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Organochlorine Residues in Wolves' Viscera from Galicia (Northwest Spain)

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Organochlorine compounds such as those pertaining to the family of DDTs, used as pesticides in agriculture and polychlorinated biphenyls (PCBs) which are used as non-flammable oils in many commercial products have penetrated all ecosystem (Bucholski et al., 1996); Marsili and Focardi, 1996). Due to properties such as high fat solubility, high chemical stability and low volatility, these class of compounds are subject of biomagnification in food chain. Although in the main time many countries have banned the use of these chemicals, they are currently present in variety of biological matrices. The presence of organochlorine pesticides and PCBs in mussels (Alvarez et al., 1996), fishes (Pastor et al., 1996), water birds (Mora, 1996), etc. has been the subject of a number of works reported during the past two decades but a little information is available on contaminant burdens in terrestrial wildlife, being specially scarce organic residue data for wolves from North West Spain.

Wolf populations have declined dramatically in Spain. The decline has been linked with authorized huntings and epidemics because this specie has no natural enemies (Bárcena, 1990; Instituto Geográfico Agostini, 1993). Thus, in the terrestrial environment, mammals are continuously exposed to the contaminants though variety of sources such us contaminated food. Wolves have a long life-span with leads to a long-term accumulation in their tissues. These factors cause prolonged exposure to organochlorine compounds after restriction on the use of some them (Longanathan, 1993). In the present study variations in organochlorine concentrations of total PCBs, total DDTs and PCB/DDT ratios with tissue, age, sex and area are studied in wolves (*Canis lupus,* L) from Galicia. The objective was to determine the degree of chlorinated hydrocarbon exposure of this specie.

MATERIALS AND METHODS

Samples were collected, chosen and were at our disposal from Laboratory of Parasitology of Institute of Investigation and Food Analysis in Santiago de Compostela (Spain). They determined age and sex, too. Samples

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consisted of spleen, liver, muscle, kidney and suprarenal which were cut by scalpel in a glass plate and then each viscera sample was frozen prior to its lyophilization.

The analytical method has been recently described (Carril et al., 1996, 1997) and it was used without any modifications.

An aliquot (approximately, 0.5 g accurately weighed) of lyophilized sample was extracted with dichloromethane (DCM):hexane (1:1). The DCM: hexane was then evaporated to dryness by using a stream of nitrogen in order to determine lipid content and redissolved in hexane; the sample was then applied to a silica minicolumn and eluted with 10 ml hexane. The obtained fraction was analyzed by gas chromatography using electron-capture detector (ECD). The recoveries of the method have been investigated and were found to vary between 73 and 90% for ΣDDT and ΣPCB, respectively.

ΣDDT is the sum of p,p'-DDE, o,p'-DDT and p,p'-DDT. ΣPCB were quantified by using an Aroclor-based standard.

All results are presented as arithmetic means. For the calculation of means, samples with residue levels below detection limits ware assigned a value of 0.

Differences in levels of organochlorines between tissues, age, sex and areas were analyzed by using a one-way-ANOVA.

RESULTS AND DISCUSSION

Twelve individual wolves were analyzed for ΣPCB and ΣDDT. Five were considered adults (≥ 1 year old; 4 females, 1 male) and 7 subadults (< 1 year old; 4 females, 3 males).

Fifty-six tissue samples, consisting of 11 spleen, 12 liver, 10 muscle, 11 kidney and 12 suprarenal samples were examined.

Raw data before combining all the PCBs and all the DDTs and levels of ΣPCB, ΣDDT along with ratio ΣPCB/ΣDDT in viscera are shown in Tables I and II respectively. Concentrations are reported on lipid weigh basis (LW).

Of the fifty-six visceras analyzed, forty-nine (88%) contained DDTs although o,p'-DDT was not detected in over half of wolves. The lowest concentrations of this investigated compound group were recorded in suprarenal and muscle although low concentrations were also recorded in liver.

Levels of ΣPCB ranged from ~7 mg kg^{-1} LW in liver and spleen to 16.9 mg kg^{-1} in muscle. These concentrations were lower than ΣDDT in spleen (3-

times lower) and kidney (4-times lower); muscle-and suprarenal contained levels of PCBs > 10 mg kg^{-1} considered indicative of toxicity and < 1 $mg.kg^{-1}$ for ΣDDT.

Table 1. Raw data before combining all the PCBs and all the DDTs (ND: not detecteable

	ΣDDTs	ΣPCBs
spleen 2	0.60	2.96
spleen 3	253.81	6.06
spleen 4	ND	3.31
spleen 5	0.11	2.09
spleen 6	ND	4.59
spleen 7	ND	3.14
spleen 8	0.60	6.12
spleen 9	0.59	3.32
spleen 10	0.28	5.31
spleen 11	0.36	3.00
spleen 12	20.41	44.51
liver 1	3.36	7.63
liver 2	3.08	5.07
liver 3	2.89	7.88
liver 4	1.51	3.22
liver 5	1.54	4.47
liver 6	12.28	11.68
liver 7	9.89	14.80
liver 8	6.43	10.77
liver 9	2.01	5.93
liver 10	2.25	6.09
liver 11	4.25	6.99
liver 12	1.25	4.43
muscle 1	0.86	15.59
muscle 3	0.08	17.07
muscle 5	0.11	11.93
muscle 6	0.30	16.00
muscle 7	ND	5.46
muscle 8	ND	6.48
muscle 9	0.16	9.87
muscle 10	ND	11.93
muscle 11	0.54	18.66
muscle 12	ND	9.08
kidney 1	3.22	5.36
kidney 2	ND	3.59
kidney 4	5.59	11.58
kidney 5	25.71	11.18
kidney 6	5.90	8.30
kidney 7	1.76	7.36
kidney 8	8.07	11.04
kidney 9	7.26	4.44
kidney 10	411.11	15.63
kidney 11	9.44	19.01
kidney 12	4.31	7.73
suprarenal 1	0.08	5.67
suprarenal 2	0.05	17.93
suprarenal 3	0.32	7.46
suprarenal 4	0.19	7.63
suprarenal 5	0.24	19.13
suprarenal 6	0.02	22.30
suprarenal 7	0.03	6.58
suprarenal 8	0.08	12.05
suprarenal 9	0.08	9.32
suprarenal 10	0.34	28.68
suprarenal 11	0.04	8.86
suprarenal 12	0.05	10.39

Table 2. CPCB levels, ΣDDT levels and ΣPCB/ΣDDT ratio in wolf viscera' (mg kg^{-1}LW)

	SPLEEN	LIVER	MUSCLE	KIDNEY	SUPRARENAL
ΣPCB	7.67	7.41	16.90	9.56	13.0
ΣDDT	25.16	4.23	0.21	43.85	0.14
ΣPCB/ΣDDT	0.30	1.75	82.04	0.23	92.2

Positive correlations between ΣPCB and ΣDDT were found in liver samples but there were no correlations in the other samples. It may due to the fact that the study sample size was fairly small.

Figures 1-3 summarize age class, sex and location of recovery of the twelve wolves as well as levels of ΣPCB and ΣDDT that they contained.

Levels of CPCB were similar between subadults and adults but ΣDDT concentrations were significantly higher in older age-group; so these last results have revealed an age-dependent accumulation. It may due to subadults have had less time to reach pharmacokinetic equilibrium with dietary residues (Elliot, 1996).

The low ΣPCB/ΣDDT ratio (max 1.87 in subadults) indeed suggest that these wolves are avoiding industrialized Galician zones while no significant differences appears to exist between sexes (ANOVA; all $p > 0.01$).

Levels of ΣPCB and ΣDDT in were similar in males and females.

In relation to sample sites, mean concentrations of ΣPCB are similar but although ΣDDT in wolves from Lugo did not differ significantly from those in Pontevedra, they were found ~6-times higher than samples from Orense.

It is difficult to compare the present results with other studies on the subject since little information is available, specially in Galician context. The lack of historical data and comparative studies to detect trends in contamination levels and effects of contamination in wolves makes it difficult to draw any conclusion in this respect.

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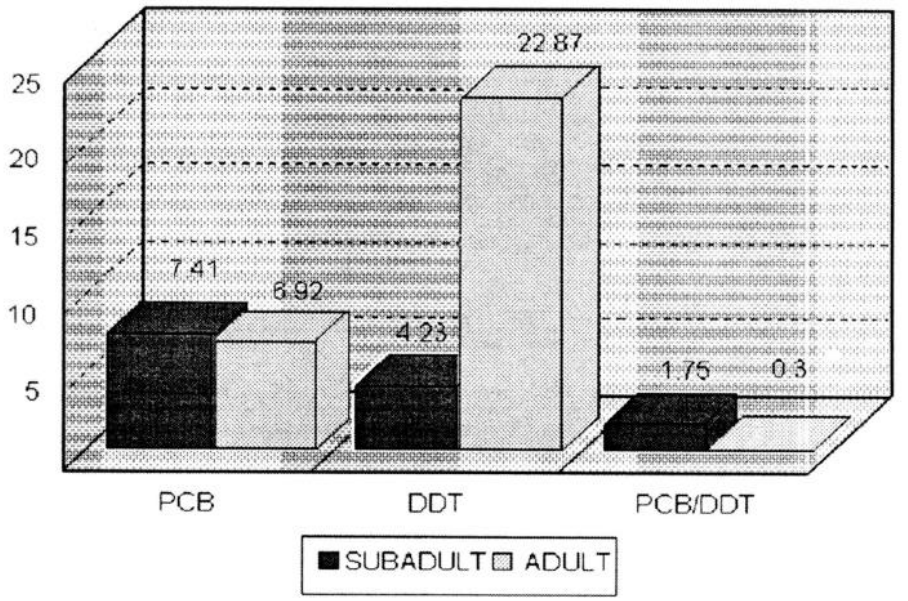

Figure 1. Levels of PCB, DDT and PCB/DDT ratio on age basis

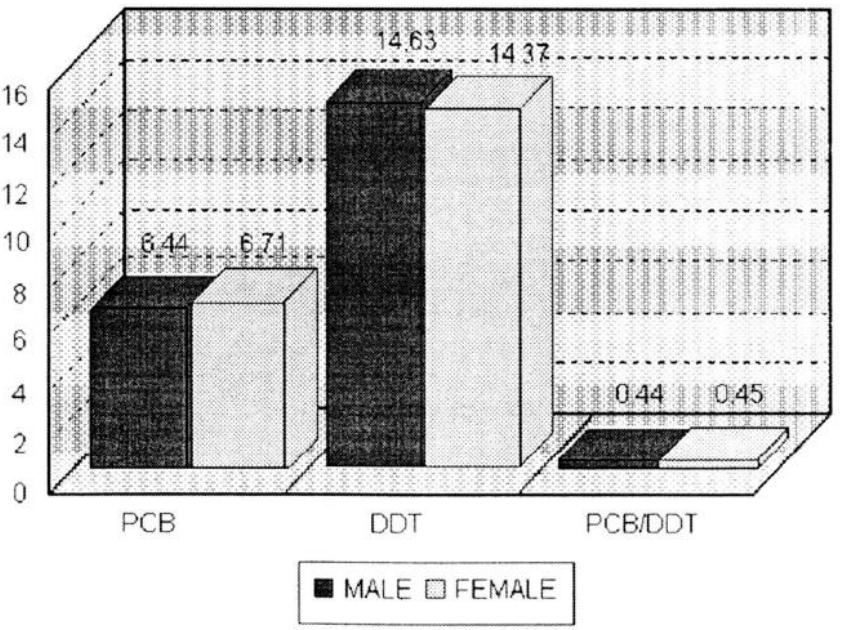

Figure 2. Levels of PCB, DDT and PCB/DDT ratio on sex basis

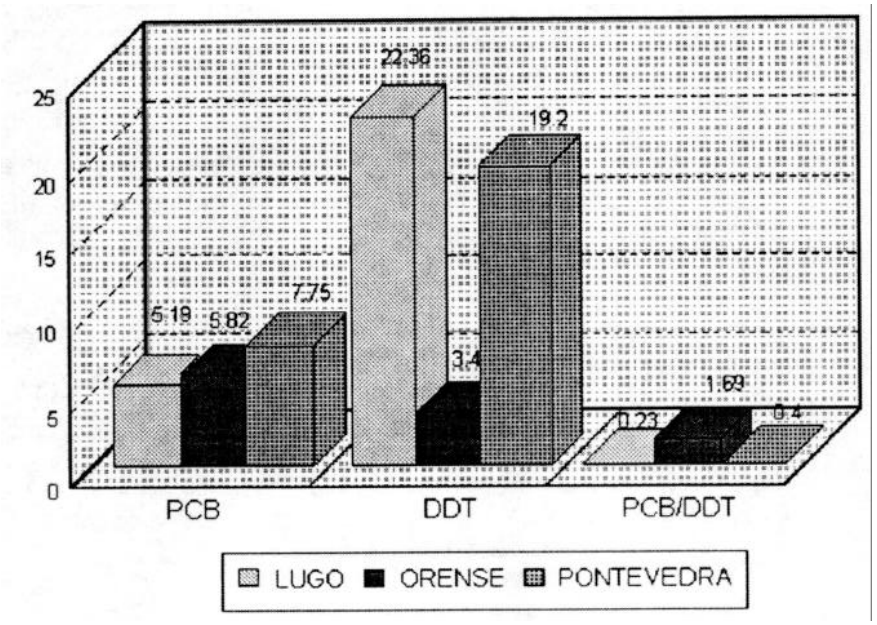

Figure 3. Levels of PCB, DDT and PCB/DDT ratio on area basis

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