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Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids

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ABSTRACT

In the present study, we have used salmon embryos whose continuous exposure to waterborne PFOA or PFOS at 100 μg/L started as freshly fertilized eggs, and lasted for a total of 52 days. PFOS and PFOA were dissolved in methanol (carrier vehicle) whose concentration never exceeded 0.01% of total tank volume. Samples were collected at day 21, 28, 35, 52, 49 and 56 after the start of the exposure. Note that days 49 and 56 represent end of exposure and 1 week after a recovery period, respectively. Tissue bioaccumulations were determined by HPLC/MS/MS, steroid hormones, fatty acids (FAs) and lipids were determined by GC–MS, while mRNA expression levels of genes were determined by qPCR in whole body homogenate. We observed that PFOS and PFOA showed a steady increase in whole body burden during the exposure period, with a slight decrease after the recovery period. Calculated somatic indexes showed that PFOA produced increases in heart-, thymus-, liver- and kidney somatic indexes (HSI, TSI, LSI and KSI). PFOA and PFOS exposure produced significant decreases in whole body dehydroepiandrosterone (DHEA), estrone and testosterone at sampling day 21 and a strong increase of cortisol and cholesterol at the end of recovery period (day 56). PFOA and PFOS effects differed with DHEA and estrone. While PFOS decreased DHEA levels, PFOA produced an increase at day 49, and while PFOS decreased estrone, PFOA produced a slight increase at day 56. We observed changes in FA composition that predominantly involved increases in FA methyl esters (FAMEs), mono- and poly-unsaturated FA (MUFA and PUFA) and a decrease in n-3/n-6 PUFA ratio by both PFOA and PFOS. Particularly, an increase in – pentadecenoic MUFA (15:1), two n-3 PUFAs α-linolenic acid [ALA: 18:3 n3] and eicosapentaenoic acid [EPA: 20:5 n-3] and n-6 PUFA: arachidonic acid [ARA: 20:4 n6], docosapentaenoic acid (DPA) by PFOA and PFOS were observed. These effects were associated with changes in mRNA expression of FA elongase (FAE), Δ5-desaturase (FAD5) and Δ6-desaturase (FAD6) genes. In summary, the changes in hormonal and FA profiles may represent cellular and/or physiological adaptation to continuous PFOS and PFOA exposure by increasing membrane fluidity, and/or overt developmental effects. The present findings provide some potential insights and basis for a better understanding on the possible mechanisms of PFCs toxicity in fish.

1. Introduction

Perfluoroalkyl acids (PFAAs) and their precursors are used in various industrial and consumer products such as oil and water repellents for textiles and food packaging, surfactants, insecticides, and aqueous fire-fighting foams, and have been manufactured for over 50 years (Moody et al., 2002; Prevedouros et al., 2006). For example, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) does not have natural sources, but have been widely used since World War II with environmental persistency (3M, 1999; Olsen et al., 2005). In Norway, PFOS and PFOA has been shown to occur widely in freshwater systems, although their water concentrations are lower compared to other industrialized nations (Fjeld et al., 2005). Despite the production and use of PFAAs for the past 60 years, concerns have accumulated regarding their environmental hazards, together with human and wildlife exposures (Kannan et al., 2002, 2004; Butenhoff et al., 2006; Houde et al., 2006; Kärrman et al., 2006; Calafat et al., 2007; Fromme et al., 2007).

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PFOA and PFOS accumulate in the liver where they inhibit glutathione peroxidase, a selenoprotein essential for thyroid hormone conversion (Hagenaars et al., 2008) and suppress growth (Du et al., 2009), and are known peroxisome proliferators. Besides their role as direct modulators of lipid β-oxidation, PFOA and PFOS have been reported to modulate cytochrome P450 (CYP) dependent drug, steroid and xenobiotic metabolizing enzymes and their regulatory genes (Yeung et al., 2007; Hagenaars et al., 2008; Krøvel et al., 2010). In humans, PFOA and PFOS have also been detected in serum samples from the general population of USA (Olsen et al., 2003) and in human milk and blood samples from China (So et al., 2006) and in human serum from Japan (Harada et al., 2007). Toxicological studies on adult laboratory animals (Cynomolgus monkeys, rats and mice) have shown that repeated dosing with PFOS produced several adverse health effects, including metabolic wasting, hepatomegaly, and decline in triacylglycerols (TG) and/or total cholesterol (TC) levels (Seacat et al., 2003; Thibodeaux et al., 2003). A positive association between plasma PFOA and PFOS levels and all lipids, except high density lipoprotein cholesterol (HDL-C) has been reported in adult residents of West Virginia, who drank PFOA-contaminated water (Steenland et al., 2009). A positive correlation between PFOA and cholesterol has been reported in several studies (Olsen et al., 2000, 2003; Olsen and Zobel, 2007; Sakr et al., 2007a,b; Costa et al., 2009; Frisbee et al., 2009).

The fatty acid (FA) composition of neutral lipids generally reflects the dietary sources (Bell et al., 2003). FAs in fish tissues are present in different lipid classes, with different functions. There are two classes of polyunsaturated fatty acids (PUFAs) – namely omega-3 (n-3) and omega-6 (n-6), based on the location of the first double bond in the third (ω-3) or sixth (ω-6) position from the methyl end of the aliphatic carbon chain (Stephensen, 2004). Of the omega-3 FAs, α-linolenic acid (ALA, 18:3n-3) can be desaturated and elongated to first form eicosapentaenoic (EPA, 20:5n-3) and thereafter docosapentaenoic (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Both EPA and DHA are synthesized from ALA by marine microorganisms and accumulate in seafood and fish, which is the primary human source of EPA and DHA. Although seafood is a good source of protein and is low in saturated fats, most beneficial effects of fish consumption and fish oils are usually attributed to the omega-3 PUFAs (Siddiqui et al., 2004). The precursor molecule in the omega-6 family is linoleic acid (LNA, 18:2n-6) that can be elongated and desaturated to form arachidonic acid (ARA, 20:4n-6) and docosatetraenoic acid (DTA, 22:4n-6). ARA is the precursor for some prostaglandins, thromboxane, leukotrienes and other biologically active substances. Thus, omega-6 FAs are considered as proinflammatory, whereas the omega-3 FAs are considered anti-inflammatory (Calder et al., 2002).

Although several reports have documented the environmental distribution of PFAAs, information on their toxicity, especially developmental effects and toxic mechanisms, is not well understood. For example, free radicals such as hydroxyl radical are capable of initiating chain reactions in lipid domains that result in lipid peroxidation (Bascetta et al., 1983). Peroxidation of membrane lipids perturbs membrane fluidity, permeability, ion and solute transport. Thus, lipid peroxidation plays an important role in oxidant-induced early cell injury (Salahydden, 1995). The most pronounced toxic effects of PFOA are interference with lipid metabolism, such as increasing peroxisomal FA β-oxidation and inducing acyl-CoA oxidase activity, which catalyzes the first and limiting-step in FA oxidation (Kawashima et al., 1989). PFOS can also increase membrane fluidity and inhibit gap junction intracellular communication (Hu et al., 2002). Exposure of adult rats to ammonium perfluorooctanoate and perfluorododecanoic acid (PFDoA) produced effects on their endocrine system by respectively decreasing and increasing testosterone and estradiol levels (Biegel et al., 1995; Shi et al., 2007) and modulating many genes involved in cholesterol transport and steroidogenesis (Shi et al., 2007).

To our knowledge, there are no studies on the comparative uptake and bioaccumulation of PFOS and PFOA and their association with cellular lipid and hormone levels in wildlife, and knowledge appears to be non-existent for fish at sensitive developmental stages (egg and embryo). Furthermore, eggs accumulate high levels of PFCs (Giesy and Kannan, 2001) and no studies have looked at high levels of PFCs in eggs with developmental outcomes. In a rare example, two studies that include PFOS and PFOA, environmentally relevant concentrations of these PFC were injected into the eggs of domestic white leghorn chickens (*Gallus gallus domesticus*), and the accumulation and effects in the livers of the developed embryos were examined showing that hepatic PFOS concentrations increased concomitantly with the dose (O'Brien et al., 2009a). In another study, hepatic accumulation was highest for PFOA (4.5 times) compared to perfluoroundecanoic acid (PFUdA) and perfluorodecane sulfonate (PFDS) (O'Brien et al., 2009b). Therefore, the present study was designed with the objective of investigating possible differences in bioaccumulation patterns of PFOS and PFOA with parallel effects on cellular/organ development, FAs and lipid homeostasis and whole body levels of sex steroid hormones, using Atlantic salmon exposed from egg stage immediately after fertilization. Our hypothesis is that exposure of salmon eggs/embryo to PFOA and PFOS will produce changes in endocrine and physiological responses that are integral for optimal developmental processes and overt health condition.

2. Materials and methods

2.1. Reagents and standards

Dehydroepiandrosterone (DHEA), estrone, estradiol-17β, testosterone, 3β-hydroxypregn-5-en-20-one (pregnenolone), pregn-4-one 3,20 dione (progesterone), cortisol, pregnan-20-one-17-hydroxy, corticosterone, cholesterol, methanol, ethanol, hexane, ethyl acetate, diethyl ether, chloroform, hydrochloric acid, potassium hydroxide (all of analytical grade), N-methyl-N-tri-methylsilyltrifluoroacetamide (MSTFA), trimethyliodosilane (TMIS), chloroform, methanol, KCl, $CaCl_2$, NaCl, $MgCl_2$, PUFA1 and PUFA2 fatty acids were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). SPE cartridges (Bond Elut C8, Bond Elut Si and Bond Elut NH2, 500 mg, 3 mL each), 20 mL polypropylene reservoirs and a vacuum manifold (VAC ELUT 10) were from Varian (Darmstadt, Germany). Water-saturated ethyl acetate was prepared freshly by adding 675 mL water to 25 mL ethyl acetate. For the salmon feeding study, perfluoro-n-octanoic acid (PFOA) and sodium salt of perfluoro-1-octane sulfonic acid (PFOS) (linear, technical grade were purchased from Alfa Aesar (Karlsruhe, Germany). For the determination of PFOA and PFOS in tissues, PFOA standard (50 μg/mL in methanol) and sodium PFOS salt standard (50 μg/mL in methanol), internal standard of perfluoro-n-[1,2,3,4-13134 C4]octanoic acid (MPFOA) (50 μg/mL in methanol) and sodium perfluoro-1-[1,2,3,4-13135 C4]octane sulfonate (MPFOS) (50 μg/mL in methanol) were purchased from Wellington Laboratories (Guelph, ON, Canada). Trizol reagent for ribonucleic acid (RNA) purification and TA Cloning kit were purchased from Invitrogen Corporation (Carlsbad, CA, USA). IScript cDNA synthesis kit, iTAQ™SYBR® Green Supermix with ROX, an Immun-Star WesternC Chemiluminescent Kit were purchased from 139 Bio-Rad Laboratories (Hercules, CA, USA).