

# DDT and HCH in Liver Fat of Cormorants

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## Abstract

Eighty cormorants from the area of Ostroda and Mrągowo were used for this study. The analyses were carried out between 1993-1996. Each year 10 two-year-old birds were shot (5 females and 5 males) in two nesting grounds. On average, the amount of concentrated DDT in cormorant liver fat was 4.914 mg/kg and  $\gamma$ -HCH was 0.039 mg/kg of wet mass. The average amount of DDT in subsequent years varied and ranged from 2.515 to 7.252 mg/kg; however, the differences were statistically insignificant. The females accumulated higher levels of DDT than the males, on average up to 8.796 mg/kg. The differences in DDT amounts concentrated in liver fat of both cormorant groups in different years appeared to be insignificant.

Liver concentrations of  $\gamma$ -HCH in the observed period most frequently showed tendency to increase, from the level of 0.012 in 1993 to 0.106 mg/kg in 1995. This value decreased in 1996 to 0.003 mg/kg. Higher amounts of  $\gamma$ -HCH in liver fat were recorded in birds originating from the Mrągowo area, excluding the individuals shot in 1995. It was shown that the males accumulated higher levels of  $\gamma$ -HCH in their livers than the females (years 1993, 1994 and 1996).

The presented study shows the continuous threat of the analyzed xenobiotic agents to water fowl and the need for continuous monitoring of water fowl in respect of their intoxication by DDT and HCH.

**Keywords:**  $\Sigma$ DDT,  $\gamma$ -HCH, liver, cormorant.

## Introduction

Hexachlorocyclohexane (HCH) and DDT exhibit insecticidal properties, high stability in the natural environment and high biological activity towards animals [18]. These agents were withdrawn from the market over 20 years ago; however, they have been circulating in the form of original compounds or stable metabolites and accumulating in the environment [4, 14, 17, 23]. In animals, these agents, to a large extent, tend to accumulate in the fat tissue favoured by their high solubility in fat [18]. These xenobiotic agents have an exceptional tendency to accumulate in organisms in a food chain [4, 18,

20]. Therefore, in scientific literature there are numerous references to waterfowl feeding on fish [5, 8]. Moreover, the accumulation of DDT and HCH in wild birds including the black cormorant resulted in reproduction disorders exhibited by "thin egg shells" embryo mortality and increased hatchling mortality [3, 6, 13, 23].

One of the significant disadvantageous effects of the mentioned chlorohydrocarbons on animals is that they damage intercellular connections. Such communication disorders between cells leads to a control loss over cell growth and diversification [4]. These agents are suspected to cause the promotion of carcinogenicity. Due to such properties, future carcinogenic processes should be considered even at low human and animal exposure to chlorohydrocarbons [2, 4, 11, 22].

The aim of this paper was to determine the vulnerabi-

lity of cormorants from the Olsztyn area to intoxication with DDT and HCH by analyzing the level of these agents in bird liver fat. Moreover, due to the fact that the cormorant as a fish-feeding bird is the last link in the food chain of the pond and lake ecosystem, contamination of the bird's fishing grounds could be determined.

## Materials and Methods

Eighty 2-year-old black cormorants (*Phalacrocorax carbo*) were used for the experiment. Twenty birds (5 females and 5 males from Ostroda and Mragowo) were studied each year from 1993 to 1996. The birds were shot to reduce the species population between 1993 and 1996 from the area of ponds and lakes situated near Ostroda (O) and Mragowo (M). The cormorant population in Poland is reduced by a certain number in summer (after the inoculation period) as determined by the Minister of the Environment and Natural Resource Protection. The water reservoirs, being the cormorant hunting ground in the area of Mragowo, are surrounded mainly by crop fields and some forests. Mainly forest ponds and lakes and only some crop fields are the hunting ground for the cormorants from the Ostroda area.

Three-gram liver samples were taken from the cormorants immediately after the shooting for subsequent analysis of  $\Sigma$ DDT and  $\gamma$ -HCH concentration. The material was sealed in polyethylene bags and stored frozen. The liver tissue was defrosted directly before analysis.

The analyzed compounds were extracted from the liver samples along with fat with the use of petroleum ether and acetone. After concentration in a rotary evaporator and removal of the remaining solvents the fat samples were weighed with the accuracy of 0.001g, dissolved in n-hexane and purified with the use of sulphur acid mixture. The polychlorinated biphenyl (PCB) were separated from the chlorohydrocarbons by means of column chromatography on Cellite 545 and Silica Gel and by chromium oxidation according to Gorecki et al. [9, 10]. The separation and quantitative assay of  $\Sigma$ DDT and  $\gamma$ -HCH was carried out by gas chromatography with the system incorporating PYE Unicam 104 apparatus and the Philips recorder for recording the results.

The resulting compounds were identified by the retention time comparison of peaks for the analyzed extracts with the relevant peaks of the standard solution (Aroclor 1260). The quantitative calculations were completed by comparing the height of the main peaks in the analyzed extract.

The distribution parameters of particular values were determined in the statistical evaluation of the results. To evaluate the significance of the differences between the mean values the following tests were used: Duncan, Student-Newman-Keuls, F-Fisher-Snedecor.

## Results

DDT. The concentrations of  $\Sigma$ DDT in cormorant liver fat in the particular years of the experiment and by bird sex and origin are included in Table 1.

During the four-year experimental period, the concentration of  $\Sigma$ DDT in all bird liver fat was on average 4.914 mg/kg. The average concentration was high due to the high concentration of  $\Sigma$ DDT in a few birds which ranged between 15.457 mg/kg and 30.745 mg/kg of wet mass. Most livers contained the agents at lower levels than the mentioned average. However, the mean value was 2.903 mg/kg  $\Sigma$ DDT, which indicates that half of the individuals exhibited the  $\Sigma$ DDT concentration in the liver at a level lower than the mean value. Between 1993 and 1995 the  $\Sigma$ DDT level increase was statistically significant. A clear decrease in  $\Sigma$ DDT concentrations between 1995 and 1996 was not statistically significant.

$\Sigma$ DDT levels in female liver fat significantly increased from year to year throughout the study period. In cormorant males,  $\Sigma$ DDT concentrations in subsequent years ranged insignificantly. The difference tendencies (increasing and decreasing) between both sexes were not stable. The differences were sometimes large; however, statistically insignificant except the difference occurring in 1994. Females were then intoxicated more than males.

Considering the hunting ground location, the average  $\Sigma$ DDT concentration in individuals from group O in 1993 was 4.603 mg/kg. In 1994, the level of the analyzed compound decreased significantly; however, that change was statistically insignificant similarly to the differences in subsequent experimental years. The average  $\Sigma$ DDT con-

Table 1. Average concentrations of  $\Sigma$ DDT (mg/kg of wet mass) in cormorant liver fat between 1993 - 1996 by bird sex and origin (n = 5).

Year	$\Sigma$ DDT				
	cormorants total	females	males	group O	group M
1993	3.475 <sup>a</sup>	2.061	4.814	4.603 <sup>A</sup>	2.272 <sup>AB</sup>
1994	2.515 <sup>b</sup>	2.989 <sup>A</sup>	2.041 <sup>B</sup>	1.604 <sup>A</sup>	3.426 <sup>BB</sup>
1995	7.252 <sup>c</sup>	6.720	7.784	9.013	5.491
1996	6.453	8.796	4.111	5.228	7.678

<sup>a, b, c</sup> - the mean values marked with small letters differ statistically significantly at  $p < 0.01$  vertically.

<sup>A, B</sup> - the mean values marked with capital letters differ statistically significantly at  $p < 0.01$  horizontally.

Group O - cormorants from the Ostróda area.

Group M - cormorants from the Mragowo area.

Table 2. Average concentrations of  $\gamma$ -HCH (mg/kg of wet mass) in cormorant liver fat between 1993 - 1996 by bird sex and origin (n = 5).

Year	$\gamma$ -HCH				
	cormorants total	females	males	group O	group M
1993	0.012 <sup>a</sup>	0.009 <sup>aA</sup>	0.015 <sup>aB</sup>	0.008 <sup>aA</sup>	0.016 <sup>aB</sup>
1994	0.034 <sup>b</sup>	0.027 <sup>bA</sup>	0.041 <sup>bB</sup>	0.013 <sup>bA</sup>	0.055 <sup>bB</sup>
1995	0.106 <sup>c</sup>	0.128	0.084	0.138	0.074
1996	0.003	0.000	0.005	0.002 <sup>A</sup>	0.004 <sup>B</sup>

<sup>a,b,c</sup> - the mean values marked with small letters differ statistically significantly at  $p < 0.01$  vertically. <sup>A, B</sup> - the mean values marked with capital letters differ statistically significantly at  $p < 0.01$  horizontally. Group O - cormorants from the Ostroda area. Group M - cormorants from the Mrągowo area.

centration in the cormorants from group M in 1993 was 2.272 mg/kg in liver wet mass. The  $\Sigma$ DDT level increased significantly in a subsequent year in this group of birds. In subsequent years,  $\Sigma$ DDT concentrations in livers of birds shot in the area of Mrągowo increased; however, the differences were statistically insignificant. The differences between the cormorant groups in particular years varied: in 1993 the livers of the O individuals were more intoxicated with  $\Sigma$ DDT than the livers of group M; in 1994 the situation was reversed. The mentioned differences appeared to be statistically significant. However, higher concentration of  $\Sigma$ DDT in the liver of group O birds than group M birds in 1995 and the reverse correlation in 1996 were statistically insignificant.

HCH. The concentration of  $\gamma$ -HCH in cormorant liver fat between 1993 and 1996 were presented in Table 2.

The average concentration of  $\gamma$ -HCH in liver fat in all cormorants between 1993 and 1996 was 0.039 mg/kg ( $\pm$  0.066 mg/kg). Taking into account the asymmetry coefficient ( $A = 2.2681 > 0$ ) and the middle value (median = 0.009 mg/kg) it appeared that the majority of the birds had lower-than-average concentrations of  $\gamma$ -HCH in the liver. In half of the analyzed birds the concentration of  $\gamma$ -HCH was lower than the mentioned medium value.

Between 1993 and 1995, the average concentration of  $\gamma$ -HCH in cormorants significantly increased. In 1996 the concentration of the analyzed xenobiotic agent decreased statistically insignificantly.

Comparing the average concentrations of  $\gamma$ -HCH in the livers by sex in subsequent years it was found that in 1993 there was a significant difference between females and males. Statistically significant increase of  $\gamma$ -HCH concentrations occurred in both females and males between 1993 and 1994. In 1994 was also important due to the statistically significant difference between both sexes. Then male individuals accumulated higher levels of the agent in comparison to the females. The livers of both sexes obtained in 1995 contained the highest levels of  $\gamma$ -HCH compared to those obtained in other years of the analyzed period. In the last year of the experiment, a statistically insignificant decrease of the analyzed toxic agent was recorded for both sexes. The females did not contain  $\gamma$ -HCH in their livers and the males accumulated it at 0.005 mg/kg.

Considering the origin of birds in 1993, a significant difference in the amount of the accumulated  $\gamma$ -HCH in the liver was recorded between the individuals for group O and those from group M. In 1994, in the case of both groups a statistically significant increase in  $\gamma$ -HCH residue in livers was found. The difference between both groups that occurred in 1994 was also statistically significant. Such correlations were similar also in 1995, when the cormorants obtained from Ostroda had 0.138 mg/kg  $\gamma$ -HCH in their livers and the individuals originating from Mrągowo only 0.074 mg/kg. However, the above difference was statistically insignificant. In 1996, the concentration of  $\gamma$ -HCH in both bird groups decreased. The livers of the cormorants from group O contained 0.002 mg/kg of  $\gamma$ -HCH and from the other group it was 0.004 mg/kg. The occurring difference was statistically significant.

## Discussion

It was then determined that the  $\Sigma$ DDT concentration in cormorant liver fat was 4.914 mg/kg of fresh mass and the average level of  $\gamma$ -HCH in the analyzed samples was 0.039 mg/kg. For comparison, the concentrations of DDT and  $\gamma$ -HCH in the livers of grey heron originating from the Olsztyn area ranged from 0.238 to 1.456 mg/kg and from 0.082 to 0.184 mg/kg, respectively [21]. The analyzed cormorants originating from the same area accumulated significantly higher DDT amounts than the mentioned herons; however, the accumulated amounts of  $\gamma$ -HCH in the former were smaller.

In different regions of the world, where both DDT and HCH are still in use or have been banned recently (South Africa), the recorded amounts of the analyzed xenobiotic agents are higher. The liver samples of wild geese feeding in South African rivers contained from 22.7 to 48.6 mg/kg  $\Sigma$ DDT [8]. The results obtained by other authors were similar and it was found that although DDT concentrations in living organisms is still high, it changes every year and generally decreases [3, 15, 16, 21]. The levels of animal intoxication with DDT and HCH in countries such as the USA (where these agents were banned much earlier), is lower than in the Warmia and Masuria region. King et al. [12, 13] found that the

amount of the described xenobiotic agents decreased in the cormorants from Galveston Bay in Texas between 1980 and 1986. In 1986, cormorant carcasses still contained from 0.8 to 2.5 mg/kg DDT and from 0.1 to 0.6 mg/kg  $\gamma$ -HCH. The above values of DDT are similar to those obtained during our own observations; however, the values of  $\gamma$ -HCH were significantly higher. Significant decreases in DDT and  $\gamma$ -HCH amounts in slaughter and wild animals were also recorded in Poland [4, 15, 16, 20, 21]. In many analyzed samples (particularly those taken from domestic animals) the described xenobiotic agents were not found [15, 16].

The concentration of  $\gamma$ -HCH and  $\Sigma$ DDT in liver fat of the cormorants originating from the Olsztyn area between 1993 and 1996 varied. Cormorants nesting in the vicinity of Mrągowo were more intoxicated with  $\gamma$ -HCH than birds originating from the Ostroda area. The  $\Sigma$ DDT concentration levels in the birds of both groups did not differ significantly. Due to relatively high residues of DDT and continuous concentrations of  $\gamma$ -HCH in cormorant liver fat there is the need for permanent monitoring of intoxication with the mentioned xenobiotic agents of wild animals. The presented facts show the existence of chlorinated hydrocarbon reservoirs in the Olsztyn natural environment and their continuous threat to wild animals.

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