Toxic and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl (CB-77) and Clophen A50 on eider ducklings (Somateria mollissima) in a semi-field experiment.


Published in: Environmental Pollution

DOI: 10.1016/0269-7491(94)90005-1

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
TOXIC AND BIOCHEMICAL EFFECTS OF 3,3',4,4',5'-TETRACHLOROBIPHENYL (CB-77) AND CLOPHEN A50 ON EIDER DUCKLINGS (Somateria mollissima) IN A SEMI-FIELD EXPERIMENT*


*Department of Toxicology, Agricultural University, PO Box 8000, 6700 EA Wageningen, The Netherlands
bNetherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

(Received 10 May 1993; accepted 2 August 1993)

Abstract
In this study the possible toxic and biochemical effects of one intraperitoneal dose of 5 or 50 mg kg⁻¹ of 3,3',4,4',5'-tetrachlorobiphenyl (CB-77) or 50 or 200 mg kg⁻¹ of Clophen A50 (CloA50) on 28-day-old eider ducklings (Somateria mollissima) were investigated. After ten days, no significant differences could be observed in any of the toxic and biochemical parameters studied, apart from ethoxyresorufin (EROD) activity, when comparing group average values of the dosed and control animals. However, significant correlations were observed at day 10 after exposure between the individual internal PCB concentration and body weight gain and beak length growth (negative correlations in the CloA50 groups); relative liver weight and cytochrome P4501A activity (positive correlations in CB-77 and CloA50 groups); plasma thyroid-hormone and hepatic retinoid levels (negative correlations in CB-77 groups); and plasma retinol levels and the ratio plasma retinol/hepatic retinyl palmitate (positive correlations in CB-77 groups only). Animal activity was significantly reduced in the group that received 50 mg CB-77 kg⁻¹. These observations indicate that eider ducks are a sensitive species to PCB toxicity and may be at risk for development of adverse health effects in relatively highly contaminated areas such as the Waddenzee.

Keywords: Eider duck, PCB, vitamin A, thyroid hormone, semi-field experiment

INTRODUCTION
Polychlorinated biphenyls (PCBs) are ubiquitous environmental toxicants which accumulate especially in aquatic food chains. Of the total world production of PCBs, about 60% is still in use and it is calculated from literature data that 11–17 tonnes of PCBs still enter the North Sea each year (Klamer et al., 1991). Becker et al. (1991) found that PCB levels in eggs of various shore birds were increased 2–6-fold in 1987 compared with levels in 1981.

PCBs are known to induce a wide spectrum of toxic effects in laboratory animals (Goldstein & Sate, 1989; McConnell, 1989). In fish-eating birds, exposure to PCBs and related compounds has been associated with effects such as impaired reproduction (Hoffman et al., 1986, 1987; Kubiak et al., 1989; Van den Berg et al., 1993), morphological abnormalities (Hoffman et al., 1986, 1987), thymus atrophy and immunotoxicity (Nikolaidis et al., 1988; Andersson et al., 1991), hypovitaminosis A and/or hypothyroidy (Moccia et al., 1986; Spear et al., 1989; Van den Berg et al., 1993), behavioural changes (Kubiak et al., 1989; McArthur et al., 1983; Tori & Peterle, 1983), and loss of body weight (Koeman et al., 1973; Gilbertson, 1989). Most of these studies are, however, fieldwork in which it is impossible to make a distinction between effects caused by PCBs and those caused by other substances such as polycyclic aromatic hydrocarbons (PAHs). Some of the results of these field studies have been supported by experimental observations.

PCDDs, PCDFs and some PCBs initiate their effects, at least partially, through binding to the Ah-receptor followed by the induction of specific gene products such as cytochrome P4501A1 and 1A2. The Ah-receptor-mediated effects are induced by the parent compounds. However, reports have been published recently that indicate that the toxicity of PCBs may partly be due to Ah-mediated production of hydroxylated PCB-metabolites which interfere with the thyroid hormone and vitamin A transport system (Brouwer, 1991).

Blue mussels (Mytilus edulis) are the main source of PCB exposure for eider ducks (Broman et al., 1990). During the growth period, a single bird consumes every 24 h more than 3000 specimens of Mytilus of 15–25 mm in size. Among other substances, extreme PCB levels of 890–2400 μg g⁻¹ fat were measured in blue mussels close to the Dutch shore (Klamer et al., 1991). From earlier research it is known that female eider ducks (Somateria mollissima) are especially at risk for development of PCB toxicity because they do not feed during incubation, leading to a strong increase in blood concentration (Koeman & van Genderen, 1972). A
reduction of about 65% in the size of the breeding population of eiders on Vlieland (an island in the north of the Netherlands) was observed in the period 1988–1990. The question addressed in this study concerns whether the eiders are sensitive to PCB toxicity.

Eider ducks were experimentally exposed to a single i.p. dose of a planar PCB congener, 3,3',4,4'-tetrachlorobiphenyl (CB-77), or to a commercial PCB mixture, Clophen A50 (CloA50), under semi-field conditions. Several toxic and biochemical parameters were investigated.

MATERIALS AND METHODS

Animals

Newly hatched eider ducklings were caught on the isle of Vlieland, in the Dutch Waddenzee. They were kept on the isle of Texel at the Netherlands Institute for Sea Research (NIOZ), in two large open-air cages. Each cage consisted of a seawater section of area 49 m², 0.6 m deep, that was flushed through with filtered seawater, and a terrestrial section of 9.75 m² with a shelter and heat lamp. The animals were walked daily, to keep them in a good condition.

After 14 days, the eiders were individually numbered with an aluminium leg ring. Their weight and beak length (culmen midline) were measured daily before feeding. Food (poultry pellets, Koopmans BV, Leeuwarden) was available ad libitum during daytime until 10 p.m. Fresh water was available at all times.

Treatment

After an acclimation period of 27 days, the animals were divided among five groups and received an extra plastic leg ring with their group colour. Ten animals were dosed once by i.p. injection with corn oil [5 ml kg⁻¹ body weight (bw)] as a vehicle, six animals with 5, ten with 50 mg CB-77 kg⁻¹ bw, six animals with 50, and ten with 200 mg CloA50 kg⁻¹ bw dissolved in 5 ml corn oil kg⁻¹ bw. For practical reasons, the animals from the low-dose groups were dosed one day later than the animals in the other groups.

Blood was collected from the superficial plantar metatarsal vein at days 0, 1 and 7 after exposure. At day 10, the animals were killed under ether anaesthesia through heart puncture. Wing length was measured, and blood, liver, thymus, brain and adipose tissue were collected. Liver, thymus and brain weight were recorded immediately. After decapitation, the skull length was measured from the tip of the beak to the basioccipital condyle. Blood was centrifuged at 1000 g for 10 min and plasma was stored at −20°C. Liver was immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

PCB analyses in adipose tissue

For adipose tissue analyses, a piece of (approx. 60 mg) abdominal fat was used to extract and measure CB-77 levels and to determine the pattern of PCB congeners (CloA50 groups) by GC–ECD (analytical CPSil 8 column, 50 m × 0.25 mm i.d., Chrompack, The Netherlands), as described by Everaarts et al. (1991). Concentrations of CB-77 are expressed as μg g⁻¹ lipid. Total concentrations of CloA50 congeners are converted to toxic equivalences (TEQs) using international TEF (toxic equivalence factors) as described by Safe (1990).

Not all congeners can be separated on the analytical CPSil 8 column used. As a consequence, TEQ estimates have to be based on a limited number of congeners. None of the non-ortho-substituted PCBs (CBs -77, -126 and -169) can be determined. Of these congeners, only CB-126 is measurably present in CloA50 (Schulz et al., 1989). Of the mono-ortho-substituted PCBs present in CloA50, CB-105 and CB-118 can be determined unequivocally. CB-156, however, is not separated from CB-171. The ratio between CB-156 and CB-171 in CloA 50 is 1.43 : 0.50. As the height of the combined peak showed a constant ratio with respect to CB-180 in the eider ducklings in this experiment (Rozemeijer et al., 1991), it is assumed that the original ratio in the CloA50 mixture is maintained during the experimental period of 10 days. The measured concentrations of CB-156 will therefore be multiplied by a factor of 0.74 for TEQ calculations. The mono-ortho-substituted PCBs have TEF values of 0-001 (Safe, 1990). Of the di-ortho-substituted congeners, only CB-128, CB-138 and CB-170 are present in relatively high concentrations in CloA50 (Schulz et al., 1989). CB-128 is well separated. CB-138 co-elutes with CB-158, but since they have identical TEFs (0-00002) the concentration measured as CB-138 equivalents is applied for the calculation of TEQs. CB-170 co-elutes with CB-190 (Schulz et al., 1989). The same reasoning as for CB-156/171 is valid for these two pairs of congeners. The correction factor for measured concentrations of CB-170 is 0.65 : 0.70 = 0.93. In total, 62-4% of the total amount of TEQs present in CloA50 (Schultz et al., 1989) can be measured. To obtain an estimate of the total TEQ body burden of the CloA50-dosed animals, the observed amount was multiplied by 1.6.

EROD and PROD activities

Hepatic microsomes were prepared according to Gibson & Skett (1986), and stored in a 0.1 M sodium phosphate buffer, pH 7.4, containing 20 (v/v) glycerol, 1 mM EDTA and 1 mM dithiolthreitol in liquid nitrogen. EROD and PROD activities were determined according to Burke & Mayer (1983).

Extractions and HPLC analysis of retinoids

Aliquots of 50 μl of plasma were spiked with retinyl acetate as an internal standard and extracted with 50 μl of methanol and 100 μl of di-isopropyl ether. The ether phase was filtered over a 0.45 μm Milipore filter, dried under nitrogen gas and dissolved in 50 μl of methanol. Aliquots (20 μl) were analysed on a reversed-phase silica C₁₈ column with methanol/water (85 : 15) as eluent. Aliquots of 50 μl of liver homogenate were prepared and analysed according to the same procedures.
Effects of PCBs on eider ducklings

Table 1. Internal PCB levels in the five exposure groups of eider duck

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CB-77 5 mg kg⁻¹</th>
<th>CB-77 50 mg kg⁻¹</th>
<th>CloA50 50 mg kg⁻¹</th>
<th>CloA50 200 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEQ (ng g⁻¹ lipid)</td>
<td>0.3 ± 0.2</td>
<td>140 ± 52</td>
<td>875 ± 715</td>
<td>23 ± 17</td>
<td>129 ± 86</td>
</tr>
<tr>
<td>CB-77 (µg g⁻¹ lipid)</td>
<td>n.d.</td>
<td>14.0 ± 5.15</td>
<td>87.45 ± 71-51</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.d. Abbreviation: n.d., not determined.

as described for plasma, with the exception that the eluent composition was changed linearly in 10 min from 85:15 to 100:0 methanol/water. Retinoids were detected at 326 nm and quantified using standard curves of retinol or retinyl palmitate.

Thyroid hormones
Total thyroxine (T4) and total tri-iodothyronine (T3) levels were determined in respectively 10 and 25 µl aliquots of plasma by chemiluminescence immunoassay using commercially available kits (Amerlite assay kits, Amersham International plc, Amersham, UK).

Animal activity
Swimming and eating activities were scored in the control, the CloA50 200 mg kg⁻¹ and the CB-77 50 mg kg⁻¹ groups, by counting the number of times that the eiders crossed the footboard to the water or went eating. Activities were scored with a frequency of five times a day, for 10 min, starting three days before dosing and continuing until the end of the experiment.

Statistics
Statistical analysis of dose/effect relationships was performed by unweighted least-squares linear regression analysis. PCB levels, expressed as either mass or TEQs, and EROD activity are presented on a log scale to obtain better distribution of the values over the axes. Differences between group means were tested using the Mann–Whitney test. Differences in activity were tested with ANOVA. The acceptance level was set at P < 0.05.

RESULTS
Internal concentration of PCBs in body lipids
The mean internal PCB concentrations expressed as ng TEQ g⁻¹ lipid and µg g⁻¹ (CB-77 only) are presented in Table 1. The average TEQ values of the high-dose CloA50 group (129 ng TEQ g⁻¹ lipid) is comparable with that of the low-dose CB-77 group (140 ng TEQ g⁻¹ lipid). The individual variation is very large, up to 82% of the average value in the 50 mg CB-77 kg⁻¹ bw group.

Morphological measurements
The total body weight of the eider ducklings increased from 246–492 g (14 days old) to 949–1443 g (end of the experiment, 38 days old). This is comparable to the body weight gain of eider ducks in the field (Swennen, 1991). The beak length increased in the same period from 26.3–33.9 mm to 40.1–49.1 mm. No significant differences in group averages of total body weight gain nor increases in beak length were observed between the PCB-exposed and control groups after 10 days of exposure. In the CloA50-dosed groups, however, body weight gain in 10 days (percentage increase = 45.3–0.71 [TEQ]) and increase in beak length in 10 days (Fig. 1) were significantly negatively correlated with internal PCB concentrations expressed as pg TEQ g⁻¹ body lipid. This correlation was not seen in the CB-77-dosed eiders.

The relative liver weights (group average values) were not significantly different between PCB-exposed groups and controls. However, when the relative liver weight of each individual animal was plotted against internal CB-77 level (µg g⁻¹ lipid) or CloA50 (ng TEQ g⁻¹ lipid), significant positive correlations were observed (Fig. 2a,b).

No significant alterations were observed for relative thymus weight, relative brain weight, wing length and skull length, either compared as group averages or individually correlated with PCB internal concentrations.

Hepatic EROD activity
Cytochrome P4501A induction measured as EROD activities were dose-dependently increased in the CB-77 groups (respectively 60.2 ± 13.4 and 502.8 ± 412.9 nmol mg⁻¹ min⁻¹) compared with control values (8.8 ± 4.1 nmol mg⁻¹ min⁻¹). No significant differences were found for both CloA50-dosed groups (respectively

Fig. 1. Increase in beak length in CloA50-dose animals between day 0 and day 10 of the experiment plotted against the PCB concentration, expressed as ng TEQs g⁻¹ body lipid ($r = 0.62, P < 0.05$).
Fig. 2. Relative liver weight of eiders dosed with (a) CB-77 ($r = 0.63$, $P < 0.05$) and (b) CloA50 ($r = 0.43$, $P < 0.05$), plotted against internal PCB concentration expressed as, respectively, mg CB-77 g$^{-1}$ and ng TGEQ g$^{-1}$ body lipid.

9.7 ± 11.7 and 17.4 ± 14.0 nmol mg$^{-1}$ min$^{-1}$) compared with the control group. However, when correlated on an individual basis, the EROD activities in both the CB-77 and the CloA50 groups were significant positively correlated with internal PCB levels (respectively, log EROD = 1.19 + 0.48 log [CB-77], log EROD = 0.46 + 0.41 log [TEQ], $r = 0.70$, $p < 0.001$; and log EROD = 0.46 + 0.41 log [TEQ], $r = 0.67$, $p < 0.005$).

Thyroid hormone measurements

The average total thyroxine (T4) levels in the control group increased from 6.1 ± 1.2 nmol litre$^{-1}$ plasma at day 0 to 11.7 ± 4.2 nmol litre$^{-1}$ day 10 of the experi-

### Table 2. Plasma total thyroxine (T4) and total thyronine (T3) levels in control and PCB-exposed eider ducklings

<table>
<thead>
<tr>
<th>Parameter (nmol litre$^{-1}$)</th>
<th>Control</th>
<th>CB-77 5 mg kg$^{-1}$</th>
<th>50 mg kg$^{-1}$</th>
<th>CloA50 50 mg kg$^{-1}$</th>
<th>200 mg kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4, day 1</td>
<td>7.5 ± 2.6</td>
<td>9.1 ± 3.3</td>
<td>7.7 ± 2.2</td>
<td>9.7 ± 3.2</td>
<td>6.9 ± 1.9</td>
</tr>
<tr>
<td>T4, day 7</td>
<td>9.7 ± 2.8</td>
<td>8.3 ± 1.9</td>
<td>10.4 ± 1.5</td>
<td>7.5 ± 2.5</td>
<td>10.3 ± 3.4</td>
</tr>
<tr>
<td>T4, day 10</td>
<td>11.7 ± 4.2</td>
<td>13.3 ± 2.5</td>
<td>12.7 ± 3.5</td>
<td>13.9 ± 5.7</td>
<td>9.9 ± 3.2</td>
</tr>
<tr>
<td>T3, day 1</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>T3, day 7</td>
<td>1.9 ± 0.4</td>
<td>2.5 ± 0.3*</td>
<td>1.6 ± 0.3</td>
<td>2.7 ± 0.1*</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>T3, day 10</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3*</td>
<td>1.3 ± 0.5*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.d.

* Significantly different from control with $P < 0.05$. 
Effects of PCBs on eider ducklings

Fig. 4. Plasma retinol levels 1, 7 and 10 days after dosing with PCBs, expressed as a percentage of the control values (absolute values can be found in Table 3). For CB-77 and Clophen A50-dosed groups, low doses are (respectively) 5 and 50 mg kg⁻¹ bw, high doses (respectively) 50 and 200 mg kg⁻¹ bw. An asterisk (*) indicates significant difference from control values, with P < 0.05. , Low dose; , high dose.

Plasma total tri-iodothyronine (T3) levels decreased from 1.8 ± 0.6 to 0.9 ± 0.2 nmol litre⁻¹ over the same period. There were no significant differences in group average plasma values for T4 levels at any time point of exposure (Table 2). Group average values of plasma total T3 levels of the low-dose CB-77 and the low-dose CloA50 groups were significantly higher than control levels (respectively 132% and 142%) at day 7 of exposure (Table 2). At the same time point, a reduced plasma total T3 level compared with the control group (respectively 84% and 68%) was observed in both high-dose groups, but this difference was significant only in the CloA50 200 mg kg⁻¹ group. At day 10, plasma total T3 levels in both CB-77-dosed groups did not differ from the controls. In the low- and high-dose CloA50 groups, total T3 levels were significantly higher compared with controls (138% for the 50 mg kg⁻¹ and 163% for the 200 mg kg⁻¹ CloA50 group).

Individual plasma total T4 levels were significantly negatively correlated with internal CB-77 levels at day 10 of exposure only (Fig. 3a). Plasma total T3 levels were significantly negatively correlated with CB-77 levels at day 7 (Fig. 3b). No significant correlations of thyroid hormone levels with internal PCB concentrations were found in both CloA50-dosed groups.

Plasma retinol and hepatic retinoids

During the experimental period, the average plasma retinol levels in the control eider ducks were 2.0 ± 0.22 µg ml⁻¹. Plasma retinol levels of the PCB-dosed groups at days 1, 7 and 10, expressed as a percentage of the control values, are shown in Fig. 4. In the CB-77-exposure groups, a significant decrease to 84 and 82% of the control values is observed at day 1, of exposure, followed by a return to control levels at day 7, and a subsequent increase at day 10 to 108% for the low-dose and 127% for the high dose group. In the CB-77 groups, plasma retinol levels were significantly increased with PCB internal dose, ten days after dosing (Fig. 5a).

Hepatic retinol, retinyl palmitate and retinyl stearate levels showed large inter-individual variations (up to 95%; Table 3). The only significant difference in group averages from control values was a reduction to 65% of the retinyl palmitate level in the high-dose CB-77 group. Hepatic retinoid levels were also correlated on an individual basis with the PCB body burdens. In the CB-77-exposure groups a clear, significant, negative correlation between internal PCB levels and hepatic retinol and both retinyl ester concentrations was observed. Figure 5(b) shows this relationship for hepatic retinyl palmitate. In the CloA50-dosed animals no such relationships were found.

The average ratio between plasma retinol and hepatic retinyl palmitate is significantly different from the controls in the CB-77 50 mg kg⁻¹ group (Table 3). The ratio of hepatic retinol to hepatic retinyl palmitate was not significantly correlated with the internal PCB concentration. The ratio of plasma retinol to hepatic retinyl palmitate (µg g⁻¹ lipid) however, was significantly positively correlated with the internal CB-77 concentration (µg g⁻¹ lipid) (ratio = −15 + 24.7 log [CB-77]; r = 0.74, P < 0.05). In the CloA50-dosed animals, this ratio did
not change with the PCB body burden (expressed as TEQs).

**Animal activity**

In the control group, swimming and eating activity gradually increased over the experimental period from 10 times per animal per day (five observations of 10 min per day, for both activities) to respectively 17 and 19 times per animal per day. This increase was less in the CB-77-50 mg kg\(^{-1}\) group. Swimming activity in this CB-77 group was 79% and eating activity 77% of the control values. In the CloA50 200 mg kg\(^{-1}\) group, 10 times per animal per day (five observations of 10 min per day). This increase was less in the CB-77-50 mg kg\(^{-1}\) group. Swimming activity in this CB-77 group was 79% and eating activity 77% of the control values. In the CloA50 200 mg kg\(^{-1}\) group.

**DISSCUSSION**

In this semi-field study eider ducklings were found to be responsive with respect to Ah-receptor-mediated responses, e.g. cytochrome P4501A induction and hepatomegaly. Some changes were observed in vitamin A and thyroid-hormone levels that may be partially mediated by the Ah-receptor. Reduced growth and responses, e.g. cytochrome P4501A induction and hepatomegaly. Some changes were observed in vitamin A and thyroid-hormone levels that may be partially mediated by the Ah-receptor. Reduced growth and responses.

**Variation in internal dose**

The average internal PCB-concentrations in the body lipid of the eiders dosed with 5 mg CB-77 kg\(^{-1}\) was 140 ± 52 ng TEQ g\(^{-1}\) lipid, of the 200 mg CloA50 kg\(^{-1}\) dosed group 129-4 ± 85-9 ng TEQ g\(^{-1}\), and of the 50 mg CloA50 kg\(^{-1}\) group 23 ± 17 ng TEQ g\(^{-1}\) lipid. These levels were comparable with the levels found in yolk sacs of eggs from fish-eating birds in recent bird studies in The Netherlands. In relatively polluted cormorant yolk sacs (Biesbosch), average PCB levels of 136 ng TEQ g\(^{-1}\) lipid were found (Van den Berg et al., 1993), and in common tern (Sterna hirundo) yolk sacs from a relatively polluted colony (Slijkplaat) 40 ng TEQ g\(^{-1}\) lipid (Murk et al., 1993; Bosveld et al., in preparation). In our semi-field experiment, as well as in both field studies mentioned above, the large variation in internal dose and measured parameters was obvious. This is mainly due to large intrinsic differences between individuals from a wild species compared with laboratory animals. Even when the diet is identical for all individuals and exposure is controlled, as in our semi-field experiment, correlation of the measured parameters with the individual internal PCB concentration is essential for studying effects.

**Ah-mediated effects**

A strong P450 induction was observable in the CB-77 groups, whilst no significant induction was observed in the CloA50 groups (Rozemeijer et al., 1991). We nevertheless found a PCB-related EROD induction in both CB-77 and CloA50 groups. In both CloA50- and CB-77-dosed eiders, we also found a significant positive correlation between relative liver weight and PCB body burden. This usually coincides with P450 induction.

For a number of reasons it is difficult to compare the relationship PCB between body burden and EROD activity that we found here with the results of other studies; firstly, this is because wild birds are always exposed to various chemicals which may induce or inhibit P450 activity; secondly, it was because most of the birds used were adults, often caught during the breeding season (Walker, 1990), when the mixed-function oxidase activity has its peak (Fossi et al., 1989). We therefore only compared our results with that of one-day-old chicks of common tern and cormorant. In our experiment, the ratio of log (EROD activity) to log [CB-77] internal dose was 0.49, with the corresponding ratio to [TEQ] internal dose in the CloA50-dosed groups 0.41. In one-day-old cormorants (Van den Berg et al., 1993), the ratio of log (EROD activity) to log [mono-ortho-PCBs] was 0.49 and in one-day-old common terns 0.50 was the ratio to log [TEQ]. The eider ducklings therefore showed a P4501A-inducing potency comparable with that of the cormorant and the common tern.

The only study reported of the effects of PCBs on eiders was performed with eggs by Brunström et al. (1990). They concluded from egg injection experiments that elder embryos were rather insensitive to CB-77 and 3,3',4,4',5-pentachlorobiphenyl. They did not find significant embryonic mortality at a dose of 1 mg CB-77 kg\(^{-1}\) egg, but they did not measure biochemical

---

**Table 3. Hepatic and plasma retinoid levels in control and PCB-exposed eider ducklings**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CB-77</th>
<th>CloA50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg kg(^{-1})</td>
<td>50 mg kg(^{-1})</td>
<td>50 mg kg(^{-1})</td>
</tr>
<tr>
<td>Hepatic retinol ((\mu g) g(^{-1}) liver)</td>
<td>5.1 ± 3.6</td>
<td>9.7 ± 8.2</td>
<td>9.8 ± 5.1</td>
</tr>
<tr>
<td>Hepatic retinyl palmitate ((\mu g) g(^{-1}) liver)</td>
<td>17.0 ± 6.0</td>
<td>20.0 ± 8.2</td>
<td>11.1 ± 5.2*</td>
</tr>
<tr>
<td>Hepatic retinyl stearate ((\mu g) g(^{-1}))</td>
<td>6.9 ± 2.5</td>
<td>8.6 ± 3.8</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Plasma retinol day 1 ((\mu g) ml(^{-1}))</td>
<td>6.9 ± 2.5</td>
<td>8.6 ± 3.8</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Plasma retinol, day 7 ((\mu g) ml(^{-1}))</td>
<td>8.7 ± 4.9</td>
<td>9.5 ± 2.1</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Plasma retinol, day 10 ((\mu g) ml(^{-1}))</td>
<td>8.7 ± 4.9</td>
<td>9.5 ± 2.1</td>
<td>6.2 ± 2.2</td>
</tr>
<tr>
<td>Ratio retinol/retinyl palmitate*</td>
<td>0.11 ± 0.04</td>
<td>0.12 ± 0.07</td>
<td>0.24 ± 0.11*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.d.

* Significantly different from control with \(P < 0.05\).

* Ratio of (plasma retinol)/(hepatic retinyl palmitate).
parameters such as EROD activity or retinoid levels. It is difficult to compare the results of their egg experiment with our results with eider ducklings because no internal doses were measured in the eggs. A very rough estimation of the internal dose per gram of yolk sac lipid after injection of 2 mg CB-77 kg\(^{-1}\) egg, based on the assumption that 4% of the egg is lipid, as in cormorant eggs (Van Schaik & De Voogt, 1989), and that all PCBs will be present in the lipid, results in a PCB level of 50 mg CB-77 kg\(^{-1}\) lipid. In our experiment, such a body burden resulted in large reductions in hepatic retinoid stores and an increase of EROD activity.

Morphology

In our experiment, we did not detect any morphological aberrations like the ones related to PCBs in field studies with black-crowned night herons (Nycticorax nycticorax) (Hoffman et al., 1986) and Forster’s tern (Sterna fosteri) (Hoffman et al., 1987). One possible explanation is that, under natural circumstances, exposure already takes place in ovo, during organ formation. The eider ducklings in our experiment were dosed at the age of 28 days. They were growing rapidly (36% in 10 days), but tissue differentiation had already taken place before dosing. On the other hand, important differences between field studies and more controlled experimental studies, like our eider study, are the presence in the field of various other pollutants and confounding factors such as parasites, which can cause additional effects that may be attributed to PCBs.

We did find ±45% difference in body weight gain between the animals with low and high PCB body burdens in the CloA50-dosed groups. The mechanism of body-weight loss and reduced body weight gain is not yet elucidated.

Thyroid hormones

In the unexposed eider ducklings, plasma T3 levels decreased by about 50% during the experimental period and T4 levels increased by 90%. This is in accordance with Decuyper & Kühn (1988), who described an increase in T4 concentrations with age and weight in chicks after hatching, up to 128 days. In those chicks, plasma T3 levels increased in the first two weeks after hatching and then declined with age.

In both CloA50- and CB-77-dosed groups there was a significant decrease in plasma T3 level at day 7 related to PCB-body burdens, but not at day 10. Plasma T4 levels were significantly negatively correlated with internal CB-77 concentrations only in the CB-77 group at day 10 of the experiment. Under normal circumstances, reductions in thyroid-hormone levels will be compensated by additional secretion of thyroid hormone by the thyroid gland. We nevertheless found significant plasma T3 and T4 reductions at, respectively, 7 and 10 days after a single CB-77 dose. A reduction of thyroid-hormone levels in birds by PCBs has been demonstrated before in experiments with black-backed gulls (Larus fuscus), pigeons (Jefferies & French, 1971) and Japanese quail (Coturnix coturnix; Grässle & Biemann, 1982). In field studies a correlation between PCB-body burdens and thyroid-hormone levels has been demonstrated in common tern (Murk et al., 1993) and cormorants (Van den Berg et al., 1993).

PCBs have been reported to influence thyroid-hormone levels at two levels at least: by interference of hydroxylated metabolites with the T4 transportation system (Brouwer et al., 1990, Lans et al., 1993) and by induction of T4-glucuronidation, thus enhancing hepatic elimination of thyroid hormone (Barter & Klaassen, 1992; Beetsstra et al., 1991; Visser et al., 1993). Arguments that both mechanisms may actually be involved in eiders and other fish-eating birds have been obtained recently. From in vitro metabolism experiments (Murk et al., 1994), it was apparent that hepatic microsomes of eiders from the CloA50 and the CB-77 groups, are able to produce especially SOH-metabolites of CB-77. These metabolites are very potent in interfering with the T4 transport system (Lans et al., 1993). In a recent experiment with common tern chicks, a significant positive correlation between T4-glucuronidation and PCB body burdens was demonstrated (Murk et al., 1993).

Thyroid hormones are important regulators of physiological functions, e.g. the maintenance of body temperature (Falconer, 1984), energy metabolism, growth and differentiation of epithelia, and synchronization of reproduction and migration of birds with the seasons (Nicholls et al., 1985, Kar & Chandola, 1985; Sharp & Klandorf, 1985; Dawson, 1989a,b). However, at present it is unknown whether thyroid-hormone-related health effects may develop in fish-eating birds upon exposure to environmental levels of PCBs.

Retinoids

One day after exposure, a decrease of plasma retinol levels in the CloA50-50 mg kg\(^{-1}\) group animals and a significant decrease of plasma retinol levels in both CB-77-dosage groups was observed to approximately 80% (Fig. 4). The plasma retinol levels probably are influenced by at least two processes: an increase by enhanced mobilization from the liver and a decrease through interference of the hydroxylated metabolites with the plasma transport. In laboratory animals such as mice, a reduction in plasma retinol concentration can be observed within a day after a single dose of CB-77, followed by a recovery, and sometimes overcompensation, in the following days (Murk et al., 1991).

The hepatic retinyl palmitate levels were more than four times lower in the animals with high CB-77 body burdens than in the animals with low CB-77 levels (Fig. 5b). At the same time (day 10 of the experiment) plasma retinol levels were significantly positively correlated with CB-77 body burden (Fig. 5b). These results were not observed for the CloA50-dosed animals.

In laboratory studies with rats dosed 40 mg of 3,3,4,4',5,5'-hexabromobiphenyl (HBB) kg\(^{-1}\) bw, Spear et al. (1987) observed elevated serum retinol levels after 28 days, increased liver weight, and decreased liver...
retinol and retinyl palmitate levels. In rats dosed with 20 mg HBB kg\(^{-1}\) bw, only liver retinol and retinyl palmitate levels declined. They found in vitro elevated hydroxylation and conjugation by UDP-glucuronyl transferase of retinoic acid in liver microsome that corresponded with increased activities of P4501A. Spear et al. (1985, 1990) observed lowered liver retinol and retinyl palmitate levels in natural populations of herring gulls (Larus argentatus) from contaminated colonies, compared with relatively clean colonies. Also, in our field study with common tern chicks, we observed reduced yolk sac retinyl palmitate levels and increased plasma retinol concentrations (Murk et al., 1993).

If the processes mentioned above continued for a longer period of time, depletion of the retinoid store should be expected. This may have consequences for vitamin A-mediated physiological functions such as reproduction, differentiation of epithelia and good skin condition (Sporn & Roberts, 1983), and resistance against infections (Sijtsma et al., 1989).

**Behaviour**

In our experiment we found a significant decrease in swimming and eating activity for the CB-77-50 mg kg\(^{-1}\) animals. This effect was not found for the CloA50-200 mg kg\(^{-1}\) group. PCBs have been shown to influence bird behaviour much as parental attentiveness (Kubiak et al., 1989; McArthur et al., 1983), courtship behaviour (Tori & Peterle, 1983) and avoidance behaviour (Kreitzer & Heinz, 1974). The mechanism through which behaviour is influenced is not yet clear. For eider ducklings it is very important to react on a distress call of the females by congregating into as compact a group as possible for protection against predators (Swennen, 1989). Under natural circumstances, less active reaction could decrease the chance of survival.

**CONCLUSIONS**

From this experiment we can conclude that eider ducks are vulnerable to the toxic action of PCBs. This effect is at least partially Ah-receptor-related. Whether PCBs actually have adverse health effects on eiders in natural populations is not known. In The Netherlands, eider populations are not only exposed to various chemicals, but they are also seriously affected by mechanical destruction of mussel banks by fishing activity. This forces the eiders to eat more crabs, which contain the parasite Polymorphus botulus, leading to increased incidence of infection (Swennen, 1991). Because of intrinsic individual differences between the wild eiders in our experiment, correlation of the measured parameters with the individual internal dose is essential in studying effects. This is even more important for field studies where exposure occurs via the diet and thus the individual food choice of the animals is an extra source of variation.

**ACKNOWLEDGEMENTS**

We thank J. S. J. van de Sant, E. van Arnhem, W. Mullie, and members of the Department of Toxicology, Wageningen, for helping us out at crucial moments.

**REFERENCES**


