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Internal effect concentrations of organic substances for early life development of egg-exposed fish

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ABSTRACT

The present study investigates the likelihood that early life development of marine fish from contaminated areas is affected by maternally transferred persistent organic substances (POPs). The common sole (*Solea solea*) was used as model species. Fertilized eggs were exposed via the water until hatching, 6 days post fertilization. The newly hatched larvae were allowed to develop further under unexposed conditions until the end of the metamorphosis. Effects on the larvae were determined for the dioxin-like polychlorinated biphenyl PCB 126, the technical PCB-mixture Arochlor 1254, polybrominated diphenylethers (PBDEs), and hexabromocyclododecane (HBCD), for an artificial mixture of PCBs and PBDEs, and for 'field mixtures' extracted from sole from the North Sea and the contaminated Western Scheldt estuary. Effect levels were expressed as tissue concentrations in the newly hatched larvae at the end of the exposure period. Exposure to PCBs, PBDEs, and the artificial and field mixtures caused mortality that started to occur shortly after the larvae became free-feeding (10 days post fertilization) and continued to increase until the onset of metamorphosis, 15 days later. The effects induced by the field mixtures correlated well with the ΣPCB concentrations in the tissue of the exposed larvae. No indications were found for synergistic effects or for substantial contribution of other (unknown) substances in the field mixtures. HBCD did not induce toxic effects. As lipid normalized POP levels in fish eggs are in general comparable to the levels in the tissue of the female fish, fish tissue concentrations are indicative of the internal exposure of the developing larvae as a result maternally transferred POPs will occur in the field. In sole from the Western Scheldt estuary POP levels are about twenty times lower than the larval tissue concentration that produced 50 percent early life stage mortality. Levels in North Sea sole are an order of a magnitude lower. At more heavily contaminated sites negative effect of PCBs, especially of those with dioxin-like toxicity can be expected.

1. Introduction

Although bulk production and use have been stopped for most persistent organic pollutants (POPs), compounds like polychlorinated biphenyls (PCBs), brominated flame retardants (polybrominated diphenylethers – PBDEs, hexabromocyclododecane – HBCDs), and chlorinated pesticides (dichloro-diphenyl-trichloroethane – DDT, lindane, dieldrin, etc.) still are present in the environment (de Boer et al., 2010; Maes et al., 2008; Voorspoels et al., 2004a). As a result of the low water solubility/high lipophilicity in combination with high persistence these substances concentrate in sediments and accumulate in biota, including fish. Due to a homogeneous distribution of the POPs within the lipids in the female tissue, the lipid normalized concentration in the eggs that she produces will be comparable to the maternal tissue (Russell et al., 1999). These maternally transferred POPs could cause negative effects on the developing off-spring after fertilization (Tietge et al., 1998; Olsson et al., 1999; Nakayama et al., 2005; Ishibashi et al., 2006).

Hence the POP concentration in the tissue of the mother fish represents the minimum toxic pressure for the developping offspring. Compared to fully developed fish, larvae are relatively sensitive to toxicants (McKim, 1977; Hutchinson et al., 1998) as a concequence of the critical development of organs and tissues during this life phase. In addition the lipid normalized concentration of lipophilic substances (log Kow > 6) will further increase during larval development when the lipids stored in the yolk are depleted. This results in an exposure peak to these substances at the end of the yolk-sac stage that is the highest to be expected during the life time of the fish (Foekema et al., 2012). The sensitivity of fish early life stages for the effects of POPs has already been demonstrated by various researchers (see amongst others Henry et al., 1997; King-Heiden et al., 2012; Walker and Peterson, 1994 for dioxins; Mhadhbi et al., 2012; Usenko et al., 2011

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for PBDEs; and Sisman et al., 2007; Soffientino et al., 2010; Murk et al., 1996; Wilson and Tillitt, 1996; Zabel et al., 1995a,1995b for -dioxin-like- PCBs).

In an earlier study we determined that in a prolonged Early Life Stage (pELS) test with the common sole (*Solea solea*) the effect concentrations of dioxin-like compounds in the larvae were in the same order of magnitude as levels found in tissue of fish from highly contaminated areas. This implies that in these areas maternal transfer of such compounds can negatively affect larval development (Foekema et al., 2008). In addition to dioxin-like compounds other POPs are found in fish from polluted field locations. The present paper assesses the effect of well-known field-relevant lipophilic POPs (PCBs, PBDEs, and HBCDs) that will be maternally transferred, on early life development of *Solea solea*. These POPs have, among others, been identified as potential endocrine disrupters (Zimmer et al., 2011), including the thyroid hormone system (Brouwer et al., 1999; Noyes et al., 2011; Palace et al., 2010; Schriks et al., 2006; Yu et al., 2011) which plays an essential role in early development and (flat)fish metamorphosis (Klaren et al., 2008).

The common sole (*Solea solea*) was chosen as model species for this study as it is available from aquaculture which ensures almost year round availability of fertilized eggs. Also, sole is a common marine species along the North Sea coast, that uses the contaminated Western Scheldt estuary (Baeyens et al., 2003; De Vijver et al., 2003; Roosens et al., 2008; Voorspoels et al., 2003, 2004a, 2004b) as nursery area and feeding ground until first reproduction (Rijnsdorp et al., 1992). Further advantage of using sole is that, as a flatfish, it undergoes an obvious thyroid hormone mediated metamorphosis (Klaren et al., 2008) and thus is potentially sensitive to thyroid hormone disruption as has been described for other species (Noyes et al., 2011; Palace et al., 2010; Schriks et al., 2006; Yu et al., 2011; Gutleb et al., 1999). Last but not least, an optimized protocol is available for testing the impact of substances on the full development from a fertilized egg into a metamorphosed flat fish (Foekema et al., 2008).

In this study, first the effect concentrations were determined for the selected PCBs, PBDEs, and HBCD, and secondly a test with an artificial mixture of PCBs and PBDEs was performed in order to determine possible mixture effects. Finally, sole eggs were exposed to two 'field mixtures' of lipophilic organic pollutants extracted from tissue of sole from the North Sea and the Western Scheldt estuary. Invertebrates and fish from the Western Scheldt estuary are known to be contaminated with PCBs and brominated flame retardants (Baeyens et al., 2003; De Vijver et al., 2003; Voorspoels et al., 2003, 2004a, 2004b; Janak et al., 2005; Roosens et al., 2010), but also with other substances as perfluorooctane sulfonic acid (PFOS; De Vijver et al., 2003) and organo-chlorine pesticides (OCPs; Voorspoels et al., 2004a).

The comparison of the effects of the field mixtures with those induced by the artificial mixture, composed of only PCBs and PBDEs, indicates to what extent other (unknown) substances present in the cocktail of POPs extracted from the fish contribute to an effect on the developing larvae.

In summary, the present study aimed to assess the likelihood that maternally transferred POPs negatively affect early life development of fish from contaminated areas with special focus on PCBs, HBCD and PBDEs, and (other) POPs present in sole from the Western Scheldt estuary.

2. Materials and methods

2.1. Test organisms and seawater

Fertilized Sole eggs were produced at IMARES (location IJmuiden, the Netherlands) where a group of mature male and female soles is kept together in large spawning tanks. Spawning and fertilization were temperature-induced and took place overnight at water temperatures around 12 °C. The following morning, the fertilized eggs were transported in plastic bags with oxygen saturated seawater to the IMARES laboratory in Den Helder, the Netherlands, where the tests were performed. Together with the eggs, artificial seawater (Instant Ocean Aquarium Salt in demineralized water, salinity ~35‰) from the spawning tank was transported to the laboratory. Here, the water was stored in a container where it was continuously aerated and circulated through a particle filter and UV-disinfection system. This water was used for the start of the tests. During the test the artificial seawater was gradually replaced with natural seawater (salinity ~32‰) collected from the Eastern Scheldt, a relatively pristine bay of the North Sea, that is often used as a reference site in marine ecotoxicological research in the Netherlands (e.g. Kuiper et al., 2007; Foekema et al., 2008, 2012). For the tests presented in this paper three batches of eggs were used, one for the tests with the PCB mixture Arochlor 1254, PCB 126 and HBCD, and two others for testing the PBDEs, and the artificial and field mixtures.

2.2. Sole ELS test

The sole prolonged early life stage (pELS) test was performed according to Foekema et al., 2008, with some slight modifications to improve test performance.

Immediately after delivery at the laboratory, fertilized eggs were transferred to 5 L glass beakers and placed in a temperature controlled (15 °C) room. The beakers were left untouched for about 1 h after which non-fertilized/dead eggs had sunk to the bottom of the beaker. Exposure started approximately 12 h after fertilization by transferring 500 fertilized eggs in approximately 50–100 ml seawater to the glass 'exposure beakers' containing 2 L seawater with the desired exposure concentration.

The exposure beakers were covered with individual glass lids and kept in a temperature controlled cabinet at 14.5 ± 0.5 °C, with a 16 h photoperiod (ca. 100 LUX). During the following 5 days, about 60–75 percent of the water volume was replaced every other day with freshly spiked seawater. Dead eggs were removed daily. The first larvae hatched around 5 days post fertilization (dpf). At 6 dpf, from each exposure group, two sub-groups of fifteen larvae were randomly selected and transferred to two smaller glass beakers containing 80 ml of clean seawater. The two glass beakers per exposure group were regarded as duplicates. Transfer of the larvae was done by means of a polyethylene pipette, with as little exposure water as possible. The larvae were then allowed to develop further under similar, yet unexposed conditions. From 9 dpf onwards, when pigmentation of the eyes indicates that the larvae are about to start external feeding, they were fed ad libitum with newly hatched naupllii of *Artemia salina* on a daily basis. Every other day, approximately 75 percent of the water volume in each glass beaker was replaced with fresh seawater, and almost daily dead larvae, feces and surplus food items were removed with a polyethylene pipette when appropriate.

The development of the fish was recorded following the stages described for the development from fertilized egg to a fully metamorphosed summer flounder (*Paralichthys dentatus*) (Martinez and Bolker, 2003). At the given test conditions the first signs of metamorphosis can be observed around 25 dpf. Under normal conditions all fish completed metamorphosis around 40 dpf. Fish that completed metamorphoses were taken out of the test, narcotized and subsequently killed in a strong MS222-solution (500 mg of ethyl 3-aminonebzoate methanesulfonic acid salt, purity 98 percent, ACROS Organics (Landsmeer, The Netherlands), and 200 mg $NaHCO_3$, purity 99.0 percent, Fluka Chemika, (Zwijndrecht, The Netherlands), in 1 L seawater). The moment of completed metamorphosis (in dpf), body length, and morphological deviations, if any, were recorded.

All experiments were approved by the Animal Experimentation Board of Wageningen UR.

2.3. Chemicals, stock solutions and exposure concentrations

The technical PCB mixture Arochlor 1254 (Monsanto, St. Louis, USA) was used to prepare the stock solutions for the treatment that will be further referred to as ARO. The contribution of the different PCB congeners in this mixture was chemically analyzed (Table S1 in supplementary data). The PBDE mixture was composed of standards of five different congeners: BDE 28, 47, 99, 100 and 153 (Accustandard, New Haven, USA, purity 99.3–100 percent). The rationale behind the chosen composition (Table S1 in supplementary data) was that exposure of the eggs will result in tissue concentrations of the individual congeners with a composition that is comparable to what is found in fish tissue from the Western Scheldt estuary (Tables S3 and S4 in supplementary data). The HBCD and PCB 126 that were used in this study were obtained from Albermarle (Amsterdam, The Netherlands) and Promochem (Wesel, Germany) respectively.

For each exposure concentration a 1000× concentrated stock solution was prepared in dimethyl sulfoxide (DMSO, purity 99.9 percent A.C.S. spectrophotometric grade, supplier SIGMA-Aldrich, Zwijndrecht, The Netherlands), so that addition of 1 ml of this stock solution in 1 L seawater produced the appropriate exposure concentration in 0.1 percent DMSO. In every test series triplicate solvent controls and seawater controls were included.

The stock solution for the test with the artificial mixture was prepared by combining Arochlor-1254, PCB 126 and the PBDE mixture, in such proportions that, based on accumulation kinetics as a function of the octanol–water partition ratio (Kow), exposure results in a composition that is comparable to what is found