



HAL
open science

Estrogenic effect of dioxin-like aryl hydrocarbon receptor (AhR) agonist (PCB congener 126) in salmon hepatocytes

Anne S. Mortensen, Augustine Arukwe

► **To cite this version:**

Anne S. Mortensen, Augustine Arukwe. Estrogenic effect of dioxin-like aryl hydrocarbon receptor (AhR) agonist (PCB congener 126) in salmon hepatocytes. *Marine Environmental Research*, 2008, 66 (1), pp.119. 10.1016/j.marenvres.2008.02.041 . hal-00563016

HAL Id: hal-00563016

<https://hal.science/hal-00563016>

Submitted on 4 Feb 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Estrogenic effect of dioxin-like aryl hydrocarbon receptor (AhR) agonist (PCB congener 126) in salmon hepatocytes

Anne S. Mortensen, Augustine Arukwe

PII: S0141-1136(08)00047-0

DOI: [10.1016/j.marenvres.2008.02.041](https://doi.org/10.1016/j.marenvres.2008.02.041)

Reference: MERE 3199

To appear in: *Marine Environmental Research*



Please cite this article as: Mortensen, A.S., Arukwe, A., Estrogenic effect of dioxin-like aryl hydrocarbon receptor (AhR) agonist (PCB congener 126) in salmon hepatocytes, *Marine Environmental Research* (2008), doi: [10.1016/j.marenvres.2008.02.041](https://doi.org/10.1016/j.marenvres.2008.02.041)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Estrogenic effect of dioxin-like aryl hydrocarbon receptor (AhR) agonist (PCB congener 126) in salmon hepatocytes

Anne S. Mortensen, Augustine Arukwe*

Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway

Abstract

Previous studies have shown that aryl hydrocarbon receptor (AhR) agonists possess anti-estrogenic activities and several mechanisms have been proposed to explain the interactions between AhR and estrogen receptor (ER) signalling pathways. In the present study, we show that 3,3',4,4'-pentachlorobiphenyl (PCB126 – a dioxin-like AhR agonist) produced estrogenic responses in the absence of ER agonist, in fish in vitro system. We exposed salmon primary hepatocytes to PCB126 (1, 10 and 50 pM) and the ER-agonist nonylphenol (NP; 5 and 10 µM) singly and also in combination. Vitellogenin (Vtg) and zona radiata proteins (Zr-proteins) levels were analysed by semi-quantitative ELISA. We observed that the protein levels of Vtg and Zr-proteins were significantly induced in a concentration-specific manner in cells treated with PCB126 and NP, singly or in combination. In general, these results show a novel aspect of dioxin-like PCB effect not previously demonstrated in fish system.

Keywords: Dioxin-like PCB; Estrogenicity; Vitellogenin; *Zona radiata* protein

*Corresponding author: arukwe@bio.ntnu.no (A. Arukwe)

A majority of scientific publications on ER-AhR crosstalk have concluded that the ER-AhR crosstalk is bidirectional and that AhR agonists have negative effects on ER mediated gene and protein expressions (Mortensen et al., 2006; Safe et al., 1991). Among the models that have been used to explain the molecular basis for the anti-estrogenic properties of AhR agonists are 1) increased rate of E2 degradation, 2) decreased cellular ER levels by proteasomes 3) suppression of E2 induced transcription by AhR acting close to the estrogen response element (ERE) and 4) competition for common co-factors. Previously we observed that the anti-estrogenic activities of AhR agonists are not absolute, as AhR agonist (PCB77) was found to modulate NP effects depending on season and sequential order of exposure (Arukwe et al., 2001). Particularly, an increase of plasma Vtg and Zr-protein expression was observed in salmon exposed to combined PCB77 and NP, compared to fish exposed to NP alone. The present study was designed to investigate whether the strong AhR agonist, PCB126, showed exclusively anti-estrogenic actions in a fish in vitro system, or if the compound has the potential of inducing ER mediated protein induction.

Salmon primary hepatocytes were isolated by two-step collagenase perfusion method as previously described by Mortensen et al. (2006). Male Juvenile Atlantic salmon (*Salmo salar*) of approximately 350 g were used. The cells were exposed to 0.01% DMSO (control), NP (5 or 10 μ M) and PCB126 (1, 10 and 50 pM) singly and also in combination. Media were replaced once after 24h with fresh media containing the appropriate chemical and at the same concentrations. Cells (3 plates for each exposure group) for protein and mRNA analysis were harvested after 48h exposure and stored at -80°C.

Immunochemical assays of Vtg and Zr-protein levels were performed on cells homogenized in 200 μ L buffer (0.1 M PBS, 0.15 M KCl, 1 mM EDTA, 1 mM DTT and 10% glycerol, pH 7.6). The indirect ELISA was performed essentially by coating each well with 15 μ g of total protein. Polyclonal anti-artic char antibody and polyclonal anti-salmon Zr-proteins antibody (both diluted

1:2500 in TBS with 0.01 % BSA) and goat anti-rabbit peroxidase-conjugated secondary antibody (GAR-HRP, Bio-Rad) diluted 1:3000 and H₂O₂/*o*-phenylenediamine dihydrochloride (OPD) were used for detection at 492 nm.

The effects of NP and PCB126 on Vtg and *Zr*-protein levels in salmon hepatocytes are shown in Figure 1A and B, respectively. Exposure to PCB126 alone produced significantly higher Vtg and *Zr*-protein levels, compared to control (Fig 1A and B, respectively). Exposure to 5 and 10 μ M NP increased the Vtg and *Zr*-protein levels in a concentration-dependent manner. Combined 5 μ M NP and PCB126 concentrations, produced reductions in the level of Vtg (more so at 50 pM PCB126), compared with PCB126 alone (Fig. 1A). For *Zr*-protein, treatment with 1, 10 and 50 pM PCB126 increased *Zr*-protein levels relative to control. Exposure to 10 μ M NP in combination with 10 pM PCB126 produced higher Vtg level compared to 10 μ M NP alone.

In this study, we showed that PCB126 produced estrogenic responses in primary culture of salmon hepatocytes. Using the same experimental materials, we observed that PCB126 produced increases in mRNA levels for ER α , Vtg and *Zr*-protein that were reduced in the presence of ICI182,780 (ICI) and as expected, increased mRNA expression for AhR gene battery (Mortensen and Arukwe, 2007a). Our data are in accordance with recent reports showing that ligand activated AhR-Arnt can associate with the unliganded ER to form a functional complex that binds EREs with subsequent activation of estrogen responses (Ohtake et al., 2003). However, our data showed that high PCB126 concentration (50 pM) decreased cellular Vtg levels and suggests a possible impairment of protein synthesis or post-transcriptional/translational effects due to PCB126 toxicity. In general, the estrogenic action of PCB126 reported here is in sharp contrast with previously published data showing that AhR agonists has anti-estrogenic effects in both mammalian (Safe et al., 1991) and teleost cell culture systems (Mortensen et al., 2006). A high proportion of molecular toxicology research efforts have focused on the investigation of how complex chemical mixture exposures affect biological systems. In order to understand mechanisms; knowledge of protein-protein interactions between various nuclear receptors and transcription factors are required.

Several reports have shown that AhR ligands possess anti-estrogenic properties. It was previously reported *in vitro*, that AhR interacts directly with ER α in a ligand specific manner (Klinge et al., 1999). We have previously reported bi-directional interactions between the ER and AhR in salmon *in vitro* system (Mortensen et al., 2006). In a very recent study, we observed that inhibition of AhR by α naphthoflavone (ANF) did restore PCB77 decreased gene expression of ER signalling (Mortensen and Arukwe, 2007b). Studies of TCDD ability to bind to ER, demonstrated that this strong AhR agonist did not compete with E2 for binding to the ER (Harris et al., 1990). The transcriptional capability of basic-helix-loop-helix (bHLH)-Per-ARNT-Sim (PAS) family of transcription factors is yet to be fully understood and their individual *in vivo* functions are still subject of current discussions. For example, by showing that the AhR may be involved in regulating the development of the vascular system in liver and other organs of AhR-null mice, the developmental, metabolic, and cardiovascular phenotypes of AhR-null mice are providing important clues to the numerous functions of the AhRs (Fernandez-Salguero et al., 1995; Gonzalez and Fernandez-Salguero, 1998; Thurmond et al., 1987). Based on the present data and other reports elsewhere, the mode of estrogenic and anti-estrogenic action of AhR-mediated effects on biological systems seem to be exceptions rather rule of action. The present study contributes with novel information concerning the molecular mechanisms behind ER-AhR interactions in fish systems. In addition to more *in vitro* studies, the relevance to *in vivo* systems needs further investigation as these responses will ultimately be affected by other biological factors such as metabolism, biotransformation, bioconcentration and bioaccumulation.

Acknowledgements

This work has been support by NTNU doctoral fellowship grant to ASM and grants from the Norwegian Research Council (NFR). The authors are grateful to Marthe Braathen for her valuable help during preparation of primary hepatocyte cultures.

References

- Arukwe, A., Yadetie, F., Male, R., and Goksoyr, A. (2001). *Environmental Toxicology and Pharmacology*, 10, 5-15.
- Gonzalez, F. J., and Fernandez-Salguero, P. (1998). *Drug Metabolism and Disposition*, 26, 1194-1198.
- Harris, M., Zacharewski, T., and Safe, S. (1990). *Cancer Research*, 50, 3579-3584.
- Klinge, C. M., Bowers, J. L., Kulakosky, P. C., Kamboj, K. K., and Swanson, H.I. (1999). *Molecular and Cellular Endocrinology*, 157, 105-119.
- Mortensen, A.S., and Arukwe, A. (2007a). *Molecular Pharmacology*. submitted.
- Mortensen, A.S., and Arukwe, A. (2007b). *Chemical Research in Toxicology*, 20, 474-488.
- Mortensen, A.S., Tolfsen, C.C., and Arukwe, A. (2006). *Journal of Toxicology and Environmental Health*, 69, 1-19.
- Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., et al. (2003). *Nature*, 423, 545-550.
- Safe, S., Astroff, B., Harris, M., Zacharewski, T., Dickerson, R., Romkes, M., et al. (1991). *Pharmacology and Toxicology*, 69, 400-409.

Figure captions

Fig. 1. Modulation of Vitellogenin (Vtg; A) and zona radiata protein (Zr-protein; B) levels in primary culture of salmon hepatocytes treated for 48h with PCB-126 (1, 10 and 50 pM), singly and also in combination with NP (5 and 10 μ M). The protein levels were measured by semi-quantitative ELISA with Vtg and Zr-protein polyclonal antibodies. Data are given as mean (n=3) \pm standard error of mean (SEM). Asterisks denote significant difference compared to control and different letters denote statistical significant differences within exposure groups.

ACCEPTED MANUSCRIPT

Figure 1

