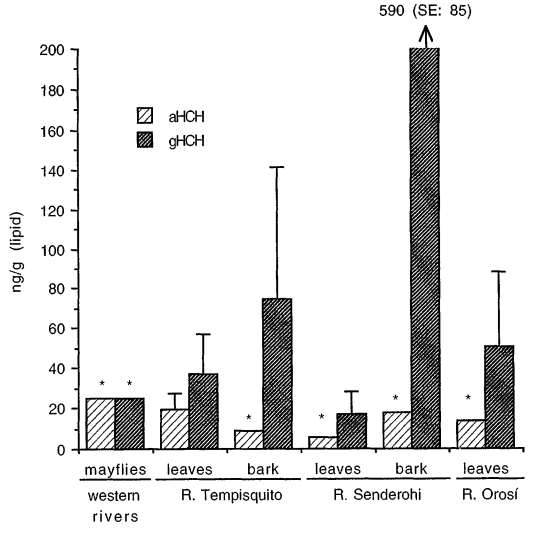

FIG. 3. Concentration (ng/g, lipid) and standard error of α- and γ-hexachlorocyclohexane (aHCH, gHCH) in samples of mayfly larvae (data composited for insects from eight western streams) and tree bark and leaves collected from catchments associated with the Volcán Orosí and Cerro Cacao on either the western, dry forest slopes (Río Tempisquito) or on the eastern, rain forest slopes (Río Senderohi and Río Orosí). Asterisks denote data below detection and were calculated as means of one half the detection limit.

weight as organic matter (6-8% ash; T. L. Bott, Stroud Center, personal communication), with components ranging widely in polarity and thus sorptive capacity. The lipid content of leaves, which is a measure of nonpolar organic components such as waxes and resins, is more predictive of sorption than total organic matter, which includes more polar, less sorptive, components such as lignocellulosic structural material. Furthermore, sorption of atmospheric contaminants occurs on the external waxy surfaces of plant tissues. Researchers therefore normalize residue levels to lipid content in order to compare sorption of atmospheric contaminants among a range of terrestrial plant species (Paterson et al. 1991, Simonich and Hites 1994a). We also present means of organochlorine content of mayfly and plant samples on a dry or wet weight basis, respectively, to facilitate comparisons with other research (Table 2).

TABLE 2. Organochlorines in global biota (ng/g).

Location	ng/g	aHCH	gHCH	HPX	aESN	bESN
Flora						
Costa Rica[1a]	d.w.	0.7	2.6	2.3	0.8	n.d.
Guatemala[2]	d.w.	0.5	0.3	n.a.	n.a.	n.a.
Amazonas[2]	d.w.	41	0.2	n.a.	n.a.	n.a.
Italy[2]	d.w.	27	10	n.a.	n.a.	n.a.
New Delhi[2]	d.w.	110	14	n.a.	n.a.	n.a.
Scandinavia[2]	d.w.	8.6	3.7	n.a.	n.a.	n.a.
Fauna						
Costa Rica[1b]	w.w.	n.d.	n.d.	1.5	1.8	5.0
Costa Rica[1b]	lipid	n.d.	n.d.	37	51	150
Costa Rica[3a]	w.w.	n.d.	n.d.	n.a.	n.a.	n.a.
Costa Rica[3b]	w.w.	n.d.–10	n.d.–380	n.a.	n.a.	n.a.
Mexico[4]	d.w.	0.3–2.6	1.5–2.7	2.2–3.2	n.d.–1.2	8.8–18
N.W. Atlantic[5]	lipid	7–46	1–8	n.a.	n.a.	n.a.
Argentina[6]	lipid	n.a.	600	700	n.a.	n.a.

Abbreviations: n.d. = not detected; n.a. = not analyzed; d.w. = dry weight; w.w. = wet weight; aHCH = α-hexachlorocyclohexane; gHCH = γ-hexachlorocyclohexane; HPX = heptachlor epoxide; aESN = α-endosulfan; bESN = β-endosulfan; EST = endosulfan sulfate; DDE = 1,1-dichloro-2,2-bis-(4-chlorophenyl) ethylene; DDT = 1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane; ALD = aldrin; DLD = dieldrin; END = endrin; EA = endrin aldehyde. [1] This study ([a] R. Tempisquito leaves and bark; [b] *E. hecuba* from eight western streams); [2] Calamari et al. 1991 (mango leaves, lichens, mosses); [3] Fyfe et al. 1990 ([a] shrimp, [b] birds); [4] Botello et al. 1994 (oysters); [5] Krämer et al. 1984 (surface fish); [6] Colombo et al. 1990 (clams, fish).

Organochlorine content of mayflies collected from each the eight streams on western slopes of the volcanic ridge could not be distinguished statistically (*t*-test, $p < 0.05$), in part because distributions of contaminants were similar and in part because only a few samples were collected from each river (median $n = 2.5$, some of which consisted of composites of individuals from the same stream to improve the detection limits). Thus, the data were pooled to determine an overall signature of organochlorine content in insects of western rivers.

Results

Means, standard errors, and medians for organochlorine pesticide content of mayfly and vegetation samples are summarized in Table 1. Salient features of the data are presented graphically in Figures 2 and 3. Mayflies collected from eight streams in primary forest on the western slopes of Volcán Orosí and Cerro Cacao contained measurable quantities of several pesticides, with endosulfans (aESN and bESN) and their metabolic or oxidative breakdown product, endosuflan sulfate (EST), dominating the organochlorine signature (Table 1, Fig. 2). EST, the breakdown product, contributed ~90% to the endosulfan content in mayflies (Fig. 2). Larvae also contained measurable concentrations of pesticide metabolites DDE and HPX but no detectable quantities of the parent compounds DDT and HEP, respectively (Table 1). ALD, DLD, and EA were present in the mayflies, but lindane (gHCH) and its isomer aHCH were not detected (Table 1, Fig. 3). No mayfly larvae were successfully analyzed from the eastern slope rivers.

Leaves and bark collected from the R. Tempisquito catchment, one of the eight study streams on the western slope of Volcán Orosí and Cerro Cacao, also contained organochlorine pesticides; however, the signature was not dominated by endosulfans (Table 1). Leaves and bark contained residues of the α isomer of endosulfan (aESN) and the breakdown product EST in roughly similar proportions, unlike the distribution present in mayflies, but no detectable residues of the β isomer (bESN, Fig. 2). Although gHCH was present in both leaves and bark, aHCH was only detectable in leaves (Fig. 3). Endrin was detectable in both leaves and

TABLE 2. Extended.

EST	DDE	DDT	ALD	DLD	END	EA
1.5	n.d.	n.d.	n.d.	3.5	2.4	2.0
n.a.	1.1	2.9	n.a.	n.a.	n.a.	n.a.
n.a.	6.5	52	n.a.	n.a.	n.a.	n.a.
n.a.	8.4	12	n.a.	n.a.	n.a.	n.a.
n.a.	21	78	n.a.	n.a.	n.a.	n.a.
n.a.	0.4	1.6	n.a.	n.a.	n.a.	n.a.
65	1.9	n.d.	2.2	3.9	n.d.	5.2
2000	67	n.d.	54	100	n.d.	150
n.a.	10	n.d.	n.a.	n.d.	n.d.	n.a.
n.a.	30-3400	n.d.-120	n.a.	n.d.-790	n.d.	n.a.
n.d.	n.d.-4.2	n.d.-5.6	1.6-6.6	n.d.	1.5-11	n.d.-4.0
n.a.	10-310	<0.1	n.a.	1-18	n.a.	n.a.
n.a.	1800	3200	n.a.	n.a.	n.a.	n.a.

bark; however, HPX, DLD, and EA were only detected in leaves. DDT, DDE, and ALD were not detected in either leaves or bark of the R. Tempisquito catchment.

Sediment samples collected from R. Tempisquito revealed only the presence of just-detectable levels of endrin aldehyde, a breakdown product of endrin. Although detection limits were low enough in general to measure pesticide loadings comparable to those measured in coastal lagoon sediments draining agricultural regions of Mexico (Botello et al. 1994), they were not low enough to detect residues expected for streams draining more pristine catchments such as those sampled in this study. Thus, sediment results will not be discussed further.

Endosulfan compounds were the dominant organochlorines in leaves collected from both catchments on the eastern slopes of Volcán Orosí and Cerro Cacao (i.e., R. Orosí and R. Senderohi), though not to the extent noted for mayflies from western catchments (see above). Conversely, although bark samples collected from the R. Senderohi catchment also contained moderate levels of aESN and EST, they were dominated by highly elevated concentrations of the pesticides DDT and gHCH (Table 1). Leaves from the same plants contained undetectable levels of DDT and only low levels of gHCH (Table 1). Leaves from the R. Orosí catchment contained moderately low levels of gHCH (Table 1, Fig. 2) and no detectable DDT or DDE. Endosulfan distributions were varied for plant tissues collected from eastern basins, with bESN dominating the signature in leaves at R. Orosí but EST the major component in both leaves and bark at R. Senderohi. Leaves and bark contained no detectable residue levels of HPX, ALD, DLD, or EA (Table 1); however, END was detected in leaves from the R. Senderohi catchment.

In general, total endosulfan (ΣESNs) content was significantly (ten-fold) higher in vegetation from the eastern slope catchment (R. Orosí and R. Senderohi) than in leaves and bark collected in the R. Tempisquito catchment on the western slopes of Volcán Orosí (t-test, $p < 0.05$). Except for lindane (gHCH), which was significantly higher in R. Senderohi bark than in vegetation from all other catchments ($p < 0.05$), there were no other statistically significant east-west differences in content of organochlorine

pesticides ($p < 0.05$). DDT was detected only in R. Senderohi bark and at very high concentrations (Table 1); however, the concentrations were not statistically different from other vegetation samples due to high variability and the low number of bark samples analyzed from the R. Senderohi catchment ($n = 3$, $p > 0.05$).

Discussion

Higher concentrations of pesticides in vegetation from catchments east of the volcanic ridge might be expected because agricultural activity, and therefore pesticide use, is more intense there. The actual presence of pesticides in stream mayflies and plant tissues of the western slope catchments containing undisturbed primary forest might be explained by the close proximity (7–10 km) of the two regions. However, the magnitude of the decrease in concentration over such a short distance is not expected. For example, only a ten-fold decrease in atmospheric polychlorinated biphenyl (PCB) concentrations was observed over a distance of 1300 km over open ocean near the northeastern shore of the United States (Harvey and Steinhauer 1974). If we assume that plant tissue content is linearly related to ambient atmospheric concentrations (Meredith and Hites 1987, Hermanson and Hites 1990, Paterson et al. 1991, Simonich and Hites 1994a), our results for endosulfan show a ten-fold decrease of atmospheric concentrations over a distance that is two orders of magnitude less than was observed for PCB concentrations over open ocean. We hypothesize that aerial transport of pesticides from east to west is the main mechanism of endosulfan contamination in our study and that the main barrier to contaminant transport in the region is the volcanic ridge. The source regions for other organochlorine pesticides are less clear.

The distribution of endosulfan components varied highly among samples, probably due to differences in environmental stability and/or differential transport of the two stereoisomers, α- and β-endosulfan, and formation of the breakdown product endosulfan sulfate. For example, in *E. hecuba* larvae, 90% of the endosulfan content was present as the metabolite EST (Fig. 2). The α isomer, which is the major component of the technical mixture, was present in the mayflies at only one third and one fortieth the concentrations of bESN and EST, respectively. In contrast, the original distribution of components in technical grade endosulfan is 64–67% α-endosulfan and 29–32% β-endosulfan, with endosulfan sulfate not listed (Worthing and Walker 1987). Data on oysters in Mexico showed a similar inverse relationship with respect to the relative concentration of the two stereoisomeric endosulfans (Botello et al. 1994).

Endosulfan composition in leaves and bark was also dominated by EST, except for leaf samples collected in the R. Orosí catchment, which were dominated by bESN and contained no detectable quantities of EST. Because the ratio of bESN:aESN was lower than that present in technical grade of endosulfan, the data suggest that the β isomer of endosulfan was more labile than the α isomer. If preferential transformation of one isomer is causing the shift in relative composition, then loss of the more labile isomer (ESN) should parallel formation of the breakdown product (EST). Thus, we regressed the log of the ratio of bESN:aESN to the log of the proportion of ΣESNs present as EST. The regression showed a significant inverse relationship $\{\log(\text{bESN}/\text{aESN}) = -0.64 \log(\text{EST}/\Sigma\text{ESNs}) - 0.82, r^2 = 0.83\}$, strongly suggesting that the metabolite EST was formed as bESN was consumed. Because the magnitude of formation of EST more than accounted for the loss of bESN, conversion of the aESN isomer must also have occurred (although to a lesser degree). Without atmospheric data, we are unable to evaluate whether this transformation occurred prior to deposition.

Elevated residues of DDT and lindane in bark samples from the R. Senderohi catchment did not match the organochlorine content of leaf tissues from the same plants. Leaves from R. Senderohi contained undetectable levels of DDT and only low levels of gHCH (Table 1). This disparity between residue content in different tissues of the same plant may reflect a historical exposure of the plants to pesticides before the current leaf growth.

Organochlorine pesticide content of aquatic insects and vegetation collected from catchments containing primary forest in northwestern Costa Rica fell in the middle of the range of residues present in worldwide biota, except for residues of DDT and DDE, which were undetectable in leaves and bark (Table 2). Lindane (gHCH) content was higher in Costa Rican veg-

etation than in samples from both Guatemala and Amazonas but lower than that of vegetation in Scandinavia, Italy, and India (Calamari et al. 1991). However, residues of the α isomer of hexachlorocyclohexane (aHCH) were present at much lower concentrations in R. Tempisquito vegetation than such residues in other global vegetation (except Guatemala). Two factors control the ratio of gHCH to aHCH in environmental samples: (1) preferential decomposition of the γ isomer (gHCH) and (2) regional use of either the pure formulation of lindane, which is composed primarily of the isomer with stronger insecticidal activity (gHCH), or the technical grade formulation, which contains 55–80% aHCH and only 8–15% gHCH (Metcalf 1955). The greater concentration of the γ isomer in vegetation of the R. Tempisquito catchment suggests that the pure formulation of lindane was used in the region and that minimal transformation of the residues had occurred because the sources of contamination were nearby.

Endosulfan residue concentrations in *E. hecuba* larvae from western catchments were comparable to residues in oysters sampled from a river draining an agricultural and industrial area of Mexico (Table 2). However, although the metabolite endosulfan sulfate was the dominant organochlorine pesticide in the larvae, it was undetected in the Mexican oysters (Botello et al. 1994). DDE residues in *E. hecuba* were at the low end of the range measured in other organisms from Central and South America (Colombo et al. 1990, Fyfe et al. 1991, Botello et al. 1994) and surface fish collected from the northwest Atlantic near Spain (Krämer et al. 1984). Hexachlorocyclohexanes and DDT, commonly detected in substantial quantities in other organisms worldwide, were not detected in *E. hecuba*. ALD, EA, and HPX were present in *E. hecuba* at similar concentrations as in oysters in Mexico (Botello et al. 1994); however, HPX content of the larvae was twenty-fold lower than that measured in clams and fish in Argentina (Colombo et al. 1990).

Organochlorine pesticide use in Costa Rica seems to be less than in other countries in the region. For example, DDT is no longer shipped to Costa Rica for use in malarial control (PAHO 1992). However, old stocks may still be in use. Thus, the presence of the metabolite, DDE, in *E. hecuba* larvae may reflect remobilization of historically applied DDT, present use of stored DDT in Costa Rica, or long-range atmospheric transport from countries in the region where DDT is still in use, e.g., the Dominican Republic, Colombia, and Venezuela (PAHO 1992), with the source region dependent on season and the dominant wind pattern. Fyfe et al. (1991) showed that migrating peregrine falcons that overwinter in Costa Rica were exposed to relatively low levels of DDT and other organochlorines compared with falcons that overwintered in countries (e.g., Peru) still using these pesticides. Fyfe et al. (1991) detected no endosulfan residues in the raptors or their prey. Also, Mora and Anderson (1991) determined that endosulfans were nondetectable in birds feeding in regions where this pesticide accounted for more than 50% of the organochlorine pesticides in use. These data suggest a rapid depuration of endosulfans by birds. In contrast, the accumulation of endosulfans in the stream mayfly larvae of this study suggests that they had limited ability to depurate or metabolize endosulfans and thus accumulated them in their lipids. Because endosulfan is still shipped to Costa Rica for agricultural use (Instituto Nacional de Biodiversidad, Costa Rica, personal communication) and is highly toxic to fish (LC_{50}[96h] 2μg/L, golden orfe, Worthing and Walker [1987]), atmospheric transport of residues to undisturbed forested catchments of Costa Rica from intensely cultivated regions seems likely, and places certain aquatic species in jeopardy.

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