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PFOS affects posterior swim bladder chamber inflation and swimming performance of zebrafish larvae

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ABSTRACT

Perfluorooctane sulphonate (PFOS) is one of the most commonly detected perfluorinated alkylated substances in the aquatic environment due to its persistence and the degradation of less stable compounds to PFOS. PFOS is known to cause developmental effects in fish. The main effect of PFOS in zebrafish larvae is an uninflated swim bladder. As no previous studies have focused on the effect of PFOS on zebrafish swim bladder inflation, the exact mechanisms leading to this effect are currently unknown. The objective of this study was to determine the exposure windows during early zebrafish development that are sensitive to PFOS exposure and result in impaired swim bladder inflation in order to specify the mechanisms by which this effect might be caused. Seven different time windows of exposure (1–48, 1–72, 1–120, 1–144, 48–144, 72–144, 120–144 h post fertilization (hpf)) were tested based on the different developmental stages of the swim bladder. These seven time windows were tested for four concentrations corresponding to the EC-values of 1, 10, 80 and 95% impaired swim bladder inflation (EC_1 = 0.70 mg L^{-1}, EC_{10} = 1.14 mg L^{-1}, EC_{80} = 3.07 mg L^{-1} and EC_{95} = 4.28 mg L^{-1}). At 6 days post fertilization, effects on survival, hatching, swim bladder inflation and size, larval length and swimming performance were assessed. For 0.70 mg L^{-1}, no significant effects were found for the tested parameters while 1.14 mg L^{-1} resulted in a reduction of larval length. For 3.07 and 4.28 mg L^{-1}, the number of larvae affected and the severity of effects caused by PFOS were dependent on the time window of exposure. Exposure for 3 days or more resulted in significant reductions of swim bladder size, larval length and swimming speed with increasing severity of effects when the duration of exposure was longer, suggesting a possible effect of accumulated dose. Larvae that were only exposed early (1–48 hpf) or late (120–144 hpf) during development showed no effects on the studied endpoints. The results demonstrate that PFOS does not affect the budding phase, and does not cause deflation of already inflated swim bladders. PFOS clearly affects processes that take place during the inflation phase and might also have an effect on the formation of the tissue layers forming the swim bladder.

1. Introduction

Due to the persistent and bioaccumulative nature of perfluorinated alkylated substances (PFAS), these compounds are being detected worldwide in wildlife and humans (Sturm and Ahrens, 2010). The aquatic environment is known as an important sink for PFAS resulting in high concentrations in aquatic organisms. Despite the phase-out of perfluorooctane sulphonate (PFOS) production in 2002 by its largest producer (3M, 2000), this pollutant is still one of the most commonly detected perfluorinated alkylated substances in the aquatic environment due to its persistence and the degradation of less stable PFAS to PFOS (Galatius et al., 2013; Lutz, 2011). PFOS is currently being used as one of the most important model pollutants to study the effects and modes of action of PFAS in general.

Exposure to PFOS is known to cause developmental effects in many organisms (Lau et al., 2004; Shi et al., 2008). Several studies have described the developmental effects of PFOS in zebrafish embryos. The main morphological effects are an uninflated swim bladder and spinal curvature. Both effects have been reported in all of the studies that included these endpoints (Huang et al., 2010;

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Hagenaars et al., 2011; Shi et al., 2008; Ulhaq et al., 2013a; Zheng et al., 2012). Spinal curvature or the impairment of swim bladder inflation can alter swimming performance and buoyancy in both juvenile and adult fish and therefore affect essential behaviour such as feeding, predator avoidance and reproduction (Gee, 1983). The effects of PFOS on larval swimming performance were already studied by Huang et al. (2010) and Ulhaq et al. (2013b) demonstrating a reduced swimming performance of larvae exposed to high concentrations while low concentrations resulted in hyperactivity. Although swim bladder inflation effects are sublethal, it is generally assumed that they affect apical endpoints that are traditionally considered relevant to environmental risk assessment such as feeding behaviour, predator avoidance, growth and reproduction. Such effects lower the probability of survival, especially in natural habitats where food resources are limited (Villeneuve et al., 2014).

As no previous studies have focused on the effect of PFOS on zebrafish swim bladder inflation, the exact mechanism leading to this effect is currently unknown. The swim bladder of zebrafish consists of two chambers separated by a narrow duct. The posterior chamber is developed and inflated around 4 dpf and operates as a hydrostatic organ to regulate buoyancy while the anterior chamber is developed and inflated around 20 dpf and functions primarily as an acoustic resonator aiding hearing (Finney et al., 2006; Robertson et al., 2007). This study focusses on the effects of PFOS on swim bladder development during the embryonic and larval stages. The following hypotheses are therefore related to the inability to inflate the posterior chamber.

The first hypothesis involves the interference with the formation of the swim bladder tissue leading to the impairment of swim bladder inflation. PFOS might affect the early development of the swim bladder which originates from an evagination of the foregut epithelium from 30 to 48 h post fertilization (hpf, see Fig. 1). Different signalling pathways are then involved in the differentiation, organization and growth of three tissue layers (epithelium, mesenchymal layer differentiating into smooth muscle and an outer mesothelial layer) during the pre-inflation phase from 48 to 72 hpf. Hedgehog signalling is required for the growth of the swim bladder epithelial cells and the differentiation of the mesenchymal cells while the Wnt/β-catenin signalling is required for the organization and growth of all three tissue layers. PFOS might disturb these signalling pathways and affect swim bladder structure (Lindsey et al., 2010; Robertson et al., 2007; Winata et al., 2009, 2010; Yin et al., 2011).

A second hypothesis is interference with the initial inflation of the swim bladder. Prior to swim bladder inflation, a sequence of behavioural patterns including adherence to the tank walls, detachment, tail flicking for upward movement and reattachment are seen in zebrafish larvae of 3–4 dpf (days post fertilization). These behaviours are necessary to reach the air–water interface after which the larvae gulp air that is transmitted by peristaltic movement through the foregut and the pneumatic duct into the gas bladder lumen to inflate the swim bladder by 4–5 dpf (Goolish and Okutake, 1999; Lindsey et al., 2010). Several studies describe tail malformations such as a spinal curvature, apoptosis in the tail region and muscle lesions after PFOS exposure (Huang et al., 2010; Hagenaars et al., 2011; Shi et al., 2008; Ulhaq et al., 2013a; Zhang et al., 2011; Zheng et al., 2012). Ji et al. (2008) demonstrated that the swim-up success in medaka larvae was decreased when the parent generation was exposed to PFOS. These effects might preclude the swim-up behaviour to reach the air–water interface and therefore result in the inability of the larvae to inflate the swim bladder. Furthermore, PFOS forms a layer on the air–water interface with the hydrophobic tails of PFOS reaching out of the water. It is known that zebrafish larvae grown in closed chambers without air–water interface show very small inflation rates (Goolish and Okutake, 1999). If the tension of the PFOS layer at the air–water interface is high enough to prevent the larvae from surface air-gulping, the effects

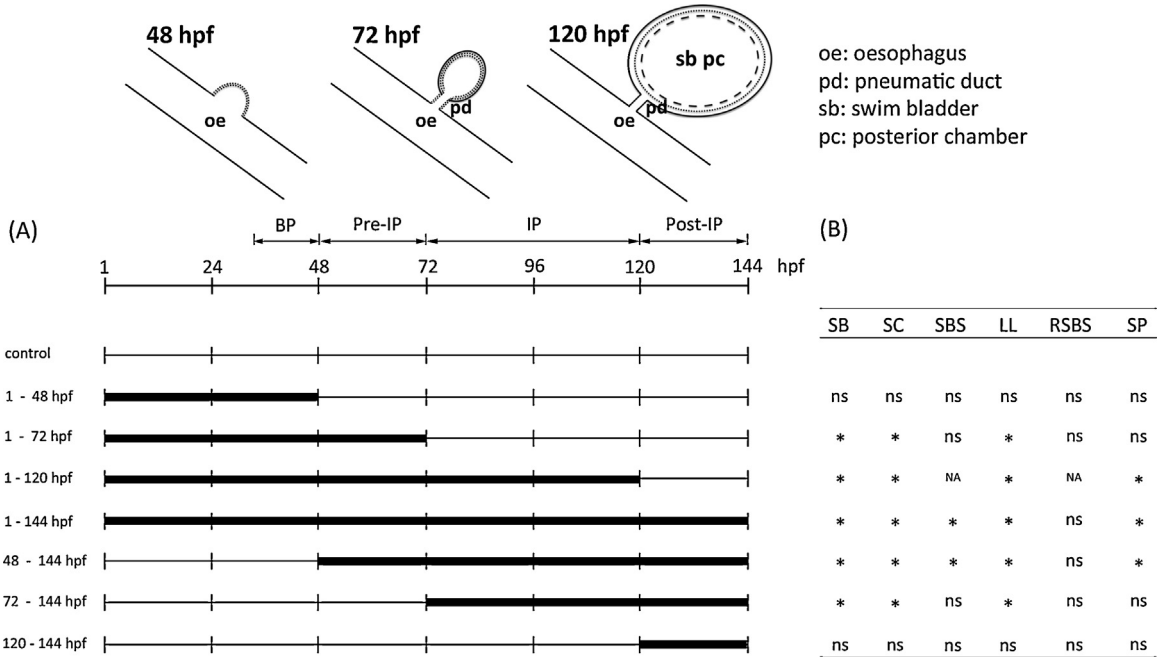

Fig. 1. (A) The seven different exposure windows were based on the developmental stages of the swim bladder. Dark grey boxes indicate the periods of exposure to PFOS. The seven exposure windows were tested for each of the four selected concentrations. $n = 42$ for each exposure window and each concentration. All experiments were ended at 144 hpf. BP: budding phase; Pre-IP: pre-inflation phase; IP: inflation phase; Post-IP: post-inflation phase. (B) A summary of the significant effects of PFOS exposure during the different time windows on the presence of swim bladder inflation (SB) and spinal curvature (SC), swim bladder surface (SBS), larval length (LL), relative swim bladder surface (RSBS) and swimming performance (SP). Asterisks represent significant effects compared to the control, 'ns' represents no significant effect and 'NA' represents the inability to perform statistical analysis due to the low number of larvae with an inflated swim bladder exposed during this time window. The illustrations on top of the timeline illustrate the different stages of the formation and inflation process (see Section 1 for more details).