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Abnormalities in eelpout *Zoarces viviparus* upon chemical exposure

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ABSTRACT

Elevated frequencies of abnormal embryos in female eelpout *Zoarces viviparus* have been demonstrated in Danish, Swedish and German monitoring programmes at certain geographic locations with high levels of anthropogenic input. Pollutants present in areas with high malformation frequencies were selected and tested in a controlled laboratory experiment for their potential to induce abnormalities among eelpout embryos upon injection into pregnant eelpout. Tributyltin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, pyrene, nonylphenol, 2,2′,4,4′-tetrabromophenylether and heptadecafluorooctanesulfonic acid were tested, either individually or combined. Generally, the chemicals were transferred to eggs and/or embryos. Some of the exposures increased the proportion of broods with more than 10% abnormal or 5% malformed embryos, although the average percentages of abnormal development were not affected. Spinal, cranial and eye deformities were evident, similarly to what is seen in nature. Some of the exposures resulted in increased percentages of females with as well a low reproductive capacity as embryos with a low condition index.

1. Introduction

The viviparous eelpout (*Zoarces viviparus*) has been designated and used as a key bioindicator of effects of harmful environmental substances in the Baltic and North Sea in international monitoring programs (HELCOM, 2008; OSPAR, 2007) under the implementation of the EU Marine Strategy Framework Directive (EC, 2008). Elevated frequencies of malformations among its embryos at certain geographic locations have been noted in monitoring programs in several Baltic countries (Bergek et al., 2012; Gercken et al., 2006; Hedman et al., 2011; Strand et al., 2004). The usefulness of the species as a monitoring organism is based upon its coastal and stationary habitat, and most importantly, its viviparous life history, where the fry stays in the ovary sac during the entire gestation period of 4–5 months (Hedman et al., 2011). This allows detection of malformations among the offspring in contrary to oviparous fish species where malformed larvae likely are lost in the environment.

In a review of Danish data on eelpout malformations it was concluded that increased frequencies were associated with high anthropogenic impact in the coastal areas (Halling-Sørensen et al., 2008; Stuer-Lauridsen et al., 2008). This supports similar observations in Germany and Sweden (Gercken et al., 2006; Vetemaa et al., 1997). It is therefore hypothesized that the malformations observed in the eelpout might be caused by environmental conditions including chemicals. Chronic exposure to various kinds of hazardous substances has the potential to induce teratogenic effects in fish embryos and larvae (Bodammer, 1993; von Westernhagen, 1988; Weis and Weis, 1989). There is therefore an urgent need of experimental evidence to link the malformations to exposure to chemicals and to pinpoint the causal agents since no information on this is available in the published literature.

In the coastal waters around Denmark female eelpout undergo vitellogenesis during spring and summer and mating takes place within a few days, fairly synchronously within the population, at the end of August or early September (Vetemaa, 1999). The ovulated eggs stay in the ovary and hatch after approximately three weeks of development. It is well documented that the development of the skeleton, muscles and organ systems is especially sensitive to teratogenic compounds in a limited period at the very beginning of the development (von Westernhagen, 1988; Weis and Weis, 1989). This corresponds well with unpublished observations from previous experiments in our laboratory which indicate that it is within the first 3 weeks after fertilization the eelpout larvae are susceptible to teratogenic effects upon chemical exposure. It is therefore

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crucial that experimental exposure to chemicals for investigation of teratogenic effects starts immediately after fertilization, within this rather narrow teratogenic window.

The Danish monitoring programme (Larsen and Strand, 2012; Strand et al., 2004) has shown that a number of pollutants tend to be present at higher concentrations at locations with increased prevalence of malformations than at locations with 'background' levels of malformations. The most obvious candidates of these chemicals or groups of chemicals are tributyltin (TBT), polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls and dioxins, but also nonylphenol (NP), brominated flame retardants (BFR) and fluorinated compounds (PFOS) might have potential as teratogens in eelpout (Larsen and Strand, 2012; Strand et al., 2004). Most of these chemicals can be transferred from the mother fish to the developing larvae and may furthermore bioaccumulate to higher concentrations as earlier life stages tend to lack or have a reduced ability to metabolize and excrete the compounds (Fent, 1991; Giesy et al., 2002; Nyholm et al., 2008; Ohji et al., 2006; Sharpe et al., 2010). Moreover, most of the compounds can reduce hatching success or delay hatching (Hose et al., 1982; Ishibashi et al., 2006; Lema et al., 2007; Nakayama et al., 2005; Shi et al., 2008).

The aim of this study was to investigate if TBT, pyrene, TCDD, nonylphenol, PFOS and BFR alone or in combination induce malformations in eelpout at environmentally realistic doses.

2. Materials and methods

2.1. Experimental animals

The eelpout were caught in seines by local fishermen in the coastal waters south of Funen, around the island of Birkholm (54′56″ N, 10′31″ E). The area is considered as fairly clean as no obvious sources of contamination are present.

The experiment was commenced immediately post ovulation and fertilization of the eelpout eggs. As the exact date of these events was not known and as there are no known procedure that ensures fertilization in captivity, wild eelpout were collected once or twice a week from a period mid-August until September to register the reproductive status of the females.

Up till August 31, no ovulated females were seen. September 3, two out of four females were ovulated and September 7 four out of four were ovulated. Eelpout for the experiments were subsequently collected September 7 and 9. The fish were transported to the University of Southern Denmark's Marine Biological Station in Kerteminde and maintained in tanks (2 m*2 m*0.32 m) supplied with running seawater until the start of exposure experiment on September 13.

2.2. Exposure chemicals

The pregnant female eelpout were exposed to chemicals or groups of chemicals which are known to be present at elevated levels in areas with an increased frequency of malformations among the eelpout. Pyrene (Sigma, product no. 82648; CAS# 129-00-0) was chosen as a representative for the PAHs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Sigma (Supelco), product no. 48599; CAS#1746-01-6) for the chlorinated dioxins, dibenzofurans and planar biphenyls, 2,2′,4,4′-tetrabromodiphenylether (BDE-47) (Fluka, product no. 33670; CAS# 5436-43-1) for the brominated flame retardants (BFRs) and heptadecafluorooctanesulfonic acid potassium salt (PFOS) (Fluka, 33829; CAS# 2795-39-3) for fluorinated compounds. Tributyltin chloride (TBT) (Aldrich, product no. T5,020-2; CAS# 1461-22-9) and nonylphenol (NP) (Fluka product no. 46405; CAS# 104-40-5) were also used.

Table 1
Exposure groups and doses of chemicals.

Group	Number of fish (start)	Exposure doses (per gram wet weight)	Tank size (l)
Control	20		~300
DK10	20	5 ng Sn-TBT + 200 ng pyrene + 10 pg TCDD	~300
DK50	20	25 ng Sn-TBT + 1 μg pyrene + 50 pg TCDD	~300
DK50+	23	25 ng Sn-TBT + 1 μg pyrene + 50 pg TCDD + 500 ng NP + 2.5 ng BFR + 500 ng PFOS	~300
Control	10		178
DK10	10	5 ng Sn-TBT + 200 ng pyrene + 10 pg TCDD	178
DK50	10	25 ng Sn-TBT + 1 μg pyrene + 50 pg TCDD	178
DK50+	10	25 ng Sn-TBT + 1 μg pyrene + 50 pg TCDD + 500 ng NP + 2.5 ng BFR + 500 ng PFOS	178
DK+	10	500 ng NP + 2.5 ng BFR + 500 ng PFOS	178
Sn-TBT	10	25 ng Sn-TBT	178
Pyrene	10	1 μg pyrene	178
TCDD	10	50 pg TCDD	178

2.3. Exposure doses

The doses of the chemicals were calculated from two different principles: 1) based on the concentrations of the chemicals in the food of eelpout and 2) based on the concentrations of the chemicals measured in eelpout from the areas with the highest frequencies of abnormalities.

The calculation of exposure doses based on the amounts consumed from food (1) assumes that a standard eelpout of 60 g eats approximately 5 g dry weight a week. We wished to expose the eelpout to the equivalent of one week's intake. We used the concentrations of the chemicals in blue mussels (*Mytilus edulis*) as a proxy for the concentrations in the food of the eelpout. Approximate concentrations (14 ng TBT, 253 ng pyrene, 0.3 ng BFR, 0.37 pg TCDD, 50 ng PFOS and 1.5 μg nonylphenol/g dry weight soft parts) were extracted from information available in the Danish Marine Monitoring Program (Larsen and Strand, 2012).

For the chemicals (TCDD, TBT, PFOS and BFR) where data were available, the doses (2) were calculated from the concentrations measured in one or several organs of eelpout. The calculated dose was supposed to bring the concentration in the eelpout from background levels to the levels found at contaminated sites (areas with high malformation frequencies). The organ concentrations used for the calculations at the most contaminated sites (Larsen and Strand, 2012) were: Ovary: 6.5 ng TBT/g, 0.9 ng BFR/g, 2.1 pg TCDD/g, 148 ng PFOS/g; liver: 5.0 ng TBT/g, 0.9 ng BFR/g, 2.2 pg TCDD/g, 10,700 ng PFOS/g and muscle 1.2 ng/TBT/g, 0.5 ng BFR/g, 2.2 pg TCDD/g and 16 ng PFOS/g (all expressed in dry weight). Average tissue proportions were assumed to be: muscle 60%, liver 2%, ovaries 3% and the rest 35% of the whole animal. The concentrations in the rest of the animal were assumed to be half of that in the muscles.

The two methods of calculation gave doses within the same order of magnitude and the final dose (from now on termed 'environmental levels') was a compromise between the two methods of calculation. The doses finally used in the experiment were factors 10 (DK10) and 50 (DK50) higher than the calculated environmental levels (Table 1).

Three compounds suspected to be main causative agents of malformations (TBT, TCDD and pyrene) were applied to groups of eelpouts individually (at levels 50 times higher than environmental concentrations) or in combination with other compounds (10 and 50 times the environmental levels) (Table 1). The three weaker candidates (NP, BFR, PFOS) were tested in combination (DK+, Table 1). A mixture of the three stronger and weaker candidates was also tested (DK50+, Table 1).