EROD- and AHH-inducing Potency and Lethality of Chlorinated Naphthalenes in Chicken (Gallus domesticus) and Eider Duck (Somateria mollissima) Embryos

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Introduction

Polychlorinated naphthalenes (PCNs) are found in diverse environmental samples worldwide. In biota collected in Sweden, PCNs seem to be as widespread as PCBs at levels similar to those of non-ortho PCBs. Some PCN congeners induce ethoxyresorufin O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities in rat hepatoma H-4-II E cells in vitro as do chlorinated dioxins and coplanar PCBs. Chicken embryos (Gallus domesticus) are extremely sensitive to coplanar halogenated aromatic compounds and very low doses of coplanar PCBs enhance hepatic EROD-activity.

The aim of this work was to study the toxic potencies of some PCNs in chicken (Gallus domesticus) and eider duck (Somateria mollissima) embryos. Hepatic EROD- and AHH-induction, mortality rates and frequencies of embryonic anomalies were determined.

Methods

A technical preparation of PCNs (Halowax 1014, 20% tetrachloronaphthalenes, 40% pentachloronaphthalenes, 40% hexachloronaphthalenes), a mixture of 50% 1,2,3,5,6,7-hexachloronaphthalene and 50% 1,2,3,4,6,7-hexachloronaphthalene (HxCN-mlx), and 1,2,3,4,5,6,7-heptachloronaphthalene (HpCN) were studied.

Chicken embryos were exposed via the air-sacs of the eggs in a 72-hour test and via the yolk-sacs for 14 days. The eider embryos were exposed via the yolk-sacs for 19 days. The compounds were dissolved in peanut oil and in an emulsion of peanut oil, lecithin and water for air- and yolk-sac injections, respectively. EROD activities were determined according to the method previously described by Pohl.
The preparation of liver microsomes and subsequent determination of AHH-activities was essentially done as described by Naf et al.\textsuperscript{9}.

Results and Discussion

In chicken embryos, the HxCN-mix and Halowax 1014 proved to have both EROD-inducing (fig 1) and embryolethal properties, while the HpCN was of low EROD-inducing potency (fig 1) and embryolethality. ED\textsubscript{50}-values for EROD-induction by the HxCN-mix and Halowax 1014 in the 72-hour test were estimated to be 0.06 mg/kg egg and 0.2 mg/kg egg, respectively. Fifty percent of the chicken embryos died (6/12) when given 3.0 mg/kg of the HxCN-mix while a similar dose of Halowax 1014 caused death in 4 out of 12 chicken embryos.

The dose-response curve for EROD-induction by Halowax 1014 displayed a decline after the maximal level was reached (fig 1). When Halowax 1014 (1.0 mg/kg egg) was coinjected with 3,3',4,4',5-pentachlorobiphenyl (PCB IUPAC #126), (0.1 µg/kg egg) no additive effects on EROD-activity were found and when the same dose of Halowax 1014 was coinjected with a dose of PCB #126 known to cause maximal induction (1.0 µg/kg egg)\textsuperscript{7}, the resulting EROD-activity was lower than that solely caused by 1.0 µg PCB #126/kg egg (fig 2). These findings indicate that Halowax 1014 has both EROD-inducing and EROD-inhibiting properties.

Hepatic EROD- and AHH activities were determined on day 18 (chicken) or day 24 (eider) of incubation in embryos exposed to 1.0 mg/kg egg via the yolk-sac on day 4 (chicken) or day 5 (eider). The HxCN-mix and Halowax 1014 induced AHH and EROD in both chicken and eider, but the induction rates were higher in the eider embryos (fig 3). The HxCN-mix and Halowax 1014 induced degenerative hepatic lesions and pericardial oedema in the chicken embryos but not in the eider embryos.
The most toxic PCNs (the HxCN-mix and Halowax 1014) tested were approximately of the same EROD-inducing potency as the most toxic mono-ortho polychlorinated biphenyls, and 1000 times less toxic and potent as EROD-inducers compared with PCB #126, previously tested by Brunström and Andersson. HpCN was considerably less toxic and exhibited a low EROD-inducing potency.

The chicken embryos were more sensitive to the hepatotoxic effects produced by Halowax 1014 and the HxCN-mix than the eider duck embryos while the eider embryos were more responsive in terms of EROD- and AHH induction.

![Figure 2. Hepatic EROD-activity in 10-day-old chicken embryos exposed to PCB #126, Halowax 1014, or a mixture of both via the air-sac on day 7 of incubation. Each bar represents the mean of 6 livers and variation is shown as S.D. The difference in activity after treatment with PCB #126 alone or with both PCB #126 and Halowax 1014 was tested with Student’s t test (** = p<0.01).](image1)

![Figure 3. Hepatic EROD and AHH induction rates in relation to control values on day 18 (chicken) or day 24 (eider) of incubation in embryos exposed to Halowax 1014, the HxCN-mix, or HpCN via the yolk-sac on day 4 (chicken) or day 5 (eider). Each bar represents the mean of 6 livers and variation is shown as S.D. Differences from the corresponding control value were tested with Student’s t test, (*) = p<0.05, (**) = p<0.01, (***) = p<0.001.](image2)
When considering the total load of xenobiotics in birds and other organisms, it is likely that PCNs can be regarded as potent toxic environmental contaminants, with the most toxic PCNs tested in this study being in the same range of toxicity as mono-ortho PCBs. There are reasons to assume that the PCNs have bioaccumulating potential in biota, because their structure indicate persistence and lipophilicity, and some of the PCNs seem to have a dioxinlike mechanism of action. Since the present uses and sources of PCNs are insufficiently known, the load of PCNs in the environment poses an unknown risk, and monitoring of these compounds should therefore be considered.

Conclusions
In chicken embryos, the HxCN-mix and Halowax 1014 proved to have both EROD-inducing and embryolethal properties, while the HpCN was of low EROD inducing potency and embryolethality. The HxCN-mix and Halowax 1014 were approximately of the same EROD-inducing potency as the most toxic mono-ortho PCBs. Halowax 1014 has indications of being both EROD-inducing and EROD-inhibiting in chicken embryos. The chicken embryos were more sensitive to the hepatotoxic effects produced by Halowax 1014 and the HxCN-mix than the eider duck embryos while the eider embryos were more responsive in terms of EROD- and AHH induction.

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References
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