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Increase of Organochlorines and MFO Activity in Water Birds Wintering in an Italian Lagoon

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The Black-necked grebe (Podiceps nigricollis) is a migratory and dispersive birds species (Cramp 1980). Its breeding areas are located in central and eastern Europe; its wintering quarters are in western Europe, around the Mediterranean, Adriatic, Black and Caspian Seas (Cramp 1980). Recently Focardi et al. (1984) have reported that specimens of Black-necked grebes accumulate polychlorinated biphenyls and mercury in their tissues during their wintering in the Northern Adriatic lagoon of Marano. During the last decade information has been gathered about the biological effect of chlorinated hydrocarbons on birds (Peakall 1970; Walker and Knight 1981) and particularly about biochemical defense mechanisms developed by several animal species (Kappas and Alvares 1975).

Residues of some chlorinated hydrocarbons and the levels of the mixed function oxidases (one of the most efficient hepatic detoxication systems) have been evaluated in specimens of Black-necked grebes collected in the lagoon of Marano during the wintering period (October and December 1984; April 1985) and are the subject of this report.

MATERIALS AND METHODS

For the analysis of chlorinated hydrocarbons a 0.1-1 g aliquot of freeze-dried homogeneous material was extracted in a Soxhlet apparatus with n-hexane. The extract was subjected to sulfuric acid cleanup followed by Florisil chromatography. The eluates were analysed with Perkin Elmer Sigma 3 gas chromatographs equipped with Ni63 Electron Capture Detectors. Glass columns packed with 4% SE-30 + 6% SP-2401 on Supelcoport and a 30-m-long SBP-5 fused silica capillary column were used.

The isolation of the microsomal fraction was made, after liver homogenization and centrifugation (12000xg for 10 minutes), by gel filtration of the supernatant fraction (Pyykko 1983). The MFO activity was tested by aldrin epoxidation (Wolff et al. 1979) and by the 7-ethoxyresorufin dealkylation (Pohl et al. 1980). For the separation of the forms of Cytochrome P-450, the microsomal proteins (quantified by the Bio-Rad Protein assay) were subjected to electrophoresis in SDS-Polyacrylamide as described by Laemmli (1970).

RESULTS AND DISCUSSION

The concentrations of hexachlorobenzene (HCB) and of pp'DDE in the tissues of grebes during their wintering period in the lagoon of Marano remain at roughly the same level (Table 1). Instead the concentrations of PCBs increase in all analysed tissues from October 1984 (arrival at the wintering quarters) through April 1985 (departure for the breeding grounds). The differences between the October and the April samples are statistically significant for the adipose tissue ($p<0.05$) and for the uropygial gland ($p<0.05$). Because of the increased concentration of PCBs during the grebe's wintering, the PCBs/DDE ratios for all the tissues averaged together change considerably from October to April (Table 1). Comparing PCBs values of April 1985 with those reported by Focardi et al. (1984) for April 1983 and April 1984, it would seem that there is a marked increase of this xenobiotic from year to year. This confirms, for the Mediterranean region, the previous findings of Lambertini et al. (1983) for a resident water bird species and of Boldreghini et al. (1983) for several migratory species breeding in Italy.

Liver sensitivity in mammals and birds to certain PCB isomers, involving an increase of Cytochrome P-450 and conseguently of MFO activity, has frequently been reported during the last decades (Allen and Abrahanson 1979; Knight and Walker 1982; Thomas et al. 1983). In our samples, the PCB congeners and isomers in the liver of grebes are mostly penta-, hexa-, hepta- and octachlorobiphenyls (Figure 1). Among the isomers there are three (2,2',3,3',4,4' - 2,2',4,4',5,5' - 2,2',3,3',4,4',5,5'), characterized by IUPAC numbers 128, 153 and 194 (Ballschmiter and Zell 1980), that are strong inducers of Cytochrome P-450 (Goldstein et al. 1977). Together with the increase of PCB residues, the hepatic mixed function oxidases, 7-ethoxyresorufin O-deethylase and aldrin epoxidase, show a significant variation between October 1984 and April 1985 ($p<0.001$ in both cases), with activities lowest upon the birds' arrival in the lagoon and highest upon their departure

Table 1. Chlorinated hydrocarbons (μg/g d.w.) in tissues ($\bar{x}$ = mean; SD = Standard Deviation; E.O.M. = Extracted Organic Matter).

	E.O.M. %		HCB		pp'DDE		PCBs		PCBs/DDE	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
October 1984										
FAT	74.7	9.9	0.33	0.13	7.08	3.39	2.14	1.41	0.31	0.14
UROPYG.	54.2	8.1	0.28	0.13	3.01	1.41	0.96	0.36	0.36	0.15
MUSCLE	9.5	1.7	0.03	0.01	0.68	0.47	0.46	0.38	0.79	0.58
BRAIN	16.2	5.2	0.05	0.03	0.61	0.46	0.76	0.92	1.20	0.91
KIDNEY	7.6	3.0	0.03	0.01	0.43	0.19	0.29	0.11	0.68	0.22
LIVER	12.5	1.4	0.04	0.01	0.46	0.32	0.18	0.06	0.39	0.25
December 1984										
FAT	80.2	5.4	0.61	0.19	10.12	8.54	4.25	3.23	0.42	0.41
UROPYG.	56.3	6.2	0.25	0.23	8.24	6.60	2.27	0.83	0.28	0.16
MUSCLE	9.4	2.1	0.11	0.04	1.12	0.83	0.56	0.20	0.66	0.49
BRAIN	18.2	4.1	0.06	0.05	1.18	0.45	1.17	0.44	0.99	0.43
KIDNEY	8.2	3.1	0.06	0.04	0.53	0.39	0.49	0.23	0.91	0.41
LIVER	10.3	1.1	0.10	0.08	0.64	0.32	0.37	0.19	0.52	0.24
April 1985										
FAT	89.3	4.6	0.58	0.85	8.13	3.82	8.42	9.12	1.05	0.81
UROPYG.	59.2	3.1	0.27	0.31	6.09	2.89	5.49	6.07	0.94	0.73
MUSCLE	10.2	2.2	0.18	0.15	0.75	0.32	0.79	0.86	1.17	0.97
BRAIN	20.4	5.0	0.08	0.06	0.64	0.53	0.92	0.67	1.42	0.74
KIDNEY	9.7	2.1	0.07	0.10	0.40	0.18	0.58	0.64	1.29	1.16
LIVER	10.4	1.2	0.09	0.12	0.49	0.27	0.82	1.05	1.68	1.49

October 1984 n = 8; December 1984 n = 5; April 1985 n = 5.

(Figure 2). However, when trying to interpret this increase, another fact should be taken into consideration, that is, the important function of these enzymes in steroid metabolism (De Bruin 1976). The increased activity of the MFO enzymes in the liver of grebes immediately before their departure for the breeding grounds also coincides with the hormonal production preceding breeding activities. A similar finding has been reported for the females of the razorbill (Knight and Walker 1982) in contrast with other findings of "no relationships between activity and sex, site, time of the year" in five other species of fish-eating birds (Walker and Knight 1981).

It is difficult to understand the actual influence of the two factors (xenobiotic and biotic) on the variations of the MFO activities in this species during the wintering period. One important finding is the similar electrophoretic configuration of the proteins of the cytochrome P-450 area of the grebes (Figure 3)

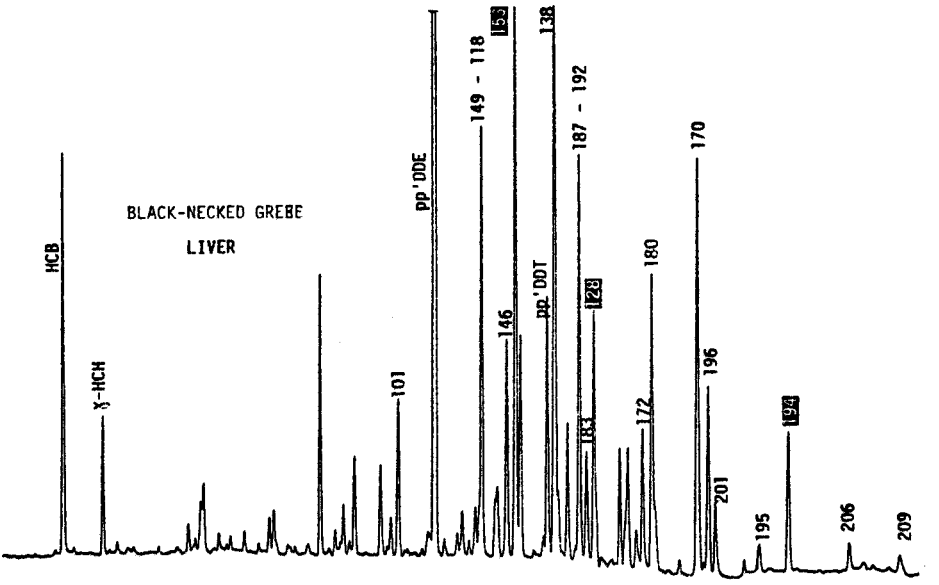

Figure 1. Capillary column gas chromatogram of a sample of liver of Black-necked grebe (April 1985). Conditions: initial temp 100°C for 10 min, 3°/min to 280°; iniector temp 220°; detector temp 280°; Carrier gas Argon/Methane (95:5); Detector 63Ni EC.

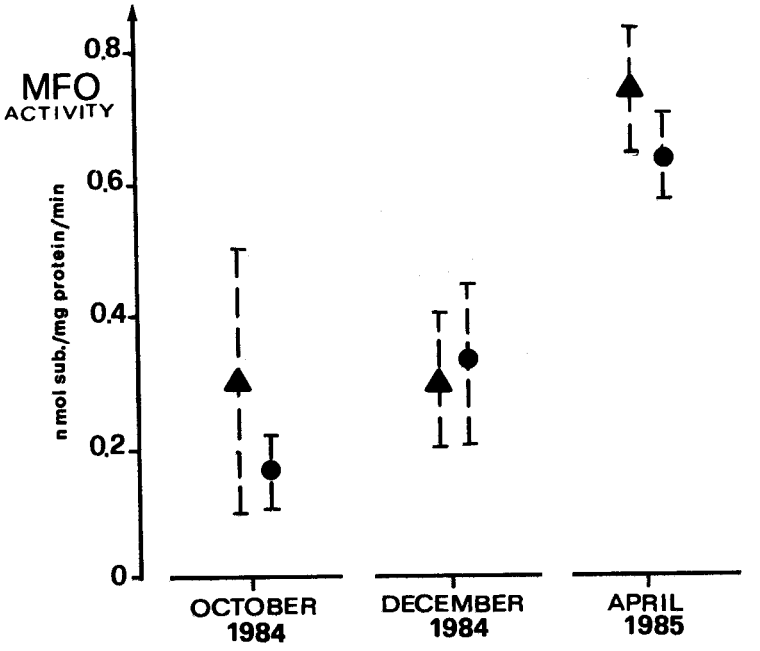

Figure 2. Hepatic 7-ethoxyresorufin o-deethylase (●) and aldrin epoxidase (▲) activity during the wintering period (mean ad SD).

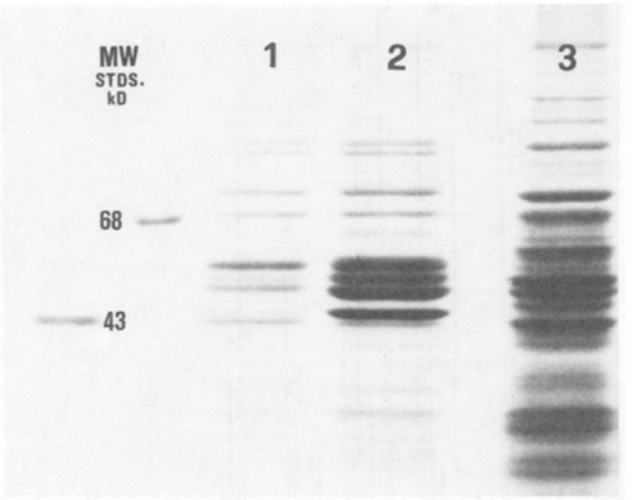

Figure 3. SDS-Polyacrylamide slab gels of the P-450 cytochromes (45-60 kD) from: 1, control quail; 2, PCB-treated quail; 3, Black-necked grebe in April 1985.

and that of specimens of Japanese quail experimentally treated in our laboratory with a commercial mixture of PCBs (Leonzio et al. 1985). This could be indirect evidence that in the liver of the specimens collected in the Marano lagoon the induction of the MFO activity is at least in part due to the PCB residues present in that area.

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REFERENCES

Allen JR, Abrahanson LJ (1979) Responses of rats exposed to polychlorinated biphenyls for fifty-two weeks. II Compositional and enzymatic changes in the liver. Arch Environ Contam Toxicol 8:191-200

Ballschmiter K, Zell M (1980) Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Fresenius Z Anal Chem 302:20-31

Boldreghini P, Fossi C, Focardi S, Leonzio C, Renzoni A (1983)

Elementi in tracce e idrocarburi clorurati in uova di uccelli raccolte nel delta del Pò (1977-1981). II° Congr Ital Ornitologia, Parma, 27-29 October 1983
Cramps S (1980) Handbook of the birds of Europe the Middle East and North Africa. Vol 1. Oxford University Press, Oxford
De Bruin A (1976) Biochemical toxicology of environmental agents. Elsevier North-Holland Biomedical Press, Amsterdam
Focardi S, Fossi C, Leonzio C, Renzoni A (1984) Uptake of contaminants by birds wintering in an italian lagoon. VIIes Journées Etud Pollut, Lucerne, 11-13 October 1984
Goldstein JA, Hickman P, Bergman H, McKinney JD, Walker MP (1977) Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem Biol Interact 17:69-87
Kappas A, Alvares AP (1975) How the liver metabolizes foreign substances. Sci Amer 232: 22-31
Knight GC, Walker CH (1982) A study of the hepatic microsomal monooxygenase of sea birds and its relationship to organochlorine pollutants. Comp Biochem Physiol 1:211-221
Laemmli UK (1970) Claveage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 227:680-685
Lambertini M, Focardi S, Fossi C, Leonzio C (1983) Il gabbiano corso (Larus audouinii) nidificante nell'Arcipelago Toscano: contaminazione da elementi in tracce e da idrocarburi clorurati. II° Congr It Ornitologia, Parma, 27-29 October 1983
Leonzio C, Fossi C, Focardi S, Milanesi C, Tiezzi A (1985) Osservazioni sulle diverse forme del citocromo P-450 indotte da xenobiotici. Atti IV° Conv ABCD, Bardonecchia, p 228
Peakall DB (1970) pp'DDT: effect on calcium metabolism and concentration of estradiol in the blood. Science 168:592-594
Pohl RJ, Fouts JR (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. Analytical Biochem 107:150-155
Pyykkö K (1983) Characterization and stability of rat liver microsomes isolated by rapid gel filtration method. Acta Pharmacol Toxicol 52:39-46
Thomas PE, Reik LM, Ryan DE, Levin W (1983) Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochrome P-450c and P-450d, by structurally diverse xenobiotics. J Biol Chem 258:4590-4598
Walker CH, Knight GC (1981) The hepatic microsomal enzymes of sea birds and their interaction with liposoluble pollutants. Aquatic Toxicol 1:343-345
Wolff T, Deml E, Wanders H (1979) Aldrin epoxidation, a highly sensitive indicator specific for cytochrome P-450 dependent mono-oxygenase activities. Drug Metab Dispos 5:301-305
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