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Organochlorine Residues in Hunted Wild Mallards in the Ebro Delta, Spain

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Determination of chemicals in tissues of some wild animal species can be of interest not only to biomonitoring selected environmental pollutants, but also for their public health implications if these animals are consumed by humans. This dual ecotoxicologic-human health interest is accomplished for example when mallard (*Anas platyrhynchos*) is the species analyzed.

Although it has an almost worldwide distribution, few studies have been published in the last 20 years using wild mallard as sentinel species of organochlorine (OC) pollutants either in Europe (Llorente et al. 1984) or in the United States (Botero et al. 1996; Cain 1981; Foley 1992; Prouty and Bunck 1986). However, farm-raised mallards released to the wild and later collected have been verified also as a successful means to monitor environmental contaminants (Custer et al. 1996; Gebauer and Weseloh 1993; Weseloh et al. 1994).

The purpose of the present study was to determine the main OC residues in three tissues of wild mallards captured in the Ebro Delta during the hunting season of 1991-92. The Ebro Delta is an alluvial plain situated in the NE of Spain, and supports one of the most important wildfowl populations of Europe. Mallard is the most abundant species in this wetland, with mean wintering populations ranging from 21,345 to 42,822 in the decade 1986 to 1995 *(A. Martínez-Vilalta, personal* comm.). Hunting of this and other waterfowl species are allowed during approximately five months per year, beginning in October, and it should be noted that mallard is the only waterfowl species that the Spanish laws permit to be sold to the public. For this reason, it is not uncommon that local restaurants include mallard in their menus.

MATERIALS AND METHODS

Forty mallards, captured by authorized hunters between October 1991 and January 1992, were included in this study. Weight and sex were determined, and different tissue samples and subsamples were collected and stored at -20°C until analysis. Some of them were used to determine the lead content in tissues, and results of this study have been previously published (Guitart et al. 1994). OC residues were analyzed in abdominal

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fat (n = 37; 3 animals were found without fat stores), liver (n = 40) and breast muscle (n = 40).

Samples (0.5 g of fat and 1.0 g of liver and muscle) were processed following previously published methods (Guitart et al. 1990), based on the homogenization of the samples with anhydrous sodium sulfate, ultrasonic extraction with n-hexane and clean-up with sulfuric acid. All the solvents and reagents used (pesticide or analytical grade) were purchased from Scharlau (Barcelona, Spain) and Merck (Darmstadt, Germany).

Analyses were carried out on a Perkin-Elmer AutoSystem equipped with a 30 m capillary column (Rtx-5 from Restek, Bellefonte, PA, USA) coupled to an electron capture detector (ECD) . Chromatographic conditions were optimized for the compounds under study, and have been previously published (Gutiérrez et al. 1997). Pesticides and polychlorinated biphenyls (PCBs) were localized using the PCBs #l and #209 (added to the samples from the beginning) as standards and using the following formula:

$$SU = 10{,}000 - \left[\left(1 + \frac{t_R\#1 - t_R\ X}{t_R\#209 - t_R\#1} \right) * 9{,}000 \right]$$

where SU are the *situation units,* and t_R the retention times (in minutes) of PCBs #l and #209, and of peak X under analysis. Taking into account that all the typical OCs elute between these PCB standards, all have SUs ranging from 1,000 to 10,000. With the appropriate BASIC software and after the injection of calibrated solutions of pesticides and PCBs, chromatographic peaks in mallard samples were easily identified with this system and quantified using PCB #209 as internal standard. Peak heights were used to calculate concentrations in samples (Gutiérrez et al. 1997).

Pesticide standards were purchased from Alltech (Deerfield, IL, USA) and Chem Service (West Chester, PA, USA). For PCBs, the Aroclor® 1248, 1254 and 1260 from Alltech were used. Individual peaks and their concentration in Aroclors, for which the correctednumbering of Ballsmitter and Zell was used (Guitart et al. 1993), were obtained from the data of Schulz et al. (1989).

Reproducibility (n = 5 to 15) and recovery (n = 5) of selected pesticides and PCBs were calculated, and were considered satisfactory; only recovery of hexachlorobenzene (HCB) was below 80% (74%). Anyway, no corrections were made for recoveries. Data are shown in terms of ng/g wet weight (WW), which has been consideredmore accurate than lipid basis for mallard tissues (Custer et al. 1996).

Data were analyzed with the SPSS/PC+ statistical program. Concentrationvalues of OCs were analyzed with the Kolmogorov-Smirnov test of normality. Sex differences were studied with the Student-t test with data transformed to logarithms (ln); correlations were also calculated using logarithms.

RESULTS AND DISCUSSION

Both pesticides and PCB concentrations showed a log-normal distribution, and this is why Table 1 shows data in terms of geometric means and 95% confidence intervals.

Results indicate that HCB and p,p'-DDE were the predominant pesticides found in all the analyzed samples. Others such as β-hexachlorocyclohexane (HCH), γ-HCH (lindane), aldrin, p,p'-DDD and p,p'-DDT, were observed in a reduced number of mallards (less than 30%;) but in all cases at concentrations 10 to 20 times lower than HCB and p,p'-DDE. For these reasons, no attempts were made to quantify these minor pollutants.

PCBs were also detected in the samples. The profile of PCB congeners found in mallard tissues resembled clearly that of the Aroclor® 1260 type. However, many minor PCB congeners seen in fat were below the limit of detection in liver and muscle samples, so it was decided to quantify only the major PCBs, i.e. #118+149, #153, #138+158+160, #l87, #180+193 and #170+190. For this reason, the ΣPCBs concentration displayed in Table 1 refers to the sum of these congeners. It should be noted that #118, #138, #153 and #180 (plus #28, #52 and #l0l, not detected in our samples) are those recommended for quantitative purposes following the criteria of International Monitoring Programs (Tuinstra et al. 1985).

Only for p,p'-DDE (Table 1) were significant differences observed between male and female concentrations. Due to this

Table 1. Geometric mean (and 95% confidence interval) of the main OC residues (ng/g WW) determined in tissues of male (n = 14) and female (n = 26) mallards (asterisks denotes significant differences between sexes [* = p<0.05, *** = p<0.005]).

♂	FAT	LIVER	MUSCLE
HCB	299 (183-487)	32.9 (21.7-49.8)	18.6 (13.6-25.3)
p,p'-DDE	466 (250-870)***	34.7 (22.9-52.5)*	18.7 (11.9-29.6)*
118+149	209 (84.5-515)	18.7 (10.9-31.8)	8.57 (4.91-15.0)
153	434 (282-669)	45.6 (27.7-75.2)	32.7 (23.3-45.9)
138+158+160	248 (161-382)	23.7 (13.9-40.3)	13.0 (9.57-17.8)
187	85.1 (52.9-137)	11.9 (5.95-23.6)	6.96 (3.97-12.2)
180+193	104 (54.9-197)	14.3 (6.37-31.9)	8.43 (4.37-16.3)
170+190	122 (76.0-194)	22.3 (14.2-35.1)	13.2 (7.92-21.9)
ΣPCBs	1310 (823-2086)	143 (86.0-237)	89.0 (61.8-128)
♀	FAT	LIVER	MUSCLE
HCB	252 (191-333)	30.7 (22.4-42.1)	13.1 (8.84-19.4)
p,p'-DDE	1180 (889-1566)***	60.3 (42.5-85.7)*	34.4 (25.7-46.0)*
118+149	214 (151-303)	16.2 (9.85-26.6)	10.4 (7.37-14.6)
153	398 (287-550)	47.0 (28.9-76.4)	25.5 (16.7-39.1)
138+158+160	202 (144-282)	27.4 (15.8-47.5)	10.5 (7.16-15.4)
187	84.5 (59.0-121)	12.8 (7.32-22.4)	6.03 (3.92-9.30)
180+193	119 (78.0-182)	11.3 (6.12-20.8)	6.43 (4.01-10.3)
170+190	114 (79.5-164)	18.4 (10.8-31.3)	8.92 (6.03-13.2)
ΣPCBs	1160 (828-1624)	153 (98.1-239)	76.4 (56.1-104)

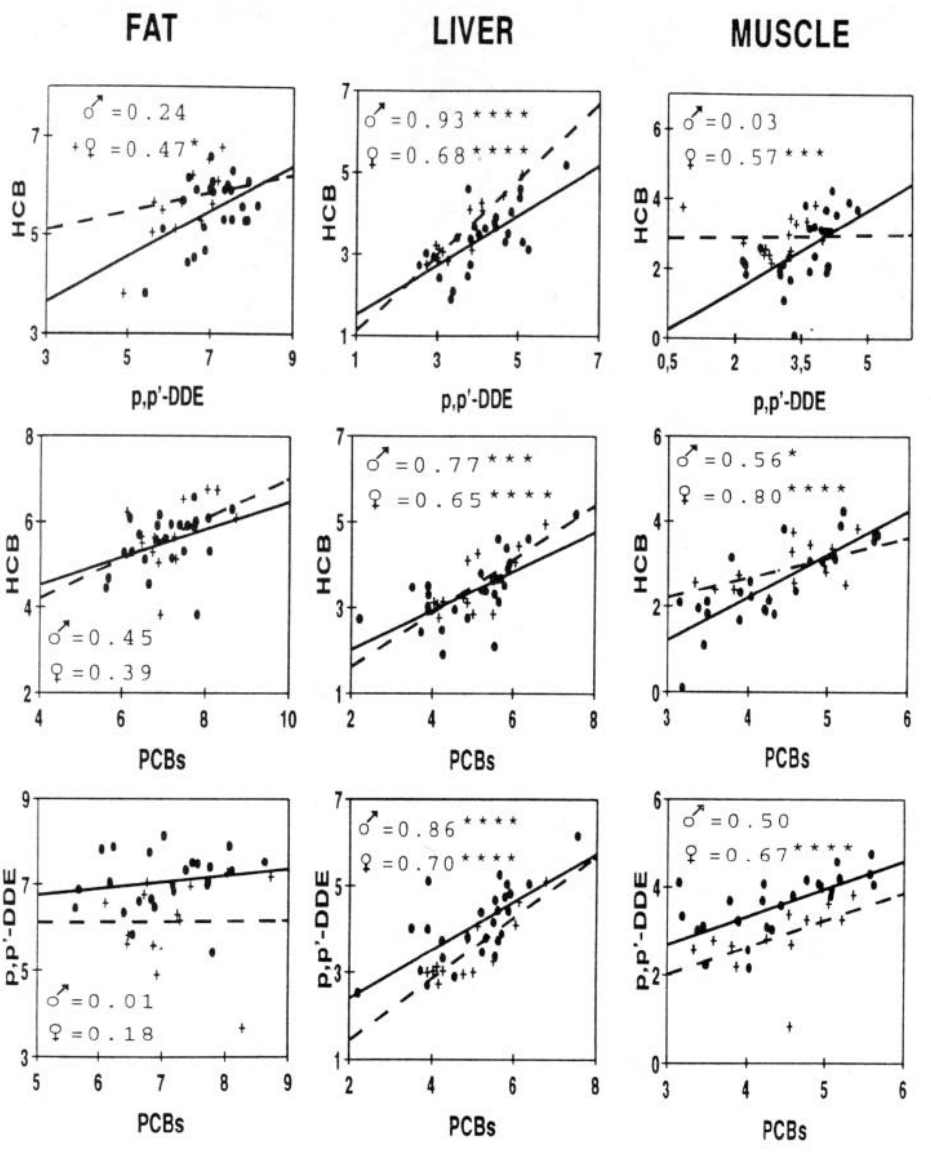

Figure 1. Relationships between HCB, p,p'DDE and PCBs concentrations in fat (n = 37) liver (n = 40) and muscle (n = 40) of male (+) and female (●) wild mallards from the Ebro Delta (* = p<0.05; ** = p<0.01; *** = p<0.005; **** = p<0.00l).

fact, Figure 1, which shows the correlation of concentrations in fat, liver and muscle between the three main types of OC residues, and Figure 2, which shows the correlation of concentrations for HCB, p,p'-DDE and ΣPCBs between tissues, are displayed separated by sexes.

The finding that HCB, p,p'-DDE and PCBs were the main residues inmallard samples was not unexpected, taking into account the

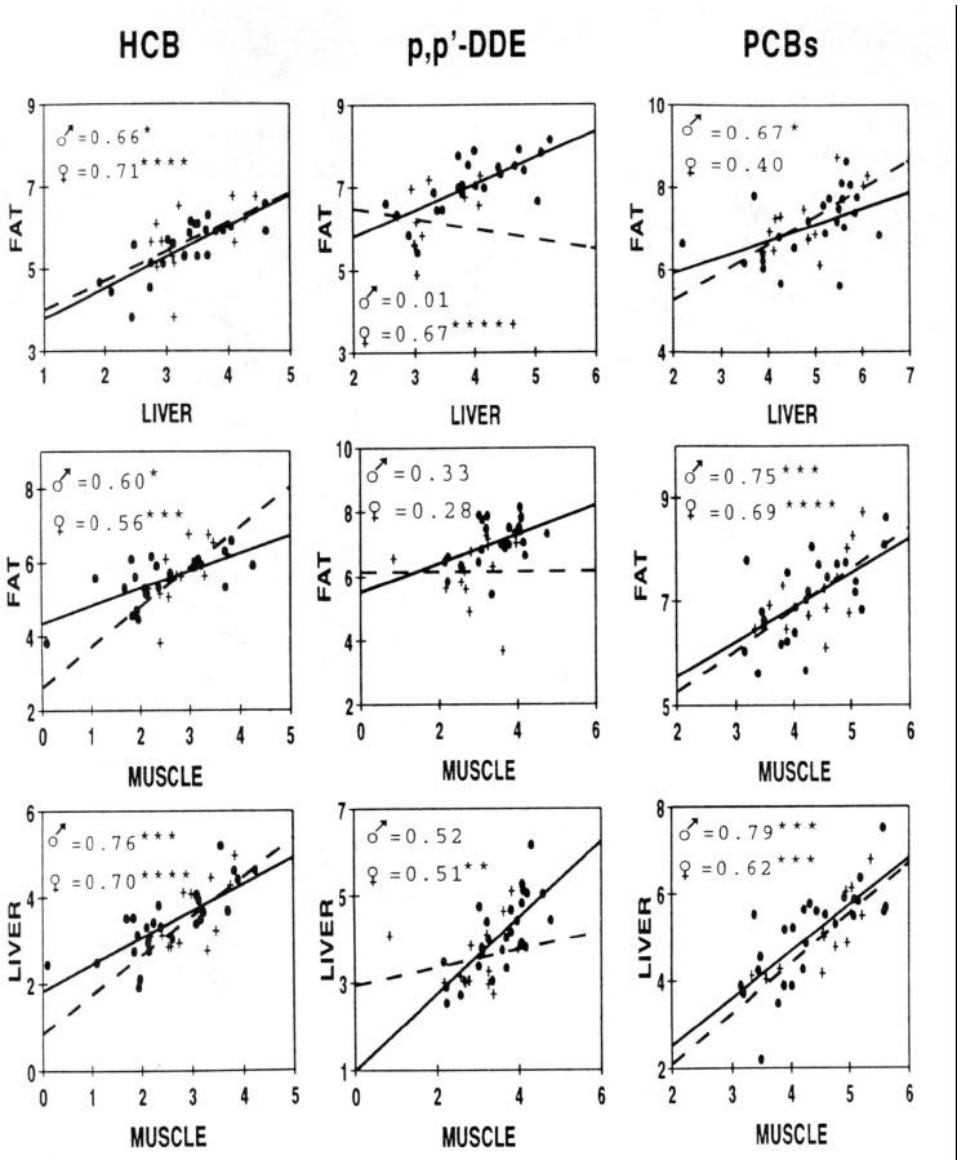

Figure 2. Relationships between organochlorine concentrations in the three tissues of male (+) and female (●) wild mallards from the Ebro Delta (* = p<0.05; ** = p<0.01; *** = p<0.005; **** = p<0.001).

traditional agricultural activity in the Ebro Delta but especially the estimated high inputs of these three types of compounds by the Ebro River (Cid et al. 1990). Mallards may be exposed to this waterborne contaminants not only through the diet based mainly on seeds, plants and invertebrates, but also while drinking, swimming, wading, preening or ingesting sediments. The source of these pollutants is more or less reflected in Figure 1, in which can be observed a general

better correlation between HCB and PCBs (source the Ebro River) than that of both with p,p'-DDE (source the Ebro River but also the older use *in situ* in the Ebro Delta of p,p'-DDT). Another interesting observation derived from Figure 1 is that, once absorbed, these lipophilic pollutants seemed to remain at levels more stable in the liver than in the other tissues. This is probably due to the high metabolic rate of breast muscle (the major and most active muscle in birds) and fluctuations in lipid stores (highly variable in content throughout the year for wildlife) (Furness 1993). In this sense, only the liver seems to be a proper alternative to other methods based on the analysis of mallard wings, which have been used for years in the United States to monitor OC pollutants in nationwide programs (Cain 1981; Prouty and Bunck 1986).

The ratio DDE/PCBs suggest a predominating industrial contamination in the zone of the Ebro Delta, which agree with results from recent studies (González et al. 1991; Pastor et al. 1996) and confirms the inversion produced since the late 70's in Anatidae captured in this zone, when this ratio was 21 (Llorente et al. 1984; 1987). The dominance of the Aroclor® 1260 type of PCB congeners in the Ebro Delta agrees with previous reports in mallards (Llorente et al. 1984) or other birds (Gonzalez et al, 1991; Gutiérrez et al. 1997; Pastor et al. 1996) from this and other nearby Spanish Mediterranean wetlands.

Although methodology and expression of results differs and thus direct comparisons are difficult to establish, it seems that PCB concentrations in mallards wintering in the Ebro Delta (Llorente et al. 1984) have been maintained more or less stable for the last l0-12 years, while those of p,p'-DDE have slightly declined. Comparing data from the Ebro Delta with published data for wild mallards from other zones of the world, in general they are in the order or are even lower than those observed in different areas of New York State (Foley 1992) and Wisconsin (Botero et al. 1996), and are similar (Gebauer and Weseloh 1993; Weseloh et al. 1994) or markedly lower (Custer et al. 1996) when compared with farm-raised animals released to the wild as sentinels.

A surprising finding was to detect that females contained more p,p'-DDE than males do. At this time we can not give a reasonable explanation for this fact, as initially one could expect no differences or levels higher in male than in female birds (Furness 1993). Also remarkable, distribution of HCB and PCBs between tissues seemed to be more homogeneous (Figure 2) than that of p,p'-DDE, which showed poorer correlation indexes.

A final consideration referred to the potential hazard for human consumers. Some of them at the Ebro Delta, and especially hunters and their families, may eat dozens of mallards during the winter season. Taking into account the mean weight of intraperitoneal fat (16 g), liver (19 g) and breast muscle (200 g) calculated in our laboratory as standard values for mallards of 1084 g body weight, the consumption of these tissues of one of these birds represents the ingestion of approximately 8, 19.5 and 39 µg of HCB, p,p'-DDE and PCBs,

respectively. If skin, muscle legs or other parts are also consumed, these figures maybe something higher. These intakes of HCB, p,p'-DDE and PCBs are probably harmless, but they greatly exceed the estimated normal daily intake (Herrera et al. 1996; Kimbrough 1995) only from a single dietary source.

Considering also that about 27% of mallards from the Ebro Delta have been found to be lead poisoned and thus can contain medium to high levels of lead (Guitart et al. 1994), the human health recommendation derived is obvious: regular consumption of wild mallards (and other waterfowl) from the Ebro Delta should be discouraged, and when done, birds should be eviscerated and the fat and skin removed before cooking and consuming. Similar guidances to this have been done before to waterfowl hunters of other polluted wetlands around the world (Botero et al. 1996; Custer et al. 1996; Foley 1992).

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