VITAMIN A REDUCTION IN EIDER DUCKLINGS *(Somateria mollissima)* EXPOSED TO POLYCHLORINATED BIPHENYLS (CB-77 AND CLOPHEN A50)

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ABSTRACT
Eider ducklings (*Somateria mollissima*) kept under semi-field conditions were exposed to one intraperitoneal dose of a planar polychlorobiphenyl (PCB), 3,3',4,4'-tetrachlorobiphenyl (CB-77) or a commercial PCB-mixture Clophen A50 (CloA50). After ten days the animals were sacrificed and the internal dose and various biochemical parameters were measured. In both CB-77 and CloA50 groups cytochrome P450IA activity and relative liverweight were increased. In CB-77 dosed animals hepatic retinol and retinylesters were reduced and plasma retinol levels were elevated. These effects were not found in CloA50 dosed eiders.

INTRODUCTION
Wild eider ducks consume more than 3,000 specimens of blue mussels (*Mytilus edulis*) every 24 hours during the growth period[1]. Extreme PCB-levels of 890-2400 µg/g fat were measured in blue mussels close to the Dutch shore, so high exposure is to be expected. Especially female eiders are vulnerable to PCB toxicity because they do not feed during incubation, which results in a strong increase in blood concentration of polyhalogenated aromatic hydrocarbons[2]. We investigated the possible adverse effects of PCB exposure to eider ducks (*Somateria mollissima*) under-semi field conditions. This paper deals with effects on hepatic and plasma vitamin A levels in relation to P450IA activity.

METHODS
Animals and treatment: Newly hatched, wild eider ducklings were kept in large open air cages, flushed through with seawater. They were fed Poultry pellets (Koopmans BV, Leeuwarden) and fresh water was available *ad libitum*. After an acclimatization period of 27 days, the animals received one i.p. injection with either cornoil (5 ml/kg bw., 10 eiders) as a vehicle, 5 mg (6 animals) or 50 mg (10 animals) of CB-77/kg bw., or 50 mg (6 animals) or 200 mg (10 animals) CloA50/kg bw. dissolved in cornoil. Blood was collected at days 0, 1, and 7 after exposure. At day 10 the animals were killed, liver was weighed,

frozen in liquid nitrogen and stored at -80°C until further analysis. Blood was centrifuged at 1000 g for 10 minutes and plasma was stored at -20°C. ***Hepatic EROD activities*** were measured according to Burke & Mayer[3].

PCB analyses were performed with GC-ECD[4] (analytical SE54 column, 50 m x 0.32 mm I.D., Chrompack, the Netherlands). Total concentrations of PCB congeners in the CloA50 dosed animals are converted to toxic equivalences (TEQs) using international TEF factors[5]. Due to separational problems we only could measure 62.4% of the pcbTEQs present, the result was multiplied by 1.6[6]. ***HPLC analysis of retinoids*** was performed according to Brouwer et al.[7] with aliquots of 50 µl plasma or liver homogenate on a reversed phase silica C18 column. Retinoids were detected at 326 nm.

Statistics: dose effect relationships were tested with unweighed least squares linear regression analysis. PCB levels and EROD activity were used on a log scale. Differences between group means were tested using the Mann-Whitney test. The acceptance level was set at $p<0.05$.

RESULTS AND DISCUSSION

As is obvious from the table, a large variation in internal dose and measured parameters was observed in our semi-field experiment. The extent of this variation was a surprise to us, as the animals were dosed i.p. and were fed with a similar diet. As a result most biochemical and toxic effects were only visible when correlated with individual PCB body burdens, but not with comparison of group averages[6]. EROD activities of both CB-77 groups were significantly higher than the control groups. This was not the case for both CloA50 dosed groups (see table)[4]. However, when correlated on an individual basis, the EROD activities in both the CB-77 and the CloA50 groups significantly increased with internal PCB-levels (data not shown). In both CloA50 and CB-77 dosed eiders we found a significant positive correlation between PCB-body burden and relative liverweight.

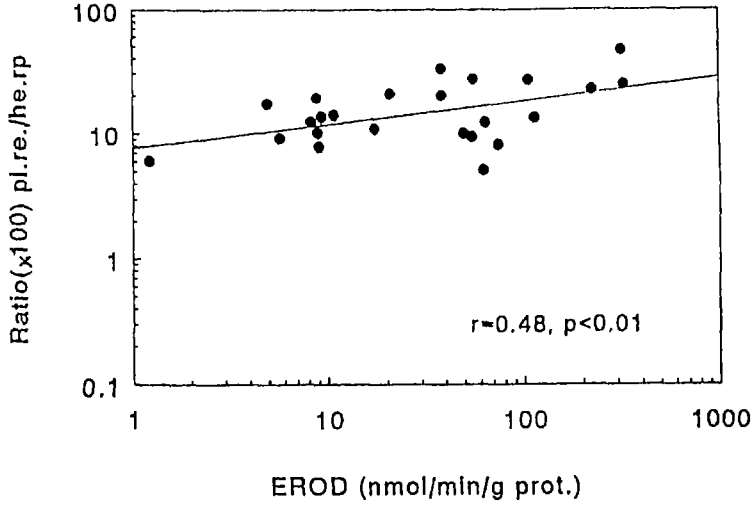
A significant negative relationship between PCB levels and hepatic retinol, retinyl stearate and retinyl palmitate was observed in the CB-77 exposure groups, but not in the CloA50 dosed animals[6] However, the CloA50 dosed animals had a much lower body burden than the CB-77 dosed animals (see table). In both CB-77 exposure groups plasma retinol levels were decreased significantly to 84% and 82% of the control values one day after 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. At day 10 the plasma retinol levels of the CB-77 dosed eiders significantly increased with PCB-body burdens. The figure shows a significant correlation between the ratio plasma retinol/hepatic retinylpalmitate and EROD activity for CB-77 dosed and control eiders, suggesting an P4501A related mechanism. In the CloA50 dosed groups a significantly negative correlation was only observed between plasma retinol and levels of pcbTEQ/g lipid) at day 1.

At least two Ah-linked mechanisms can influence vitamin A levels. 1): induction of P450 enzmes and UDPglucuronyltansferase that may speed up hepatic metabolism and mobilisation of vitamin A leading to an increase in plasma retinol levels, and 2): P450-

Average internal PCB-level, EROD activity and hepatic retinylpalmitate level in PCB exposed and non-exposed elder ducks.

Parameter	Control	PCB-77 5 mg/kg	PCB-77 50 mg/kg	CloA50 50 mg/kg	CloA50 200 mg/kg
pcbTEQ (ng/g lipid)	0.3 ± 0.2	140 ± 52 ***	875 ± 715 ***	23 ± 17 *	129 ± 86 *
EROD (nmol/mg protein)	9 ± 4	60 ± 13 **	503 ± 413 **	10 ± 12	17 ± 14
Hep.Ret.palm. (µg/g liver)	17 ± 6	20 ± 8	11 ± 5 *	19 ± 9	20 ± 4

* significantly different from control with $p<0.05$; ** with $p<0.01$; *** with $p<0.005$



Ratio plasma retinol/hepatic retinylpalmitate against EROD-activity of CB-77 dosed and control eiders.

mediated production of hydroxylated PCB-metabolites which interfere with vitamin A transport system leading to a decrease in plasma retinol levels[7,8]. From an *in vitro* metabolism experiment it was apparent that eiders (from the CloA50 and the CB-77 groups) are able of producing especially the very potent 5-OH-metabolites of CB-77[8]. The significant decrease of plasma retinol levels in Clophen dosed animals at day 1 suggests interference of hydroxylated PCB metabolites with the plasma transport of retinol, resulting in an increased loss of retinol. P450 related reduction of hepatic retinylpalmitate and retinol levels

and increased plasma retinol levels was also observed in laboratory studies with 3,3',4,4',5,5'-hexabromobiphenyl/kg dosed rats[10], and in natural populations of herring gulls (Larus argentatus) from contaminated colonies compared to relatively clean colonies[11]. In one day old common tern chicks with relatively high PCB body burdens plasma retinol levels were significantly higher and yolksac retinylpalmitate levels lower than from tern chicks with low body burdens[12].

In conclusion, these data indicate that eider ducks are responsive to PCB toxicity and show cytochrome P450 related retinoid reduction.

LITERATURE

1 Broman, D., Naf C., Lundbergh I. and Zebuhr Y., 1990. An in situ study on the distribution, biotransformation and flux of polycyclicaromatic hydrocarbons (PAHs) in an aquatic food chain (Seston-*Mytilus edulis* L.-*Somateria mollissima* L.) from the baltic: an ecotoxicological perspective. Environ. Toxicol. Chem., 9: 429-442.

2 Koeman, J.H., and van Genderen H., 1972. Tissue levels in animals and effects caused by chlorinated hydrocarbon insecticides, chlorinated biphenyls and mercury in the marine environment along the Netherlands coast. Marine pollution and dea life, Fishing New (Books) Ltd, Surrey, pp.428-435.

3 Burke, M.D., Mayer R.T., 1983. Differential effects of phenobarbitone and 3-methylcholanthrene induction on hepatic microsomal metabolism and cytochrome P-450 binding of phenoxazone and a homologous series of its alkyl ethers (alkoxyresorufins). Chem.-Biol. Interactions 45: 243-258.

4 Rozemeijer, M.J.C., Boon J.P., Duiven P., Meer J. van der, Sant J.S.J. van der, Swennen C., Murk A.J., Berg J.H.J. van der e.a., 1991. The effect of 3,3',4,4'-tetrachlorobiphenyl and clophen A50 on the hepatic monooxygenase system of eider ducklings (Somateria mollisima) with indications for structure related biotransformation of CB-congeners. Mar. Env. Research 34: 207-213.

5 Safe, S., 1990. Polychlorinated biphenyls (PCBs), Dibenzo-p-Dioxins (PCDDs), Dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21(1) 51-88.

6 Murk A.J., Van den Berg J.H.J., Fellinger M., Rozemeijer M.J.C., Swennen C., Duiven P., Boon J.P., Brouwer A., Koeman J.H.. 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. Submitted to Env. Poll.

7 Brouwer, a., Blaner W.S, Kukler A., Berg van den K.J., 1988. Study on the mechanism of interference of 3,4,3',4'-tetrachlorobiphenyl with the plasma retinol-binding proteins in rodents.

8 Lans, M.C., Klasson-Wehler, E., Willemsen, M., Meussen, E., Safe, S., Brouwer, A., 1993. Structure dependent competitive interaction of hydroxychlorobiphenyls, dibenzo-p-dioxins and dibenzofurans with human transthyretin. Chem.-Biol. Interactions, in press.

9 Murk, A.J., Brouwer A., Klasson wehler, E., Morse D.C., 1993. In vitro metabolism of 3,4,3',4'-tetrachlorobiphenyl by microsomes of six wildlife species compared to the rat. Submitted for presentation at Dioxin 1993.

10 Spear, P.A., Garcin H. and Narbonne J.F., 1987. Increased retinoic acid metabolism following 3,3',4,4',5,5'-hexabromobiphenyl injection. Can. J. Physiol. Pharmacol, 66: 1181-1186.

11 Spear, P.A., Moon T.W. and Peakall D.B., 1985. Liver retinoid concentrations in natural populations of herring gulls (*larus argentatus*) contaminated by 2,3,7,8-tetrachlorodibenzo-p-dioxin and in ring doves (*Streptopelia risoria*) injected with a dioxin analoque. Can. J. Zool., 64: 204-207.

12 Murk, A.J., Spenkelink, A., Brouwer, A., Van Kampen, M., Bosveld, A.T.C., Gradener, J., Van den Berg, M., 1993. Effects of PCBs, PCDDs and PCDFs on reproductive success, and morphological, physiological and biochemical parameters in chicks, of the common tern (Sterna hirundo). Part II: laboratory study. Rijkswaterstaat, Den Haag, 60 pp.