Delayed teratogenic effects of Aroclor 1254 and PCB 126 in frog embryos in a newly developed prolonged-FETAX assay

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Introduction

Over the last decades a world-wide trend of decreasing amphibian populations in different types of habitats has been observed (1,2,3). In addition to physical threats such as habitat destruction and increased UV-radiation, environmental pollution with persistent substances belonging to the group of polyhalogenated aromatic hydrocarbons (PHAHs) is suspected to be one part of the puzzle. In previous investigations, teratogenic effects of heavy metals, pesticides and several toxicants were observed in the South African clawed frog (*Xenopus laevis*) by means of the FETAX assay (Frog Embryo Teratogenic Assay-*Xenopus*) (4,5,6). Our objectives were to study short-term effects of PCBs using the classical FETAX assay and delayed effects on the development and metamorphosis of *Xenopus laevis* with a recently developed prolonged-FETAX assay.

Animals, Material and Methods

Adult *Xenopus laevis* were obtained from the Department for Experimental Zoology, Catholic University of Nijmegen, The Netherlands. FETAX assays were performed as described elsewhere (7,8). The technical PCB-mixture Aroclor 1254 was used in concentrations ranging from 1.1 nmol/ml up to 1.2 mmol/ml and the non-ortho congener PCB 126 in a concentration range of 17.1 pmol/ml up to 15.5 µmol/ml all dissolved in dimethylsulfoxide (DMSO). Final DMSO concentrations were 0.5% in the PCB exposed and the vehicle control group.
For the prolonged-FETAX assay groups of 100 animals per concentration were exposed to PCB 126 (7.7 pmol/ml, 0.64 nmol/ml, 6.4 µmol/ml) in duplicate for a 96 hours period according to the standard FETAX procedure and were thereafter transferred into bigger aquaria. Animals were not further exposed to PCBs until the termination of the experiment after 80 days. Aquaria were checked every day for dead animals. Larvae found dead were fixed and scored for malformations under a stereomicroscope throughout the experiments (8). Animals which have successfully undergone metamorphosis (stage 65/66) were sacrificed, weighed and scored for malformations.

Results and Discussion

FETAX assay

Neither Aroclor 1254 nor the single congener PCB 126 had an effect on rate of malformations, growth and development in the FETAX assay. The only effect was depigmentation of animals exposed to Aroclor 1254. The EC50 for depigmentation was found to be 64.4 µmol/ml. Using proposed TEF values (9) a concentration of 64.4 µmol/ml (or 19.3 mg/ml) Aroclor 1254 is equivalent to a TEQ concentration of 2.95 µg/ml which is a factor 10^3 higher than the EC50 of ≥3 µg/liter found for TCDD in other amphibian species such as leopard frog (*Rana pipiens*) and green frog (*Rana clamitans*) (10). The difference may be explained by antagonistic action of certain di-ortho substituted PCBs on the dioxin-like action of congeners present in the technical mixture (11). A possible explanation for the depigmentation of amphibian larvae is an alteration of retinoid metabolism due to PHAHs (12). Retinoic acid inhibits tyrosinase activity and melanin synthesis in different melanoma cells (13). Depigmentation may therefore give evidence for a disturbance of retinoid homeostasis.

Prolonged-FETAX assay

Five days after the last PCB 126 exposure mortality increased sharply in the highest dose group (6.4 µmol/ml PCB 126). 58 animals or 29% died within the first ten days and 95 (47.5%) died over the whole experimental period. Three weeks after the end of exposure tadpoles started to become pale and showing swimming disorders in the 0.64 nmol/ml PCB 126 group. A steadily increased mortality resulted in 43 dead animals (21.5%) over the whole experimental period. In the group exposed to 7.7 pmol/ml PCB 126 a total of 21 animals died showing similar symptoms. In addition sudden mortality without preceding symptoms was observed in the DMSO treated group (n=91) and in the control group (n=95) after the aquaria have been cleaned by staff members using Latex-gloves starting with these two groups. Shortly after that, such acute mortality of *Xenopus laevis* as a result of exposure to Latex-gloves was described by others (14).

A dose-related increase in the rate of malformations was found in the groups exposed to PCB 126 (Fig. 1) whereas only 7.3% and 8.4% of dead animals in the control and DMSO treated group showed malformations.

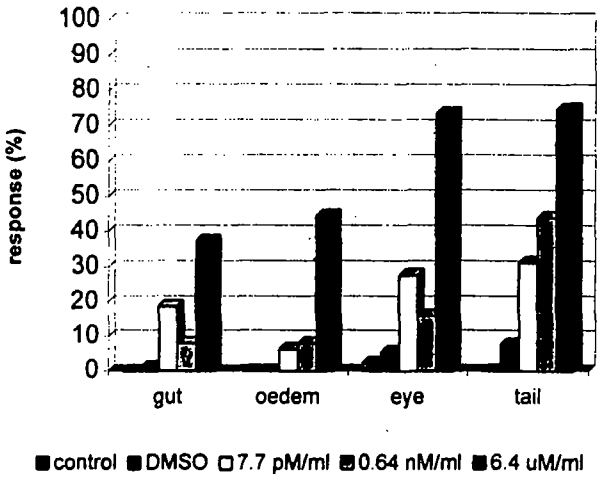

Fig. 1. Percentage of dead tadpoles of *Xenopus laevis* with malformations as a result of 96-hours exposure to PCB 126 (prolonged-FETAX)

This effect was highest in the group exposed to 6.4 µmol/ml PCB 126 with 93.2% of malformed animals. Oedema, misformed eyes and tail, and lack of gut coiling were the most prominent observed malformations (Fig. 2). Eye malformations included reduction in size, failure of the choriod fissure to close, rupture of the optic cup and irregular depigmentation.

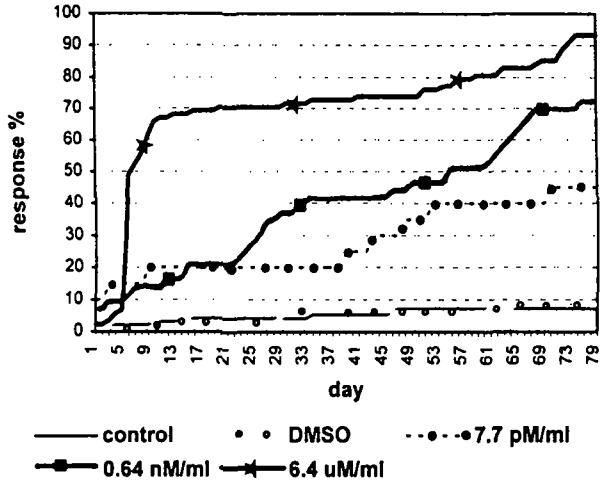

Fig. 2. Presence and type of malformations in dead tadpoles of *Xenopus laevis* as a result of 96-hours exposure to PCB 126 (prolonged-FETAX)

Tail deformities and oedema have been described earlier in amphibians as a result of 4-day exposure to technical PCB-mixtures such as Aroclor 1016, 1242, and 1254 (15) and axial malformations have also been related to treatment of *Xenopus laevis* embryos with retinoids (16). It was shown that the induction of a thyroid hormone receptor in early *Xenopus laevis* embryos was associated with hormone-dependent abnormalities such as head deficiencies, and misformed eyes (17). PCBs are known to alter retinoid and thyroid homeostasis (13,18). Disturbance of these two pysiological important parameters may explain at least in part the malformations.

To summarise our results we can state that:

- The prolonged-FETAX assay reveals long-term effects of early exposure to PCB 126 whereas no effects were visible in the 96-hours exposure period of the FETAX assay.
- PCB 126 induced teratogenic effects in *Xenopus laevis* tadpoles in a delayed and dose-dependent manner.

Our results strongly suggest that PCBs are able to alter normal amphibian development and that presently used early-life-stage tests in amphibians are not suitable for substances with low acute toxicity. The long-term impact of PCBs on amphibians on the population level cannot be judged with present knowledge of amphibian population dynamics.

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