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Organochlorine Residues in Spanish Common Pipistrelle Bats (*Pipistrellus pipistrellus*)

A. Guillén,[1] C. Ibáñez,[1] J. L. Pérez,[1] L. M. Hernández,[2] M. J. González,[2] M. A. Fernández,[2] R. Fernández,[1]

[1]Doñana Biological Station (CSIC), Apartado 1056, 41080 Sevilla, Spain
[2]Institute of Organic Chemistry (CSIC), c/ Juan de la Cierva 3, 28006 Madrid, Spain

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During this century, drastic declines of bat populations in Europe and USA have been reported. There seem to be three determining factors: general and dramatic changes on natural habitats brought about by men, loss of roosts, and massive use of pesticides. Organochlorine pollution and bat die-offs have been found to be related (Clark et al. 1978; Geluso 1976). Dramatic declines of populations while organochlorine pesticides were used have been shown through well documented population histories (Brosset et al. 1988).

Regulation of agricultural use of DDT was first stated in Spain in 1971. In 1975 the use of aldrin, dieldrin, endrin, heptachlor and chlordane was banned. The use of DDT and other organochlorines was also severely restricted. Nowadays law allows the use of several organochlorines (for example, the lindane group) for timber preservation, individual treatments of damaged trees and applications in some crops (M.A.P.A. 1990). In 1988 we took on several studies with the aim of assessing levels and possible effects of organochlorines on spanish bats populations and some results have been published elsewhere (Guillén et al. 1991, Fernández et al. 1993, Hernández et al. 1993).

The objectives of this study were: (1) assessing the levels of organochlorine compounds in common pipistrelles (Pipistrellus pipistrellus) in Spain; (2) evaluating the patterns of residues in areas with different use of land (related to different pesticide use) after the ban or regulation of most of these chemicals; (3) obtaining some information about differences among sexes and ages.

MATERIALS AND METHODS

Specimens used for pesticide residue analysis were obtained from four Spanish areas with different organochlorine pesticides pressure. Pesticide use and main uses of land within a 25 km radius area around the capture sites were the following: (1) Cameros Mountains (La Rioja): 90% of the area is covered by oak, beech and pine forests, scrubland and pastures, never sprayed with pesticides; (2) Aljibe Mountains (Cádiz and Málaga): 81% cork and oak tree forest, scrubland and pastures sporadically sprayed with insecticides in order to avoid forest pests, mainly Gipsy Moth (Lymantria dispar); (3) Antequera basin (Málaga): 80% dry farming land, mainly covered with olive trees, but also some grain and sunflower

Correspondence to: A. Guillén

Table 1. Frequency (percentage of samples), recoveries (mean±SD), and ppm wet weight and ppm lipid weight (geometric mean followed by range in brackets) of the organochlorine pollutants found in 81 common pipistrelles from Spain. H. epox. = Heptachlor epoxi; DBF = Dichlorobenzophenone).

Compound	Frequency	Recoveries	ppm wet weight	ppm lipid weight
α-HCH	66.6%	82.14 ± 2.36%	0.002 (N.D.-0.104)	0.008 (N.D.-0.550)
β-HCH	82.7%	86.42 ± 1.89%	0.013 (N.D.-2.859)	0.100 (N.D.-35.023)
γ-HCH	65.4%	88.17 ± 1.65%	0.002 (N.D.-0.035)	0.008 (N.D.-0.868)
δ-HCH	97.5%	84.28 ± 1.23%	0.029 (N.D.-0.303)	0.308 (N.D.-3.318)
H. epox.	96.3%	92.73 ± 0.75%	0.008 (N.D.-0.160)	0.085 (N.D.-2.189)
Dieldrin	70.3%	93.53 ± 0.04%	0.005 (N.D.-0.362)	0.028 (N.D.-5.890)
DBF	55.6%	92.43 ± 0.81%	0.003 (N.D.-0.054)	0.012 (N.D.-8.442)
DDE	100%	92.29 ± 0.87%	0.382 (0.014-35.360)	4.864 (0.226-437.60)
TDE	32.1%	95.23 ± 0.67%	0.002 (N.D.-0.202)	0.004 (N.D.-3.217)
DDT	24.7%	103.01 ± 0.49%	0.002 (N.D.-0.580)	0.003 (N.D.-1.589)
PCBs	100%	102.36 ± 4.24%	0.530 (0.067-7.264)	6.341 (0.428-212.90)

crops, where pesticides are used extensively; (4) Water meadows of Aranjuez (Madrid): 58% dry farming lands, mainly grain crop, but also some olive trees and vineyard, extensively treated with pesticides, 12% irrigated land crops supporting intensive pesticide treatments; this area is located close to the metropolitan area of the city of Madrid, as well as to areas of industrial activity.

The studied species, the common pipistrelle (Pipistrellus pipistrellus), is a non-migrating species and the most common bat within the Iberian peninsula. It is the only European bat which has not been included into the 'Red lists' of none of the European countries (Stebbings 1988). From 1988 to 1990, 81 individuals were captured. These few specimens were captured in different sites within each of the four areas, during different sampling periods (spring, summer, autumn), so as not to be detrimental to any of the populations of this species. All bats appeared to be healthy, and they were full grown and capable of flying when collected. Cartilaginous epiphyseal plates in finger bones of young bats and pelage colour (Anthony 1988) were regarded for qualitative aging (young, adult) of animals in the field. Only up to three months old young can be identified through these methods, so juveniles and adults were only differentiated in summer samples.

The selected specimens were treated and samples analized according to methods described in Hernández et al. (1993). All the residues are expressed in ppm (mg/kg). A value of half the minimum detectable limit (0.5 µg/kg) was assigned to all the samples with no detectable residues for the statistical calculations. Total contents (µg) were obtained multiplying contents in ppm (mg/kg) by weight in grams. In a primary inspection, the distributions of contents in ppm and total contents in µg, for each compound, showed no-normality, and adjusted to a log-normal distribution, so a decimal logarithmic transformation was applied. Due to inequal number of data per cell and to the fixed character of the studied factors (sex-age and differently treated localities), the ANOVA with type III calculation of sum of squares from GLM procedure of SAS statistical package was used to analize the studied effects. Tukey's Studentized Range test (α=0.05) was used to detect the non-homogeneous groups.

Table 2. Two-way ANOVA for log-transformed residue contents of organochlorine compounds, for sex-age and locality factors in summer samples.

Dependent variable	Source of variation	SS	d.f.	MS	F	P	
logΣHCH	SEX-AGE	0.253	2	0.126	1.66	0.204	NS
	LOCALITY	5.556	3	1.852	24.31	0.000	
	SEX-AGE*LOCALITY	0.447	5	0.089	1.17	0.339	NS
logΣCD	SEX-AGE	3.359	2	1.679	6.20	0.005	
	LOCALITY	8.581	3	2.860	10.57	0.000	
	SEX-AGE*LOCALITY	2.221	5	0.444	1.64	0.172	NS
logΣDDT	SEX-AGE	7.349	2	3.675	16.13	0.000	
	LOCALITY	22.477	3	7.492	32.88	0.000	
	SEX-AGE*LOCALITY	0.000	5	0.000	0.00	1.000	NS
logPCBs	SEX-AGE	2.905	2	1.453	9.45	0.000	
	LOCALITY	6.706	3	2.235	14.54	0.000	
	SEX-AGE*LOCALITY	0.909	5	0.182	1.18	0.336	NS

RESULTS AND DISCUSSION

Geometric mean and range of organochlorine residues found in bat samples are shown in Table 1. Other chlorinated compounds (aldrin, heptachlor, mirex and methoxychlor) were not present in any of the bats analyzed. According to the data summarized in Table 1, organochlorine insecticide concentrations increased in this order: hexachlorocyclohexanes (ΣHCH=α-HCH+β-HCH+γ-HCH+δ-HCH) < cyclodienes (ΣCD= dieldrin+heptachlor epoxide) < DDT group (ΣDDT= dichlorobenzophenone+DDE+TDE+DDT).

Organochlorine contents are in general in the same range that those detected in the other bat species (Rhinolophus ferrumequinum and Miniopterus schreibersii) studied in the Iberian Peninsula, except for Miniopterus schreibersii have more ΣDDT residues (Guillén et al. 1991, Hernández et al. 1993). In the Iberian Peninsula Pipistrellus and Rhinolophus undertake just local displacements along the year, but Miniopterus is a strong flyer and migrant, for the differences can be related to the different ecology. Concerning ΣDDT, averages and ranges are similar to those detected by other authors in the same bat genus in other countries after DDT restrictions for agricultural use. Thus, Reidinger (1976) reported 1.4 ppm (median residues, wet weight) of DDE in carcasses of P. hesperus in Arizona. In Germany, Braun (1986) found 0.8-6.5 ppm of DDE in P. pipistrellus and 0.2-3.2 ppm in P. nathusii (wet weight), and Nagel and Disser (1990) 4.6 ppm in P. pipistrellus (arithmetic mean of fat weight in carcasses of 12 adult females (1.88 ppm) and 11 juveniles (7.66 ppm)). Nevertheless, PCBs levels are lower than those found by Braun (1986) in P. pipistrellus and P. nathusii (3-46 ppm body wet weight), and by Disser and Nagel (1989) in a maternity colony of P.pipistrellus (32.4 ppm arithmetic mean fat weight in carcass). Anyhow, differences among localities are significant (Table 2), and the residues of ΣDDT (0.26-35.53, $\bar{X}$=8.10 ppm wet weight) in samples from Antequera are in the same range with those reported by Jefferies (1972) from British common pipistrelles (2.58-13.2, $\bar{X}$=7.02 ppm wet weight) when DDT was still being used in Britain (however, the DDT/DDE ratio he found was close to 100 times ours).

The residues found are in general very far from reported lethal levels (1300 to 1500 ppm brain wet weight of Aroclor PCB in Myotis lucifugus, Clark and Stafford 1981; 66000 ppm body fat weight of DDE in Tadarida brasiliensis, Clark

Table 3. Contents of organochlorine compounds for sex-age groups (m=adult males, f=adult females, y=young), in summer, for all localities grouped together. Arithmetic mean (Mean) and standard deviation (SD) of log-transformed residue contents (ppm wet weight) are shown. Residue mean (RM) and total content (TC) are antilogarithms from corresponding log-transformed values means. Homogeneous groups (H.Gr.) in Tukey's Studentized Range test (α=0.05) for log-transformed ppm values are joined in columns with asteriscs. (N), the number of samples, is different for ppm values and total contents because the weights of some individuals were missing.

Dependent	Sex-Age	Mean (N)	SD	H.Gr.		RM	TC (N)
logΣHCH	m	-1.419 (11)	0.285	*		0.038	0.146 (7)
	f	-1.309 (21)	0.469	*		0.049	0.248 (17)
	y	-1.227 (18)	0.471	*		0.059	0.248 (18)
logΣCD	m	-1.887 (11)	0.844	*	*	0.013	0.137 (7)
	f	-1.982 (21)	0.673	*		0.010	0.062 (17)
	y	-1.415 (18)	0.553		*	0.038	1.161 (18)
logΣDDT	m	-0.667 (11)	0.462	*		0.215	1.135 (7)
	f	-0.795 (21)	0.821	*		0.160	1.197 (17)
	y	0.041 (18)	0.942		*	1.099	4.602 (18)
logPCBs	m	-0.035 (11)	0.492		*	0.923	4.709 (7)
	f	-0.506 (21)	0.530	*		0.312	1.817 (17)
	y	-0.008 (18)	0.572		*	0.983	4.114 (18)

and Kroll 1977, and 79000 ppm in Myotis lucifugus, Clark and Stafford 1981). Nevertheless, according to Clark and Prouty (1977), PCBs may reduce metabolic rate in bats at these environmental levels. The reproductive processes of some mammals, as mink, ferret (Aulerich and Ringer 1977; Bleavins et al. 1980) and common seal (Reinjders 1986), are highly sensitive to PCBs. Also, in mammals such as the mink, reproductive failure occurs with low levels of DDT and DDE in their diet (Aulerich et al. 1973). In any case, the scarce information available (Clark 1981) on chronic and sublethal effects of these compounds on bats makes it difficult to know whether the found levels have negative effects or not, but the concentrations are far below any one proved to be lethal or harmful.

A two-way ANOVA was performed for log-transformed residue levels in ppm of ΣHCH, ΣCD, ΣDDT and PCBs, classified in sex-age categories (adult males, adult females and young) and sites. For this analysis, only individuals caught in summer (when young individuals are distinguishable) were used. In no case was the interaction between sex-age and locality a significant effect ($P<0.05$). Both factors were significant for all the dependent variables except for ΣHCH (for this group of pesticides, locality is the only significant effect) (Table 2). Highest residue contents were always present in young, followed by males. Using Tukey's test, differences between adult males and females were not detected either in ΣDDT or in ΣCD contents. Neither in ΣCD nor in PCBs contents were differences detected between adult males and young, also using Tukey's test (Table 3). The analysis was also performed for total contents in µg, with similar results (ΣDDT: sex-age: $P=0.624$, locality: $P=0.000$, sex-age*locality: $P=0.220$; ΣCD: sex-age: $P=0.037$, locality: $P=0.000$, sex-age*locality: $P=0.283$; ΣCD: sex-age: $P=0.017$, locality: $P=0.000$, sex-age*locality: $P=0.098$; PCBs sex-age: $P=0.058$, locality: $P=0.000$, sex-age*locality: $P=0.252$), except for sex-age effect in PCBs contents, which probability rose slightly over the 0.005 level. Using Tukey's test with PCBs contents, differences were not detected either between males and females, or between males and young (geometric means derived from log-transformed residue means are shown in Table 3).

Table 4. Organochlorine residues classified into sex (m=males, f=females) and locality (1=Cameros, 2=Aljibe, 3=Antequera, 4=Aranjuez) groups. Mean and SD of logarithmic values used in the ANOVA are shown, as well as residue mean (RM, geometric mean derived from logarithms mean). Homogeneous groups (H.Gr.) in Tukey's Studentized Range test (α=0.05) are joined in columns with asterisks.

Dependent	Factor	Level	N	Mean	SD	H.Gr.	RM
logΣHCH	SEX	m	33	-1.050	0.093		0.089
		f	30	-1.193	0.092		0.064
	LOCALITY	1	18	-1.385	0.088	*	0.041
		2	15	-1.206	0.079	*	0.062
		3	17	-0.579	0.132	*	0.264
		4	13	-1.351	0.094	*	0.045
logΣCD	SEX	m	33	-1.917	0.114		0.012
		f	30	-2.062	0.132		0.009
	LOCALITY	1	18	-2.250	0.132	*	0.006
		2	15	-2.312	0.081	*	0.005
		3	17	-1.968	0.129	*	0.010
		4	13	-1.267	0.232	*	0.054
logΣDDT	SEX	m	33	-0.318	0.122		0.481
		f	30	-0.737	0.136		0.183
	LOCALITY	1	18	-1.106	0.100	*	0.078
		2	15	-0.675	0.139	*	0.211
		3	17	0.284	0.126	*	0.520
		4	13	-0.570	0.189	*	0.269
logPCBs	SEX	m	33	-0.267	0.090		0.541
		f	30	-0.445	0.102		0.359
	LOCALITY	1	18	-0.453	0.084	*	0.353
		2	15	-0.572	0.102	*	0.268
		3	17	-0.519	0.079	*	0.303
		4	13	0.260	0.192	*	1.820

A two-way ANOVA was performed for log-transformed residue levels classified into sex and locality. Young were removed for this analysis, where only adult specimens (more than three months old) collected throughout the year were used. For PCBs (sex: P=0.004, locality: P=0.000, sex*locality: P=0.548), ΣCD (sex: P=0.025, locality: P=0.000, sex*locality: P=0.207) and ΣDDT (sex: P=0.001, locality: P=0.000, sex*locality: P=0.590) the effect of both factors is significant, but not their interaction. For ΣHCH (sex: P=0.133; locality: P=0.000; sex*locality: P=0.027) the interaction is a significant effect, showing that this two factors affect jointly the variable, and it is not possible to test separate effects. Females show consistently less quantities of every compound analyzed (Table 4).

The geographical pattern of residues was as expected (Table 4). The highest levels of ΣHCH and ΣDDT were detected in Antequera, the area with an extensive dry farm use. PCBs and ΣCD appeared in higher levels in Aranjuez, the area being the closest to industrial activity places (sources of PCBs) and with more vegetable gardens (where ΣCD were mostly used). Hernández et al. (1993) found the same pattern for other bat species in Spain. This unequal distribution is probably due to a different use at present, in ΣHCH and PCBs cases. For DDE and ΣCD unequal distribution is probably due to the high persistence of residues not circulating long after the insecticide use, so that the DDE/DDT ratios ($\bar{X}$=1257.69, 9.95-26550) show that illegal use probably has not been common during the last years.

Females organochlorine transference to foetuses and young via placenta and milk is a known fact for some bat species (Clark and Lamont 1976a,b; Clark 1981).

These processes result in a net 'excretion' of organochlorine compounds in female bats during pregnancy and nursing, and a heavy intake in foetuses and suckling young, evaluated in Pipistrellus pipistrellus in 91% and 83% of the female accumulated residues for PCBs and ∑DDT (Disser and Nagel 1989; Nagel and Disser 1990). According to these authors, males can never eliminate residues in that way, so the highest levels would be expected in males, followed by young and females. According to our data, however, the juveniles show higher amounts of residues than adults throughout the year (both in ppm body wet weight and total content) (table 3). That pattern disagrees with the explanation given by the above mentioned authors, who suggested that all the residues found in juveniles come from those accumulated by the mother during the year after parturition. A mechanism which may account for higher residue levels in juveniles could be high proportions of fats in nursing females' bodies and transmission of lacteous lipids determining a faster intake way from environment to the young through the mother. Moreover, half-time of these toxic substances could be higher in young individuals, due to inmaturity of catabolic and excretion mechanisms. For example, Ballatori and Clarkson (1982) report that in some mammals the bile duct does not become functional for some time after birth, thus being unable to excrete toxics, such as mercury.

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