EFFECTS OF PCBs ON COMMON TERN (*STERNA HIRUNDO*) BREEDING IN DUTCH ESTUARIES: MONITORING AND EXPERIMENTAL DOSING STUDIES.

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INTRODUCTION

Estuarine systems all over the world are of high ecological value. They are a resort and feeding ground for many species. The contamination of our watersystems with persistent halogenated aromatic hydrocarbons (PHAHs), such as polychlorinated biphenyls (PCBs), polychlorinated dibezo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) will exhibit effects on wildlife especially in these systems where contaminated sediments will be deposited at a relatively high rate (Evers *et al* 1993). Once deposited the contaminants in the sediment may enter the foodchain and will finally accumulate in toppredators of the aquatic system, such as fish eating birds. To select a relatively sensitive species for monitoring the effects of PHAHs in the field, *in vitro* experiments were performed studying the Ah-receptor mediated induction of cytochrome P450 1A (CYP1A) in cultured livercells of various species (Bosveld et al 1996). These studies revealed that the common tern (*Sterna hirundo*) is relatively sensitive towards PHAHs when compared with the herring gull and the black headed gull, two alternative species for monitoring effects on fish eating birds in The Netherlands. Eggs of the common tern were collected at eight different locations with a varying level of contamination. The eggs were hatched in the laboratory and the effects on breeding success and embryonal development were related to PHAH concentrations in the residual yolk. The highest average PHAH concentrations were found in eggs from the Slijkplaat in the Haringvliet, which is the sedimentation area of the rivers Rhine and Meuse. Significant concentration-effect relationships were found between PHAH concentrations in the yolk and egg volume, incubation period, and both EROD and PROD activity (Bosveld et al 1995). Fractionated regression analysis revealed that PCBs showed to be the major contributive components and that PCDDs and PCDFs only played a minor role in causing the observed effects. In terms of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalencies (TEQs) the *non-ortho*-(NO)PCBs (IUPAC #77, #126 and #169) were the most important and contributed on average for 77% to the total TEQ concentration, when calculated using TEFs derived from egg injection studies with chicken (Bosveld et al 1995). In absolute numbers the *di-ortho*-(DO)PCB 22,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) was the most important. However this congener is known to have no Ah-receptor mediated CYP1A inducing activity in chicken (Kennedy et al 1996) and is as such no important contributant to the total TEQs in these samples.

Based on the previous findings experimental feeding studies were designed to esthablish the effects of the most potent NO-PCB 3,3',4,4',5-pentachlorobiphenyl (PCB 126) alone or in combination with the DO-PCB 153 on the early post hatching life stage of the common tern.

The aim of the studies was to give evidence to a *causal* relationship between the effects observed in the field and the concentrations of a specific dioxin-like PCB in the food. In addition it was aimed to study the importance of *post natal* exposure in relation to *in ovo* exposure.

MATERIALS AND METHODS

In june 1993 (experiment A) and in june 1994 (experiment B) eggs were collected from a reference breeding colony of which the PHAH contamination level was found to be low in our earlier study (Bosveld et al. 1995). The eggs were incubated and hatched in the laboratory. After hatching the chicks were divided among the different dose groups, each consisting of five birds. Each group was housed in a 90 x 60 x 60 cm (wxdxh) perspex cage. The birds were fed fish that was previously catched and frozen. Each day a portion of fish was thawed and individual fishes were injected with a PCB solution in peanut oil (10 μl/g fish). In experiment A the fish was additionally contaminated with PCB 126 alone, resulting in concentrations of 0, 0.1 and 1 ng PCB 126/g food in the respective dose groups A/0, A/.1 and A/1. In experiment B the food was contaminated with PCB 126 in combination with PCB 153 (1:1000). Concentrations in the food for the respective dosegroups B/0, B/.1, B/.3, B/1 B/10 and B/33 were 0, 0.1. 0.3, 1.0, 10 and 33 ng PCB 126 combined with 1000 times higher amounts of PCB 153 per μl oil. Animals were sacrificed after 21 days. The birds were narcotisized using diethylether. Blood was taken by hearth-puncture. Blood was centrifugated and the plasma was frozen at -80°C for thyroid hormone analysis (experiment B only). Immediately after death the liver was removed, divided in four parts and stored at -80°C. One part was used for residue analysis and one part was used for microsome preparation and MFO enzyme activity measurements. Microsomal EROD, MROD, PROD and BROD activities were measured. In addition the thyroid, thymus and bursa of fabricius were dissected and weighed. Detailed methods have been published earlier (Bosveld et al 1995) or will be described elswhere.

RESULTS

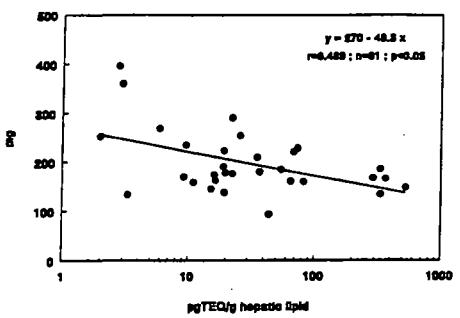
Internal doses

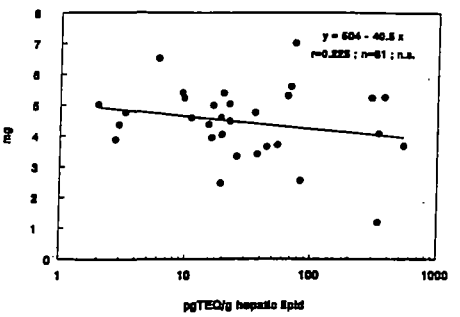
Residue analysis showed that the PCB 126 concentrations in the livers of the birds from dosegroup A/.1 and A/1 were significantly increased by a factor 10 and 100 respectively, compared to the controls (A/0). In the experiment with a combination dose of PCB 126 and PCB 153, the concentrations of the added PCBs were not significantly increased in the livers of the birds from dosegroup B/.1 and B/.3 compared to the controls B/0. The concentrations in dosegroup B/1, B/10 and B/33 were increased 3, 23 and 159 times respectively, compared to the controls (B/0). PCB 77 concentrations varied little among the dosegroups but varied considerable between the two experiments. In experiment A, in all dosegroups, PCB 77 concentrations were 0.5 ± 0.1 ng/g liver. In experiment B the concentrations were approximately a factor one hundred higher (ranging from 28 ± 31 to 83 ± 72 ng/g liver). These differences are reflected in the $TEQ_{NO\text{-}PCB}$ concentrations in the livers. $TEQ_{NO\text{-}PCB}$ in experiment A ranged from 0.02 ± 0.006 (A/0) to 1.8 ± 0.5 ng/g (A/1). $TEQ_{NO\text{-}PCB}$ in the controls of experiment B was 1.4 ± 1.3 ng/g. $TEQ_{NO\text{-}PCB}$ in dosegroups B/.1, B/.3 and B/1 were not increased significantly compared to the controls. In dosegroup B/10 and B/33 $TEQ_{NO\text{-}PCB}$ was increased 2.7 and 14.2 times compared to the controls (see Table 1).

Residue analysis from a random sample from the food (fish) showed that the background PCB 126 concentrations of the fish in experiment A and B were comparable (36 ± 3 pg/g fish), but that PCB 77 concentrations varied considerably among the two experiments and were on average four times higher in the fish of experiment B (0.3 ± 0.03 and 1.2 ± 0.7 respectively).

Table 1. PHAH concentrations (in ng/g fresh weight) in livers from experimentally dosed common terns. TEQ_{NO-PCB} concentrations are calculated as the sum of the concentrations of PCB 77, PCB 126 and PCB 169, multiplied by their respective TEFs (0.02, 0.1, and 0.001 respectively). NA = not analyzed.

Dosegroup	PCB 77	PCB 126	PCB 169	PCB 153	TEQ_{NO-PCB}
A/0	0.5±0.1	0.1±0.03	0.09±0.03	NA	0.02±0.006
A/.1	0.5±0.1	1.5±0.3	0.07±0.03	NA	0.2±0.05
A/1	0.5±0.1	17.6±3.7	0.11±0.05	NA	1.8±0.5
B/0	65±61	1.2±0.9	0.10±0.10	83±55	1.4±1.3
B/.1	83±72	1.9±1.5	0.08±0.05	90±11	1.8±1.6
B/.3	55±29	1.9±0.6	0.09±0.03	266±121	1.2±0.6
B/1	28±31	3.4±1.0	0.09±0.07	980±142	0.9±0.7
B/10	49±35	28.1±6.3	0.19±0.17	8748±2509	3.8±0.8
B/33	42±24	191.0±48.0	0.39.16	44690±32948	19.9±4.9





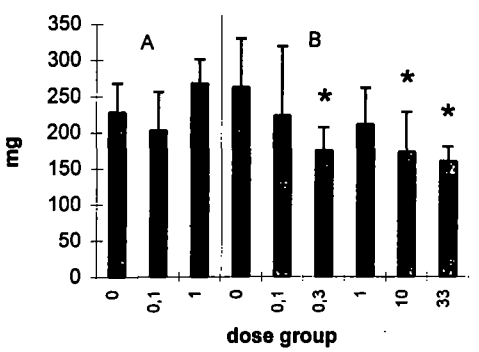


Fig. 1. Relationship between the external dose (bottom) or internal dose (top) and the weight of the bursa fabricius. For external dose levels see section Materials and Methods. Groups significantly different ($p<0.05$) from controls are marked *.

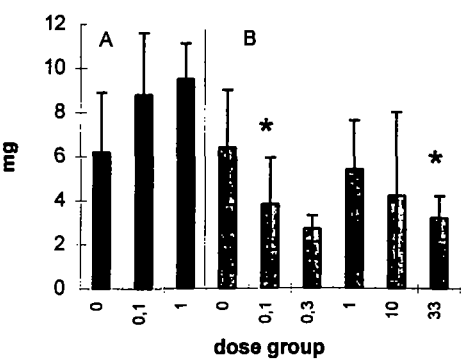


Fig. 2. Relationship between the external dose (bottom) or internal dose (top) and the weight of the thyroid. For external dose levels see section Materials and Methods. Groups significantly different ($p<0.05$) from controls are marked *.

Bodyweight and organ weights

In both experiments no significant differences were observed between the average bodyweights among the various dosegroups. Other growth related parameters such as the length of the tarsus, tibia, femur and wing showed no consistent dose-related decrease either. At the organ level dose-related negative effects were found on the bursa of fabricius in dosegroup B/.3, B/10 and B/33. When the data are plotted on an individual bases, a significant ($p<0.05$) negative correlation was found between TEQ concentrations in the liver and the weight of the bursa fabricius (see Fig. 1). At the group level also an effect on the thyroidweight was found. Compared to the controls the thyroidweight was significantly reduced in dosegroup B/.1, B/.3, and B/33. Individual analysis of the data showed no significant correlation between the internal dose (TEQ_{PHAH} in liver) and the thyroid weight (Fig. 2).

Hepatic microsomal alkoxy resorufin metabolism

The PCB 126 contaminated diet (experiment A) resulted in a significant 2.4 times increase of the EROD activity in the highest dose group (A/1) when compared with the controls (A/0). When PCB 126 was given in combination with PCB 153, a significant ($p<0.01$) 2 times increase in microsomal EROD activity was found in dosegroup B/10 compared to the control group B/0. In dose group B/33 EROD activity was 10 times increased ($p<0.001$) (see Fig. 3). Microsomal MROD activities were elevated in a similar way. PROD activities were induced to the same extend but absolute activities were approximately 30 times lower than for EROD and MROD. BROD activity showed no differences between the controls and the high dosed birds (data not shown).

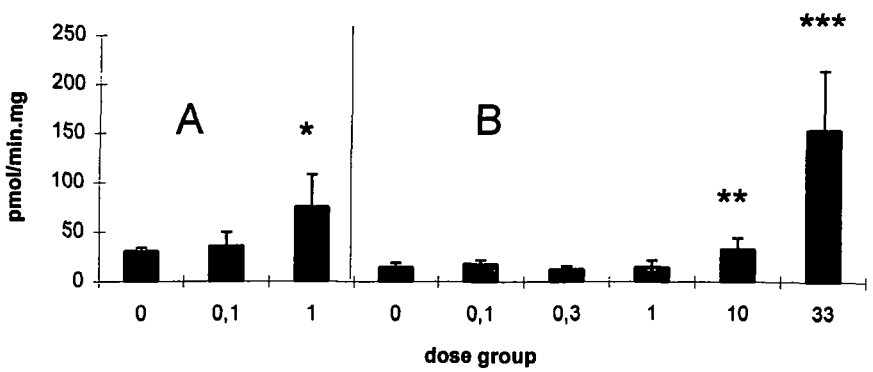


Fig. 3. Effect of PCB 126 alone (A) or in combination with PCB 153 (1:1000) (B) on the hepatic microsomal EROD activity in common tern chicks. For exposure levels in the various dose groups see section Materials and Methods. Significant differences with the controls are marked * when $p<0.05$), ** when $p<0.01$ or *** when $p<0.001$.

Hormone deregulation

Bloodsamples from experiment B (combined dose) were analysed for total thyroïd hormone (TT4) concentrations in the plasma. Concentrations in dosegroup B/33 were significantly ($p<0.05$) lower than the controls (B/0). When the data were plotted on an individual bases, a significant ($p<0.05$) negative correlation was found between the internal dose (TEQ_{PHAH} in liver) and the plasma TT4 concentrations (see Fig.4).

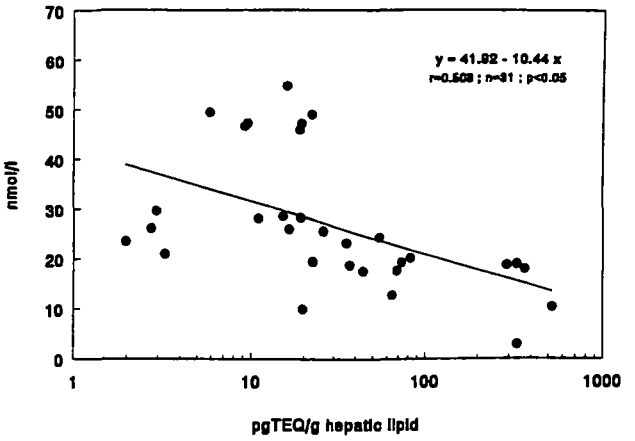


Fig. 4. Relationship between internal dose (pg TEQ_{PHAH} in liver) and the concentration of thyroid hormones (TT4) in the plasma.

DISCUSSION AND CONCLUSIONS

Post natal exposure to PCB 126 in combination with PCB 153 (1:1000) resulted in a significant induction of hepatic EROD, MROD and PROD activities, reduced plasma thyroid hormone concentrations and reduced weight of the bursa fabricius. Following exposure to PCB 126 alone, a significant induction of EROD activity was found in birds on a diet with fish additionally contaminated with 1 ng PCB 126/g fish. Expressed in TEQs this dose equals 100 pg TEQ/g in the diet (plus 11 pg TEQ/g background PHAHs in the fish). This dose resulted in an internal TEQ concentration of 1.8 $\pm$ 0.5 ng/g liver (fresh weight) and an EROD activity of 75$\pm$33 pmol/mg.min. It is noticible that the internal TEQ concentration in the controls of experiment B equals the concentrations found in the high dosed group from experiment A (1.4$\pm$1.3 and 1.8$\pm$0.5 ng/g liver respectively). Control EROD activity in experiment B was 15$\pm$4 pmol/mg.min. Absolute comparisons among the two experiments is difficult due to the different background levels in the fish used for feeding the birds, the possible differences in egg quality between the breeding seasons 1993 and 1994 and the EROD measurements in two successive years. As a result no conclusions could be drawn regarding the antagonistic or synergystic effects of PCB 153 when given in a combination with PCB 126.

The effects on the weight of the bursa of fabricius indicate a possible deregulation of the immune system. Nikolaides et al (1988) found that in chicken, *in ovo* exposed to 3,3',4,4'-tetrachlobiphenyl (PCB 77) -and with an induced EROD activity-, a reduction in the weight of the bursa of fabricius coincided with a reduction of the number of B lymphocytes. Based on the combined results it can be suggested that common terns feeding on fish containing PHAHs equivalent to 3 ng TEQ/g are at risk for malfunctioning immune responses.

The highest combination dose (B/33) resulted in a significant decrease of thyroid hormone concentrations in the plasma. This drop coincided with a significantly decreased weight of the thyroid gland. Analysis of our data on a individual level showed that TT4 concentrations were, but thyroid weights were not significantly correlated with the internal dose (see Fig. 2 & 4). This indicates that no causal relationship between these two effects exist.

When the results of our present study are compared with the results from our previously reported monitoring study (Bosveld et al 1995, Murk et al 1995) it is suggested that the effects of PCBs on EROD induction are comparable following *in ovo* exposure and *post natal* exposure. In our field monitoring study EROD was found to be induced 3.4 times in hatchlings from eggs from the Haringvliet colony compared to the reference colony. Based on results by van Hattum (1993) an average TEQ concentration in fish in the Haringvliet of 40 pg/g was calculated, which is in the same order of magnitude as the 100 pg TEQ/g that was present in the diet that resulted in an 2.4 times induction of EROD activity in the three week old chicks.

In our field monitoring study no dose-related effects on the weight of the bursa fabricius and the concentrations of thyroid hormones in the plasma were found. In our experimental feeding study, birds on a diet containing PCB 126 and PCB 153 equivalent to 3 ng TEQ/g showed a significant reduction in the weight of the bursa of fabricius and the thyroid hormone (TT4) concentrations in plasma. These results suggest that in the field, in contaminated areas, effects on the hormone homeostasis and immune functioning may be present, but these are not detected in our monitoring study (Bosveld et al 1995).

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