DISTRIBUTION PATTERN OF ORGANOCHLORINE COMPOUNDS IN FIVE TISSUES OF BUBULCUS IBIS NESTLINGS (AVES, ARDEIDAE) FROM THE EBRO DELTA, NORTHEAST SPAIN

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VIE ET MILIEU, 1984, 34 (1) : 21-26

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X. RUIZ, G.A. LLORENTE and J. NADAL

Càtedra de Zoologia Vertebrats,
Facultad de Biologia de la Universitat de Barcelona,
Av. Diagonal, 645, 08071 Barcelona, Spain

PCBs
PESTICIDES
HÉRON GARDE-BŒUF
DELTA DE L'EBRE
CROISSANCE

RÉSUMÉ. — Analyse du point de vue de la contamination par des composés organochlorés d'une série d'échantillons formés par le pool de subéchantillons de tissus provenant de sept poussins de Héron garde-bœuf *(B. ibis)* du delta de l'Ebre, âgés de 10 à 14 jours. Les tissus analysés sont les suivants : musculature de vol, foie, graisse, rein et cerveau. Les résultats montrent que la distribution quantitative et qualitative des composés organochlorés n'est pas homogène et doit être interprétée en fonction de la dynamique physiologique générale de l'organisme et du rôle spécifique que joue chacun des tissus dans celle-ci, ainsi que des affinités lipidiques particulières des composés organochlorés.

PCBs
PESTICIDES
CATTLE EGRET
EBRO DELTA
GROWTH

ABSTRACT. — Samples obtained from a pool of tissue and organ subsamples of 7 cattle egret chicks from the Ebro Delta have been analysed in search of organochlorine compounds. All specimens were between 10 and 14 days old. Tissues pooled were : flight muscle, liver, adipose tissue, kidneys and brain. Results show that the qualitative and quantitative distribution of the organochlorine compounds are not homogeneous. This should be interpreted according to the overall physiological dynamics of the organism and the specific role of each tissue, together with the peculiar affinity that the different pollutants might present.

INTRODUCTION

Pollutant residue levels in wildlife are of obvious interest in themselves but, in the present study, the interest of the survey lies in the attempt to analyse the significance of the concentrations found in subsamples of tissues or organs clearly affected by biological factors. We should point out that, while great attention has been paid to the variability in results that analytical procedures in themselves may introduce (Holden, 1970, 1973) only occasionally has the intrinsic variability of the tissue sample — due to the physiological conditions of the species at the moment of sampling — been taken into account.

Llorente *et al.* (1982) have shown the influence that ecological factors such as the trophic type of the species have on pollutant levels in such special tissues as the nervous type. The present survey aims to demonstrate the influence of global physiological dynamics on the distribution pattern of organochlorine residue levels in five tissues (those most commonly found in the literature) both from the quantitative and qualitative standpoint.

It should be borne in mind that the tissue or organ subsamples are the basic elements for interpreting changes over a period of time or for comparing pollutant residue levels among different vertebrate populations. To ignore the fluctuations in the tissues or organs that may be caused by the special physiological dynamics revealed by the species at the moment of capture, or the specific role that the said tissues are carrying out regarding the characteristic lipid metabolism of such dynamics, is almost the same as saying that the comparison is not valid.

This line of thinking is not new, since Holden (1975) had already expressed his concern about the fact that most surveys are carried out on the basis of inadequate sampling, and that the intrinsic variability thus introduced in the sample disqualifies the results for comparative purposes and, ultimately, their proper evaluation.

For this reason, the present survey has been carried out on a sample within an overall physiological framework which clearly affects the fat metabolism in one direction : the mobilization of lipidic compounds to supply the energy requirements of the newly formed or developing tissues.

MATERIAL AND METHODS

Since 1977, the cattle egret has been an all-year round resident species in the Ebro Delta. The samples used in the present survey come from seven chicks whose morphometrics are shown in table IA. They were caught in their nest in July 1981, at the nesting colony on the Aufacada lagoon, one of the most important nesting places for this species on the Ebro Delta (Ruiz *et al.*, 1982).

From the growth studies carried out on this population (Ruiz, 1982), we can deduce from the morphometrics of the sampled chicks that their age falls within the second week of life, ranging from 10 to 14 days, at which time such semialtricial birds are still strictly altricial or nidicolous.

The specimens were sacrificed at the colony site and immediately sent to the laboratory, where they were deep frozen at – 18 °C and preserved in this state until the time of analysis. Following the proposal of Bernhard (1976) samples of the following tissues were pooled : flight muscle, liver, adipose tissue, kidneys and brain. We have followed the analysis procedures described by Holden & Marsden (1969) with the modifications specified by Llorente *et al.* (1983), and Aguilar (1983) in those aspects concerning PCB-DDT interference.

The pesticides analysed are the following : cyclodienies (Heptachlor, Heptachlor epoxide, Aldrin and Dieldrin); DDTs (pp'DDE, pp'TDE (DDD), pp'DDT and all of them as the sum of DDTs); PCBs (Aroclor 1254 and 1260).

The lowest level of detection used was 10 ppb for cyclodienies and pp'DDE; 20 ppb for pp'TDE and pp'DDT and 80 ppb for PCBs. In tables, N.D. means not detected. Analysis procedures took place on a series 2700 Varian Aerograph apparatus, furnished with a Ni 63 E.C.D., a column 2 m long and 1/4 inch internal diameter with 4 % DC-200 and 6 % QF-1 on Chromosorb WAWDMCS of 80-100 mesh, and a Varian CDS 111 computer of areas.

Table I. — A, Morphometrics of *B. ibis* nestlings caught in the Aufacada Lagoon. W = weight in grams; L.C. = Culmen length, in mm ; L.T. = Tarsus length; L.M.T. = Mid Toe length; L.W. = Wing length; B, organochlorine compound levels in ppm, given on a fat basis, obtained in the analysis of *B. ibis* nestling subsamples. Figures given correspond to the averages of all the samples analysed. N.D. = not detected.

A	W.	L.C.	L.T.	L.MT.	L.W.
1	195.-	26.6	36.9	47.-	50.-
2	211.-	29.7	42.9	52.-	60.-
3	183.-	25.85	36.-	46.-	51.-
4	252.8	39.3	45.-	57.5	77.-
5	256.6	30.05	46.5	58.-	69.5
6	266.-	32.6	47.-	58.-	77.-
7	198.3	26.-	35.8	49.5	51.-

B Organochlorine compound	Flight muscle	Liver	Adipose tissue	Kidney	Brain
Heptachlor	N.D.	0.25	0.02	0.02	0.02
Aldrin	N.D.	0.01	0.04	0.02	N.D.
H. Epoxide	N.D.	N.D.	0.16	0.15	N.D.
Dieldrin	N.D.	N.D.	0.19	0.30	N.D.
Cyclodienics	–	0.26	0.41	0.49	0.02
pp'DDE	1.51	3.52	3.07	19.19	0.61
pp'TDE	–	–	0.28	1.50	–
pp'DDT	–	–	0.27	1.85	–
DDTs	1.51	3.52	3.62	22.54	0.61
PCBs	–	3.31	1.35	9.61	–
% Cyclodienics	–	3.66	7.56	1.50	2.85
% DDTs	100	49.64	67.47	69.05	97.14
% PCBs	–	46.68	25.05	29.44	–
Total in PPMs	1.51	7.09	5.38	32.64	0.63
% Lipid extraction	1.26	2.24	50.77	1.77	2.91

RESULTS

The results obtained in the analysis are given in table IB, the levels of each pesticide and its structural group being expressed in terms of ppm fat basis. The figures given correspond to the averages of all the samples analysed from each tissue.

DDTs are the most ubiquitous group and, quantitatively, they represent the most important part of the total organochlorine pollutant load in all of the five tissues. Cyclodienes are not present in the flight muscle and their percentage is higher in the adipose tissue than elsewhere, although it is in the kidneys that such pesticides reach their highest absolute levels. Moreover, cyclodienes are, as a whole, the components with least quantitative incidence in the total load of organochlorines detected.

PCBs, all matching the Aroclor 1260 pattern, reveal higher levels but they are only present in liver, adipose tissue and kidneys.

The percentage incidence of each structural group in the total load of organochlorine pollutants for each tissue is represented in figure 1. In figure 2, the distribution of organochlorines in the tissues analysed is shown where it is possible to compare the relative pollutant concentrations, expressed in lipid basis, that each one of them accumulates. Thus, the kidneys are the tissues which show the highest level of contamination by organochlorines, followed by the liver, the adipose tissue, the muscle and brain.

DISCUSSION AND CONCLUSIONS

Both cyclodienes and DDTs are fitosanitary products still used in the Ebro Delta zone, proved

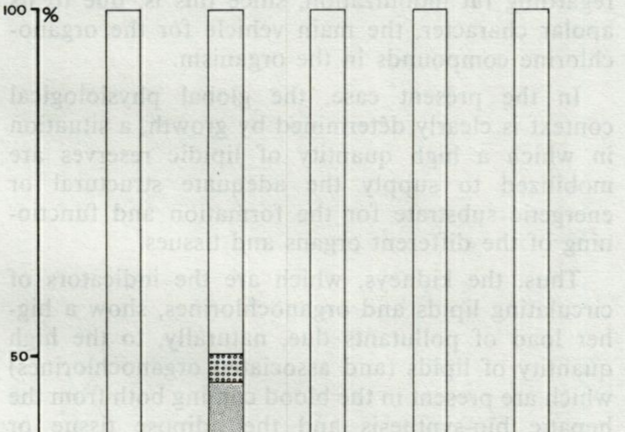

Fig. 1. — Percentage incidence of each structural group in the total load of organochlorine pollutants for each subsample.

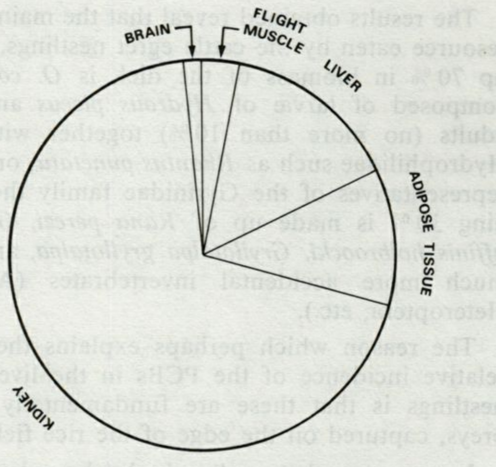

Fig. 2. — Percentage distribution of total ppm in the tissues analysed showing the relative pollutant concentrations accumulated by each one.

by the presence, although in small quantities, of insecticides such as Heptachlor, Aldrin, and pp'DDT in the samples. The experimental evidence of other studies carried out in this area, both on eggs (Nadal, 1981; Ruiz *et al.*, 1983) and bird tissues (Ruiz *et al.*, 1979; Ruiz, 1982; Ruiz *et al.*, 1982; Ruiz *et al.*, in press; Llorente *et al.*, 1982; Llorente *et al.*, 1983) as well as personal observation, are concomitant with a recent use of these products in the agricultural dynamics of the area, mainly rice, fruit, and vegetable growing (Ruiz *et al.*, 1981).

The PCB residues come mainly from the industrial activity located up-river on the course of the Ebro, spills from which are carried down-river towards the Delta.

The incidence of these compounds in the Delta's birds and wildlife depends to a large extent on their degree of ichthyophagy (Ohlendorf *et al.*, 1979), which in the case of the cattle egret favours a greater pressure from the DDTs because of its manifestly landbased habits as compared to other Ardeidae (Ruiz 1982; Ruiz *et al.*, 1983).

It is also worth considering whether the residues found in the cattle egret nestlings come from the lipidic reserves in the egg, or else correspond to new entries from their food intake.

In general the residues of organochlorine compounds found in the tissues of *B. ibis* nestlings correspond perfectly with the residues in eggs, pp'DDE being the most ubiquitous together with the PCBs. The DDT/PCB ratio favours the former group of compounds both in eggs and in nestlings and in both types of sample the cyclodienes make up the residues with least quantitative incidence. The food habits of the cattle egret nestlings has been studied on the basis of the gastric contents of the same specimens caught in order to carry out this study (Ruiz, 1982; Ruiz & Llorente, in press).

The results obtained reveal that the main trophic resource eaten by the cattle egret nestlings, making up 70 % in biomass of the diet, is *O. coleoptera,* composed of larvæ of *Hydrous piceus* and some adults (no more than 10 %) together with other Hydrophilidae such as *Rhantus punctatus* or smaller representatives of the Gyrinidae family the remaining 30 % is made up of *Rana perezi, Gambusia affinis holbroocki, Gryllotalpa gryllotalpa,* and some much more accidental invertebrates (Araneida, Heteroptera, etc.).

The reason which perhaps explains the greater relative incidence of the PCBs in the liver of the nestlings is that these are fundamentally aquatic preys, captured on the edge of the rice fields.

In any case the results obtained prove that the distribution of organochlorine compounds among the five tissues analysed is not homogeneous, either quantitatively or qualitatively.

From the quantitative standpoint, the behaviour of PCBs should be stressed, since they are only present in liver, fat and kidneys, but are absent in the flight muscle and brain. Recently, Schneider (1982) has shown that PCBs do not display the same behaviour in relation to the different kinds of lipids which are usually grouped under the heading of « fat » in the literature concerning pollution. Thus, PCBs show a clear affinity for metabolic lipids, while structural ones have a lesser capacity for containing them in solution. Possibly this should be related to the presence in the structural lipids of polar radicals and to the strong lipophilic character of the PCBs, even when compared with the organochlorine compounds.

Taking into account the above considerations, the presence of PCBs in liver and adipose tissue is reasonable, because the former has been found to be responsible for 95 % of the fat bio-synthesis in the avian body (Hazelwood, 1972; Griminger, 1976) and the latter is the main storing site for metabolic lipids. In the case of the kidneys the situation is different, since they are considered to be representative of the fat levels circulating in the blood (and then of the associated organochlorine pollutants) (Walker, 1975). The blood circulating lipids are both metabolic and structural whether they come from the liver, the adipose tissue or the ingested food, and this explains the presence of PCBs in the kidneys.

Following this line, the absence of such compounds in the brain is easily explained, because this tissue is essentially made up of structural lipids. The flight muscle needs more extensive considerations, since it can usually be considered one of the most important consumers of metabolic lipids in the avian body, in order to obtain from their oxidation the energy required for the development of flight.

In the present case the absence of PCBs should be concurrent with the absence of this kind of lipidic molecules. This can only be explained by considering that the samples on which we have worked came from chicks between 10 and 14 days old. The Cattle egret *(Bubulcus ibis)* is a semialtricial bird, which means that it displays strictly altricial behaviour during the first stages of its life (2 weeks) and behaves later as an altricial "sensu lato". Thus, it escapes from the nest at the least sign of alarm or even behaves in this way spontaneously, without any clear reason, although it comes back to the nest to be fed by the parents until the moment when it attains its independence at the end of its growing period (50-60 days) (Siegfried, 1972; Weber, 1975; pers. obs.).

During its strictly altricial stage, to which period the sample corresponds, the flight muscle is only poorly developed, being at a growing phase in which it is not yet functional and has a lipid supply based mainly on structural lipids in order to constitute the muscular cell membranes of which it is made up.

Looking now at the unequal quantitative distribution of organochlorines in the tissues, it is observed that kidneys are those most heavily polluted, followed by liver, adipose tissue, muscle ands brains. In order to understand such results, it is important to take into account the physiological context of the sampled specimens, as well as the role performed by each one of the tissues considered in relation to global physiological dynamics and, most important, regarding fat mobilization, since this is, due to its apolar character, the main vehicle for the organochlorine compounds in the organism.

In the present case, the global physiological context is clearly determined by growth, a situation in which a high quantity of lipidic reserves are mobilized to supply the adequate structural or energetic substrate for the formation and functioning of the different organs and tissues.

Thus, the kidneys, which are the indicators of circulating lipids and organochlorines, show a higher load of pollutants due, naturally, to the high quantity of lipids (and associated organochlorines) which are present in the blood coming both from the hepatic bio-synthesis and the adipose tissue or directly absorbed from ingested food.

Regarding the liver, Ruiz *et al.*, (1982) showed, in an adult population of cattle egrets *(B. ibis)* from the Ebro Delta, that a significant relationship existed between the lipidic concentration (measured through the extractable fat percentage) and the organochlorine residue levels detected in it, and that the higher the amount of lipids, the higher the levels of pollutants detected. From this it is reasonable to deduce that this organ holds the second highest pollutant load, because in such a physiologically stressed stage as the growing phase, it is necessary to synthetize a lot of lipids, therefore the liver becomes very active and it contains a high quantity of them.

The adipose tissue is, naturally, the most suitable kind for containing the organochlorine compounds dissolved in it and its position, therefore, in relation to the muscle or brain is logical. Nevertheless, it is significant that it is less contamined than the liver or the kidneys. In relation to this we must bear in mind on the one hand that a high proportion of the lipids elaborated by the liver — and thus the associated pollutants — are directly used, never managing to accumulate in the reserve tissues; on the other hand, the relative proportion of dissolvent (fat) is much higher in this tissue than in the others, a factor which will also account for the levels observed.

The flight muscle is still an immature tissue, and it should present a higher percentage of water in its constitution, related to its degree of functional maturity (Ricklefs, 1979; Ricklefs & White, 1981). Moreover, its lipidic components should be essentially structural, and this implies some discrimination in the capacity for containing organochlorines, especially the PCBs (Schneider, 1982). This should probably also affect DDTs and cyclodienecs, although the compounds of these groups, and specially the DDTs, seem to show much more ubiquitous behaviour. Up to now, no information similar to that obtained by Schneider (1982), for the PCBs is available for the remaining compounds. In any case closer and more specific studies will be required for a complete understanding of this phenomenon.

The levels in brains are usually much lower than in the rest of the body. Such a pecularity has permitted consideration of this organ as the most reliable indicator of mortality for organochlorine toxicity (Stickel, 1973), since it seems to be, by nature, protected against the accumulation of these pollutants. However, for one particular contaminant, the dieldrin, and for one avian family, the ardeidae, Ohlendorf *et al.* (1979, 1981) have been able to establish a significant relationship between the levels in this organ and the rest of the bird's body while for other pollutants this has not been possible (Brown, 1978 and the references given by this author).

The existence of an effective hematoencephalic barrier is open to discussion since Hunzicker & Hazelwood (1970) and Hazelwood (1972) showed that it does not exist in chicks of the domestic hen while in adults it can be considered a porous barrier, enabling the transfer of high molecular weight substances (molecules similar to insulin).

In conclusion, the brain's lipidic composition should considerably affect the capacity for association which the different organochlorine compounds display, and this results in an unequal representation of structural groups, essentially excluding PCBs and possibly making the entrance of DDTs and cyclodienics difficult. These considerations, together with the relatively low lipidic metabolism of this organ means that the possibilities of accumulation are lower here than in the rest of the avian body. From the above said, it is commonly found that, as is our case, the brain is the organ least affected by organochlorine compounds although it proves the most sensitive to them, since one of the main toxic effects of these compounds is axonic deregulation, which produces well known effects such as uncontrolled shaking followed by passivity and apathy.

Finally it is worth noting that the distribution of pollutant levels is related to the overall physiological dynamics of the specimen sampled. In this sense, the levels of contaminants from avian or mammalian tissue subsamples should be considered cautiously, since they are only suitable for comparison when proceeding from specimens with the same (or very similar) physiological characteristics. This should be specially stressed, since researchers rarely plan their sampling from such a standpoint and biological and physiological data are often lacking in their publications.

ACKNOWLEDGEMENTS. We would like to express our acknowledgement to Mr. A. Aguilar for the translation and useful comments, and to Mr. J. Beattie from the British Institute of Barcelona, for his revision of the final English text.

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