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ORGANOCHLORINE RESIDUES IN NORMAL AND LEAD POISONED GREATER FLAMINGOS: RELATIONSHIPS WITH THE FATTY ACID COMPOSITION

Keywords: Organochlorine residues, lead poisoning, *Phoenicopterus ruber*, liver, biomarkers, fatty acids.

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ABSTRACT

Livers from 65 greater flamingos (*Phoenicopterus ruber roseus*) collected in three Spanish wetlands were analyzed for organochlorine (OC) residues and fatty acid (FA) composition. Some of these birds were lead poisoned (n=52), as determined in a previous study published elsewhere. The effects of OC levels and normal/abnormal lead levels on the FA composition were analyzed with ANOVA tests in order to establish possible influences among them. High lead concentration was found to increase the relative proportions of 16:0 and 18:1 n-9, and to decrease the proportions of 18:0, 18:2 n-6 and 20:3 n-6. Main OC residues in liver samples were highly chlorinated PCBs (range 211-17,289 ng/g wet weight) and pp'-DDE (11-10,268 ng/g WW), and it was determined that PCBs influenced positively the relative proportions of 18:0, 20:4 n-6 and 22:4 n-6, and negatively the 16:0 and 16:1 n-7 proportions. These results are discussed and compared with previously published data on the use of FAs as biomarkers of lead and OC exposure.

INTRODUCTION

Greater flamingos (*Phoenicopterus ruber roseus*) are typical inhabitants of the Palearctic zone[1], and they are frequently observed in high numbers in some Spanish wetlands[2]. As protected species in many countries, few studies using this emblematic bird have been published reporting either biochemical[3] or ecotoxicological data[4,5]. For this reason, when an outbreak of lead poisoning killed approximately one hundred greater flamingos in two close important wetlands in the East of Spain[6], it offered an exceptional opportunity to carry out interesting analyses using a highly significant number of individuals of this species.

As the fatty acid (FA) composition as possible biomarker of organochlorine (OC) pollutant exposure in striped dolphins (*Stenella coeruleoalba*) had been explored recently[7], it was considered interesting to repeat the same protocol using now this avian species as the animal model. To our knowledge, detailed studies on liver FA composition in greater flamingos have not been published before, nor any on the possible OC/FA relationships. Moreover, lead liver levels were determined in all these animals as a part of a broad study carried out in Spanish wetlands[6,8], and we also used these data from free-ranging greater flamingos in order to confirm or reject the effect of lead on the liver FAs which has been described in chicks (*Gallus domesticus*) studied under laboratory conditions[9].

MATERIALS AND METHODS

Animals

The number of greater flamingos examined in this study was 65; 57 of them were collected in El Fondo Natural Park and Salinas de Santa Pola Natural Park, two very close wetlands of the province of Alacant (E Spain), while the other 8 were from the Ebre Delta Natural Park, province of Tarragona (NE Spain). Sex was determined upon the examination of the gonads at necropsy (30 males, 22 females, 13 unknown), and age was determined by plumage characteristics (33 unmature, 23 adult, 9 unknown). The 57 greater flamingos from Alacant were collected during 1992-94, and 52 died by lead poisoning (liver Pb levels >75 μ/g DW) due to ingestion of lead shot; the other 5 birds died by other causes (liver Pb ≤7.0 μg/g DW)[6]. The 8 birds of Tarragona were collected between 1993 and 1994, and died due to traumatisms caused by illegal shooting (liver Pb <5.0 μg/g DW). All the carcasses included in the present protocol were well preserved, and the entire liver was withdrawed and stored in polypropylene containers at -20°C until analysis.

Solvents and reagents

All the solvents and reagents were pesticide residue grade or similar, and were purchased from Merck (Darmstad, Germany) and Panreac (Montcada i Reixac, Spain). Quantitative solutions of Aroclor 1260 were obtained from Alltech (Deerfield, IL, USA), and pure standards of several individual polychlorinated biphenyls (PCB) congeners and pesticides were purchased from Promochem (Wesel, Germany). Free FA and FA methyl esters (FAMEs) were obtained from Sigma (St Louis, MO, USA), Supelco (Bellefonte, PA, USA) and TCI (Tokyo, Japan).

Analysis of liver samples

The whole livers were homogenized and aliquot parts were used for lead[6], and for FAMEs and OCs determinations. Analyses of FAs were carried out as described previously[7], using the 13:0 FA as internal standard.

For OC analysis, samples were concentrated to approximately 100 μl (never to dryness) under a gentle stream of N_2 prior to injection in the gas chromatograph. Samples were analyzed on a Perkin-Elmer AutoSystem, equipped with a Rtx-5 (crossbonded 95% dimethyl-5% diphenyl polysiloxane) open tubular column of 30 m, 0.53 mm ID and 1.0 μm film thickness (Restek, Bellefonte, PA, USA), coupled to an electron capture detector (ECD). The temperature program was: 145°C initial, then run to 297°C at a rate of 2.5°C/min. Mobile phase (He) was set at a linear velocity of 25 cm/s. Make-up gas (N_2) was adjusted at a flow of 60 ml/min; injector and detector temperatures were 320 and 330°C. In the case of PCBs, the corrected Ballschmiter and Zell nomenclature system[10] has been utilized throughout this paper, and for calibration purposes the quantitative Aroclor 1260 composition reported by Schulz et al.[11] was used. The retention times of PCBs #1 and #209, added as internal standards, and pp'-DDE, were used to localize all the other OC peaks; PCB #209 was used for quantitative purposes. Recovery of the method, based on the major eight Aroclor 1260 peaks (n = 3) and eight acid resistant pesticides (n = 3), ranged from 50.75% (β-hexachlorocyclohexane) to 91.35% (PCBs # 202+171+151).

Statistics

Concentration and percentage values for FAMEs and OCs are expressed as arithmetic means; some OCs concentrations were found to be log normal distributed, but arithmetic means were maintained in order to compare with values reported before[4,5]. Statistical comparison between sexes and ages of the FAMEs and OCs were based on a two-way ANOVA test, and comparison between localities (El Fondo-Santa Pola vs. Ebre Delta) was based on a Student t-test (two-tailed). In order to evaluate the relationships between OCs concentration (ΣPCBs [sum of all PCBs] and/or ΣDDTs [sum of pp'-DDE, pp'-DDT and pp'-DDD], both transformed to logarithms) and metal poisoning (lead poisoned-non lead poisoned birds) with FAMEs composition, ANOVA tests were used; calculations were carried out with the statistical package SPSS/PC+ on an IBM computer.

RESULTS AND DISCUSSION

The methodology used in the present study do not differ significantly from that used in our previous work with striped dolphins[7]. The only remarkable changes consisted in the substitution of the FA used as internal standard (13:0 instead of 12:0), and a new chromatographic column (of the same stationary phase) for OCs analysis. However, this time the recoveries for OCs were not as good as those found in dolphin tissues, and this could be initially explained by the lower concentration of OCs in livers of greater flamingos, which forced to a concentration of the volumes of the samples before

GC/ECD analysis. Although care was taken in order to avoid concentration to dryness, the recovery of some of the more volatile OCs (mainly β-hexachlorocyclohexane) were in this study impaired if compared with those reported in our previous work.

Table 1 shows the levels of OC residues found in livers of greater flamingos. All these birds showed measurable levels of pp'-DDE, which represented 84% of ΣDDTs (823 ± 1,658 ng/g WW). Except pp'-DDE and their parent compound pp'-DDT, no other OC pesticide were found with a mean value >50 ng/g. There are few articles in the literature reporting OC levels in this species, so comparisons are difficult to establish. However, our results with pp'-DDE are in the range of those reported for three greater flamingos of the Doñana National Park (SW Spain)[4], but lower than those determined in birds collected in India[5]. This is not unexpected as in this Asiatic country pp'-DDT is still widely used to control malaria mosquitoes, as is the case for some African countries. Bearing in mind that greater flamingos from the Palearctic zone migrate to Africa[1], it is suggested that the great predominance of ΣDDT over the rest of pesticides indicates an African origin, although the high ratio pp'-DDE/pp'-DDT seems do not support recent exposures to pp'-DDT itself. Anyway, the concentrations detected in these birds are far below those reported to cause impairment of reproductive function in black ducks[12].

The pattern of PCB congeners, as happened before with striped dolphins of the Spanish Mediterranean coast[7], was more similar to an Aroclor 1260 than to an Aroclor 1254 or a mixture 50:50 of both products (Figure 1). Concentrations were higher than those reported before for greater flamingos in Spain[4], but again much lower than those observed for cormorants after exposition to a Clophen 60 mixture that causes toxic effects[13]. Ratio ΣDDTs/ΣPCBs was 0.39 (range 0.06-3.5), reflecting a predominant industrial origin of OC pollutants; it is important to note that in a study published almost a decade ago[14] using three duck species from the Ebre Delta, the situation was inverse. This probably can be explained better

TABLE 1

Organochlorine levels (ng/g WW) in livers of greater flamingos from El Fondo-Santa Pola (n = 57) and Ebre Delta (n = 8) Natural Parks, Spain.

ORGANOCHLORINE	MEAN	SD	RANGE (min-max)
α-Hexachlorocyclohexane	4.19	5.04	ND[a]-16.3
Hexachlorobenzene	10.2	15.4	ND-110.
β-Hexachlorocyclohexane	26.1	53.9	ND-277.
γ-Hexachlorocyclohexane	1.45	6.49	ND-50.3
Heptachlor	16.7	12.1	ND-50.2
Aldrin	6.95	11.9	ND-86.4
Heptachlor-epoxide	27.7	14.6	ND-89.9
pp'-DDE	758.	1,561.	11.5-10,268
pp'-DDD	13.5	24.0	ND-135.
pp'-DDT	51.8	96.4	ND-736.
ΣPCBs	2,235.	3,171.	211-17,289

a) ND = Not detected.

by a continuous decline of environmental ΣDDT contamination, banned since the 1970's in many industrialized countries, than by a rise in PCB contamination levels.

No significant differences in OC concentrations were detected between sex, age or localities. The latter it is not unsuspected as populations of greater flamingos in the wetlands of the Spanish Mediterranean coasts have probably the same origin[2], and their diets probably do not differ. Factors like sex or age are normally considered to influence OC levels in birds[12], although data is sometimes controversial[15].

Relative composition of main liver FAs (concentrations >0.5%) is given in Figure 2. Major components were 18:1 n-9 (oleic acid), 16:0 (palmitic), 18:0 (stearic), 20:4 n-6 (arachidonic) and 18:2 n-6 (linoleic), all of which accounts

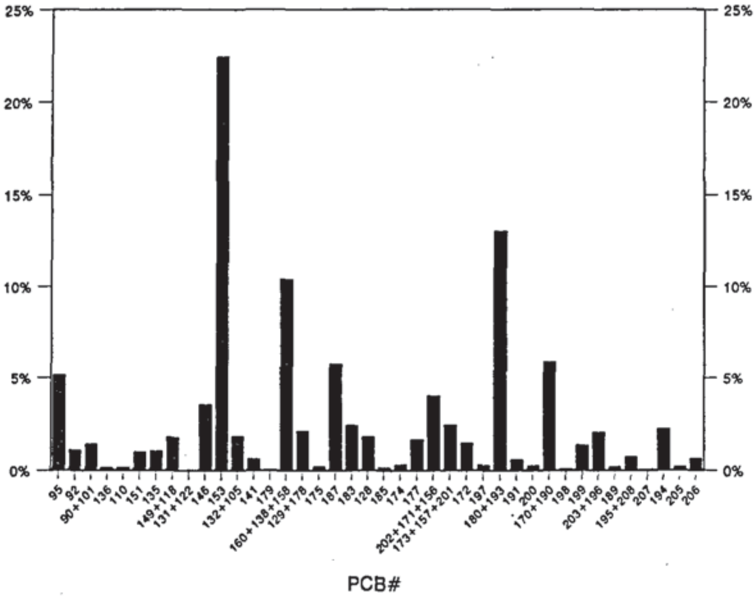

FIG. 1. ***Pattern of PCBs detected in livers of greater flamingos from Spain.***

approximately for 85% of ΣFAMEs. There were no statistical differences between sex or age, nor between localities when only non lead poisoned birds were considered, on the FAMEs composition. However, there were some remarkable differences between lead poisoned and non lead poisoned birds (shown in Figure 2), indicating that lead had influenced the FA composition of the liver. Moreover, apart from the data shown in Figure 2, other significant differences found were the following: ΣFAME (34,193 vs. 26,353 μg/g wet weight, $p<0.005$), saturated (13,171 vs. 10,145 μg/g WW, $p=0.001$) and monounsaturated (9,592 vs. 6,475 μg/g WW, $p<0.005$) concentrations were higher for lead poisoned birds, and the ratio polyunsaturated/monounsaturated (1,25 vs. 1,64, $p=0.001$) was lower. It is important to note that Donaldson and coworkers[9] reported as one of the more remarkable findings a decrease of linoleic

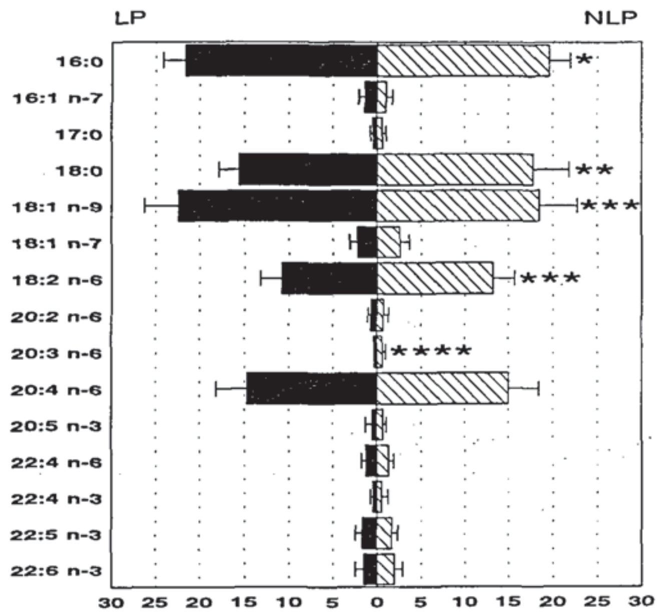

FIG. 2. Relative composition (%, mean and SD) of liver FAs of greater flamingos. Statistical differences between the lead poisoned (LP) and the not lead poisoned (NLP) groups are indicated with asterisks (= $p<0.05$, ** = $p<0.01$, *** = $p<0.005$, **** = $p<0.001$).*

acid in liver lipids of experimentally lead exposed chicks, which agree with the present results; however, no significant effect was observed with arachidonic acid, which in total liver lipids of lead poisoned chicks were found to increase[9]. Nevertheless, in general our results supports the involvement of free radicals (and thus lipid peroxidation) in lead poisoning[16], and confirms that lead induces fatty degeneration of bird's liver[17]. This results are notable because the inherent difficulties to observe in the field effects which are established in controlled laboratory conditions, even in experiments involving the same species.

The existence of the variable "lead poisoned-non lead poisoned" and the fact that the OC levels were much lower than in striped dolphins[7], makes the interpretation of the possible effect of OCs on the FA composition of greater flamingos' liver quite difficult. However, the statistical analysis performed are supposed to overcome this problems, and the results of this test indicates that ΣDDTs influenced only the relative proportion of very minor FAs (concentration $<0.5\%$). On the other hand, the effect of ΣPCBs were more prominent; it influenced positively 18:0 ($p<0.05$), 20:4 n-6 ($p<0.05$) and 22:4 n-6 ($p<0.05$), and negatively 16:0 ($p<0.05$) and 16:1 n-7 (palmitoleic, $p<0.01$), as the more significant effects on the main FA. It should be remarked that some of these effects agree with previous results obtained with wild[7] or laboratory mammals[18,19], while some others are inverse to the effects previously published. The only conclusion derived from this part of the study is that initially it is not unexpected that OCs may influence FA composition of lipids, but that there is a need of more work in this topic in order to clarify the magnitude (and positive or negative signs) of these effects, the physiological and biochemical significance, and possible differences in response among species.

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