A COMPARATIVE LABORATORY BREEDING STUDY WITH CORMORANTS (PHALACROCORAX CARBO) FROM TWO DUTCH COLONIES WITH A DIFFERENT DEGREE IN CONTAMINATION *.

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ABSTRACT

Cormorant eggs were collected from two colonies in the Netherlands with known differences in reproductivity and exposure to chlorinated aromatic hydrocarbons. These eggs were hatched in an incubator. Embryonal metabolism and several biochemical parameters in the hatchling, i.e. cytochrome P-450 induction, thyroid hormone and vitamine A status were measured. A number of relevant PCBs was measured in the yolk sac of the embryos, showing large differences between both colonies.

INTRODUCTION

In 1987 and 1988 a field reproductivity study was done in seven breeding colonies of the Cormorant Phalacrocorax carbo in the Netherlands. This study indicated that birds breeding in the highly contaminated delta of the river Rhine and Meuse (Biesbosch area) had a significant lower breeding succes, than those breeding in less contaminated parts of the Netherlands. The reproductive succes in the Biesbosch area, 0.55 fledglings/nest, was extremely low in comparison with the other colonies in which 1.18 to 2.16 fledglings/nest were raised in 1988. Differences were also observed in the survival of the hatchlings. In 1988, 1.11 eggs/nest hatched in the Biesbosch colony from which only 0.55 (50%) survived. In contrast an average of 1.74 hatchlings survived out of 2.13 hatchlings/nest (80%) in one of the less contaminated colonies, the Oude Venen (Friesland). It was a striking observation, that mortality of most of the hatchlings in the Biesbosch colony occured during the first two weeks after birth. During this period the hatchlings have digested their yolksac completely. The main lipid content of the hatchlings is found in the yolksacs, which are formed from the lipid reserves of the female, thus containing significant amounts of lipophilic microcontaminants.

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Based on these results and the known distribution of the aquatic pollution in the Netherlands, it was suggested that microcontaminants especially PCBs, PCDDs and PCDFs could be the causal agents for this low reproductive succes.

EXPERIMENTAL

In the breeding season of 1989 a laboratory study was performed, in which the embryonal development of the Cormorant was studied from two colonies with a different degree in contamination. 36 Eggs were collected in colonies from the western (Biesbosch area) and northern part (Oude Venen, Friesland) of the Netherlands.

The Biesbosch and Oude Venen colonies were situated in areas with respectively a high and low degree of contamination with chlorinated hydrocarbons, like PCBs.

These eggs were hatched in an incubator under specified laboratory conditions, which will be published separately. During the breeding period the conductance, which is comparable with the porosity of the eggshell and embryonal metabolism were measured.

Within 24 hours after hatching the young Cormorants were sacrificed. Blood was collected and liver, yolksac, thymus and bursa were removed from the body for further analysis.

After hatching, several morphological measurements, body and organ weights were determined of the young Cormorants.

Bloodplasma was used for vitamin A (retinol) and thyroidhormone (total and free thyroxin and total triiodothyronin) analysis.

Livers were used to measure total cytochrome P-450 concentration, ethoxyresorufin-O-deethylation (EROD) and pentoxyresorufin-O-depentylation (PROD) activity. EROD and PROD were used as markers for the activity of isoenzymes from the cytochrome P-450I and II group.

PCB analysis, including the major mono-ortho substituted congeners, were done on the yolksac of embryos from both colonies.

RESULTS AND DISCUSSION

The conductance of the eggshell was found similar for both colonies. However, metabolism of the developing embryo (O_2 consumption and CO_2 production) was 25 - 30% higher in the eggs from the more contaminated colony (Biesbosch). Total O_2 consumption and CO_2 production was obtained by using a computer curve fitting program and subsequent area intergration. See table 1.

Table 1. Metabolism of Cormorant embryos during the hatching period expressed as total CO_2 and O_2 exchange in ml.

Colony:	O_2 consumption	CO_2 production
Biesbosch (n=4,m=13)	4668	3401
Friesland (n=6,m=20)	3778	2529

(n=number of eggs used, m=total number of measurements)

In general, body and organ weights tended to be lower from the more contaminated Biesbosch colony, but due to large individual differences these were not statistically significant.

Plasma retinol concentrations were found similar in both groups of hatchlings, but striking differences were observed in thyroid hormone concentrations.

Young Cormorants from the more contaminated Biesbosch colony had approximately 50 % less thyroidhormones in their plasma, than those from the lesser contaminated colony in Friesland. See table 2.

Table 2. Average plasma thyroidhormone and retinol concentrations in young Cormorant hatchlings.

Colony:	free thyroxin (pmol/l)	total thyroxin (nmol/l)	total triiodothyronin (nmol/l)	retinol (ng/ml)
Biesbosch (n=5)	1.94 ± 0.54	4.7 ± 3.1	0.65 ± 0.39	0.108 ± 0.020
Friesland (n=11)	3.65 ± 2.45	8.8 ± 4.5	1.34 ± 0.84	0.110 ± 0.028

Average EROD activity was found to be 45 % higher in the livers of the young Cormorants from the contaminated colony, but due to large individual variation this difference was not statitistically significant. Differences in average total cytochrome P-450 and PROD activity were also observed, but again with high individual variations. See table 3.

Table 3. Average total cytochrome P-450, EROD and PROD activity in the livers of Cormorant hatchlings

Colony:	Cytochrome P-450 nmol/mg protein	EROD nmol/mg protein.min	PROD nmol/mg protein.min
Biesbosch (n=5)	0.18 ± 0.07	0.70 ± 0.28	0.034 ± 0.012
Friesland (n=11)	0.12 ± 0.04	0.48 ± 0.31	0.046 ± 0.018

In order to obtain some preliminary information about the degree of PCB contamination, we analyzed yolk sacs from embryos at different stages of development for six standard PCBs. These analysis showed that eggs from the Biesbosch colony contained on the average at least five times higher PCB concentrations, than those from the Friesland colony. Concentrations for these six standard PCBs are given in table 4.

Table 4. PCB concentrations in the yolk sac of the Cormorant embryo expressed in mg/kg lipidweight.

PCB congener		Biesbosch (n=7)	Friesland (n=11)
2,4,4'	(28)	4.7[1] ± 3.6[2] [1.8 - 11.2][3]	1.9 ± 1.2 [<0.4 - 3.6]
2,2',5,5'	(52)	3.5 ± 4.0 [<0.4 - 8.2]	< 0.1
2,2',4,5,5'	(101)	22.7 ± 12.7 [6.2 - 45.7]	4.0 ± 2.2 [<1.3 - 5.6]
2,2',3,4,4',5'	(138)	265.8 ± 70.5 [145.1 - 331.1]	63.5 ± 39.3 [8.5 - 162.8]
2,2',4,4',5,5'	(153)	352.6 ± 92.2 [204.0 - 464.4]	87.1 ± 51.0 [11.2-214.2]
2,2',3,4,4'5,5'	(180)	149.1 ± 33 8 [114.4 - 184.5]	32.0 ± 17.8 [<2.3 - 69.7]

([1]=average; [2]=standard deviation; [3]=minimum - maximum value)

In addition a number of mono-ortho PCBs was also detected in the yolksacs. Concentrations of these PCB congeners were also in the mg/kg lipidweight range for the higher contaminated Biesbosch colony. These PCB congeners are of particular toxicological interest because of their dioxin like mechanism of action, which can involve cytocochrome P-450I (EROD) induction and alterations in thyroidhormone and vitamine A metabolism. Concentrations of the major mono-ortho PCBs are presented in table 5.

Table 5. Concentrations for the major mono-ortho PCBs in the yolk sac of the Cormorant embryo expressed in mg/kg lipid weight

		Biesbosch (n=7)	Friesland (n=12)
Colony:			
2,3,3',4,4'	(105)	24.0[1] ± 5.9[2] [15.7 - 31.1][3]	7.7 ± 4.4 [<3.2-19.5]
2,3'4,4',5	(118)	126.4 ± 37.7 [63.6 - 167.4]	37.4 ± 21.8 [4.1 - 90.3]
2,3,3',4,4',5	(156)	16.1 ± 4.7 [9.5 - 24.4]	4.8 ± 3.6 [<1.8 - 15.1]
2,3,3',4,4',5'	(157)	5.3 ± 1.9 [<3.2 - 6.0]	3.8 ± 2.4 [<1.4 - 6.2]
2,3',4,4',5,5'	(167)	12.5 ± 4.4 [8.1 - 20.5]	3.8 ± 2.8 [<1.1 - 11.1]

([1]=average; [2]=standard deviation; [3]=minimum - maximum value)

In view of the PCB congeners present in the yolk sac of the young Cormorants from both colonies, we suggest that the commercial PCB formulation Aroclor 1254 was a major contamination source for the Cormorant and its food.

At present PCB, PCDD and PCDF analysis are in progress from those hatchlings from which biochemical measurements were obtained. These measurements may clarify the individual variations, which were observed within a population of the same colony.