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Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*)

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In this study the effect of polychlorinated biphenyl (PCB)-contaminated fish on plasma retinol (vitamin A) and thyroid hormone concentrations, i.e. sensitive indicators of PCB-intoxication, were investigated in the common seal *Phoca vitulina*. Seals fed fish from the Wadden Sea (high-level PCB contamination) had significantly lower concentrations of plasma retinol, total (TT4) and free thyroxin (FT4) and triiodothyronin (TT3) as compared to seals fed fish from the north-east Atlantic (low-level PCB contamination). The PCB-induced reduction in plasma retinol levels disappeared when seals on a Wadden Sea fish diet were subsequently fed Atlantic Ocean fish. It is suggested that reduced plasma retinol and thyroid hormone levels, which may result in an increased susceptibility to microbial infections, reproductive disorders and other pathological alterations, are critically involved in the recently reported reproductive disorders and the lethal viral infections in seals and other marine mammal populations in the Baltic, North Sea and Wadden Sea.

Key words: Polychlorinated biphenyl; Seal; Vitamin A; Thyroid hormone; Plasma

INTRODUCTION

Environmental pollutants, such as the persistent polychlorinated biphenyls (PCBs) have been implicated as possible causative agents of the drastic reduction in the common seal *Phoca vitulina* population in the Dutch part of the Wadden Sea (Reijnders, 1980; Reijnders, 1981). Fish from the Wadden Sea contaminated with PCBs, mainly as a result of PCB pollution from the river Rhine (Koeman et al., 1969; Duinker and Hillebrand, 1979; Duinker et al., 1982a,b, 1984), were reported to cause reproductive failure in common seals (Reijnders, 1986).

Studies on the mechanism of PCB-induced toxicity in experimental animals have

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indicated an important role of vitamin A and thyroid hormones (Brouwer and Van den Berg, 1986; Brouwer et al., 1986). Exposure of rats and mice to either commercial mixtures of PCBs, or to single, toxic PCB-congeners resulted in drastic reductions of plasma retinol and thyroxin concentrations (Brouwer et al., 1985; Brouwer, 1987, 1989). The in tandem reduction of plasma retinol and thyroxin levels appeared to be a consequence of interference of a PCB-metabolite with the plasma transport protein complex for both ligands (Brouwer and Van den Berg, 1986; Brouwer et al., 1988). A reduced plasma retinol concentration is indicative for the development of a vitamin A deficiency, which may result in an increased susceptibility to viral infections (Hof and Wirsing, 1979; Nauss et al., 1979; Shenai et al., 1985), reproductive disorders (Thompson et al., 1964; Moore, 1967) and other pathological alterations.

In this study, the possible impact of fish containing different levels of PCBs on vitamin A and thyroid hormone concentrations of common seal populations was investigated in plasma samples from a previously reported study, designed to investigate reproductive effects of pollutants (Reijnders, 1986).

MATERIALS AND METHODS

Animal treatment

Two groups of 12 female common seals *Phoca vitulina* were fed a diet containing different levels of pollutants for almost 2 yr. Group 1 was fed fish (predominantly plaice, flounder and dab) from the western part of the Wadden Sea. Group 2 received fish (mainly mackerel) from the north-east Atlantic. The fish were caught in four lots in the same season and exactly the same location, randomized by complete mixing and stored at −28°C (for detailed information, see Reijnders, 1986). The dietary intake was comparable with respect to nutritional quality for both groups of seals.

Residue analysis for aldrin, dieldrin, endrin, heptachlor, hepox, α, β, γ-hexachlorocyclohexane, pentachlorobenzene, hexachlorobenzene, *p,p'*-dichlorodiphenyl-dichloroethylene (DDE), *op'*-dichlorodiphenyldichloroethane, *pp'*-dichlorodiphenyl-dichloroethane and PCBs (isomer specific) showed statistically significant differences between the two diets for PCBs and *pp'*-DDE. A detailed report on residue analysis of the fish diets has already been reported earlier (Boon et al., 1987). The average daily intake was estimated to be 1.5 mg of PCBs and 0.4 mg *pp'*-DDE in the high contaminated diet and 0.22 mg of PCBs and 0.13 mg of *pp'*-DDE in the low contaminated diet.

The seals were allowed to mate during this time-period by alternating three males between both groups. Two time-points were selected during the pregnancy period, June '83 and September '83, for blood sampling and for determination of the reproduction success. The number of pregnant and non-pregnant seals were recorded and have been reported upon earlier (Reijnders, 1986). At the end of the 2-yr dietary exposure period, the group 1 seals (high level of exposure) were subsequently fed fish from the Atlantic Ocean for half a year before being released into the environment. During this period an additional time-point for blood sampling was chosen (September '86). Blood was stored as plasma at −20°C until analysis.

Analysis of plasma retinol concentrations

Plasma retinol concentrations were determined by a reversed phase (C_{18}) high performance liquid chromatography (HPLC) method as reported earlier (Brouwer et al., 1985). Briefly, retinol was extracted (overnight at −20°C) from 100-μl aliquots of plasma by a 300-μl mixture of methanol and diisopropyl ether (1:2). The diisopropyl ether extracts were collected and injected (10 μl) on a silica-C_{18} high-speed column (3 cm, 3 μm beads, Perkin Elmer). Retinol was detected by a UV-detector (Gilson Model 116) set at 326 nm, while data manipulation was performed by an integrator (Gilson data master model 620). Retinyl acetate was used as an internal standard for recovery determinations.

Radioimmunochemical analysis of plasma thyroid hormone concentrations

Plasma total and free thyroxin (TT4 and FT4) and triiodothyronin (TT3) were determined by standard radioimmunochemical methods (Amerlex-M, Amersham, UK).

Statistics

The statistical test used in this study was Student's *t* test for comparison of two populations of data with unequal sample sizes. Differences between the two populations having a P value of ≤ 0.05 were considered to be statistically significant differences.

RESULTS

Exposure of seals to fish from the Wadden Sea (high PCB levels) resulted in a drastic reduction in plasma retinol concentrations (Fig. 1) as compared to plasma retinol levels of seals fed fish from the Atlantic Ocean (low PCB levels). This reduction in plasma retinol concentrations was observed in both pregnant and non-pregnant seals at the two time-points, June '83 and September '83, e.g. 55% and 30–40% reduction, respectively. The reduction in plasma retinol concentrations was restored to normal levels when the seals on a Wadden Sea fish diet were subsequently fed the Atlantic Ocean fish for half a year (September '86).

Significant reductions of plasma TT4, FT4 and TT3 concentrations (Fig. 2) were also observed at the June '83 time-point in seals fed Wadden Sea fish as compared to seals on the Atlantic Ocean fish diet. The TT4 and FT4 concentrations were however less reduced than the plasma retinol concentrations, which may be a consequence of a compensation by the thyroid gland (increased hormone synthesis) to restore plasma TT4 levels. Plasma TT3 concentrations were significantly reduced by 50–60% at the first sampling-time during pregnancy (June '83) only and was of the

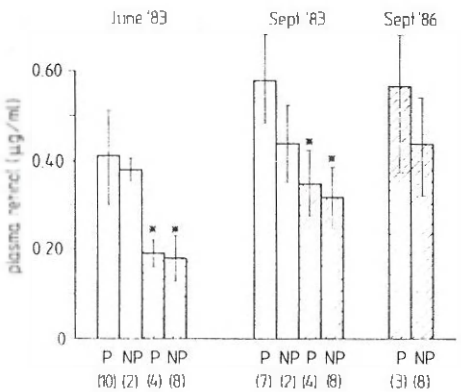

Fig. 1. Effect of PCB-contaminated fish on plasma retinol concentrations of pregnant (P) and non-pregnant (NP) common seals. The experimental setup was as described in 'Materials and Methods'. Blood was withdrawn from the seals at several time-points and retinol concentrations were determined in 100-µl aliquots of plasma by HPLC according to a previously published method (Brouwer et al., 1985). Blank columns represent seals fed low level PCB-contaminated fish from the Atlantic, while hatched columns represent seals fed high level PCB-contaminated fish from the Dutch Wadden Sea. The data are expressed as mean ± SD. * Significantly different from seals fed Atlantic fish at the same time-point of analysis ($P < 0.05$, Student's t test). Numbers in parentheses indicate the number of animals in each group.

same magnitude as the plasma retinol reduction. No alterations in thyroid hormone concentrations were observed at the September '83 time-point. The reduction in TT4 levels combined with the more drastic reduction in plasma TT3 concentrations are indicative for development of a hypothyroidic status in the seals fed fish from the Wadden Sea.

DISCUSSION

The observed, in tandem reduction in plasma retinol and TT4 concentrations in common seals is most probably caused by PCBs in the diets. Similar reductions in plasma retinol and thyroxin concentrations were observed in rats following exposure to 3,4,3',4'-tetrachlorobiphenyl (TCB), a model toxic congener of PCBs (Brouwer and Van den Berg, 1986; Brouwer et al., 1986). A mono-hydroxy PCB metabolite was found that bound with high affinity to the thyroxin-binding site of transthyretin (TTR) (Brouwer, 1987). Moreover, the complex formation between retinol-binding protein (RBP) and TTR, i.e. the plasma protein complex responsible for the transportation of both retinol and thyroxin to target organs, was drastically reduced by the TCB-metabolite (Brouwer et al., 1988). In the meantime, similar results were obtained in rats following exposure to a commercial PCB-mixture, Aroclor 1254, e.g. one of the major environmental pollutants in the Wadden Sea (Brouwer, 1989). The

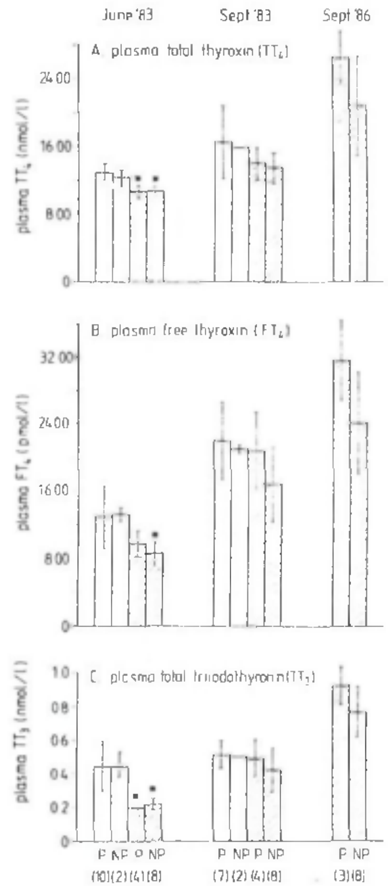

Fig. 2. Effect of PCB-contaminated fish on plasma total thyroxin (TT4), free thyroxin (FT4) and total triiodothyronin (TT3) concentrations in pregnant (P) and non-pregnant (NP) common seals. The experimental setup and blood withdrawal times were as described in 'Materials and Methods'. TT4, FT4 and TT3 concentrations were determined by standard radioimmunoassays. Blank columns represent seals fed low level PCB-contaminated fish from the Atlantic, while hatched columns represent seals fed high level PCB-contaminated fish from the Dutch Wadden Sea. Data are expressed as mean ± SD. * Significantly different from seals fed Atlantic fish at the same timepoint of analysis ($P < 0.05$, Student's t test). Numbers in parentheses indicate the number of animals in each group.

data obtained from this study suggest that in seals a similar mechanism of interference in vitamin A and thyroid hormone metabolism is operative.

A reduced concentration of plasma retinol directly influences the delivery of retinol to the target epithelia. Persistent reduction of plasma retinol levels may result in the development of a vitamin A deficiency, with accompanying symptoms, such as retarded growth, reduced reproductive success, dermal and ocular alterations and increased susceptibility to infections (Hof and Wirsing, 1979; Nauss et al., 1979; Shenai et al., 1985; Thompson et al., 1964; Moore, 1967; Wolbach and Howe, 1925; Green and Mellanby, 1928). In fact, the reduced plasma levels of retinol and thyroid hormones were accompanied by a significantly reduced reproductive success in seals fed Wadden Sea fish as reported earlier (Reijnders, 1986). These data suggest an important role of vitamin A in PCB-induced reproductive failure in common seals. Furthermore, vitamin A plays an important role in resistance to microbial infections, therefore it is conceivable that the reduced plasma retinol concentrations caused by PCB-contaminated fish from the Dutch Wadden Sea may be accompanied by a weakened condition and increased susceptibility to viral infections of the common seal present in these waters.

An important practical consequence of these results is that plasma retinol and thyroid hormone reductions may be used as sensitive biological effect monitor parameters for PCBs and related environmental pollutants.

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