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Organochlorine Insecticide and PCB Residues in Two Bat Species from Four Localities in Spain

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Use of organochlorine compounds in the last decades produced an accumulation of these chemicals in many ecosystems all over the world. Specifically, organochlorine pesticides and PCBs are well known as persistent contaminants which accumulate in the upper trophic levels of food chains.

Bat populations in many parts of the world are declininng. Although many factors may be responsible, the use of organochlorine insecticides in agriculture is demonstrated to be one cause. Thus, studies have related bat population declines to insecticides (Geluso et al. 1976; Clark et al. 1978; Frank et al. 1980).

In Spain there have been drastic declines in populations of bats, and twenty-five out of twenty-seven species were declared endangered species (ICONA 1986). Organochlorine insecticides were used widely in Spain until partial bans in 1977, but residues are still found in environmental samples (Hernández et al, 1988; González et al. 1991). Since DDT was legally restricted, lindane has become important as a substitute.

Our objectives were (1): to evaluate whether organochlorine residues were involved in bat population declines in Spain; (2): to evaluate regional patterns of residues; (3): to compare biological accumulation of residues between species.

MATERIALS AND METHODS

This study was carried out in four ecologically diverse locations: (a) La Rioja: 90% covered by oak, beech and pine forests, scrubland and pastures, never sprayed with insecticides; (b) Málaga: 80% dry farming land, mainly covered by olives, but also some grain and sunflower crop, where insecticides are used extensively; (c) Cádiz: 81% cork and oak tree forest, scrubland and pastures sprayed sporadically with insecticides to avoid forest pests mainly gypsy moth (Lymantria dispar); (d) Aranjuez (Madrid): 58% dry farming land, mainly grain crops, but also some olives and vineyards, with extensive insecticide

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treatments; 12% irrigated land crops with intensive insecticide treatments; this location is near Madrid city metropolitan area and industrial activity areas.

Thirty-nine bats were caught in 1989-1990: twenty Schreiber's bats (Miniopterus schreibersi) and nineteen greater horseshoe bats (Rhinolophus ferrumequinum). All bats were alive and appeared healty when collected; all bats were killed by separation of cervical vertebrae, sexed, weighed, their forearms measured, age determined, and frozen at -18°C until preanalytical treatment was performed. For the preanalytical treatment, specimens were thawed prior to dissection. In dissection, wings and feet were removed first. The head was severed at the base of the skull. The part of the body remaining was analyzed as "carcass".

Samples were homogenized, extracted and cleaned following methods described previously (Hernández et al. 1988). Eluates were analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with Ni 63 Electon Capture Detector. A 30 m long capillary column covered with RSL-200 was used. Chromatographic conditions as follow: detector 280°C; injector 300°C; temperature program, isothermal phases at 180°C (1 min) and 250°C (30 min), with intermediate temperature increase rate of 2°C/min. A 50 m capillary column covered with BP-5 was used for confirmation of identity of the organochlorine residues. Peaks were identified on the integrator by retention times; tolerance allowed was ± 0.05 min. The peaks were measured by area counts given by the integrator (minimum count 500). Quantitation was done comparing the peak areas in the sample with those in corresponding standards. PCBs were quantitated by comparing the peak areas from seven major peaks with those of Aroclor 1260. Recoveries of organochlorine compounds ranged from 82-103%, but the residue data in the Tables were not adjusted on the basis of these recoveries. Minimum detection limit was 0.01 μg/g. All the residues are expressed in μg/g.

RESULTS AND DISCUSSION

Geometric mean and range of organochlorine residues in 39 bat samples are shown in Tables 1 and 2. All of the 39 carcasses analyzed contained residues of heptachlor epoxide, dieldrin, p,p'-DDE, and PCBs. In twenty Schreiber's bat samples, 100% had quantifiable amounts of δ-HCH and p,p'-TDE, 90% of β-HCH, 85% of γ-HCH, 80% of dichlorobenzophenone, 70 of α-HCH, and 55% of p,p'-DDT. In nineteen greater horseshoe bat samples, 89.4% had detectable amounts of γ-HCH and δ-HCH, 79% of β-HCH, 73.6 of α-HCH, 63.1% of dichlorobenzophenone, 52.6% of p,p'-TDE, and 31.6% of p,p'-DDT. No residues of other chlorinated insecticides included in the analytical survey were detected (aldrin, heptachlor, mirex, and methoxychlor). According to the data summarized in Tables 1 and 2 it appears that insecticide concentrations decreased in the order DDT group > cyclodienes > hexachlorocyclohexanes in Schreiber's bats and in the order DDT group > hexachlorocyclohexanes > cyclodienes in greater horseshoe bats.

Table 1. Geometric mean and range of organochlorine pollutants in 20 Schreiber's bats from Spain (μg/g wet weight in carcass).

Compound	Locality			
	La Rioja(5)	Málaga(5)	Cádiz(5)	Aranjuez(5)
α-HCH	<0.01	<0.01	<0.01	<0.01
	N.D.-<0.01	N.D.-<0.01	N.D.-<0.01	N.D.-<0.01
ß-HCH	<0.01	0.07	0.05	0.03
	N.D.-0.04	0.02-0.22	N.D.-0.30	<0.01-0.16
γ-HCH	<0.01	<0.01	<0.01	<0.01
	N.D.-<0.01	N.D.-<0.01	N.D.-0.01	N.D.-<0.01
δ-HCH	0.04	0.03	0.09	0.04
	0.02-0.09	0.01-0.13	0.03-0.14	0.02-0.10
H. ep.	0.07	0.06	0.12	0.13
	0.01-0.24	0.02-0.19	0.02-0.37	0.01-1.94
Dieldrin	0.11	0.08	0.06	0.18
	0.03-0.26	0.03-0.27	0.02-0.24	0.03-2.07
DBP	0.01	0.06	0.04	0.01
	N.D.-0.05	0.04 -0.10	0.01-0.22	N.D.-0.20
p,p'-DDE	2.67	8.33	6.83	0.65
	0.12-8.29	2.93-23.53	1.79-19.34	0.11-20.79
p,p'-TDE	0.03	0.02	0.01	<0.01
	<0.01-0.11	<0.01-0.08	<0.01-0.02	<0.01-0.04
p,p'-DDT	<0.01	<0.01	0.11	<0.01
	N.D.-<0.01	N.D.-0.12	0.04-0.26	N.D.-0.18
PCBs	0.63	0.42	0.56	1.05
	0.53-0.80	0.11-2.06	0.37-0.93	0.53-1.95
p,p'-DDE*	22.08	64.67	106.5	8.07
	1.16-65.93	27.25-221.5	32.31-419.8	1.21-196.0
PCBs*	3.92	3.68	8.68	13.04
	1.18-6.46	1.01-17.62	4.12-15.47	9.63-18.42

( ) = number of samples; N.D. = Not detected; H. ep. = Heptachlor epoxide; DBP = Dichlorobenzophenone; * = μg/g lipid weight in carcass.

DDE is the predominant residue found in our bats. As expected, the residue levels varied considerably, even within the same species from the same locality. Differences in age, sex, nutritional status and food source among individuals probably contributed to this observed variance. Organochlorine compound accumulation in bats appears to be influenced by the same factors that affect accumulation in other organisms. The bats contained higher levels of p,p'-DDE and PCBs in their carcasses and probably accumulated these residues through exposure in food.

Average of organochlorine compounds according to locality are given in Table 3. Our results revealed that bats from Málaga had the highest residues of ΣDDT. These data reflect previous and extensive use of p,p'-DDT. For the Schreiber's bat, La Rioja samples usually had the smallest amounts of all insecticides, whereas for the greater horseshoe bat, the Cádiz samples had the smallest amounts of all organochlorine compounds, PCBs included; the Cádiz data reflect the absence of local pollution sources (industrial or agricultural). Residues of PCBs were highest at Aranjuez for both species; the data

Table 2. Geometric mean and range of organochlorine pollutants in 19 greater horseshoe bats from Spain (μg/g wet weight in carcass).

Compound	Locality			
	La Rioja(5)	Málaga(6)	Cádiz(5)	Aranjuez(3)
α-HCH	<0.01	<0.01	N.D.	0.01
	N.D.-<0.01	0.03-0.25	N.D.-<0.01	<0.01-0.13
ß-HCH	0.02	0.09	<0.01	0.01
	N.D.-0.11	0.03-0.25	N.D.-<0.01	<0.01-0.13
γ-HCH	<0.01	<0.01	<0.01	<0.01
	<0.01-0.01	N.D.-<0.01	<0.01-0.01	N.D.-0.01
δ-HCH	0.03	0.04	0.01	0.06
	N.D.-0.08	0.02-0.12	N.D.-0.06	0.05-0.06
H.ep.	0.09	0.03	0.01	0.05
	0.03-0.56	0.01-0.23	0.01-0.09	0.02-0.22
Dieldrin	0.01	0.02	<0.01	0.06
	N.D.-0.16	<0.01-0.06	N.D.-0.01	0.03-0.25
DBP	<0.01	<0.01	<0.01	0.02
	N.D.-0.03	N.D.-0.03	N.D.-<0.01	0.01-0.03
p,p'-DDE	0.03	0.17	<0.01	0.07
	<0.01-0.08	0.05-0.78	<0.01-0.01	0.04-0.16
p,p'-TDE	<0.01	0.03	N.D.	<0.01
	N.D.-0.15	<0.01-0.14	N.D.	N.D.-<0.01
p,p'-DDT	<0.01	0.01	N.D.	<0.01
	N.D.-0.02	N.D.-0.18	N.D.	N.D.-0.15
PCBs	0.57	0.26	0.14	0.75
	0.17-1.70	0.17-0.52	0.10-0.16	0.32-1.38
p,p'-DDE*	0.73	1.64	0.10	0.60
	0.06-6.95	0.55-4.87	0.05-0.28	0.19-4.55
PCBs*	15.46	2.55	3.26	6.27
	2.59-113.2	1.43-5.43	1.06-19.74	0.10-40.19

( ) = number of samples; N.D. = Not detected; H. ep. = Heptachlor epoxide; DBP = Dichlorobenzophenone; * = μg/g lipid weight in carcass.

correspond strongly to land use for the area, with industrial and urban activities surroundings.

In order to verify these assertions an analysis of variance (ANOVA) was used: (a) no significant differences were found in Schreiber's bats or for Σ CDin greater horseshoe bats; (b) significant differences were found in Σ HCH, Σ DDT, and PCBs concentrations in greater horseshoe bats. Average of organochlorine compounds according to species are given in Table 4.

Schreiber's bats have more residues of organochlorine insecticides and PCBs than have greater horseshoe bats; no significant differences were found except for Σ DDT. The differences in residue levels between the two bat species do not seem related to differences in feeding habits, since both of them are insect eating bats. The differences may be due to smaller dietary intake and/or more efficient excretion of organochlorine compounds in greater horseshoe bat than in Schreiber's bat. Likewise, the scouring field is higher in Schreiber's bat than in greater horseshoe bat.

Table 3. Average of organochlorine compounds in bats by locality.

Schreiber's bat					
Compound	Locality				
	La Rioja	Málaga	Cádiz	Aranjuez	p
Σ HCH	0.07	0.15	0.28	0.10	ns
Σ CD	0.25	0.19	0.26	1.14	ns
Σ DDT	4.85	13.93	8.97	5.11	ns
PCBs	0.64	0.67	0.59	1.15	ns
Greater horseshoe bat					
Compound	Locality				
	La Rioja	Málaga	Cádiz	Aranjuez	p
Σ HCH	0.10	0.17	0.03	0.12	*
Σ CD	0.21	0.08	0.02	0.19	ns
Σ DDT	0.08	0.36	0.01	0.12	*
PCBs	0.81	0.29	0.14	0.89	*

Σ HCH = α + ß + γ + δ-HCH; Σ CD = Heptachlor epoxide + dieldrin; Σ DDT = p,p'-DDE + p,p'-TDE + p,p'-DDT + dichlorobenzophenone; ns = not significant; * = $p < 0.05$.

Table 4. Average of organochlorine compounds in bats by species.

Compound	S's bat (20)	G.H. bat (19)	p
Σ HCH	0.15	0.11	ns
Σ CD	0.46	0.12	ns
Σ DDT	8.21	0.16	**
PCBs	0.76	0.48	ns

Σ HCH = α + ß + γ + δ-HCH; Σ CD = Heptachlor epoxide + dieldrin; Σ DDT = p,p'-DDE + p,p'-TDE + p,p'-DDT + dichlorobenzophenone; S's bat = Schreiber's bat; G.H. bat = Greater horseshoe bat; ( ) = Number of samples; ns = not significant; ** = $p < 0.1$; residues in µg/g.

In order to verify these assertions an analysis of variance (ANOVA) was used: (a) no significant differences were found in pollutant concentrations in La Rioja, Málaga, and Aranjuez, except Σ DDT in La Rioja and Málaga; (b) significant differences were found in Cádiz and Σ DDT in La Rioja and Málaga. Among organochlorine compounds measured in bats from wild populations, p,p'-DDE, p,p'-DDT, dieldrin, and PCBs are usually the most abundant. Table 5 shows the estimated minimum lethal concentrations in carcass of p,p'-DDE, p,p'-DDT, dieldrin, and PCBs capable of causing mortality in four bat species. In this study, geometric mean andrange of p,p'-

Table 5. Estimated minimum lethal carcass concentrations of p,p'-DDE, p,p'-DDT, dieldrin and PCBs in four bat species.

Species	DDE	DDT	Diel.	PCBs	Ref.
Tadarida brasiliensis	66,000[a]				1
Myotis lucifugus	79,000[a]				2
Pipistrellus pipistrellus		45[b,c]			3
Myotis lucifugus		470[a]			4
Myotis grisescens			390[a]		5
Myotis lucifugus				190,000[a]	2
Myotis lucifugus				5,400[b]	2

[a] = μg/g lipid weight; [b] = μg/g wet weight; [c] = DDE + DDT; 1: Clark and Kroll (1977); 2: Clark and Stafford (1981); 3: Jefferies (1972); 4: Clark et al. (1978); 5: Clark (1981).

DDE and PCBs (μg/g lipid weight) and p,p'-DDT and dieldrin (μg/g wet weight) are given in Tables 1 and 2. The highest concentration of p,p'-DDE, p,p'-DDT, dieldrin, and PCBs among the thirty-nine bats analyzed in this work are much lower than the lowest lethal level reported in Table 5. However, this comparison is risky, since bat species differ in their sensitivity to organochlorine compounds; thus, Eptesicus fuscus is much less sensitiv than Myotis lucifugus to p,p'-DDT (Clark et al., 1978), whereas Myotis lucifugus is slightly less sensitive than Tadarida brasiliensis to p,p'-DDE (Clark and Stafford 1981).

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