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[1,2]. In biota collected in Sweden, PCNs seem to be as widespread as PCBs at levels similar to those of non-*ortho* PCBs[3]. 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[4]. Chicken embryos *(Gallus domesticus)* are extremely sensitive to coplanar halogenated aromatic compounds[5,6] and very low doses of coplanar PCBs enhance hepatic EROD-activity[7].

The aim of this work was to study the toxic potencies of some PCNs in chicken *(Gallus domesticus)* and elder 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-mix**), 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

and Fouts[8]. The preparation of liver microsomes and subsequent determination of AHH-activities was essentially done as described by Näf et al.[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_{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.

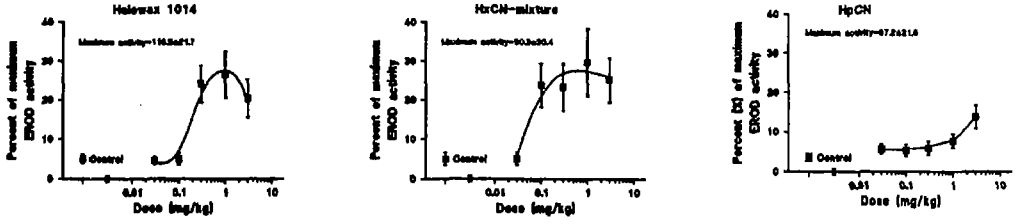

Figure 1. Hepatic EROD-activity (expressed as percent of maximal EROD-activity) in 10-day-old chicken embryos exposed to various PCNs via the air-sac on day 7 of incubation. Maximal EROD-activity (pmol x mg liver^{-1} x min^{-1}) was obtained by exposing embryos to 1 μg PCB #126/kg egg. Each point represents the mean of 6 livers and variation is shown as S.D.

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)[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[7]. 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.

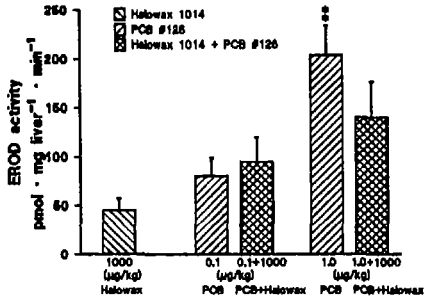

Figure 2. Hepatic EROD-activity in 10-day-old chicken embryos exposed to PCB #126, Halowax 1014, or a mixture of both via the air-sac on day 7 of incubation. Each bar represents the mean of 6 livers and variation is shown as S.D. The difference in activity after treatment with PCB #126 alone or with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

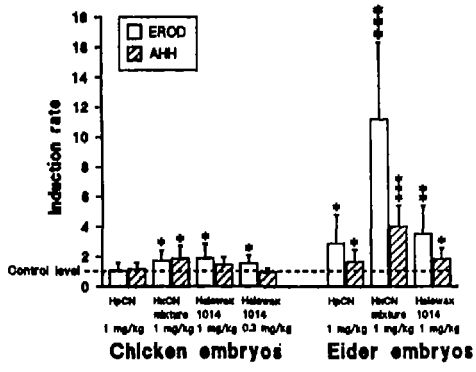

Figure 3. Hepatic EROD and AHH induction rates in relation to control values on day 18 (chicken) or day 24 (eider) of incubation in embryos exposed to Halowax 1014, the HxCN-mix, or HpCN via the yolk-sac on day 4 (chicken) or day 5 (eider). Each bar represents the mean of 6 livers and variation is shown as S.D. Differences from the corresponding control value were tested with Student's *t* test, (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

The two HxCNs studied are found in Halowax 1014 in an amount of around 1% of the total quantity of PCNs in Halowax 1014 (as determined by GC/MS). Therefore, the relatively high toxic potency of Halowax 1014 cannot be explained by its content of the two HxCNs. The effect has to be caused by other tetra-, penta- or hexachlorinated naphthalenes present in Halowax 1014.

The concentration of HxCNs in guillemot eggs collected in 1987 in the Baltic sea[3] was 200 times lower than the 1 ppm dose of the HxCN-mix which was given in the experiments (on a lipid weight basis). At this dose, the eider embryos showed an 11-fold induction of EROD compared to control values. The lowest observed effective dose for EROD-induction by the HxCN-mix in chick embryos exposed via the air-sac was 0.1 ppm, which is only 20 times higher than the HxCN concentration found in guillemot eggs.

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.

Acknowledgements

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