AN ABSTRACT OF THE THESIS OF

Lisa Duong for the degree of Master of Science in Environmental Science present on February 20, 2008.
Title: Assessment of Fluorinated Chemicals (FCs) on Developmental Toxicity in Embryonic Zebrafish.

Abstract approved:

_____

Robert L. Tanguay

Fluorinated chemicals (FCs) have been used since the 1950s in many industrial and commercial applications because of their unique properties such as chemical inertness, resistance to heat and their ability to repel water and oils. Concerns regarding potential environmental or human health risk from FCs exposure have emerged due to these chemicals being persistent in the environment and can bioaccumulate in animal tissues. Early life stages are often sensitive to chemical insult, so it is essential to determine if FCs are developmentally toxic. 41 structurally diverse FCs, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), were screened for potential developmental toxicity using an embryonic zebrafish bioassay. In initial studies, developmental responses to waterborne exposure to FCs were determined using a range of concentrations (0.01-200 ng/mL), including levels that are relevant to the environment. The majority of the FCs tested did not elicit any adverse developmental responses. However, increased embryonic malformations were observed in animals exposed to 17 FCs. Since the embryonic dose cannot be inferred from waterborne exposures, six FCs that are most commonly studied were also microinjected into the embryonic zebrafish with three doses (0.00002, 0.00024, 0.00245 mg/kg). Three of these six FCs (PFOA, PFDA and PFHxA) elicited embryonic malformations, including mortality and head defects only at certain concentrations. A subsequent microinjection study at higher concentrations was conducted to determine if a structure-response relationship exist. Exposure to three carboxylated and two eight-carbon FCs did lead to embryonic malformations, but not a definite structure-response relationship. These results demonstrate that FCs are generally not developmentally toxic. Collectively, these studies indicate the

power of the zebrafish model to conduct structure- and dose- response relationships and developmental toxicity studies.

Assessment of Fluorinated Chemicals (FCs) on Developmental Toxicity in Embryonic Zebrafish

by
Lisa Duong

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented February 20, 2008
Commencement June 2008

Master of Science thesis of Lisa Duong presented on February 20, 2008.

APPROVED:

Major Professor, representing Environmental Science

Director of the Environmental Sciences Program

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lisa Duong, Author

ACKNOWLEDGEMENTS

I would like to thank my advisor, Robert Tanguay for all the guidance, support and the numerous opportunities presented to me. A special thanks to Andrew Blaustein for being a great mentor and friend. His knowledge, encouragement and faith in me have been my inspiration. I would also like to thank Abby Benninghoff for her guidance through these studies. Others who have provided support and deserve acknowledgement are my lab members: Stacey Harper, Lijoy Mathew, Sumitra Sengupta and Kate Saili. Stacey, thank you for all the time you spent helping and guiding me. A special thanks to Jane La Du for keeping the lab running and the fish happy.

I am grateful for the love and support from my family. Jasmine Reyes, David Ly and Lewis Sam are friends who are now a part of my family. Their endless friendships have enabled me to keep my perspective on life. I would like to thank Khanh Luu, my mom, for all the confidence she had in me. My siblings, Lynda and John, for always being there to guide me through the tough times and reminding me about what matters most in life. Lastly, my greatest appreciation and love goes to my fiancé, Hao Truong. His everlasting faith in me and my ambitions have made all of this possible. I am lucky to have found such a supportive and amazing partner. Thank you for everything you have done throughout this endeavor and believing in me every day.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

LIST OF APPENDIX TABLES (Continued)

LIST OF APPENDIX TABLES (Continued)

CHAPTER 1 – INTRODUCTION

Fluorinated Chemicals

Fluorinated chemicals (FCs) are a family of compounds that consist of a carbon backbone, typically 4-14 carbons in length, with multiple carbon-fluorine bonds and a charged functional moiety (primarily carboxylated, sulfonated or phosphonated) [1]. This class of compounds has been used since the 1950s in industrial and commercial applications ranging from water-, soil- and stain resistant coating for clothing fabrics, leather, upholstery and carpets. FCs have also been used as oil-resistant coatings for paper products approved for food contact, electroplating, electronic etching bath surfactants, photographic emulsifiers, aviation hydraulic fluids, fire-fighting foams, paints, adhesives, waxes and polishes [2, 3]. Their extensive use can be attributed to the strength in the carbon-fluorine bonds, stability at high temperatures, being nonflammable and not subject to photolysis or metabolized [4]. These unique properties have made these FCs popular for consumers and render most of these chemicals ideal surfactants [5]. The most effective surfactant property is the eight-carbon chemicals [1]. The two well-known FCs with eight-carbon backbone are perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS). Although these physical properties make FCs ideal for the consumer, their unique properties causes multiple environmental problems and they are resistant to biodegradation [4].

FCs can be produced by Simons Electrochemical Fluorination (SEF) or telomerization. Simons Electrochemical Fluorination uses organic fluorochemicals that are fueled by electric current causing hydrogen atoms on the carbon backbone to be replaced by fluorine atoms [1]. This process was primarily utilized by 3M, the dominant global producer of PFOA-related chemicals, where the starting product, perfluorooctane sulfonylfluoride (POSF) is used to produce a range of different products, such as perfluorooctane sulfate (PFOS), *N*-methyl and *N*-ethyl perfluorooctanesulfonamidoethanol (*N*-MeFOSE, *N*-EtFOSE respectively). SEF also generates fluorinated molecules of various carbon chain lengths as by-products [6] and a mixture of linear, branched and cyclic isomers [1]. The telomerization process is used

by all major companies other than 3M [1, 2]. It involves telomerizing tetrafluoroethylene units, which always yields straight chain alcohols that are converted into final products for commercial application [1].

Environmental Relevance and Concerns

In the past, 3500 metric tons (in 2000) of PFOS and 500 metric tons (in 2000) of PFOA were produced [1]. Research demonstrating that PFOS was widespread in the human population and in wildlife led the 3M company to announce that it was discontinuing production of perfluorooctanoyl chemistry and related products, including PFOA [7] in May 2000. This occurred prior to United States Environmental Protection Agency requesting them to cease production [8]. Although 3M has ceased production of perfluoroalkylated substances, it has replaced the perfluorooctyl chemistry with the butyl equivalent, which is reported to be less bioaccumulative [6]. The combination of historical use, production and the physical-chemical properties of FCs have made them ubiquitous and highly persistent. FCs are present everywhere in the environment and are found in blood samples of humans from various countries as well as in biota collected in relatively remote locations [9].

FCs have been detected in a variety of environmental matrices around the world. These matrices include surface waters, air, sludge, soil, sediments, and ice caps [1]. FCs have been identified in tissues of aquatic and terrestrial organisms, including humans [8]. Previous studies have demonstrated that these volatile FCs are released during manufacturing and application processes [10]. The levels of PFOA in the air are typically in the part per trillion (ppt) range, but higher levels of PFOS, PFOA and PFHxS (ranging from parts per billion [ppb] to parts per million [ppm]) have been reported after accidental release of fire-fighting foam [11]. PFOA detected in the air ranged from 0.00000414 - 0.0000532 ppb among different Japanese cities [12] to 0.007 - 0.05 ppb in sampling area near fluoropolymer manufacturing facilities in United States [13]. In soils, sediments, waste water and sludge, PFOS and PFOA were detected in various countries [14]. Two months after firefighting foams were used to extinguish fires at major oil storage facilities following an earthquake in southern

Japan, soil concentrations were 0.00287 mg/kg. Prior to this instance, dry soil levels ranged from 0.000017 to 0.7 mg/kg [1].

Since 2000, many countries began biomonitoring the human population for FCs. In most cases, occupationally exposed workers have serum levels of both PFOA and PFOS approximately one order of magnitude higher than the general population [1]. In the general population of the United States, FCs have been detected in serum, breast milk, liver, seminal plasma and umbilical cord blood [15]. These chemicals have also been detected in human milk and blood samples in China (PFOS: 0.045 - 0.36 ng/mL, PFOA: 0.047 - 0.21 ng/mL [in milk], PFOS: 52.7 ng/mL, PFOA: 1.59 ng/mL [in blood samples]) and Japan [16]. PFOA levels from pooled blood samples from US blood banks ranged from 3 to 17 ng/mL [17]. FCs including PFOS, PFOA and PFHxS were detected in all serum sample pools in Australian urban and rural regions ranging from 13 - 29 ng/mL for PFOS, 5.0 - 9.9 ng/mL for PFOA and 2.7 - 19 ng/mL for PFHxS [9].

FCs have also been detected in the serum and plasma of wildlife. Giesy and Kannan (2001) [18] reported the global distribution of PFOS in frozen wildlife liver and blood samples. PFOS has been detected in livers of mink *(Mustela vison)* (970 - 3680 mg/kg wet weight) [18] and in blood plasma of bald eagles (13 - 2220 ng/mL) [19]. High concentrations of PFOS were detected in the liver (7760 mg/kg wet weight) from plaice (*Pleuronectes platessa)* [20] and carp *(Cyprinus carpio)* (up to 9031 mg/kg wet weight) in Belgium [21]. In North America, surveys have found the highest FC liver concentration in mink (*Mustela vision)*, bottle-nosed dolphins *(Tursiops truncates)*, polar bear *(Ursus maritmus)*, ringed seal *(Pusa hispida)* and Brandt's Cormorant [18]. In Europe, the highest FC liver concentrations reported were in ringed seal, eels and cod [22]. In Asia the Common Cormorant had the highest FC liver concentrations [22]. In general, ranges in wildlife serum or plasma levels were <3 – 100 ng/mL for PFOS and 0.05 – 4.25 ng/mL for PFOA [18].

Animal Model

Currently, there are limited data on the developmental toxicity of FCs. Without this information, it is difficult to determine the potential adverse health hazards to humans. The available data are from studies of rodents and nonhuman primates. One rodent and nonhuman primate study demonstrates that FCs increased liver weight, reduced body weight and cholesterol [3]. A two year PFOS study in Sprague-Dawley rats revealed an increase in hepatocellular adenomas at high doses of 20,000 ppb in the diet [23]. PFOA and PFOS in rats and mice have induced developmental toxicity and other adverse effects *in vivo*. These effects include reduction of fetal weight, induction of cleft palates and cardiac abnormalities [24].

The two most well studied FCs are PFOA and PFOS. In hepatoxicity studies with these two FCs, rats had enlarged livers and hepatocellular adenomas [1]. Cell lines, mice, rats and Cynomolgus monkeys are typical models for short-term studies to investigate hepatoxicity. Developmental toxicity has been assessed in rats exposed to PFOS throughout pregnancy with dose-dependent deleterious effects observed in newborns [1]. Lau *et al*. [25] reported that all pups were born alive and active, however at 10 mg/kg of PFOS (the highest dose group), newborns became pale, inactive and moribund within 30-60 minutes and died soon afterwards. At 5 mg/kg, neonates became moribund. Over 95% of these offspring did not survive the first day of postnatal life and only a few pups reached puberty. These developmental toxicity studies on PFOA and PFOS demonstrate that there is the potential for FCs to induce developmental malformations. However, these studies used high doses not normally detected in the environmental, biota or human serum. This poses the question of the relevance to the detected environmental concentration and serum level of the FCs.

Proposed Mechanism of Action

Presently, the proposed mode of action for developmental toxicity is through activation of peroxisome proliferator-activated receptor alpha (PPAR-α). PPARs are members of the nuclear hormone receptor super family of ligand-activated transcription factors [26]. The activation of PPAR by PFOA and PFOS alter enzyme activities and affect changes in gene expression (PFOA: [24], PFOS: [27]). FCs may activate PPAR-α by two mechanisms: binding directly to the receptor after heterdimerization of PPAR-α and retinoid X receptor (RXR) (i.e.: as ligands) or indirectly by perturbing lipid metabolism and transport in a manner that stimulates the synthesis and/or release of endogenous PPAR ligands (Figure 1) [27]. Luebker *et al.* [28] demonstrated that FCs displace endogenous ligands from liver fatty acid binding proteins, acyl-CoA derivatives and variety of other hydrophobic molecules within hepatocyte. FCs could also displace the fatty acids, which stimulates the activation of PPAR-α [27].

An alternative mechanism of action for FCs is that it mimics or antagonizes the effects of hormones, alter the pattern of synthesis and metabolism of hormones or modify hormone receptor levels [29]. Endocrine disrupting chemicals may interfere with processes such as synthesis, secretion, transport, binding, action or elimination of natural hormones that are responsible for the maintenance of homeostasis, reproduction, developmental and/or behavior of an organism [30]. Preliminary studies suggest that FCs act like estrogen, the primary female-sex hormone, and when FC enters a cell, it causes isoforms of the estrogen receptor (ER) to dimerize to create a ligand-binding domain (LBD). When bound by FC it blocks the site from estrogen. The complex then binds directly onto estrogen response elements (ERE) of DNA. This binding modulates transcription of target genes. Benninghoff *et al.* [31] demonstrated that certain FCs (including PFOA and PFOS) triggered juvenile trout to produce vitellogenin, a protein normally produced only by female animals during egg laying. They found that the apparent estrogen mimics bound to the receptors and block the hormone from its site of action (Figure 2). Repercussion from the FCs mimicking estrogen can result in disruption of the organism's homeostasis and lead to developmental toxicity.

Zebrafish *(Danio rerio)* as an ideal model

The available animal models to assess the developmental toxicity of FCs are limited. A new *in vivo* model to rapidly assess the diverse effects of FCs needs to be developed. The model will need to be able to assess at early life stages because animals are generally most sensitive to chemical insult during early development [32-36]. The current rodent models are inadequate for testing FCs because it is difficult to assess earlier development, the length of development is long and it is not possible to observe the effects of the compounds non-invasively, as development occurs in the mother. Embryonic zebrafish *(Danio rerio)* would be an appropriate model to serve as a screening tool to assess the numerous FCs found worldwide. Animals are generally most sensitive to chemical insult during early development, which makes this the ideal life stage to assess the effects of FCs. Embryonic zebrafish will serve as a good model system because they are vertebrates that share many cellular, anatomical and physiological characteristics with other vertebrates [35]. Female zebrafish produce several hundred eggs a week, which allows for large sample sizes. Being able to produce numerous eggs also makes it possible to evaluate the toxicity of abundant FCs within a short amount of time. The embryos also develop rapidly, offering additional technical advantages [37]. Neuronal plate formation occurs at 10 hours, followed by organogenesis at 24 hours, which compared to a rat occurs at 9.5 days and 5-6 days respectively. The first heartbeat occurs at 30 hours for the zebrafish and 10.2 days for rats. Finally, a significant advantage of the embryonic zebrafish model is that the externally developing embryos are clear, allowing for non-invasive assessments of effects over the course of development.

Rationale

Utilizing the many advantages of embryonic zebrafish was enabled me to rapidly investigate the potential developmental toxicity of FCs. My hypothesis is FCs are developmentally toxic, therefore 1) when embryonic zebrafish are exposed to FCs at a early life stage, physiological developmental malformations will be observed and 2) there will be a

dose and structure-response relationship. Selection of the 41 FCs to test the hypothesiswas based on the carbon backbone length and functional groups of the chemicals. Ten FCs with carboxylated functional groups, 20 FCs with sulfhydrol groups, two with alcohol groups, two with hydroxylated groups, three with alkene functional groups and four mixtures were analyzed (Table 1).

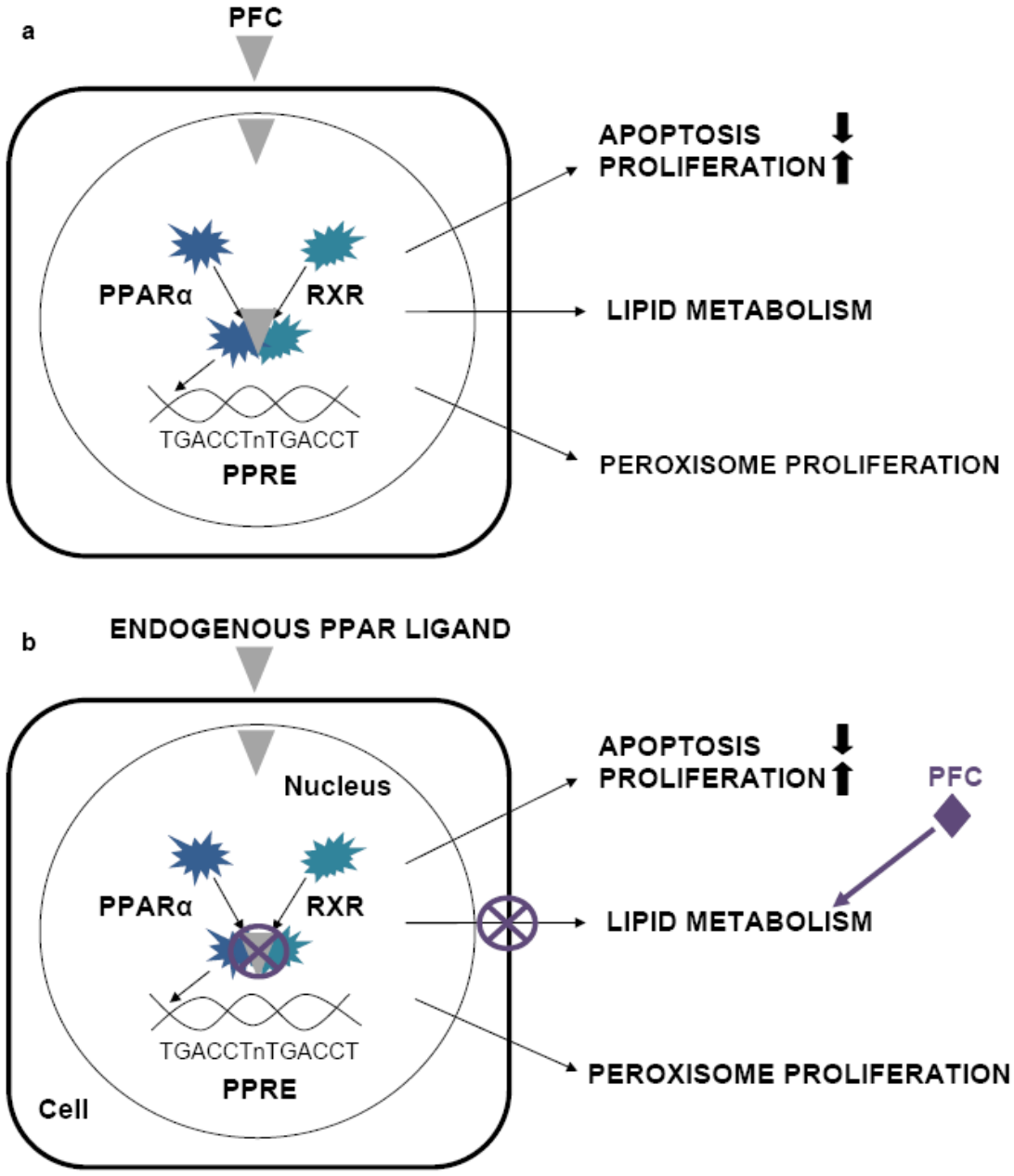


Figure 1. Proposed mechanism of action of PPAR-α.
(a) FCs enter the cell and cause heterdimerization of PPAR-α and retinoid X receptor (RXR), which binds with the FC, onto the PPRE DNA element. The PPRE consists of a direct repeat of two copies of a TGACCT-like sequence separated by a single base. Binding modulates transcription of target genes resulting in changes in peroxisome proliferation, lipid metabolism, growth and apoptosis. (b) FCs perturb lipid metabolism, thereby stimulating the release of the endogenous ligand causing a change in or elimination of peroxisome proliferation, lipid metabolism, growth and apoptosis. Figures adapted from Holden *et al [38].*

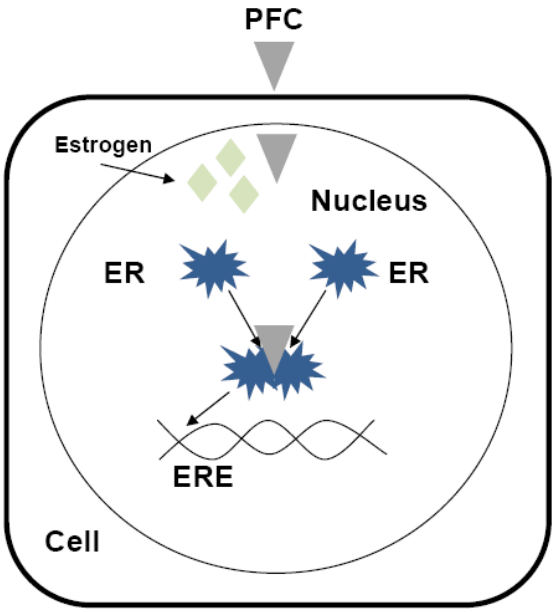


Figure 2. Proposed mechanism of action of Estrogen Receptor (ER)
FCs enter the cells and cause the ER isoforms to dimerize to create a ligand-binding domain (LBD). The FC then binds to LBD and blocks the active site from estrogen. The complex then binds directly on to the ERE element of DNA. This binding modulates transcription of target genes.

CHAPTER 2 – MATERIALS AND METHODS

Zebrafish Maintenance and Embryo Collection

The AB strain of embryonic zebrafish werereared and kept at standard laboratory conditions at 28°C on a 14 h light/ 10 h dark photoperiod in dosing solution. Dosing solutions consisted of reverse osmosis (RO) water supplemented with a commercially available salt solution (0.6% Instant Ocean). Water conditioning and environmental quality was maintained according to the manufacturer's instructions and *The Zebrafish Book* [39]. Zebrafish were group spawned in a breeding basket to allow for easy collection of embryos the following morning. The embryos were collected and staged as described in Kimmel *et al.* [37]. After the completion of the studies, embryos were photographed using a Nikon Coolpix 5000 digital camera.

Chemicals

The FCs selected were from donations from industry and purchased from Sigma Aldrich or VWR (Table 1). Depending on the experimental design and desired concentration, the chemical solution used either a dosing solution or dimethyl sulfoixde (DMSO) as a solvent depending on the specific FCs water solubility. If the chemical was not soluble in water, or precipitated out at a certain concentration, DMSO was necessary. Our lab has demonstrated that an embryo elicited no developmental deformities at 1% DMSO when exposed to the solvent in water[35]. When directly delivered into the yolk sac, any concentration above 0.1% DMSO caused developmental defects not attributed to the chemical.

Waterborne Exposure

A broad range concentration (0.01-10 ng/mL) screening of 39 FCs was completed. FCs 1-7, 9-18, 20, 24-27 (Table 1) were made into chemical solutions with a dosing solution. FCs 1-4 were provided as mixtures and used as the stock solution. A stock concentration and a series of serial dilutions was made for the remaining listed FCs above,

FC 21-23, 28-41 were not soluble in water and required the use of DMSO to get the chemicals into solution. A serial dilution was made in 100% DMSO that is 100 times more

concentrated to allow for a 1:100 fold dilution with the dosing solution to create a serial dilution with a final DMSO concentration of 1%.

A higher concentration assessment was completed by exposing embryonic zebrafish to 14 FCs by water to 25-200 ng/mL. The stock concentration solution for FC 29-31 and 33 was made with 100% DMSO as the solvent. Solutions of FC 1-3, 5, 8, 10, 15, 17, 19, 22 were made with dosing solution. The solutions for FC 29-31 and 33 was 100 times more concentrated and then diluted 1:100 times in fish water to create 1% DMSO solutions.

Microinjection

FCs (6, 8, 10, 19, 20 and 33) testing solutions were prepared for direct delivery into the embryonic yolk sac. The solutions used for microinjection contained less than 0.1% of DMSO, if the chemicals are not soluble in the dosing solution. A serial dilution for each FC was made with 100% DMSO as the solvent rather than the dosing solution to prevent solubility issues. The resulting solution was then diluted 1:1000 with dosing solution for each concentration.

Experimental Design

Waterborne Exposure

Embryos were allowed to develop until five hours post fertilization (hpf) after which unfertilized and abnormal eggs were discarded. Prior to exposing embryos directly to FCs, the chorion, an acellular envelope surrounding the embryos, was enzymatically removed with pronase to increase bioavailability and avoid barrier effects. This is a standard practice when using embryonic zebrafish to evaluate chemicals at early life stages for developmental toxicity [35, 40]. Embryos were dechorinated at five hpf in a petri dish filled with 25mL of fish water and 0.05 mL of 50,000 ng/mL pronase (Fluka, a Sigma Company, cat # 81750). The petri dish was swirled continuously for 10 minutes. Dosing solution containing pronase was removed and replenishing with fresh dosing solution for an additional 10 minutes. The dechorinated embryos were then exposed at 6hpf individually in wells of a 96-well plate to 0.1 mL of a FC solution or an appropriate vehicle control, fish water or 1% DMSO. Exposures began at six hpf to ensure coverage of gastrulation and organogenesis, which are the periods of development most well conserved among vertebrates.

The initial 39 FCs were waterborne exposed to four concentrations at a broad range (0.01 – 10 ng/mL). These concentrations were selected based on the level of FCs detected in the environment and in human blood serum. At each concentration, seven embryos were exposed and one embryo placed in each individual well. For the 14 FCs waterborne exposed at higher concentrations (25 - 200 ng/mL), four concentrations were tested. For each concentration, 24 embryos (n=24) were used with one embryo per well.

Microinjection

Embryos were collected and staged as described in Kimmel *et al* [37]. Three to four hpf embryos were aligned in troughs imbedded in a 1% agarose plate filled with dosing solution as described by *The Zebrafish Book* [39]. Embryos were injected with FCs or the appropriate vehicle control directly into the yolk (Figure 4) [35]. PFDA, PFHxA, MeFOSEA, PFOA, PFOS, and PFDS stock concentration had 100% DMSO as the solvent and were diluted 1:1000 in dosing solution. 0.01 mL of each testing solution was aliquot into 1.5 mL centrifuge tube. 0.002 mL of phenol red served as an indicator that the injected solution was present in the yolk. The testing solutions were loaded with a 20P gel pipette tip into a needle pulled from Flaming/Brown Micropipette puller (Sutter Instrument Co.). Capillary tubes (CATALOG # bf120-94-10, Sutter Instrument Co.) with O.D -1.2mm, I.D-0.94mm and 10 cm length served as the needle. Each embryo received a known milligram (mg) of a FC by being direct delivered into the yolk sac to create a desired injection dose. An average wet weight of an embryo was 0.00000094 kg, which was determined by weighing a conical tube with minimal fish water and 50 embryos and dividing it by 50.

After injections, the embryos were transferred to individual wells in a 96-well plate (N=24), filled with 0.1 mL of dosing solution.

Evaluations

After an exposure was completed, a daily evaluation assessed embryonic development. At 24, 48, 72 and 96 hpf, embryos were evaluated for viability, developmental progression and spontaneous movement. At 120hpf (5 days) embryos were first assessed for behavioral endpoints, then euthanized (with a 50% tricane solution) to evaluate for morphological and developmental endpoints. Morphological endpoints included larval morphology (e.g. body axis, eye, snout, jaw, otic vesicle, notochord, heart, brain, somite, fin, morphometrics and pigmentation), while behavioral endpoints were spontaneous movement (sp. movement), touch response and motility. Developmental endpoints consisted of embryo viability, developmental progression and hatch delay (only for microinjected embryos).

Statistical Analysis

Analyses used SigmaStat SigmaPlot (SPSS Inc, Chicago, IL). A one-sided Fisher's exact test was used to determine differences between control and treated groups at a $p<0.05$. Dose response significance was determined using one-way ANOVA ($p<0.05$).

Embryonic Zebrafish (EZ) Metrics

Embryos were assessed at 120 hpf and abnormalities in body axis, eye, snout, jaw, otic vesicle, notochord, heart, brain, somite, fin, yolk sac, trunk, circulation, pigment, swim bladder were binary scored (present or absent). Behavioral endpoints (motility, tactile response) were also binary scored for exposed embryonic zebrafish prior to euthanization. An EZ (embryonic zebrafish) metric was developed in the Tanguay lab and applied to each FC concentration based on the lethal and sublethal effects [40]. The scale for EZ metrics was derived based on severity of morbidity. For instance, a zebrafish can survive without a swim bladder, therefore the rank value would be relatively low (0.1), but it would be difficult to survive with an improperly developed heart, so the appropriate rank value would be high (0.6). EZ metrics takes into account the type and frequency of a sublethal effect in addition to mortality elicited from the exposure (ranking of the hierarchy of effects displayed in Table 2, the morbidity and mortality index). An EZ metric value for each concentration takes the number of animals that exhibited an effect and multiplies it by the appropriate ranking for the observed sublethal effect. This gives a relative comparison of the FCs effects on the integrated living systems.

Table illustrates the EZ metric evaluation and toxicity scale. An EZ metric value below 5 indicates low level of toxic potential, which means that the chemical at that concentration is most likely benign. The value 5 was determined based on the average value of the vehicle control; therefore, if a chemical possessed an EZ metric value of less than 5, it would resemble, developmentally and morphologically, the unexposed. When the EZ metric value falls within 5 to 15 for a chemical, there is a moderate level of toxic potential and the chemical would be a suspect FC that will need more testing before it can be categorized as

developmentally toxic. The upper limit (15) was determined from the highest level of toxicity on the EZ metric scale of 24. When the value is higher than 15, the FC has a high level of potential toxicity and will require further developmental investigation. High values indicate that the majority of the embryos elicited higher rank effects demonstrating that at certain concentrations, FCs can interfere with the development.

Table 1. Fluorinated chemicals sources.
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

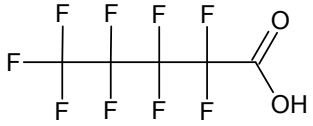
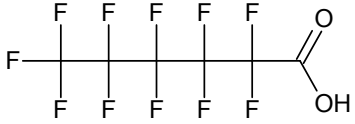
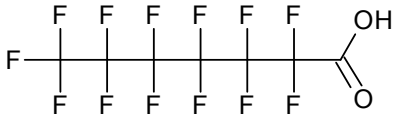
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
1	Zonyl® FSA fluoro-surfactant	FSA	Not Available – Mixture of: 25% fluorosurfactant, 37.5% isopropyl alcohol, and 37.5% water	65530-69-0[A]	Aldrich
2	2-(Perfluoroalkyl)ethanol	FSN	Not Available – Mixture of: 40% fluorosurfactant, 30% isopropyl alcohol, and 30% water	65545-80-4 [A]	Aldrich
3	Zonyl™ FSE Fluoro-surfactantl	FSE	Not Available – Mixture of: 14% fluorosurfactant, 24% ethylene glycol, and 62% water	83653-37-6 [A]	Aldrich
4	Zonyl® FSK	FSK	Not Available – Mixture	80449-64-5 [A]	Fluka
5	Perfluoro-pentanoic acid	PFPA		2706-90-3	Aldrich
6	Perfluoro-hexanoic acid	PFHxA		307-24-4	DBI
7	Perfluoro-heptanoic acid	PFHpA		375-85-9	Aldrich

Table 1. Fluorinated chemicals sources (Continued).
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

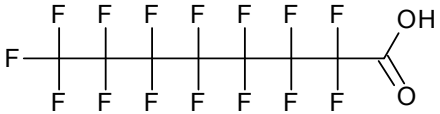
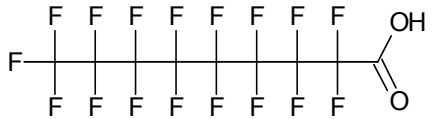
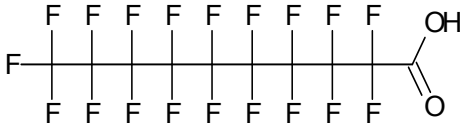
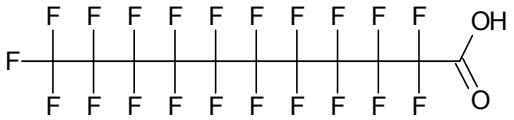
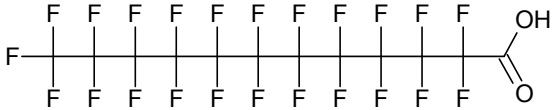
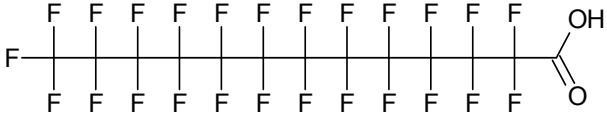
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
8	Perfluoro-octanoic acid	PFOA		335-67-1	Aldrich
9	Perfluoro-nonanoic acid	PFNA		375-95-1	Aldrich
10	Perfluoro-decanoic acid	PFDA		335-76-2	Aldrich
11	Perfluoro-undecanoic acid	PFUnDA		2058-94-8	Aldrich
12	Perfluoro-dodecanoic acid	PFDoDA		307-55-1	Aldrich
13	Perfluoro-tridecanoic acid	PFTrDA		72629-94-8	Aldrich

Table 1. Fluorinated chemicals sources (Continued).

Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

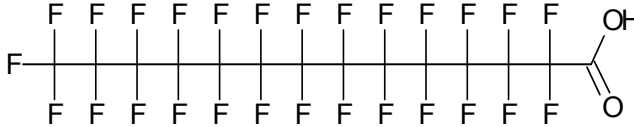
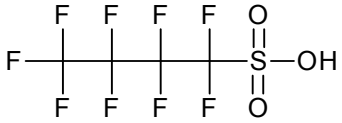
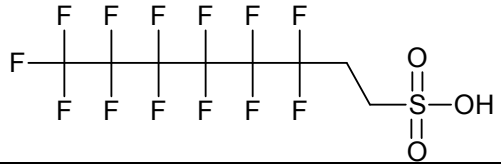
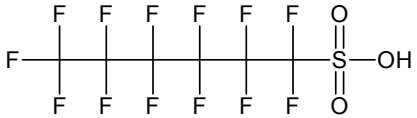
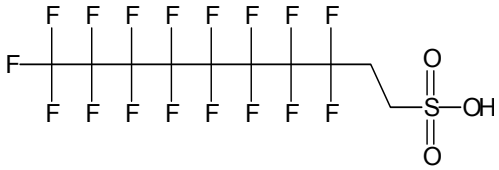
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
14	Perfluoro-tetradecanoic acid	PFTDA		376-06-7	Aldrich
15	Perfluoro-butane sulfonate	PFBS		29420-49-3	DBI
16	6:2 Fluorotelomer sulfonate	6:2 FtS		27619-97-2	Apollo
17	Perfluoro-hexane sulfonate	PFHxS		3871-99-6	DBI
18	8:2 fluorotelomer Sulfates	8:2 FtS		NR	DBI

Table 1. Fluorinated chemicals sources (Continued).
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

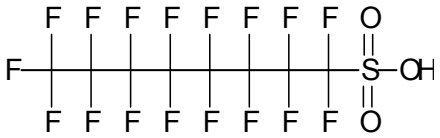
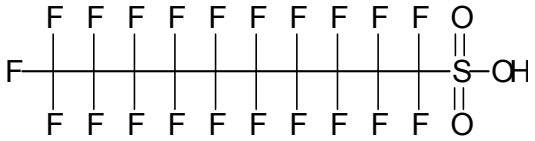
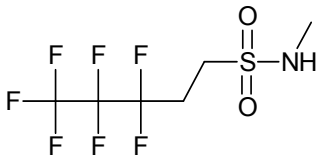
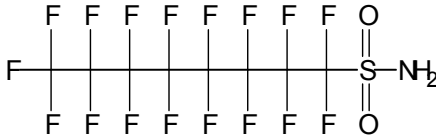
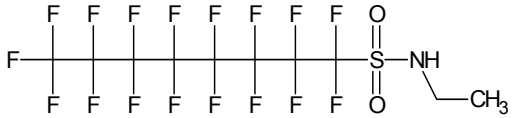
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
19	Perfluoro-octane sulfonate	PFOS		2795-39-3	Aldrich
20	Perfluoro-decane sulfonate	PFDS		67906-42-7 (NH4)	Aldrich
21	N-methyl perfluoro-butane sulfonamide	MeFBSA		NF	DBI
22	Perfluoro-octane sulfonamide	FOSA		NF	DBI
23	N-ethyl perfluoro-octane sulfonamide (sulfluramid)	EtFOSA		4151-50-2	ABCR

Table 1. Fluorinated chemicals sources (Continued).

Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

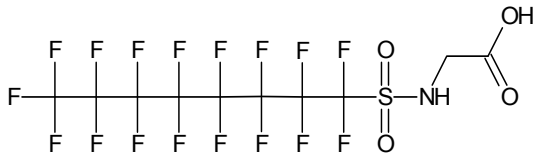
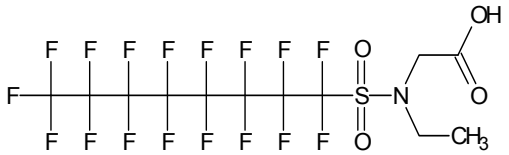
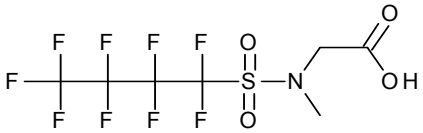
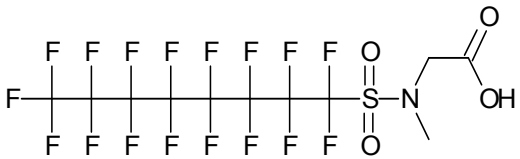
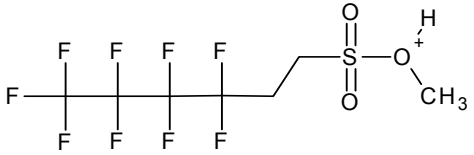
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
24	N-acetyl-perfluoro-octane sulfonamide	FOSAA		NR	DBI
25	N-ethyl perfluoro-octanesulfonamido acetic acid	EtFOSAA		NR	DBI
26	N-methyl perfluoro-butane sulfonamido acetic acid	MeFBSAA		NR	DBI
27	N-methyl perfluoro-octanesulfonamido acetic acid	MeFOSAA		NR	DBI
28	N-methyl perfluoro-butane sulfonamido ethyl	MeFBSE		NR	DBI

Table 1. Fluorinated chemicals sources (Continued).
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

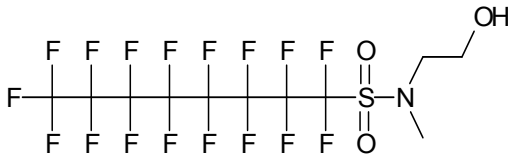
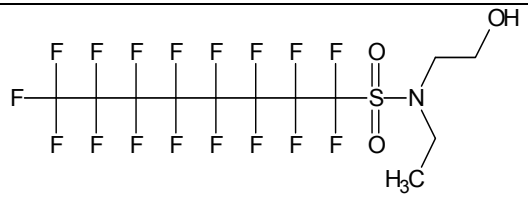
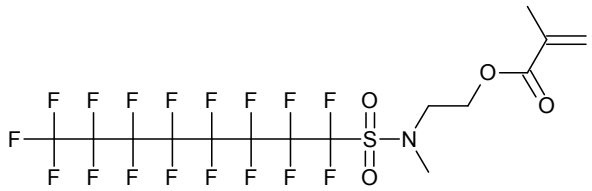
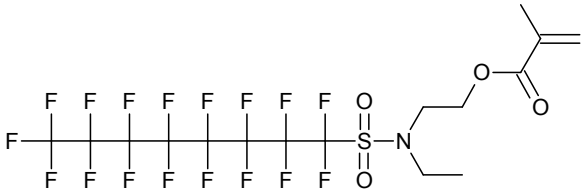
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
29	N-methyl perfluoro-octane Sulfonamide-ethanol	MeFOSE		24448-09-7	DBI
30	N-ethyl perfluoro-octane sulfonamido ethanol	EtFOSE		1691-99-2	DBI
31	N-methyl perfluoro-octane sulfonamido ethyl methacrylate	MeFOSEMA		NR	DBI
32	N-ethyl perfluoro-octane sulfonamido ethyl methacrylate	EtFOSEMA		376-14-7	Acros

Table 1. Fluorinated chemicals sources (Continued).
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

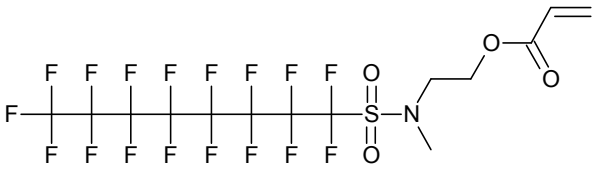
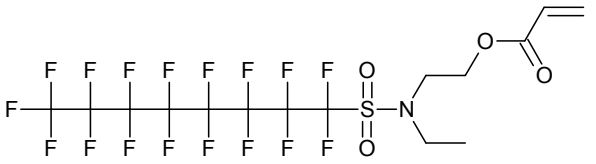
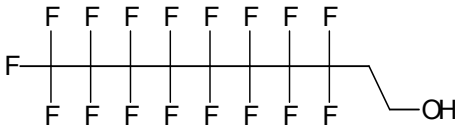
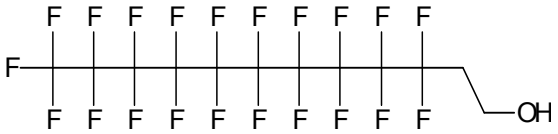
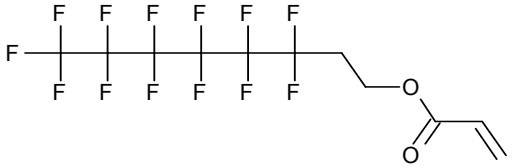
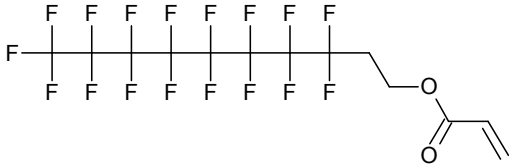
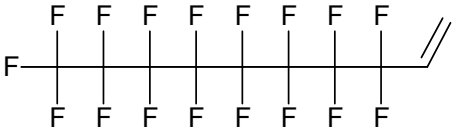
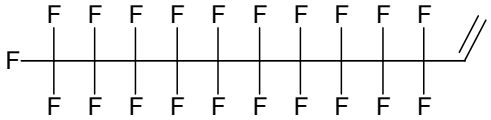
Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
33	N-methyl perfluoro-octane sulfonamido ethyl acrylate	MeFOSEA		25268-77-3	DBI
34	N-ethyl perfluoro-octane sulfonamido ethyl acrylate	EtFOSEA		423-82-5	Acros
35	8:2 Fluorotelomer Alcohol	8:2 FtOH		678-39-7	Oakwood
36	10:2 Fluorotelomer Alcohol	10:2 FtOH		865-86-1	SynQuest

Table 1. Fluorinated chemicals sources (Continued).
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

Chem. #	Chemical Name	Abbreviation	Chemical Structure	CAS Number	Source
37	6:2 Fluorotelomer acrylate	6:2 FtOAcr		17527-29-6	Aldrich
38	8:2 Fluorotelomer acrylate	8:2 FtOAcr		27905-45-9	Aldrich
39	8:2 Fluorotelomer Olefin	8:2 FtENE		21652-58-4	Aldrich
40	10:2 Fluorotelomer Olefin	10:2 FtENE		30389-25-4	SynQuest
41	12:2 Fluorotelomer Olefin	12:2 FtENE		67103-05-3	SynQuest

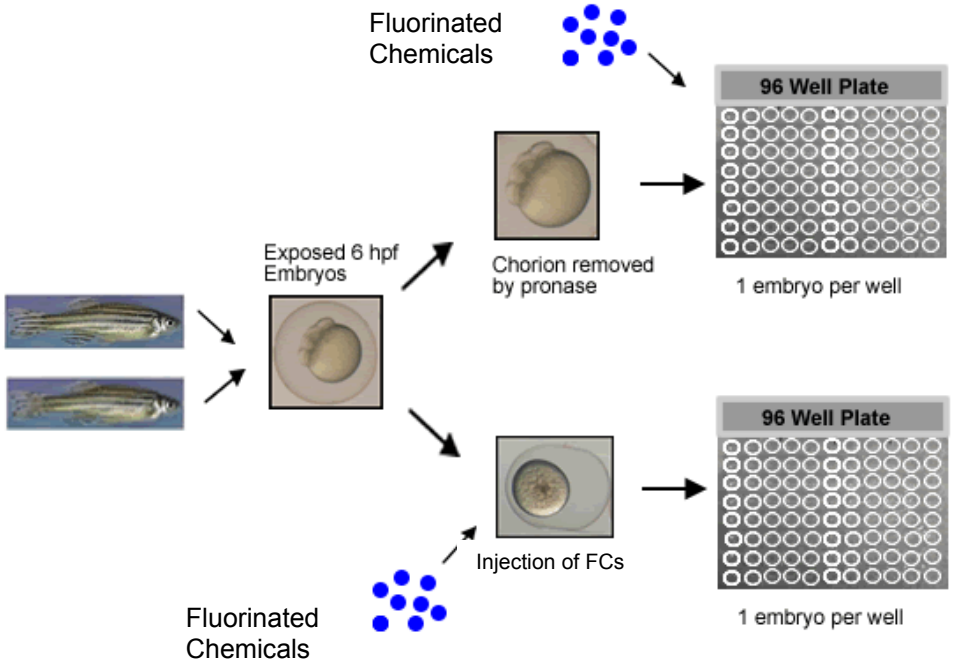


Figure 3. Proposed overview of experimental design.
Experimental design for both waterborne exposure and microinjections.

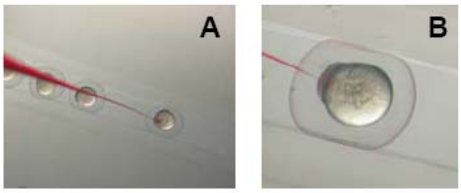


Figure 4. Injection technique.
(A)Single cell stage embryos placed in channels prior to microinjection (suspended in phenol red solution) with finely pulled needles.
(B)Close-up image of single one-cell embryo just after single injection.

Table 2. Morbidity and mortality index

Ranking	Effect
1	mortality
0.6	heart
0.6	brain
0.5	yolk sac edema
0.4	notochord
0.4	axis
0.3	trunk
0.3	delayed development
0.2	circulation
0.2	eye
0.2	jaw
0.2	sp. movement
0.1	somite
0.1	motility
0.1	touch response
0.1	snout
0.1	otic
0.1	fin
0.1	pigment
0.1	swim bladder

Table 3. EZ metric evaluation and toxicity scaling

EZ Metric	Level of toxic potential	Interpretation
≤ 5	Low	Most likely benign
5 to 15	Moderate	Suspect FC, requires complete testing
> 15	High	Requires further testing of developmental toxicity

CHAPTER 3 – RESULTS

FCs at Low Concentrations Elicited No Developmental Malformations

At low concentrations (0.01, 0.1, 1.0, 10 ng/mL), most of the 39 FCs did not display statistically-significant developmental malformations with the exception of PFDS, EtFOSAA, MeFOSAA, 6:2 FtS, PFDoDA, FSN, PFPA, PFHxS (Table 4). The eight FCs induced adverse effects at certain concentrations, but did not produce a 100% incidence rate (Figure 5). Table 5 illustrates developmental malformations observed at certain concentrations. PFDS elicited yolk sac edema (YSE), notochord, pectoral fin (p-fin), pericardial edema (PE) and head malformations (p=0.040; Fisher's exact test) at 1 ng/mL. Body axis malformation was observed for EtFOSEAA at 0.1 ng/mL (p=0.038), MeFOSAA at 0.01 ng/mL (p=0.038), 6:2 FtS at 0.1 ng/mL (p=0.038), PFDoDA at 0.01 and 0.1 ng/mL (p=0.038), FSN at 10 ng/mL (p=0.038) and PFPA at 10 ng/mL (p=0.016). PFHxS exposed at 1 ng/mL elicited p-fin, PE malformations and lack of touch response (TR) (p=0.016).

The EZ metric value for each concentration ranging between 0.01-10 ng/mL does not exceed 15, except for PFDS at 1 ng/mL (EZ = 19.55), and MeFOSEA at 0.01 and 1 ng/mL (EZ = 15.13, 18.19 respectively) (Table 6). This demonstrates that most of the chemicals at concentrations ranging from 0.01 to 10 ng/mL did not have a significant impact on embryonic developmental compared to those that were unexposed. 37 FCs had EZ metric values less than 5, which indicate that the overall consequence to the health of the animal was not severe. Figure 6 illustrates a representative of a 120hpf zebrafish after continuous exposure to PFUnDA, which did not elicit significant malformations.

Some FCs at Higher Concentrations Induce Pericardial Edema

For further investigation on potential FC toxicity, 14 FCs were exposed to embryonic zebrafish by water at higher concentrations (25, 50, 100, 150 or 200 ng/mL). Of these 14 FCs, nine elicited an increase of body axis, pericardial edema, eye, snout and jaw malformations or delay in touch response (Figure 7). To identify statistically-significant

increases in malformations, a Fisher's exact test was ran. Table 7 displays the developmental malformations observed at concentrations ranging from 25-200 ng/mL. FSA exposed at 200 ng/mL induced a statistically-significant increase in body axis malformations (p=0.034), while PFBS elicited adverse effects of the heart at 50 and 100 ng/mL (p=0.024 and p=0.011 respectively). Both MeFOSE (p=0.05) and PFOS (p=<0.001) at 100 ng/mL induced an increase in PE. At 100 ng/mL, FSE produced an increase in jaw malformation (p=0.050). MeFOSEA at 25 (p=0.017), 50, 100, 200 ng/mL caused an increase in PE (p=<0.001) and snout malformation at 25 ng/mL (p=0.05). Pericardial edema was significantly increased at 100 ng/mL (p=<0.001) and 200 ng/mL (p=0.05) for MeFOSEMA and at 100 ng/mL (p=0.11) and 150 ng/mL (p=0.002) for FOSA. EtFOSE exposure elicited an increase in heart (p=<0.001), eye (p=0.011), jaw malformations (p=0.05) and lack of touch response (p=0.011) at 100 ng/mL.

Six FCs induced pericardial edema in a percentage of embryonic zebrafish at 120hpf (n=24) (Figure. 8). PE was statistically-significant increased in EtFOSE (100 ng/mL), MeFOSEA (25, 50, 100, 200 ng/mL), PFBS (50, 100 ng/mL), PFOS (100 ng/mL), MeFOSEMA (100, 200 ng/mL) and FOSA (100 and 150 ng/mL). Figure 9 displays an embryonic zebrafish exposed to PFOS at high concentration, which induced pericardial edema. This figure is a representative of what the other zebrafish exposed to EtFOSE, MeFOSEA, PFBS, MeFOSEMA and FOSA looked like at high concentrations.

The 14 FCs data were normalized using the morbidity and mortality index from the EZ metric scale (Table 2). Eight of the selected FCs did not have an EZ value greater than 15. At 100 ng/mL, MeFOSE (EZ = 19.15), EtFOSE (EZ = 22.1), FSE (EZ = 16.3) and MeFOSEMA (EZ = 15.25) had an EZ metric value higher than 15. MeFOSEA at 25, 50, 100 and 200 ng/mL had an EZ value of 21.5, 18, 19, and 17.55, respectively. PFBS had an EZ value higher than 15 at 50 ng/mL (EZ = 15.7) and 100 ng/mL (EZ = 20.75). Table 6 displays the EZ values for higher concentration waterborne exposures.

Alternative Route of Exposure

Since embryonic dose was unknown, PFOS, PFOA, PFDA, PFDS, MeFOSEA and PFHxA were microinjected at three different concentrations (0.00002, 0.00024, 0.0024 mg/kg). Of the six FCs, only three induced a significant increase in developmental malformations (Figure 10). At 0.0002 mg/kg PFDA elicited a significant number of head malformation ($p=0.005$ determined from the Fisher's exact test), while at the same concentration, PFHxA at 0.00024 mg/kg caused an increase in mortality ($p=0.011$). PFOA at 0.00024 and 0.0024 mg/kg produced a higher mortality count ($p=0.036$ and $p=0.017$ respectively).

Table 8 displays the six FCs data in terms of the EZ metric scale. Most of the chemicals at their tested concentration had an EZ metric value of less than 15, except for PFOA at 0.00024 mg/kg had an EZ value of 20.15 and PFDA at 0.00024 mg/kg had an EZ value of 16.15.

Additional microinjection concentrations were completed for four of the previously microinjected FCs, PFHxA, PFOA, PFOS and PFDA. Each chemical was microinjected at concentrations ranging between 0.01-0.12 mg/kg. Figure 11 displays the developmental malformations observed at the three injected concentrations. PFOS demonstrated a statistically-significant increase in mortality at 0.030 mg/kg ($p=<0.001$), 0.061 mg/kg ($p=0.008$) and 0.12 mg/kg ($p=0.001$). Pericardial edema was significantly increased at 0.12 mg/kg for PFOS ($p=0.011$) and 0.098 mg/kg for PFOA ($p=0.050$).

Normalizing the microinjected data of the four FCs was necessary to determine EZ metric values (Table 7). Very few FC doses induced an EZ metric value greater than 15. PFHxA at 0.036 mg/kg had an EZ metric value of 16 while PFOS at 0.030, 0.061, 0.12 mg/kg had an EZ metric value of 18.05, 15.45 and 16.95 respectively.

The data collected for both microinjection studies is complied into Table 8. The table is organized by the statistically-significant increase in the three developmental malformations (head, mortality and PE) observed at concentrations relating to environmental background, human serum levels and whole concentration in wildlife.

Table 4. Fluorinated chemicals with increased embryonic malformations.
p values were determined by the Fisher's exact test and are only displayed for those chemicals that are statistically-significant (*p*=<0.05)
Abbreviation: YSE **= yolk sac edema,** NC **= notochord,** P-fin **= pectoral fin,** PE **= pericardial edema,** TR **= touch response, * =** microinjected
p values: [1] - *p*=<0.001, [2] - *p*=0.002, [3] – *p*=0.004, [4] – *p*=0.005, [5] – *p*=0.011, [6] – *p*=0.016, [7] – *p*=0.017, [8] – *p*=0.024, [9] – *p*=0.034, [10] – *p*=0.036, [11] – *p*=0.038, [12] – *p*=0.04, [13] – *p*=0.05

Chemical	Mortality	YSE	Axis	NC	P-Fin	PE	Head	Eye	Snout	Jaw	TR
PFDA							*0.0002 mg/kg [4]				
FSA			200 ng/mL [9]								
PFDS		1 ng/mL [12]		1 ng/mL [12]	1 ng/mL [12]	1 ng/mL [12]	1 ng/mL [12]				
EtFOSAA			0.1 ng/mL [11]								
MeFOSAA			0.01 ng/mL [11]								
6:2 FtS			0.1 ng/mL [11]								
PFDoDA			0.01, 0.1 ng/mL [11]								
FSN			10 ng/mL [11]								
PFPA			10 ng/mL [11]								
PFHxS					1 ng/mL [6]	1 ng/mL [6]					1 ng/mL [6]
PFBS						50 ng/mL [8] 100 ng/mL [5]					
PFHxA	*0.0002 mg/kg [5]										
FSE										100 ng/mL [13]	
MeFOSE									100 ng/mL [13]		
MeFOSEA						25 ng/mL [17] 50,100, 200 ng/mL [1]			25 ng/mL [13]		
MeFOSEMA						100 ng/mL [1] 200 ng/mL [1]					
EtFOSE						100 ng/mL [1]		100 ng/mL [5]		100 ng/mL [13]	100 ng/mL [5]
FOSA						100 ng/mL [5] 150 ng/mL [2]					
PFOA	*0.0024 ng/mL [10] *0.0024 ng/mL [7]										
PFOS						100 ng/mL [1]					

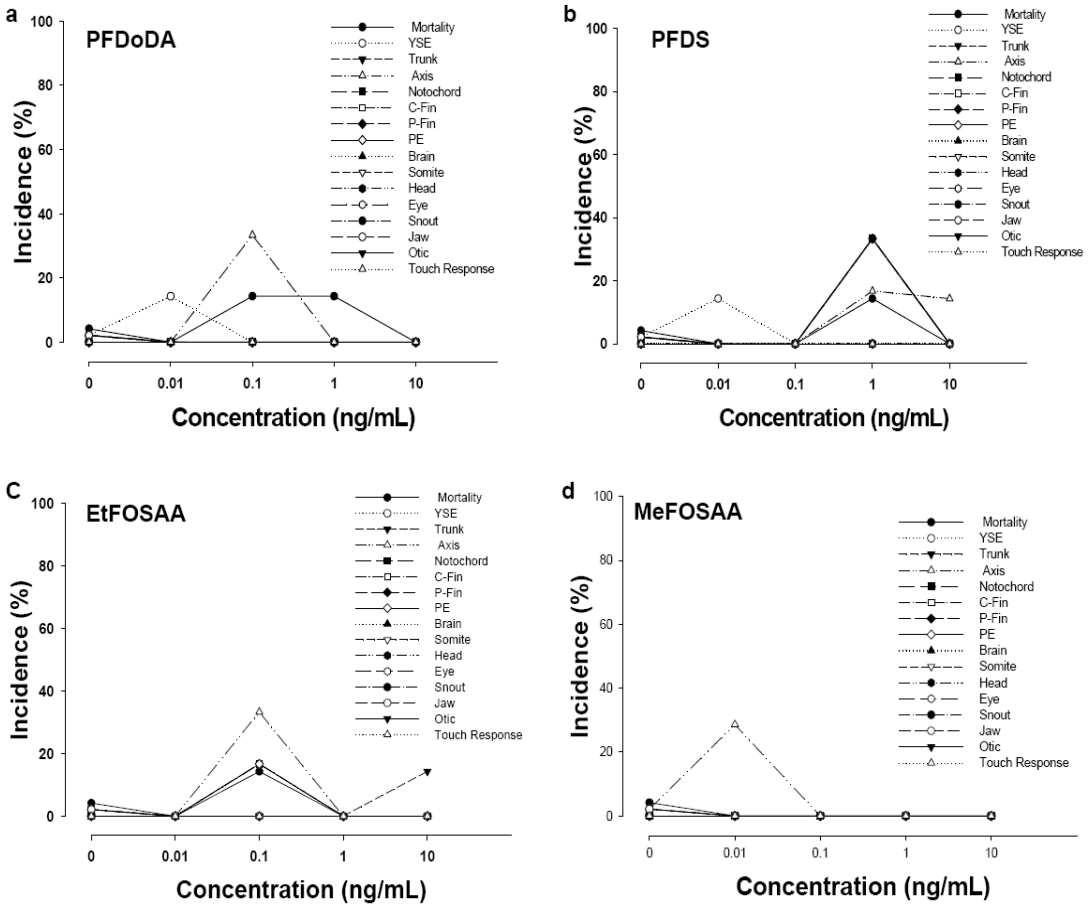


Figure 5. Zebrafish malformations elicited from exposure to 0.01-10 ng/mL of the indicated FC.

Embryos were exposed to 39 FC at 6hpf and evaluated for malformations at 120hpf. **(a, c, d)** PFDoDA, EtFOSAA and MeFOSAA exposed embryos had an increase in body axis malformation at 0.1, 0.1 and 0.01 ng/mL (respectively). **(b)** PFDS increased YSE, notochord, p-fin and PE and head malformation at 1 ng/mL.

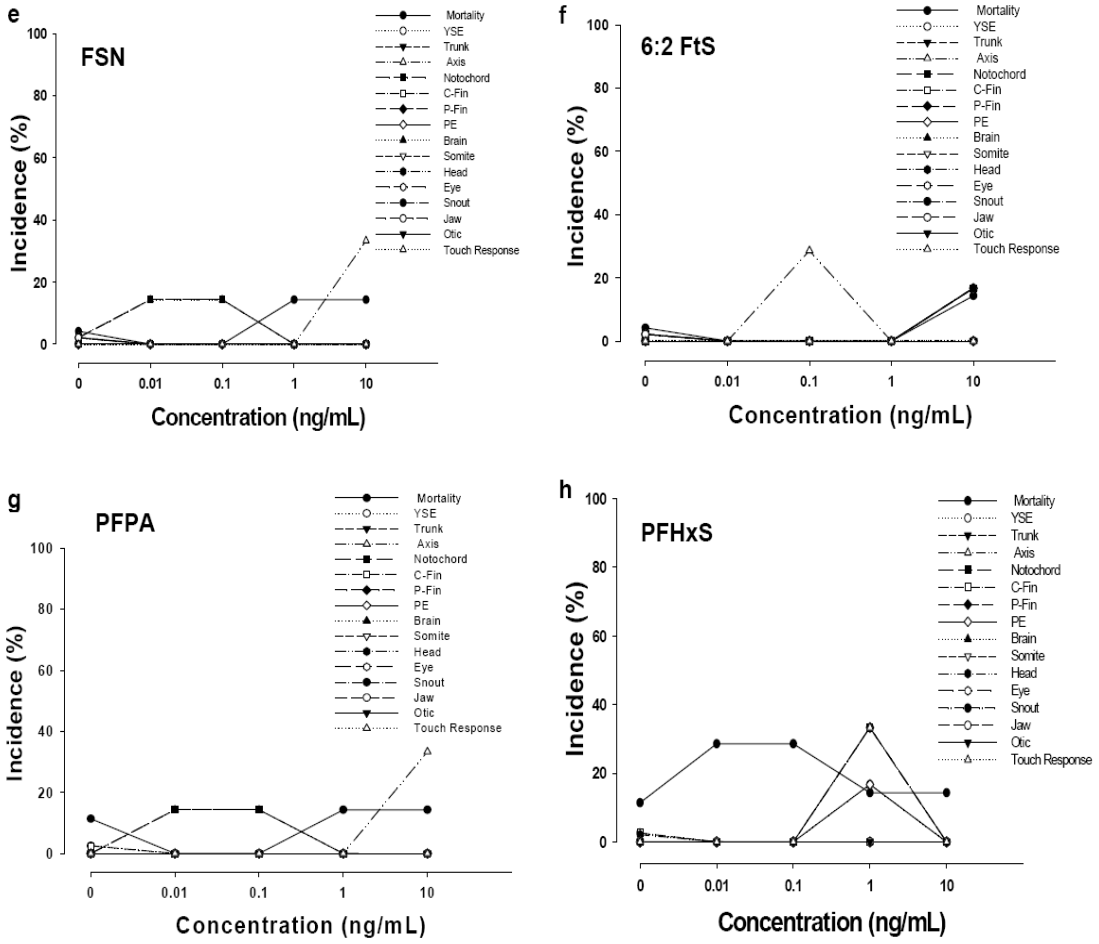


Figure 5. Zebrafish malformations elicited from exposure to 0.01-10 ng/mL of the indicated FC (Continued). Embryos were exposed to 39 FC at 6hpf and evaluated for malformations at 120hpf. **(e)** FSN caused delay in touch response at 10 ng/mL. **(f, g)** 6:2 FtS and PFPA exposed embryos has an increase in body axis malformation at 0.1 ng/mL and 10 ng/mL, respectively. **(h)** PFHxS elicited p-fin, PE malformation and no touch response at 1ng/mL.

Table 5. Developmental malformations observed at 0.01-10 ng/mL.
PFDS, EtFOSAA, MeFOSAA, 6:2 FtS, PFDoDA, FSN, PFPA and PFHxS displayed an increase in developmental malformations in yolk sac edema (YSE), notochord, pectoral fin (P-fin), pericardial edema (PE), head, body axis and lack of touch response. *p* values were determined by the Fisher's exact test.

Developmental Malformation	Chemical Name	Concentration (ng/mL)	*p* value
YSE	PFDS	1	0.040
Notochord	PFDS	1	0.040
P-fin	PFDS	1	0.040
	PFHxS	1	0.016
PE	PFDS	1	0.040
	PFHxS	1	0.016
Head	PFDS	1	0.040
Body axis	EtFOSEAA	0.1	0.038
	MeFOSAA	0.01	0.038
	6:2 FtS	0.1	0.038
	PFDoDA	0.01, 0.1	0.038
	FSN	10	0.038
	PFPA	10	0.016
TR	PFHxS	1	0.016

Table 6. Waterborne exposed fluorinated chemicals normalized for EZ metrics.

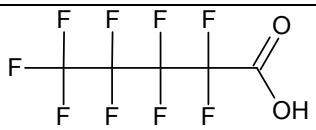
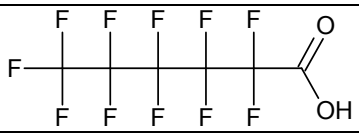
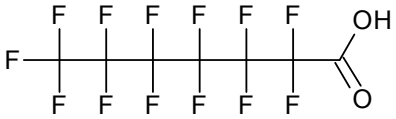
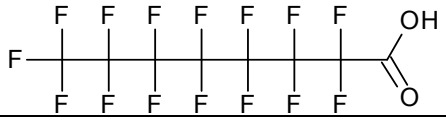
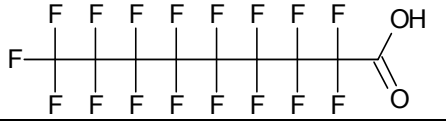
Chem. #	Abbre-viation	Chemical Structure	Con-centration	EZ Metrics
	Control Vehicle		**H_2O** **1% DMSO**	**3.38** **2.11**
1	FSA	Not Available – Mixture of: 25% fluorosurfactant, 37.5% isopropyl alcohol, and 37.5% water	0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	1.72 5.00 1.70 3.23
2	FSN	Not Available – Mixture of: 40% fluorosurfactant, 30% isopropyl alcohol, and 30% water	0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	2.72 2.72 3.23 5.95
3	FSE	Not Available – Mixture of: 14% fluorosurfactant, 24% ethylene glycol, and 62% water	0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	0 3.23 0 3.23 14.05 8.00 16.30 12.45
4	FSK	Not Available -Mixture	0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 3.23 0 0
5	PFPA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	12.20 3.23 3.74 13.40
6	PFHxA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	3.23 3.23 13.10 0
7	PFHpA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 6.80 11.00 8.16
8	PFOA		25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	5.00 0.10 5.60 1.90
9	PFNA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	6.46 3.57 3.23 8.50

Table 6. Waterborne exposed fluorinated chemicals normalized for EZ metrics (Continued).

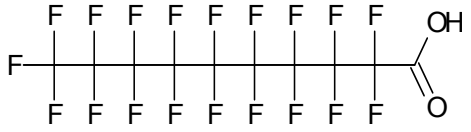
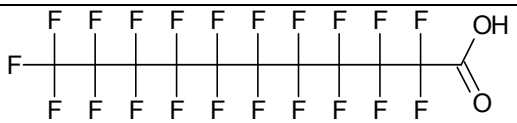
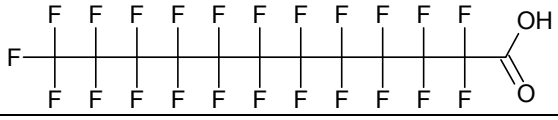
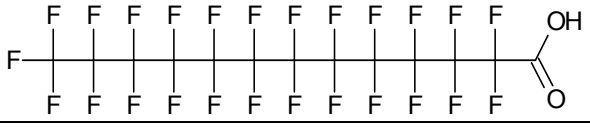
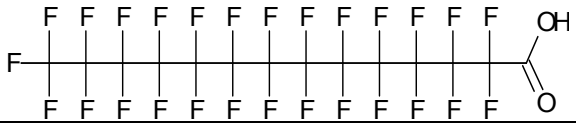
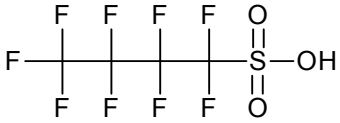
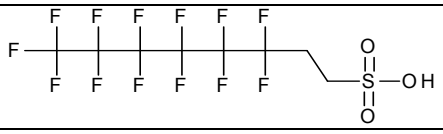
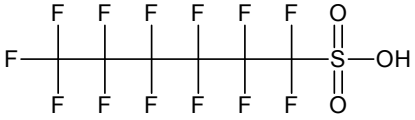
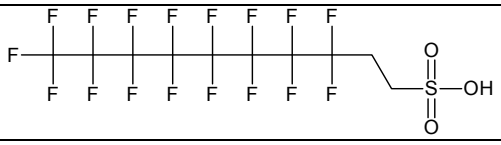
Chem. #	Abbre-viation	Chemical Structure	Con-centration	EZ Metrics
10	PFNA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	3.40 0 1.36 0 4.45 0.95 3.20 1.90
11	PFUnDA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 0 0 0
12	PFDoDA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	8.16 5.95 3.23 0
13	PFTrDA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	4.59 0 0 0
14	PFTDA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 3.4 0 7.14
15	PFBS		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	6.46 9.69 8.50 13.60 7.95 15.70 20.75 11.55
16	6:2 FtS		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 2.72 0 12.07
17	PFHxS		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	6.46 6.46 13.09 3.23
18	8:2 FtS		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	11.22 2.50 0 11.39

Table 6. Waterborne exposed fluorinated chemicals normalized for EZ metrics (Continued).

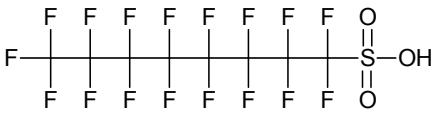
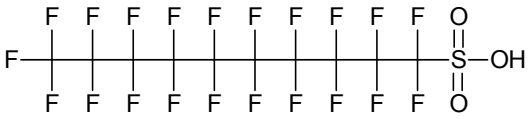
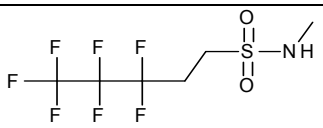
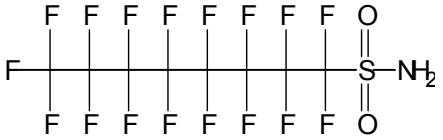
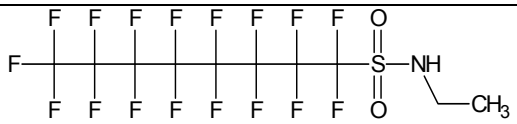
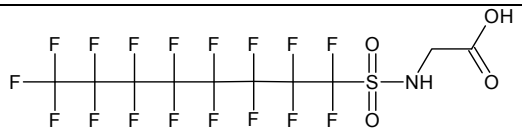
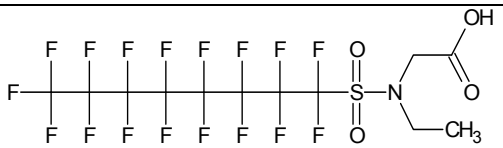
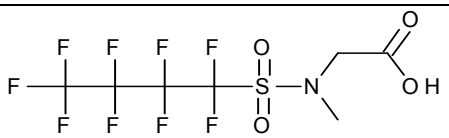
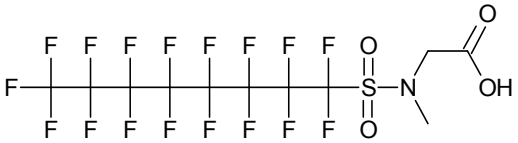
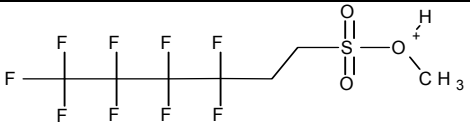
Chem. #	Abbre-viation	Chemical Structure	Con-centration	EZ Metrics
19	PFOS		25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	1.55 0.95 13.15 8.10
20	PFDS		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 0 19.55 1.36
21	MeFBSA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 0 6.46 0
22	FOSA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	0 0 3.23 0 13.50 14.70 12.35 14.35
23	EtFOSA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	2.38 0 0 0
24	FOSAA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	1.36 0 0 0
25	EtFOSAA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 11.39 0 1.02
26	MeFBSAA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	6.46 4.59 3.23 0
27	MeFOSAA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	2.72 0 0 0
28	MeFBSE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	4.59 3.23 0 2.38

Table 6. Waterborne exposed fluorinated chemicals normalized for EZ metrics (Continued).

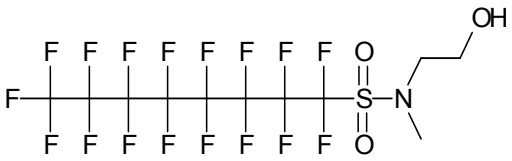
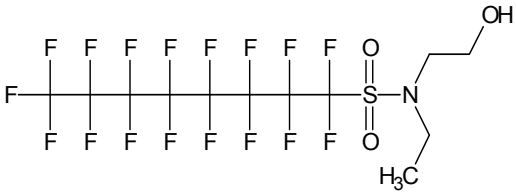
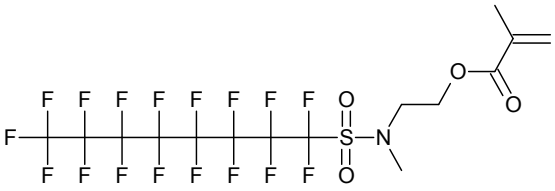
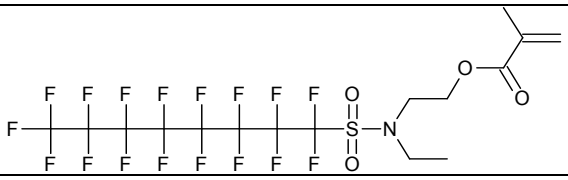
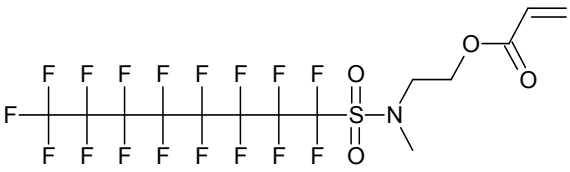
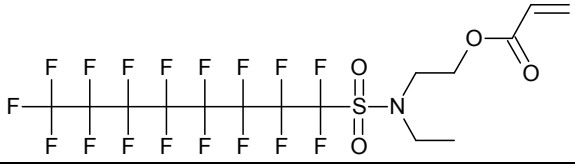
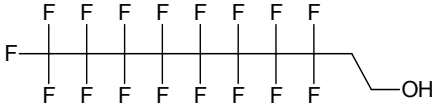
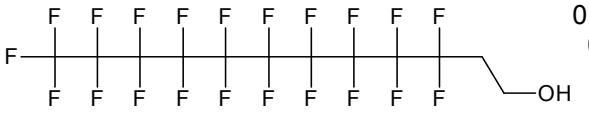
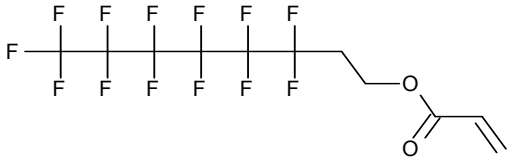
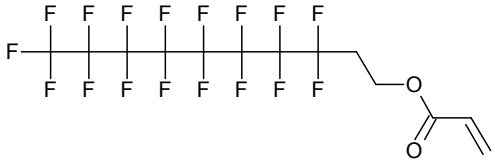
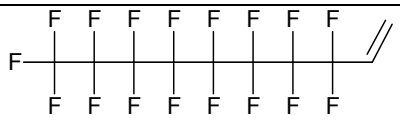
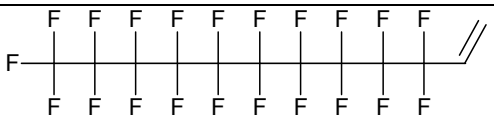
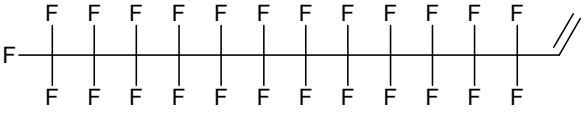
Chem. #	Abbre-viation	Chemical Structure	Con-centration	EZ Metrics
29	MeFOSE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL	2.72 0 10.20 1.36 9.40 8.95 19.10
30	EtFOSE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL	5.95 3.23 4.59 0 5.95 7.7 22.10
31	MeFOSEMA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	0 3.23 6.46 3.23 1.80 0.60 15.25 3.50
32	EtFOSEMA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 3.23 6.46 0
33	MeFOSEA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL 25 ng/mL 50 ng/mL 100 ng/mL 200 ng/mL	15.12 3.23 18.19 0 21.50 18.20 19.00 17.50
34	EtFOSEA		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	3.23 0 3.23 1.36
35	8:2 FtOH		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	3.23 1.36 1.36 0

Table 6. Waterborne exposed fluorinated chemicals normalized for EZ metrics (Continued).

Chem. #	Abbre-viation	Chemical Structure	Con-centration	EZ Metrics
36	10:2 FtOH		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 2.04 1.36 0
37	6:2 FtOAcr		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 6.46 3.23 9.86
38	8:2 FtOAcr		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	3.23 0 3.23 0
39	8:2 FtENE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	6.97 0 0 0
40	10:2 FtENE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 0 0 13.77
41	12:2 FtENE		0.01 ng/mL 0.1 ng/mL 1.0 ng/mL 10 ng/mL	0 3.23 3.23 3.23

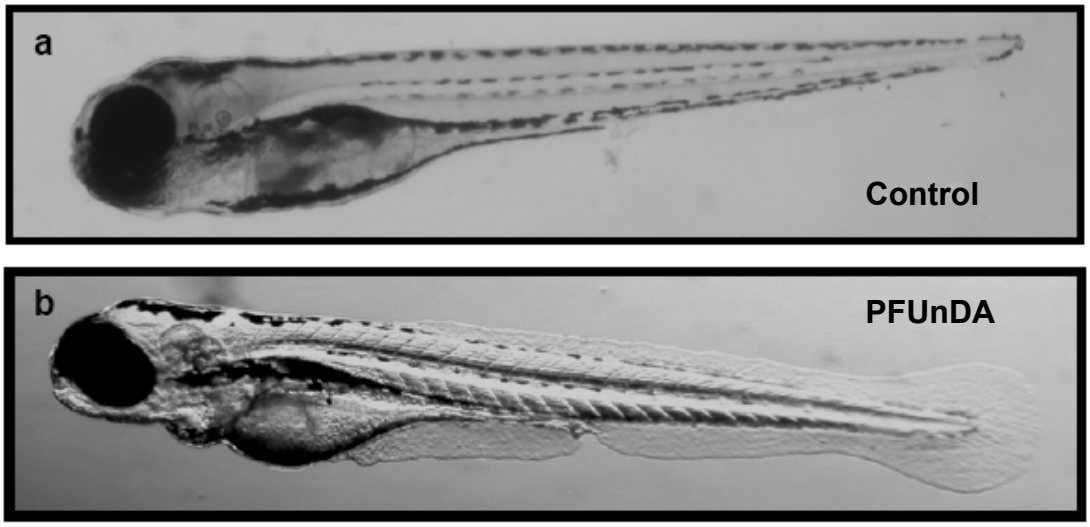


Figure 6. A 120hpf zebrafish (a) control vehicle (b) exposed to 0.01-10 ng/mL of PFUnDA.
(a) Control vehicle exposure (either dosing solution or DMSO). **(b)** Exposure of PFUnDA induced mild to no effects.

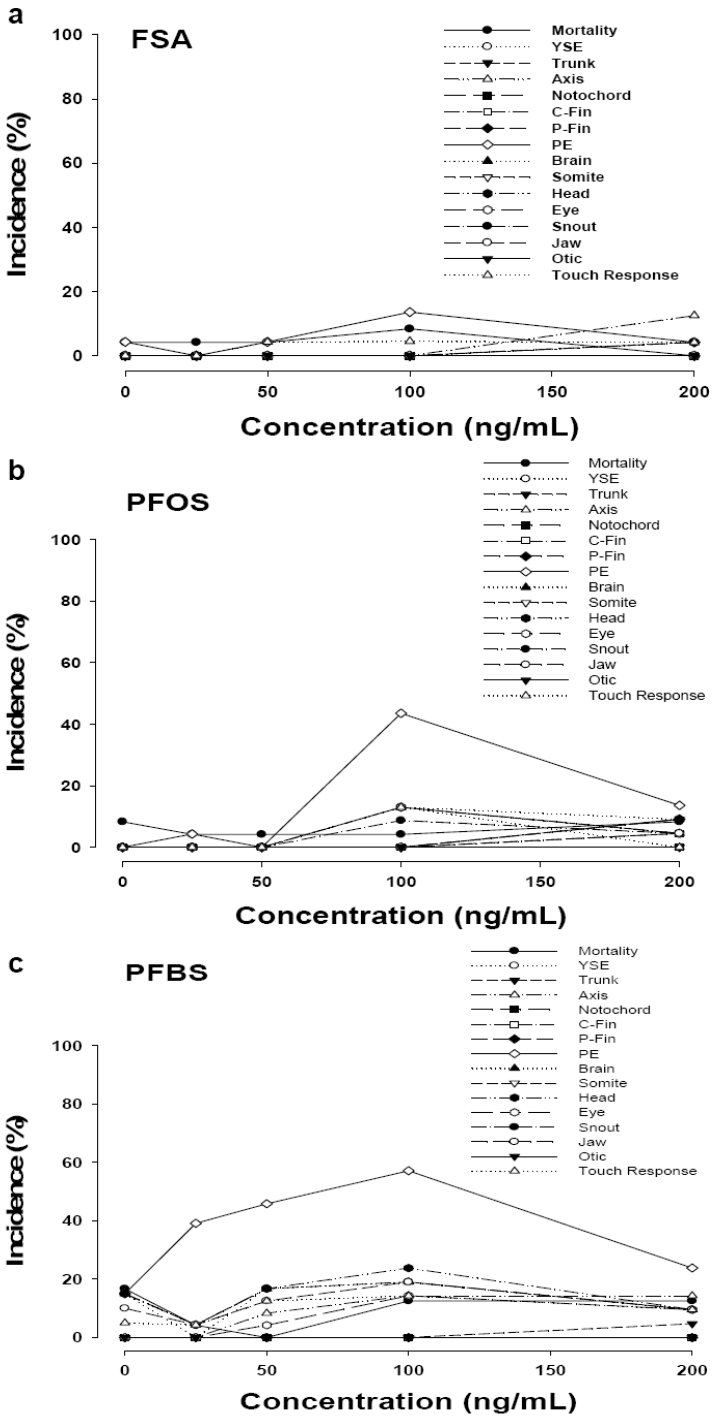


Figure 7. Zebrafish malformations elicited from exposure to 25-200ng/mL of the indicated FC.
Statistical significant was determined from a Fisher's exact test. **(a)** Increase in body axis malformation at 200 ng/mL. **(b, c)** PFOS and PFBS induced PE.

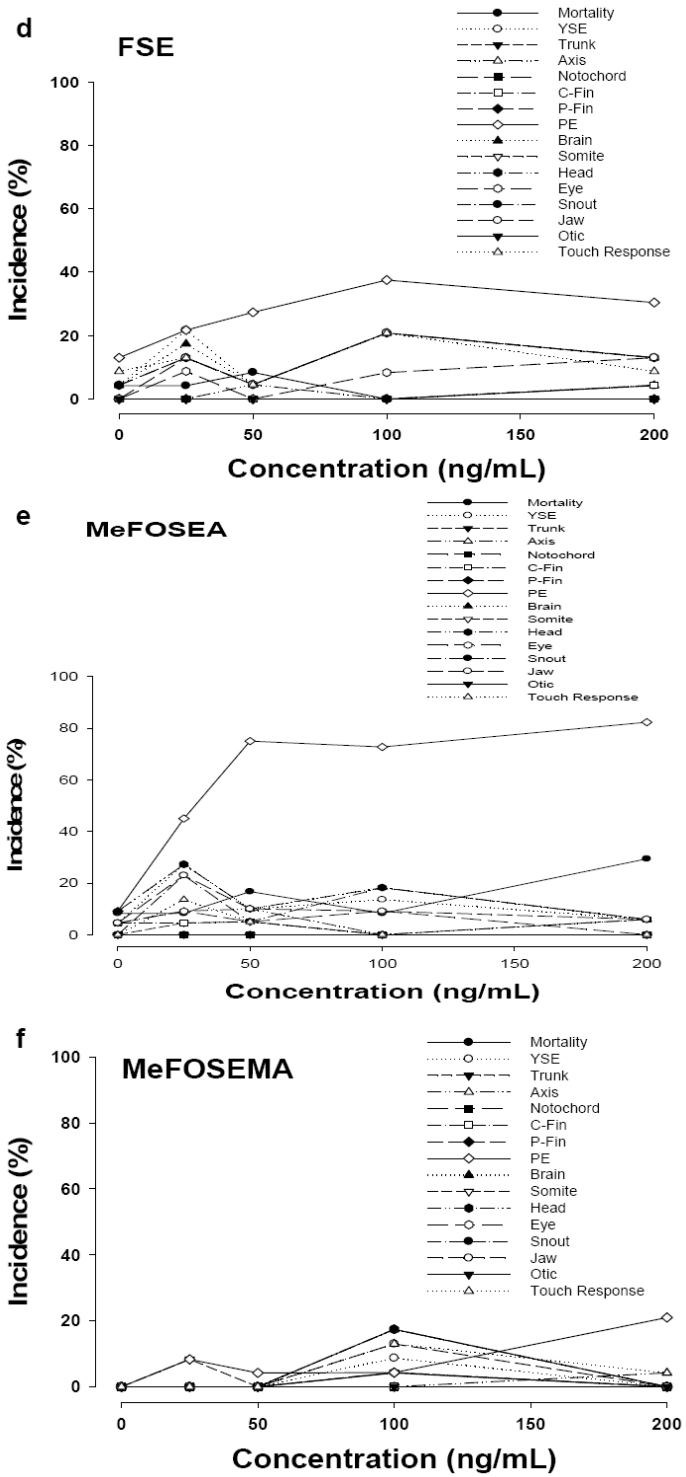


Figure 7. Zebrafish malformations elicited from exposure to 25-200ng/mL of the indicated FC (Continued). Statistical significant was determined from a Fisher's exact test. **(d)** Exposure at 100 ng/mL of FSE caused increase in jaw malformations. **(e)** PE observed at all concentrations and snout malformation at 25 ng/mL of MeFOSEA. **(f)** MeFOSEMA induced PE.

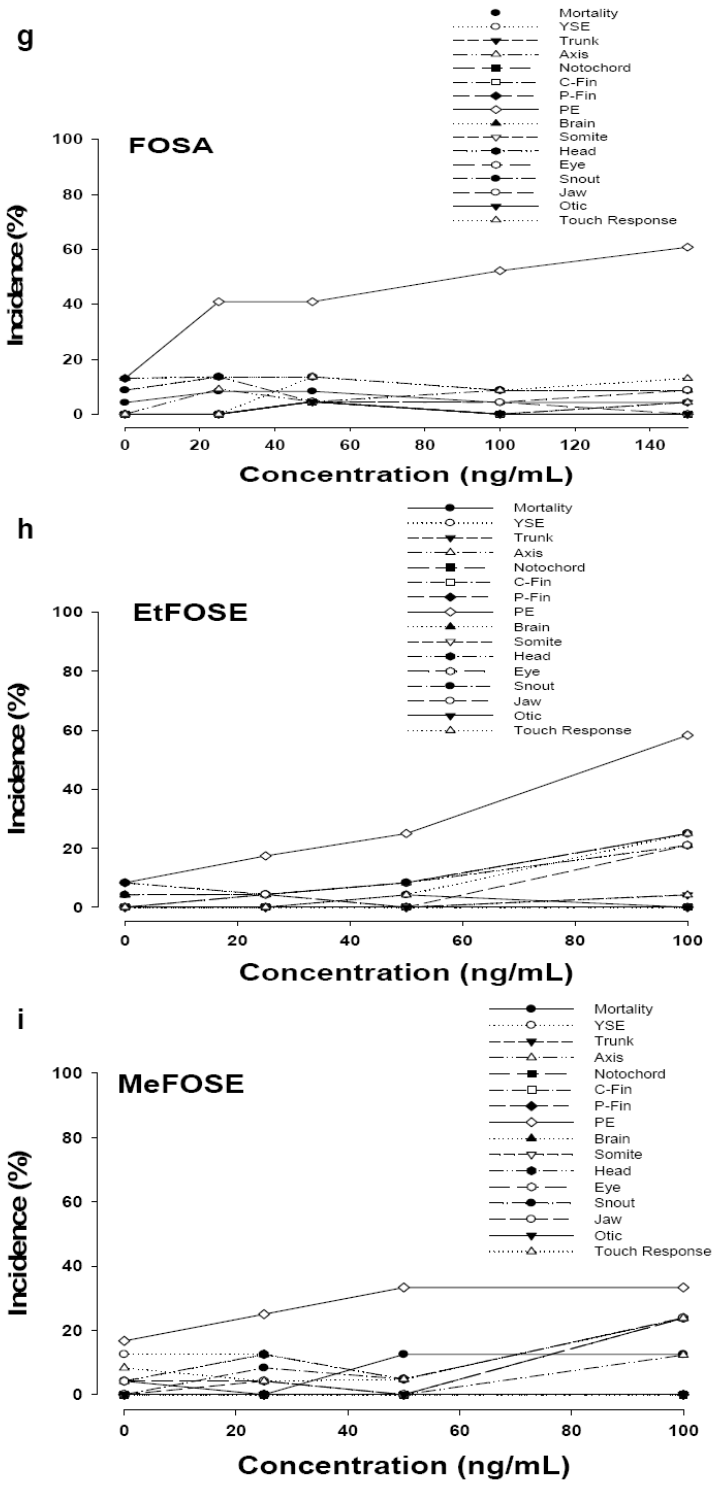


Figure 7. Zebrafish malformations elicited from exposure to 25-200ng/mL of the indicated FC (Continued). Statistical significant was determined from a Fisher's exact test. **(g, i)** FOSA and MeFOSEA induced PE. **(h)** Heart, eye and jaw malformations observed with delayed touch response for EtFOSE.

Table 7. Developmental malformations observed at 25-200 ng/mL.
FSA, FSE, FOSA, PFBS, PFOS, MeFOSE, MeFOSEA, MeFOSEMA and EtFOSE elicited an increase in developmental malformations in body axis, pericardial edema (PE), jaw, snout, eye and lack of touch response. *p* values were determined by the Fisher's exact test.

Developmental Malformation	Chemical Name	Concentration (ng/mL)	*p* value
Body axis	FSA	200	0.034
PE	PFBS	50, 100	0.024, 0.011
	MeFOSE	100	0.05
	PFOS	100	<0.001
	MeFOSEA	25, 50, 100, 200	0.017, <0.001
	MeFOSEMA	100, 200	<0.001, 0.05
	FOSA	100, 150	0.011, 0.002
	EtFOSE	100	<0.001
Jaw	FSE	100	0.050
	EtFOSE	100	0.05
Snout	MeFOSEA	25	0.05
Eye	EtFOSE	100	0.011
TR	EtFOSE	100	0.011

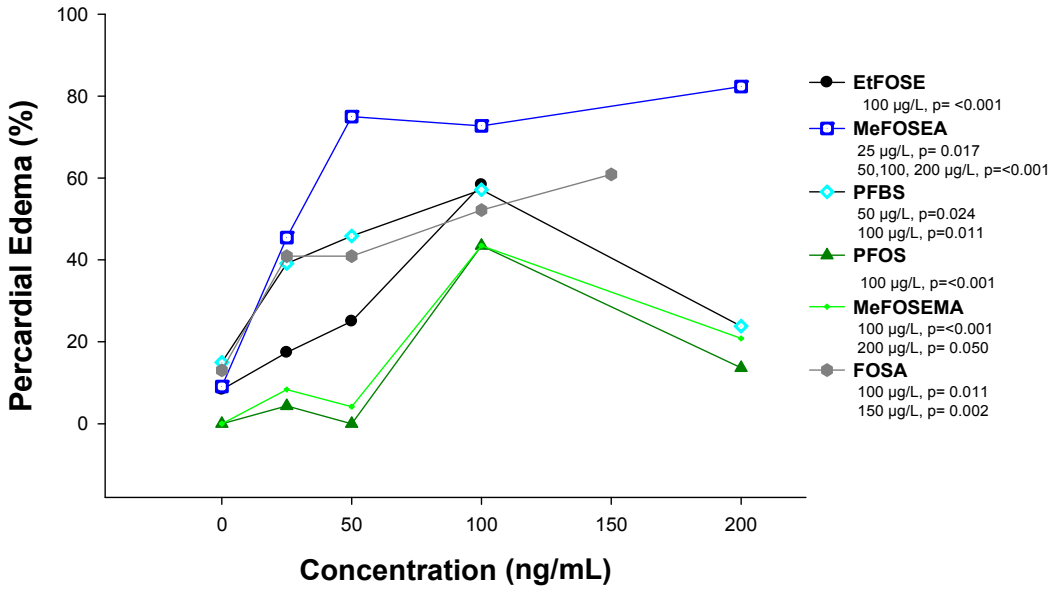


Figure 8. FCs with statistically-significant increase in pericardial edema.
EtFOSE, MeFOSEA, PFBS, PFOS, MeFOSEMA and FOSA elicited statistical significant ($p<0.05$ based on Fisher's exact test) increase at concentrations ranging from 25-200 ng/mL.

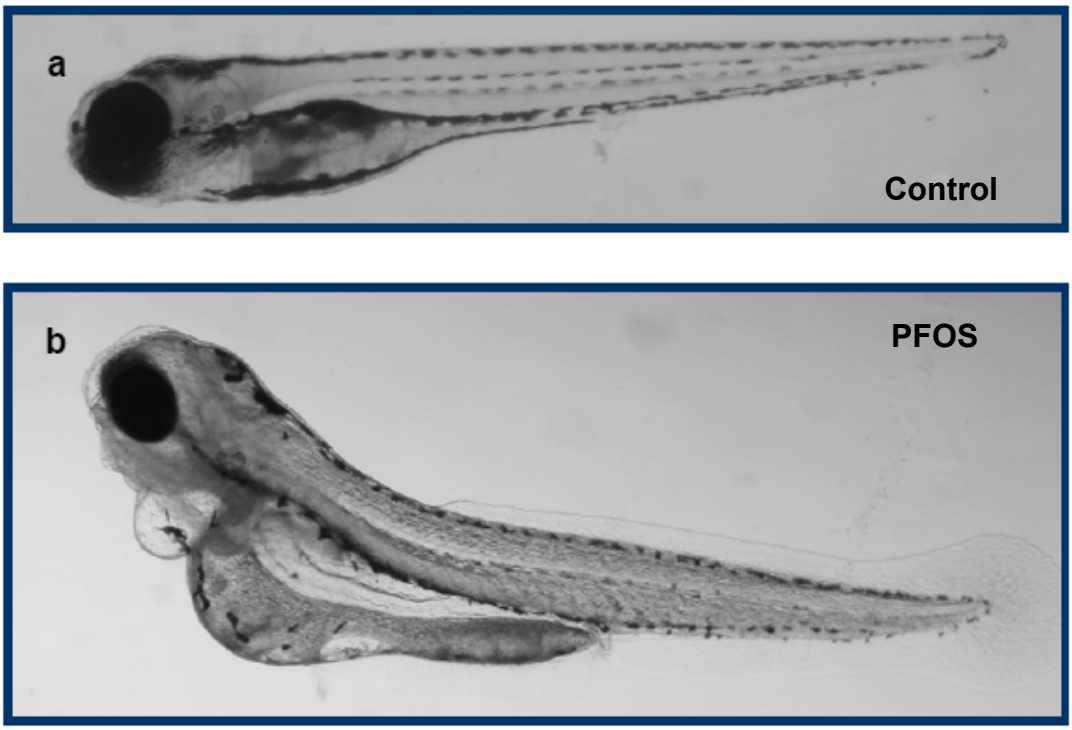
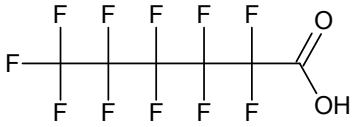
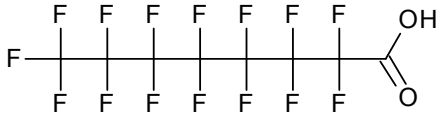
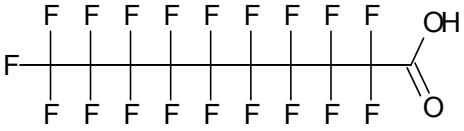
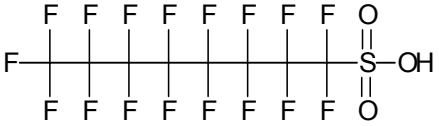
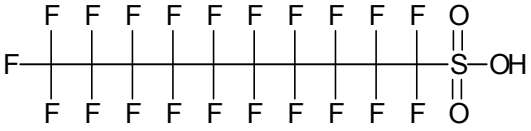
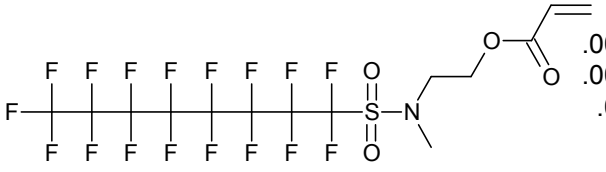


Figure 9. A 120hpf zebrafish (a) control vehicle (b) exposed to 25-200 ng/mL to PFOS. **(a)** Control vehicle exposure (either dosing solution or DMSO). **(b)** Exposure of PFOS increased heart, body axis and yolk sac malformations.

Table 8. Microinjected concentration normalized for EZ metrics.

Chem. #	Abbre-viation	Chemical Structure	Nominal Test Con-centration	EZ Metrics
	Control Vehicle		**0.1% DMSO**	**4.02**
6	PFHxA		.00002 mg/kg .00024 mg/kg .0024 mg/kg .018 mg/kg .036 mg/kg .073 mg/kg	5.7 6.2 0 13.6 15.1 6.2
8	PFOA		.00002 mg/kg .00024 mg/kg .0024 mg/kg .024 mg/kg .049 mg/kg .098 mg/kg	7.65 20.15 13.4 8.05 7.80 11.7
10	PFDA		.00002 mg/kg .00024 mg/kg .0024 mg/kg .030 mg/kg .061 mg/kg .12 mg/kg	10.7 16.15 5.75 8.95 8.05 7.95
19	PFOS		.00002 mg/kg .00024 mg/kg .0024 mg/kg .030 mg/kg .061 mg/kg .12 mg/kg	9.90 10.95 2.85 18.05 15.05 16.15
20	PFDS		.00002 mg/kg .00024 mg/kg .0024 mg/kg	9.70 3.80 8.95
33	MeFOSEA		.00002 mg/kg .00024 mg/kg .0024 mg/kg	7.70 6.55 1.90

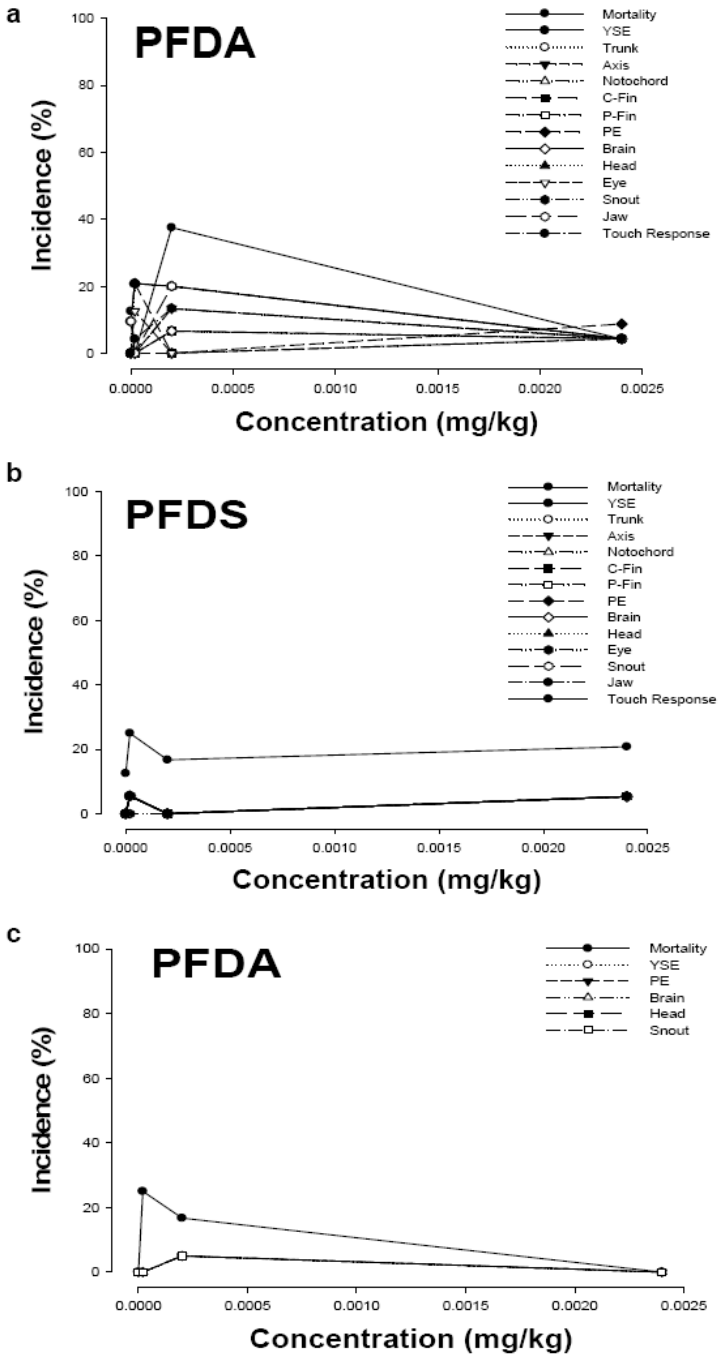


Figure 10. Zebrafish malformations elicited from microinjection of the indicated FCs at 0.00002. 0.00024, 0.0024 mg/kg.

3-4 hpf embryos were microinjected with six FCs. **(a)** PFDA at 0.00002 mg/kg elicited head malformations. **(b)** No statistically-significant malformation observed. **(c)** 0.00002 mg/kg of PFHxA induced an increase in mortality.

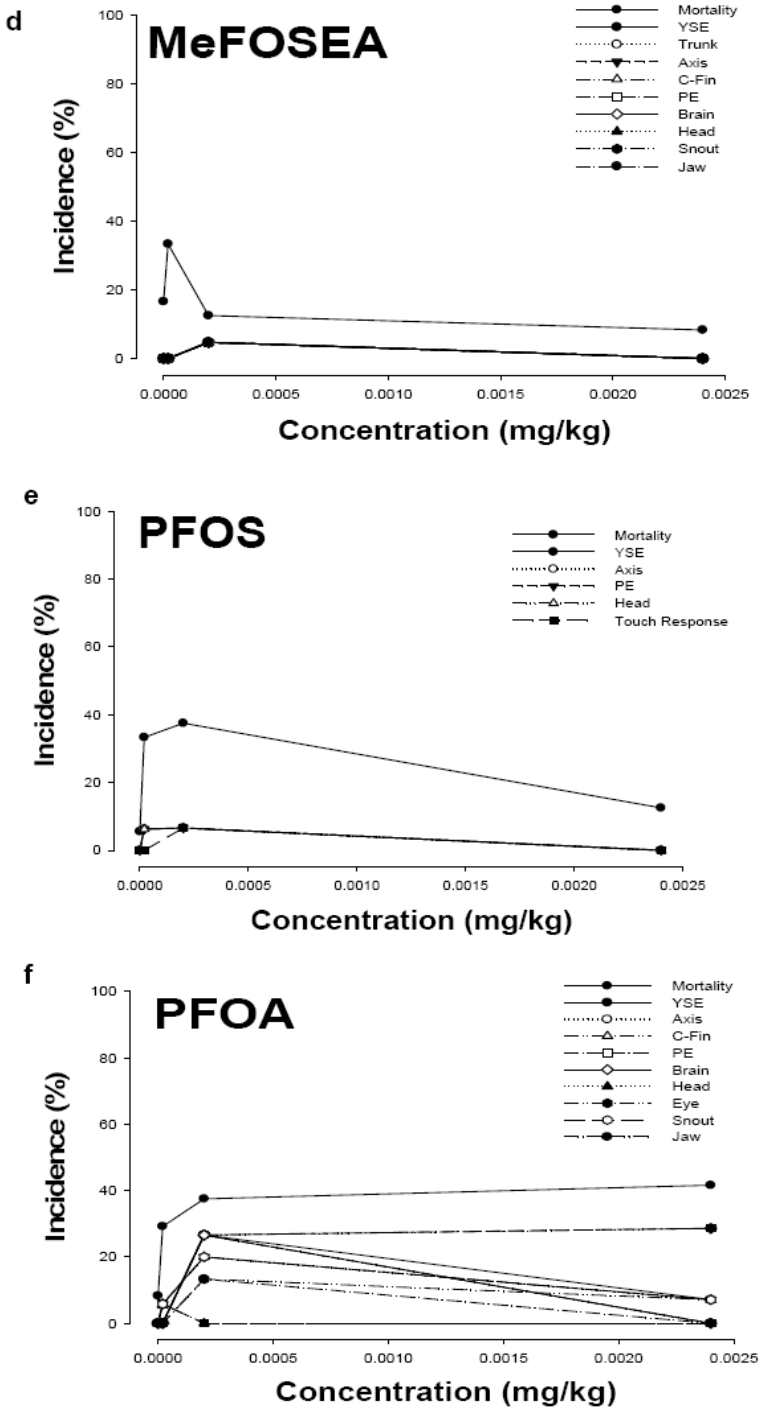


Figure 10. Zebrafish malformations elicited from microinjection of the indicated FCs at 0.00002. 0.00024, 0.0024 mg/kg (Continued).
3-4 hpf embryos were microinjected with six FCs. **(d, e)** no statistically-significant malformation observed. **(f)** 0.00024 and 0.0024 mg/kg of PFOA induced an increase in mortality.

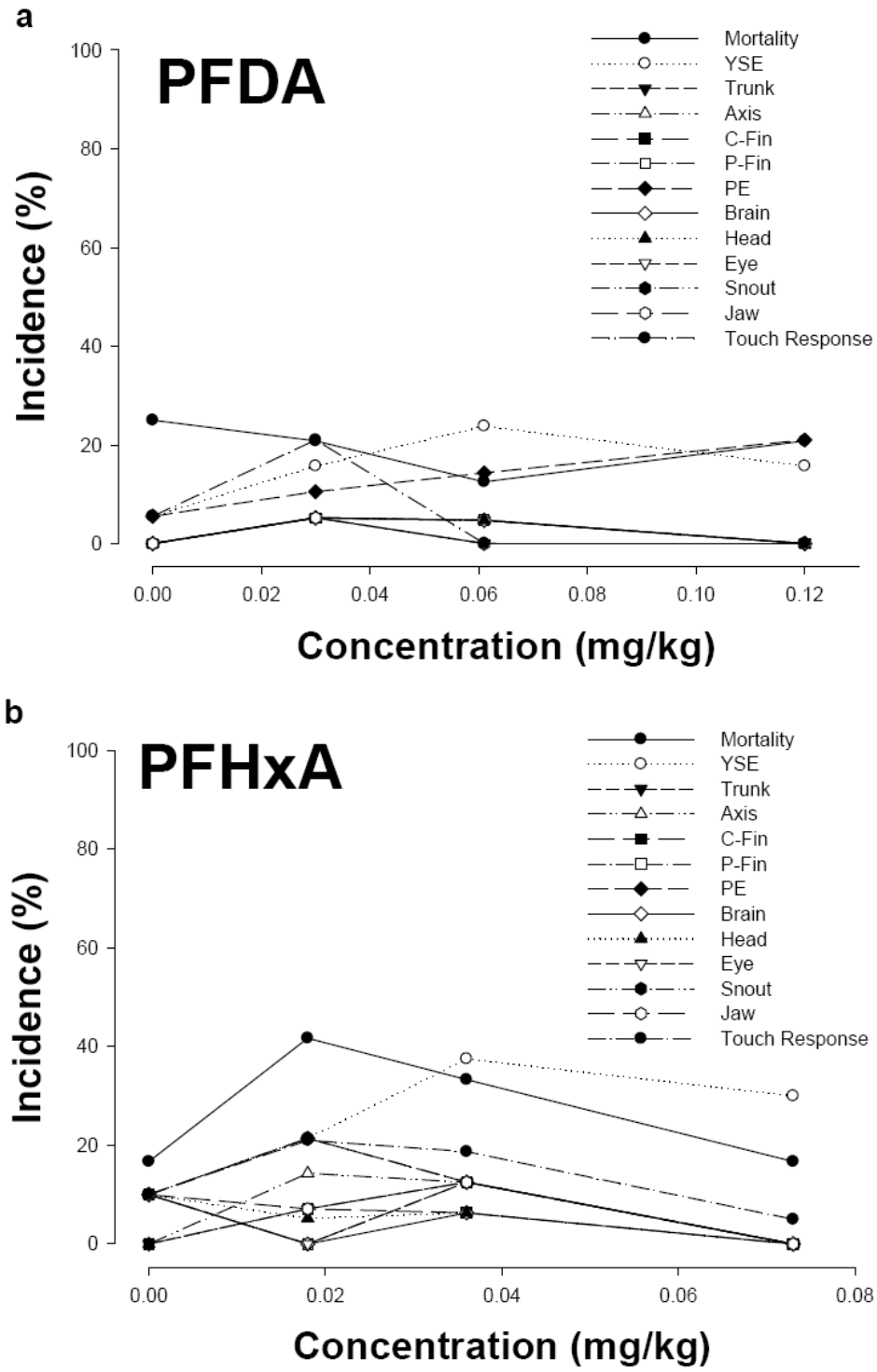


Figure 11. Zebrafish malformations elicited at higher microinjection concentrations. Further microinjection of PFDA, PFHxA, PFOA and PFOS were conducted on 3-4 hpf embryos. **(a, b)** PFDA and PFHxA did not elicit any statistically-significant malformations.

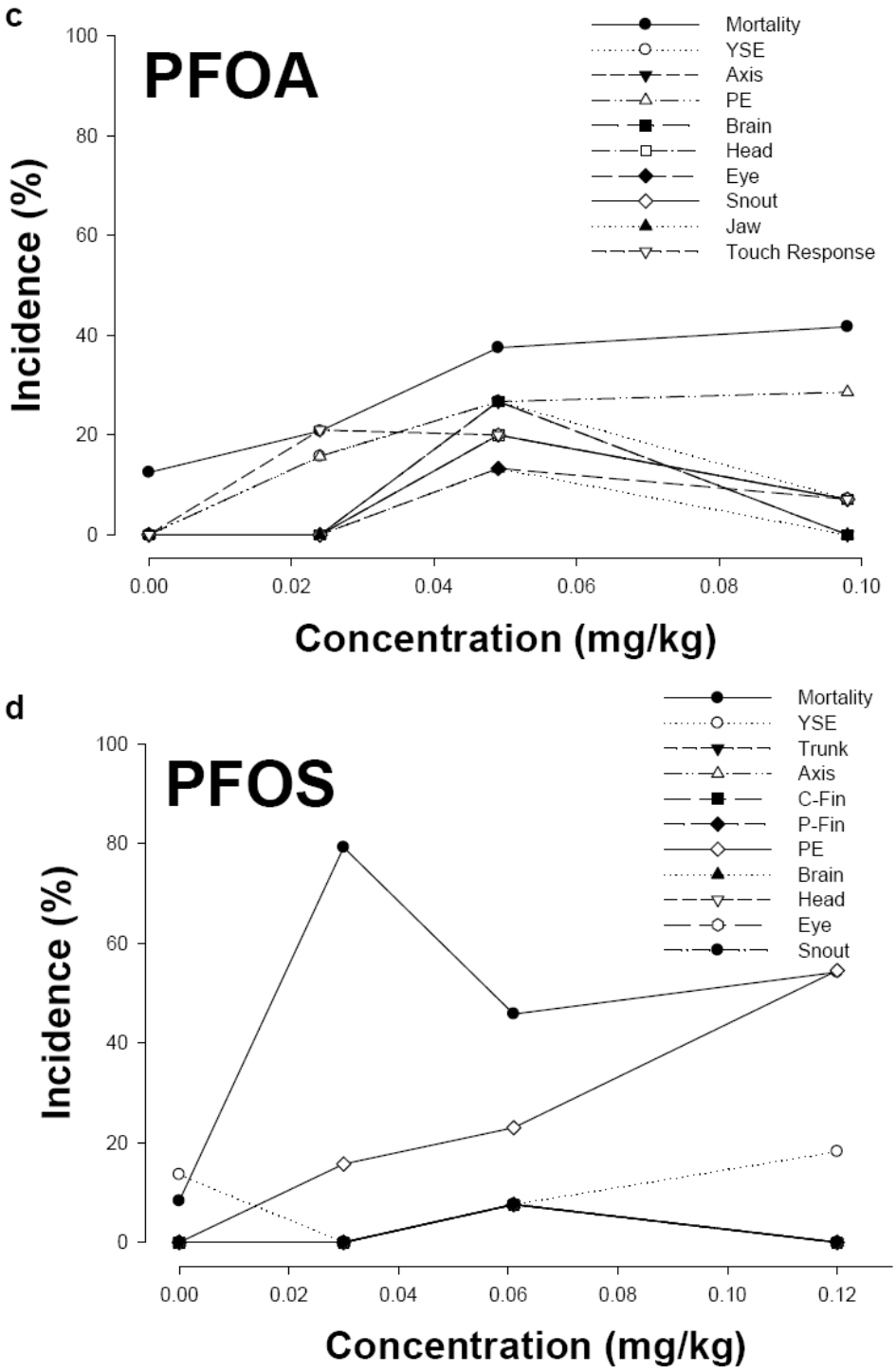


Figure 11. Zebrafish malformations elicited at higher microinjection concentrations (Continued). Further microinjection of PFDA, PFHxA, PFOA and PFOS were conducted on 3-4 hpf embryos. **(a)** Pericardial edema (PE) was significantly increased at 0.098 mg/kg of PFOA. **(b)** Mortality was increased at all concentrations and PE was increased at 0.12 mg/kg.

Table 9. Developmental malformations observed when microinjected with higher doses. PFDA, PFHxA, PFOA and PFOS elicited an increase in mortality, head and pericardial edema (PE). *p* values were determined by the Fisher's exact test.

Developmental Malformation	Chemical Name	Concentration (mg/kg)	*p* value
Head	PFDA	0.0002	0.005
Mortality	PFHxA	0.00024	0.011
	PFOA	0.00024, 0.0024	0.036, 0.017
	PFOS	0.030, 0.061, 0.12	<0.001, 0.008, 0.001
PE	PFOS	0.12	0.001
	PFOA	0.09	0.050

CHAPTER 4 – DISCUSSION

FCs demonstrated an overall low response in this model. The embryonic zebrafish is a good model for detecting environmental contaminants. Previous studies utilizing the embryonic zebrafish as an *in vivo* model to detect toxic compounds demonstrated that sodium metam and carbon fullerenes (C60) are developmentally toxic at very low concentrations. For example, sodium metam dose response curve for mortality estimated an LC50 to be 248 ng/mL at 48 hpf [32], while C60 induced significant malformations at 200 ng/mL [35]. As confirmed by various studies, this model is sensitive enough to detect toxic compounds at relatively low concentrations. This illustrates that this model is sensitive enough to detect even low-level effects in FCs. Furthermore, my results suggest that most of the FCs in my study were generally not developmentally toxic.

In this study of 41 FCs, 24 of the chemicals did not induce adverse effects. The 17 that did induce a significant increase in any assessed endpoint had a relatively low percent incidence (approximately 40% or less). Only one or two chemicals had a malformation incidence percent above that, but even for those chemicals, it never reached 100%. In this model, at low concentrations other chemicals have been able to induce 100% incidence rate of one particular abnormality [32, 33, 35]. Therefore, although the malformations are statistically significant because there was never a 100% incidence rate, it implies that the observed effect is generally not developmentally toxic. In addition, FCs that did induce statistically significant malformations only displayed one or two abnormalities, with a maximum of four. Typically, a chemical that is developmentally toxic will induce multiple abnormalities at once rather than just one or two. At higher concentrations (25-200 ng/mL), a direct dose correlation with heart malformation was observed. This observation confirms a portion of my hypothesis that certain FCs induce developmental malformations with a positive association with dose, but these concentrations are not regularly detected in the environment, biota or human serum. The data generated in this study suggets no overall dose or structure-response relationship for the chemicals in this model.

This is the first systematic study of the potential developmental toxicity of structurally diverse FCs. This study is the first to examine a large number of FCs (41) by two routes of exposures at various concentrations. At all concentrations tested in this study, no structure or dose response relationship was identified. However, an increase in pericardial edema was observed at concentrations that are 100 fold higher than at environmental and human health exposure levels, which implies that those select few FCs have the potential to perturb the pathways involved in development of the heart. An investigation of direct devliery of higher concentrations of certain FCs induced a statistically-significant increase in malformations for three FCs with carboyxlated “head” group. This demonstrates that there may be a structure-response relationship among the FCs specific to a certain route of exposure. Upon further investigation, the new data did not support the structure-response relationship for the carboxylated “head” group. However, a structure-response relationship was discovered for two FCs with eight-carbon backbone. This suggests that there may be a relationship between carbon length and induced developmental toxicity, but until other FCs with eight-carbon backbone (regardless of “head” groups) are studied, this relationship cannot be determined. Regardless, this observed potential developmental toxicity was at a concentration significantly higher than the reported concentrations in tissues and environment.

The initial waterborne exposures (0.01-10 ng/mL) were concentrations that are relevant to detected FCs in human serum level and in wildlife serum or plasma. At these lower concentrations, some FCs did produce adverse effects, predominately PE and body axis malformation. Only six of the 39 FCs induced malformations with an incidence rate of approximately 40%. At these concentration ranges, there was no dose response or structure relationship. It would be unlikely that the FCs detected in the human body would perturb pathways primarily because the concentrations at which these malformations occurred was approximately 10 fold higher than the actual level in the serum level. As a result, the concentration of FCs found in the environment may not pose concern to the development of the heart or the spine.

When embryonic zebrafish are waterborne exposed to 14 FCs at concentrations ranging between 25-200 ng/mL, which are concentrations relevant to serum levels found in human occupation exposure and in whole blood of herring gull [18], conger eel and blue gill [42] a correlation of increase concentration of certain FCs induced pericardial edema. This demonstrates that at higher concentrations, there is a dose response. This dose response for the developmental malformation of the heart observed in the embryonic zebrafish implies that at high concentrations, FCs may interfere with normal heart development. Extrapolating these data one could infer the possibility that higher concentrations of FCs could affect heart development in other systems. However, this is unlikely because these malformations were observed are high concentrations and only in humans living hear a manufacturing who have worked there for a long time [7]. Without understanding the mechanism of action for these FCs, these data cannot be extrapolated to infer assessment of these chemicals in the human population. The zebrafish model serves as a utility to connect the developmental biology with human population, but this connection cannot exist without understanding the molecular pathways and mechanism of action for this class of chemicals.

In an effort to understand the mechanism of action of the FCs, a study of direct delivery of FCs at concentrations relating to environmental background, human serum levels and whole blood concentration in wildlife (0.00002, 0.00024 and 0.0024 mg/kg) was completed. This ensures that the FCs were biologically available to the embryonic zebrafish. Uptake is an important aspect of developmental toxicity without which the chemical is unable to associate with pathways or induce malformations. In this study, one method to increase uptake was to remove the chorion, which may act as a barrier to some chemicals, while the other method was direct delivery of the FCs into the blood circulation system. The application of the first method was in the waterborne exposure study at 0.01 – 200 ng/mL, while the other method was applied by using microinjection techniques with 10 – 1000 ng/mL of FCs. For the direct injection of the FC, there was no dose response observed, but there was a limited structure relationship. To further investigate this possible structure relationship, other FCs

possessing the same structures will need to be directly injected into the embryonic zebrafish and subsequent studies to determine the mechanism of action will need to be conducted. These studies must be completed before classifying certain FCs as developmentally toxic.

In conclusion, my data suggests that the 41 FCs I investigated are generally not developmentally toxic. There was no dose or structure-response relationship observed at human or environmentally relevant concentrations to suggest that these compounds were developmentally toxic to zebrafish. Although some FCs did cause embryonic malformations, the maxium incidence rate observed was only above 80 percent for two FCs while the other FCs malformation induced an average incidence rate of less than 40%. It is difficult to declare a chemical developmentally toxic based on a 40 percent incidence rate that occurred infrequently when this model is sensitive enough to identify chemicals that induce 100 percent incidence rate at concentrations in low ng/mL. This low percent incidence trend was consistent for both routes of exposure, through waterborne and direct delivery into the biological system. A limitation in this study, which may have affected the observed developmental toxicity, was the solubility of the FCs. In the embryonic zebrafish model, developmental toxicity was relatively low at higher than observed in environmental conditions. This is the first systematic study of the potential developmental toxicity, but since it is only a descriptive study, further investigations will be required. This descriptive study is helpful in identifying the zebrafish model, as a useful tool to connect all discoveries pertaining to FCs to the potential implications to the human population. Future studies will need to include cellular and molecular work to enable results from this model to be translational and implied to human health.

CHAPTER 5 – FUTURE DIRECTIONS

Data from this study indicates that FCs are generally not generally developmentally toxic to zebrafish. However, cellular and molecular response to the FCs will need to be identified. It will require determining the molecular basis for the different FCs structure and developmental responses. It has been suggested that the activation of peroxisome proliferator-activated receptor alpha (PPAR-α) mediates several other toxic effects. To test this hypothesis in zebrafish, it will require the suppression of PPAR-α by using a moropholino then observations to see if a significant increase in toxicity occurs.

Uptake and dose will need to be determined from the tissue of the embryos water-exposed to FCs. This is crucial for the future work because lack of distribution of the FC could explain why FCs are not developmentally toxic in this model. FCs tend to bind to lipids and proteins due to their negative charge, therefore, there is a possibility that the moment the chemicals enters the biological system, it quickly bound up and did not distribute. Pharmacokinetic characteristics of FCs, especially during early developmental stages, will greatly facilitate an understanding of these chemicals dispositions and potential cellular targets. To understand the pharmacokinetics of FCs, embryonic zebrafish that are exposed to FCs through the water will have to have its tissues extracted and analyzed with analytical chemistry [43].

CHAPTER 6 – CONCLUSION

FCs are generally not overtly developmentally toxic in the embryonic zebrafish model. Compared to other chemicals (C60 and sodium metam) FCs induced low to no response. These results demonstrate the utility of the embryonic zebrafish animal model to rapidly assess the developmental toxicity of environmental and industrial chemicals. Furthermore, the versatility of this model will allow for mechanistic studies in the future.

BIBLIOGRAPHY

1. Lau, C., et al., *Perfluoroalkyl acids: a review of monitoring and toxicological findings.* Toxicol Sci, 2007. **99**(2): p. 366-94.

2. Renner, R., *Growing concern over perfluorinated chemicals.* Environ Sci Technol, 2001. **35**(7): p. 154A-160A.

3. Seacat, A.M., et al., *Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys.* Toxicol Sci, 2002. **68**(1): p. 249-64.

4. Giesy, J.P. and K. Kannan, *Perfluorochemical surfactants in the environment.* Environ Sci Technol, 2002. **36**(7): p. 146A-152A.

5. Kissa, E., *Fluorinated Surfactants adnRepellants*. 2nd ed. 2001, New York: Marcel Decker.

6. Hekster, F.M., R.W. Laane, and P. de Voogt, *Environmental and toxicity effects of perfluoroalkylated substances.* Rev Environ Contam Toxicol, 2003. **179**: p. 99-121.

7. Kudo, N. and Y. Kawashima, *Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals.* J Toxicol Sci, 2003. **28**(2): p. 49-57.

8. Allsopp, M., Santillo, D., Walters, A. & Johnston, P., *Perfluorinated chemicals: an emerging concern*. April 2005.

9. Karrman, A., et al., *Perfluorinated compounds in serum from Australian urban and rural regions.* EMG-Fluorinated Compounds, 2002: p. 780-783.

10. Stock, N.L., et al., *Polyfluorinated telomer alcohols and sulfonamides in the North American troposphere.* Environ Sci Technol, 2004. **38**(4): p. 991-6.

11. Moody, C.A., et al., *Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek.* Environ Sci Technol, 2002. **36**(4): p. 545-51.

12. Harada, K., et al., *Particle size distribution and respiratory deposition estimates of airborne perfluorooctanoate and perfluorooctanesulfonate in Kyoto area, Japan.* Bull Environ Contam Toxicol, 2006. **76**(2): p. 306-10.

13. Barton, C.A., et al., *Characterizing perfluorooctanoate in ambient air near the fence line of a manufacturing facility: comparing modeled and monitored values.* J Air Waste Manag Assoc, 2006. **56**(1): p. 48-55.

14. Wang, N., et al., *Aerobic biotransformation of 14C-labeled 8-2 telomer B alcohol by activated sludge from a domestic sewage treatment plant.* Environ Sci Technol, 2005. **39**(2): p. 531-8.

15. Karrman, A., et al., *Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden.* Environ Health Perspect, 2007. **115**(2): p. 226-30.

16. Harada, K., et al., *Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan.* Chemosphere, 2007. **66**(2): p. 293-301.

17. 3M, *The Science of organic fluorochemistry and perfluorooctane sulfonate: Current summary of human sera, health and toxicology data*. 1999.

18. Giesy, J.P. and K. Kannan, *Global distribution of perfluorooctane sulfonate in wildlife.* Environ Sci Technol, 2001. **35**(7): p. 1339-42.

19. Kannan, K., et al., *Perfluorooctane sulfonate in fish-eating water birds including bald eagles and albatrosses.* Environ Sci Technol, 2001. **35**(15): p. 3065-70.

20. Hoff, P.T., et al., *Perfluorooctane sulfonic acid in bib (Trisopterus luscus) and plaice (Pleuronectes platessa) from the Western Scheldt and the Belgian North Sea: distribution and biochemical effects.* Environ Toxicol Chem, 2003. **22**(3): p. 608-14.

21. Hoff, P.T., et al., *Evaluation of the toxicological effects of perfluorooctane sulfonic acid in the common carp (Cyprinus carpio).* Aquat Toxicol, 2003. **62**(4): p. 349-59.

22. Kannan, K., et al., *Perfluorooctanesulfonate and related fluorinated hydrocarbons in mink and river otters from the United States.* Environ Sci Technol, 2002. **36**(12): p. 2566-71.

23. 3M, *104-Week dietary chronic toxicity and carcinogenticity study with perfluoroctane sulfonic acid potassium salt (PFOS; T-6295) in rats*. 2002, 3M Company: St. Paul, Mn.

24. Kennedy, G.L., Jr., et al., *The toxicology of perfluorooctanoate.* Crit Rev Toxicol, 2004. **34**(4): p. 351-84.

25. Lau, C., J.L. Butenhoff, and J.M. Rogers, *The developmental toxicity of perfluoroalkyl acids and their derivatives.* Toxicol Appl Pharmacol, 2004. **198**(2): p. 231-41.

26. Takacs, M.L. and B.D. Abbott, *Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate.* Toxicol Sci, 2007. **95**(1): p. 108-17.

27. Shipley, J.M., et al., *trans-activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based chemicals.* Toxicol Sci, 2004. **80**(1): p. 151-60.

28. Luebker, D.J., et al., *Interactions of fluorochemicals with rat liver fatty acid-binding protein.* Toxicology, 2002. **176**(3): p. 175-85.

29. Depledge, H.M., T.S. Galloway, and Z. Billinghurst, *Effects of endocrine disrupting chemicals in invertebrates*. Endrocrine Disrupting Chemicals. 1994, Cambridge, England: Royal Society of Chemistry.

30. Vos, J.G., et al., *Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation.* Crit Rev Toxicol, 2000. **30**(1): p. 71-133.

31. Benninghoff, A.D., J.A. Field, and D.E. Williams, *Assessment of the estrogen activity of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and other*

structurally diverse perfluorinated chemicals in rainbow trout. Toxicologist CD, 2007. **96**(abstract): p. 110.

32. Haendel, M.A., et al., *Developmental toxicity of the dithiocarbamate pesticide sodium metam in zebrafish.* Toxicol Sci, 2004. **81**(2): p. 390-400.

33. Tilton, F., et al., *Dithiocarbamates have a common toxic effect on zebrafish body axis formation.* Toxicol Appl Pharmacol, 2006. **216**(1): p. 55-68.

34. Mathew, L.K., E.A. Andreasen, and R.L. Tanguay, *Aryl hydrocarbon receptor activation inhibits regenerative growth.* Mol Pharmacol, 2006. **69**(1): p. 257-65.

35. Usenko, C.Y., S.L. Harper, and R.L. Tanguay, *In vivo evaluation of carbon fullerene toxicity using embryonic zebrafish.* Carbon 2007(45): p. 1891 - 1898.

36. Teraoka, H., et al., *Muscular contractions in the zebrafish embryo are necessary to reveal thiuram-induced notochord distortions.* Toxicol Appl Pharmacol, 2006. **212**(1): p. 24-34.

37. Kimmel, C.B., et al., *Stages of embryonic development of the zebrafish.* Dev Dyn, 1995. **203**(3): p. 253-310.

38. Holden, P.R. and J.D. Tugwood, *Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences.* J Mol Endocrinol, 1999. **22**(1): p. 1-8.

39. Westerfield, M., *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)*. 2000, Eugene, OR: University of Oregon Press.

40. Harper, S.L., et al., *Proactively designing nanomaterials to enhance performance and minimise hazard.* International Journal of Nanotechnology, 2008. **5**(1): p. 124 - 142.

41. Reimers, M.J., A.R. Flockton, and R.L. Tanguay, *Ethanol- and acetaldehyde-mediated developmental toxicity in zebrafish.* Neurotoxicol Teratol, 2004. **26**(6): p. 769-81.

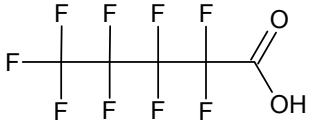
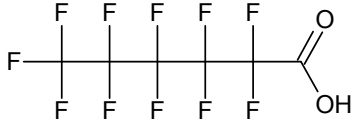
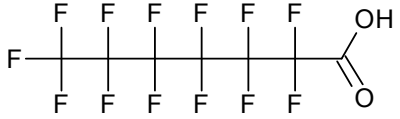
42. Taniyasu, S., et al., *A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds, and humans from Japan.* Environ Sci Technol, 2003. **37**(12): p. 2634-9.

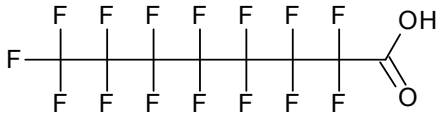
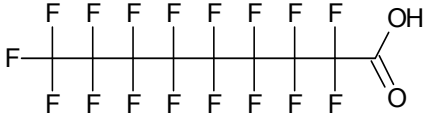
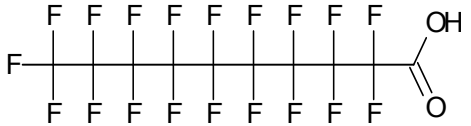
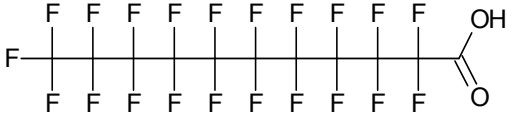
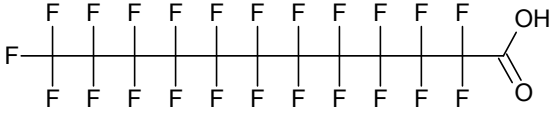
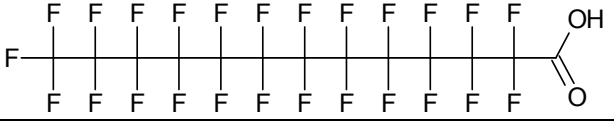
43. Lau, C., et al., *Effects of perfluorooctanoic acid exposure during pregnancy in the mouse.* Toxicol Sci, 2006. **90**(2): p. 510-8.

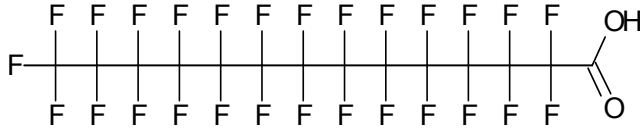
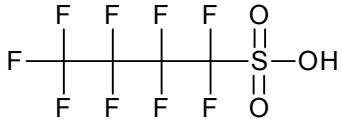
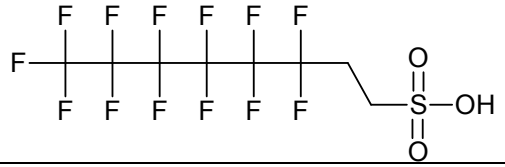
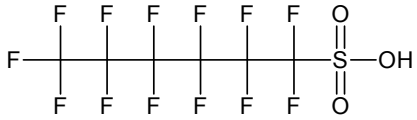
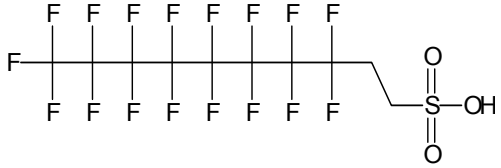
APPENDICES

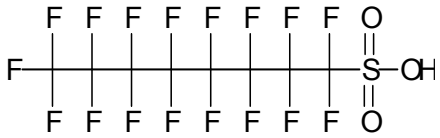
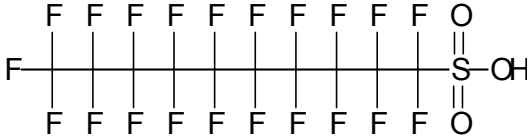
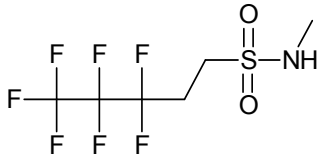
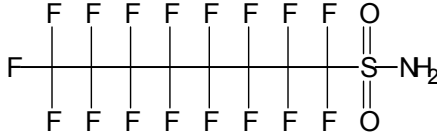
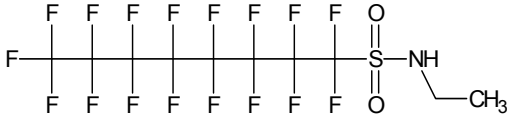
Appendix A. Chemical data on fluorinated chemicals.

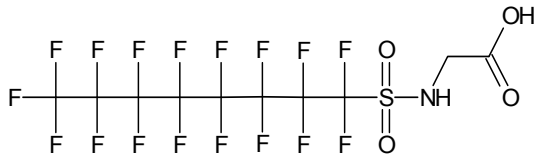
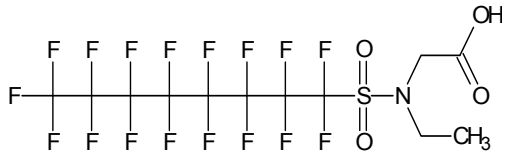
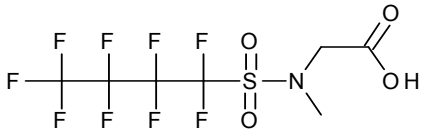
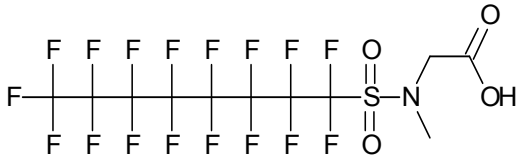
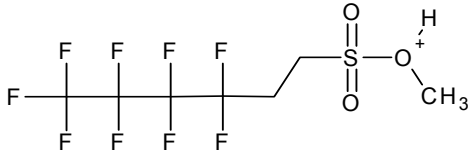
Abbreviation: [A]=not registered nationally, only at manufacturer, **NR** = not registered, **DBI** = donated by industry

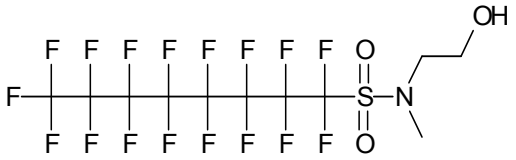
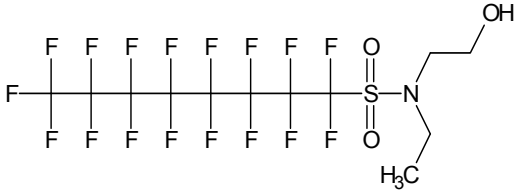
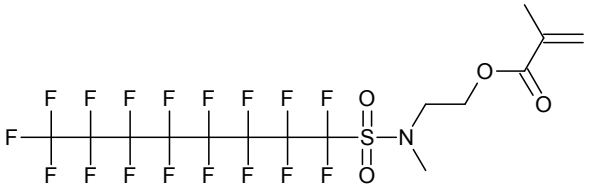
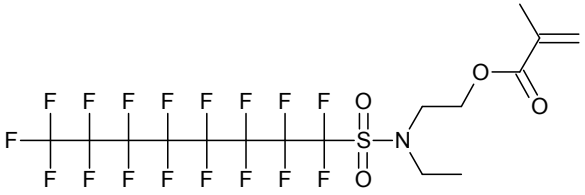
Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
1	Zonyl® FSA fluoro-surfactant	FSA	N/A	Not Available – Mixture of: 25% fluorosurfactant, 37.5% isopropyl alcohol, and 37.5% water	N/A	65530-69-0[A]	Aldrich
2	2-(Perfluoroalkyl)ethanol	FSN	N/A	Not Available – Mixture of: 40% fluorosurfactant, 30% isopropyl alcohol, and 30% water	N/A	65545-80-4 [A]	Aldrich
3	Zonyl™ FSE Fluoro-surfactantl	FSE	N/A	Not Available – Mixture of: 14% fluorosurfactant, 24% ethylene glycol, and 62% water	N/A	83653-37-6 [A]	Aldrich
4	Zonyl® FSK	FSK	N/A	Not Available – Mixture	N/A	80449-64-5 [A]	Fluka
5	Perfluoro-pentanoic acid	PFPA	4		$C_5HF_9O_2$	2706-90-3	Aldrich
6	Perfluoro-hexanoic acid	PFHxA	5		$C_6HF_{11}O_2$	307-24-4	DBI
7	Perfluoro-heptanoic acid	PFHpA	6		$C_7HF_{13}O_2$	375-85-9	Aldrich

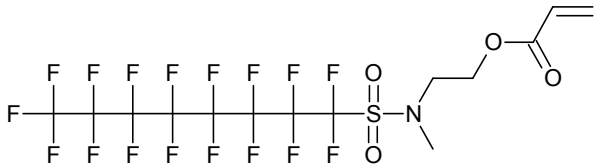
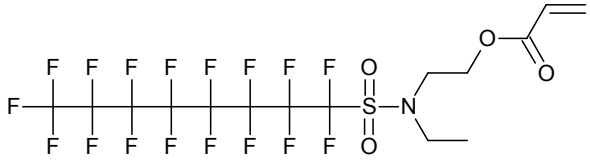
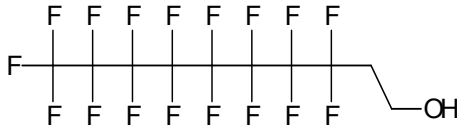
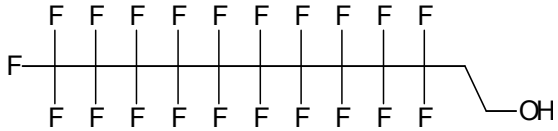
Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
8	Perfluoro-octanoic acid	PFOA	7		$C_8HF_{15}O_2$	335-67-1	Aldrich
9	Perfluoro-nonanoic acid	PFNA	8		$C_9HF_{17}O_2$	375-95-1	Aldrich
10	Perfluoro-decanoic acid	PFDA	9		$C_{10}HF_{19}O_2$	335-76-2	Aldrich
11	Perfluoro-undecanoic acid	PFUnDA	10		$C_{11}HF_{21}O_2$	2058-94-8	Aldrich
12	Perfluoro-dodecanoic acid	PFDoDA	11		$C_{12}HF_{23}O_2$	307-55-1	Aldrich
13	Perfluoro-tridecanoic acid	PFTrDA	12		$C_{13}HF_{25}O_2$	72629-94-8	Aldrich

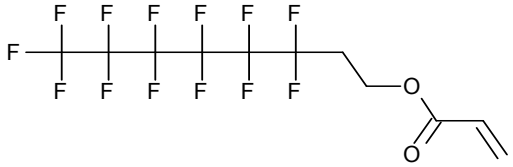
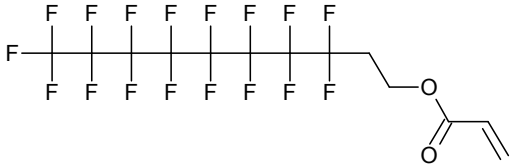
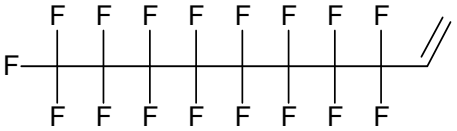
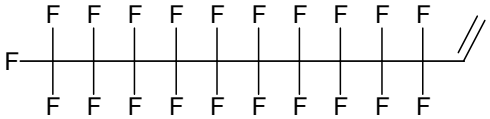
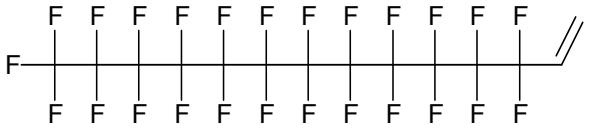
Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
14	Perfluoro-tetradecanoic acid	PFTDA	13		$C_{14}HF_{27}O_2$	376-06-7	Aldrich
15	Perfluoro-butane sulfonate	PFBS	4		$C_4HF_9SO_3$	29420-49-3	DBI
16	6:2 Fluorotelomer sulfonate	6:2 FtS	6		$C_8H_5F_{13}SO_3$	27619-97-2	Apollo
17	Perfluoro-hexane sulfonate	PFHxS	6		$C_6HF_{13}SO_3$	3871-99-6	DBI
18	8:2 fluorotelomer Sulfates	8:2 FtS	8		$C_{10}H_5F_{17}SO_3$	NR	DBI

Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
19	Perfluoro-octane sulfonate	PFOS	8		$C_8F_{17}SO_3^-$	2795-39-3	Aldrich
20	Perfluoro-decane sulfonate	PFDS	10		$C_{10}HF_{21}SO_3$	67906-42-7 (NH4)	Aldrich
21	N-methyl perfluoro-butane sulfonamide	MeFBSA	3		$C_6H_8F_7NSO_2$	NF	DBI
22	Perfluoro-octane sulfonamide	FOSA	8		$C_8H_2F_{17}NO_2S$	NF	DBI
23	N-ethyl perfluoro-octane sulfonamide (sulfluramid)	EtFOSA	8		$C_{10}H_6F_{17}NO_2S$	4151-50-2	ABCR

Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
24	N-acetyl-perfluoro-octane sulfonamide	FOSAA	8		$C_{10}H_4F_{17}NSO_4$	NR	DBI
25	N-ethyl perfluoro-octanesulfonamido acetic acid	EtFOSAA	8		$C_{12}H_8F_{17}NSO_4$	NR	DBI
26	N-methyl perfluoro-butane sulfonamido acetic acid	MeFBSAA	4		$C_7H_6F_9NSO_4$	NR	DBI
27	N-methyl perfluoro-octanesulfonamido acetic acid	MeFOSAA	8		$C_{11}H_6F_{17}NO_4S$	NR	DBI
28	N-methyl perfluoro-butane sulfonamido ethyl	MeFBSE	4		$C_7H_8F_9SO_3$	NR	DBI

Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
29	N-methyl perfluoro-octane Sulfonamide-ethanol	MeFOSE	8		$C_{11}H_8F_{17}NSO_3$	24448-09-7	DBI
30	N-ethyl perfluoro-octane sulfonamido ethanol	EtFOSE	8		$C_{12}H_{10}F_{17}NSO_3$	1691-99-2	DBI
31	N-methyl perfluoro-octane sulfonamido ethyl methacrylate	MeFOSEMA	8		$C_{15}H_{12}F_{17}NSO_4$	NR	DBI
32	N-ethyl perfluoro-octane sulfonamido ethyl methacrylate	EtFOSEMA	8		$C_{16}H_{14}F_{17}NSO_4$	376-14-7	Acros

Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
33	N-methyl perfluoro-octane sulfonamido ethyl acrylate	MeFOSEA	8		$C_{14}H_{10}F_{17}NSO_4$	25268-77-3	DBI
34	N-ethyl perfluoro-octane sulfonamido ethyl acrylate	EtFOSEA	8		$C_{15}H_{12}F_{17}NSO_4$	423-82-5	Acros
35	8:2 Fluorotelomer Alcohol	8:2 FtOH	8		$C_{10}H_5F_{17}O$	678-39-7	Oak-wood
36	10:2 Fluorotelomer Alcohol	10:2 FtOH	10		$C_{12}H_5F_{21}O$	865-86-1	Syn-Quest

Chem. #	Chemical Name	Abbreviation	Number of Fluorinated carbon	Chemical Structure	Molecular Formula	CAS Number	Source
37	6:2 Fluorotelomer acrylate	6:2 FtOAcr	6		$C_{11}H_7F_{13}O_2$	17527-29-6	Aldrich
38	8:2 Fluorotelomer acrylate	8:2 FtOAcr	8		$C_{13}H_7F_{17}O_2$	27905-45-9	Aldrich
39	8:2 Fluorotelomer Olefin	8:2 FtENE	8		$C_{10}H_3F_{17}$	21652-58-4	Aldrich
40	10:2 Fluorotelomer Olefin	10:2 FtENE	10		$C_{12}H_3F_{21}$	30389-25-4	Syn-Quest
41	12:2 Fluorotelomer Olefin	12:2 FtENE	12		$C_{14}H_3F_{25}$	67103-05-3	Syn-Quest

Appendix B. 39 FCs broad range waterborne exposure raw malformation data tables.

Abbreviation: **YSE** = Yolk Sac Edema, **NC** = Notochord, **C-Fin** = Caudal Fin, **P-Fin** = Pectoral Fin, **PE** = Pericardial Edema, **TR** = Touch Response

Table B. 1. PFDA broad range waterborne exposure raw malformation data

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-		-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 2. FSA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	1/7	-	1/7	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	1/7	-	1/7	-	-	-	1/7	-	-	-	-	-	-	-	-
1 ng/mL	-		-	1/7	-	1/7	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 3. PFDS broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	2/6	-	1/6	2/6	-	2/6	2/6	-	-	2/6	-	-	-	-	-
10 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 4. FOSAA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 5. PFTDA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	1/7	-	-	-	1/7	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	1/7	-	1/7	1/7	-	-	1/7	-	-	-	1/7	-	-	-	-

Table B. 6. PFTrDA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 7. MeFBSAA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	1/6	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 8. EtFOSAA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	1/6	-	2/6	1/6	1/6	-	1/6	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 9. MeFOSAA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	2/7	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 10. 6:2 FtS broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	2/7	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	1/6	-	1/6	1/6	1/6	-	1/6	-	-	1/6	-	-	-	-	-

Table B. 11. PFDoDA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	1/7	-	2/7	1/7	1/7	-	1/7	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	2/6	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 12. FSN broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/47	-	1/47	1/47	-	-	1/47	-	-	1/47	-	-	1/47	-	-
0.01 ng/mL	-	-	-	1/7	1/7	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	1/7	1/7	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	-	-	2/6	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 13. PFPA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	1/7	1/7	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	1/7	1/7	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	-	-	2/6	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 14. PFHxS broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	1/6	-	-	-	2/6	2/6	1/6	-	-	2/6	-	-	-	-	2/6
10 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 15. PFBS broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	3/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	1/7	-	1/7	1/7	-	-	1/7	-	-	1/7	-	-	-	-	-
10 ng/mL	-	2/7	-	2/7	2/7	-	-	2/7	-	-	-	-	-	-	-	2/7

Table B. 16. 8:2 FtS broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	1/7	-	2/7	-	1/7	-	1/7	1/7	-	-	-	-	-	-	1/7
0.1 ng/mL	3/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/7
1 ng/mL	-	1/7	-	1/7	1/7	-	-	1/7	-	-	1/7	-	-	-	-	-
10 ng/mL	-	2/7	-	2/7	2/7	-	-	2/7	-	-	-	-	-	-	-	1/7

Table B. 17. PFHpA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/5
1 ng/mL	1/7	1/6	-	-	-	-	-	1/6	1/6	-	1/6	-	-	-	-	-
10 ng/mL	-	1/7	-	-	-	-	-	1/7	1/7	-	1/7	-	-	-	-	1/7

Table B. 18. PFHxA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	1/6	-	1/6	-	-	-	1/6	1/6	-	1/6	-	1/6	-	-	1/6
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 19. FSE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 20. FSK broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 21. PFUnDA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 22. PFNA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/44	1/39	-	-	-	1/39	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	1/6	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	1/7	1/7	1/7	1/7	1/7	-	1/7	-	1/7	-	-	-	-	-

Table B. 23. 10:2 FtENE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	1/6	1/6	1/6	1/6	1/6		1/6	-	-	1/6	-	1/6	-	-	1/6

Table B. 24. 12:2 FtENE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	1/6	1/6	1/6	1/6	1/6		1/6	-	-	1/6	-	1/6	-	-	1/6

Table B. 25. 8:2 FtENE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	1/6	1/6	1/6	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 26. 8:2 FtOAcr broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 27. 8:2 FtOH broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 28. MeFOSE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	1/7	1/7	1/7	1/7	1/7	-	1/7	-	-	1/7	-	-	-	-	1/7
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 29. MeFOSEA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	1/6	1/6	2/6	1/6	1/6	-	1/6	-	-	1/6	-	1/6	-	-	1/6
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 30. MeFOSEMA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 31. EtFOSE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	2/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	2/6	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	1/6	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 32. EtFOSEA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 33. EtFOSEMA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 34. MeFBSA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 35. MeFBSE broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	1/7	-	-	1/6	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	1/7	1/7	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 36. 6:2 FtOAcr broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	2/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	1/7	1/7	1/7	1/7	-	1/7	1/7	-	-	1/7	-	-	-	-	-

Table B. 37. 10:2 FtOH broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	1/7	-	-	1/7	-	-	-	-	-	-	-	-	1/7
1 ng/mL	-	-	-	1/7	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 38. FOSA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	1/7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table B. 39. EtFOSA broad range waterborne exposure raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/36	-	-	1/35	-	-	-	-	-	-	-	-	-	-	-	-
0.01 ng/mL	-	-	-	-	-	-	-	1/7	-	-	-	-	-	-	-	1/7
0.1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix C. 14 FCs waterborne exposure at higher concentration raw malformation data tables.

Abbreviation: **YSE** = Yolk Sac Edema, **NC** = Notochord, **C-Fin** = Caudal Fin, **P-Fin** = Pectoral Fin, **PE** = Pericardial Edema, **TR** = Touch Response

Table C. 1. PFDA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 ng/mL	3/24	-	-	1/21	-	-	-	2/21	-	-	-	-	-	-	-	-
50 ng/mL	1/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 ng/mL	-	1/24	-	1/24	-	-	-	1/24	1/24	-	1/24	1/24	1/24	1/24	-	-
200 ng/mL	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table C. 2. FSA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	-	-	-	-	-	-	2/46	-	-	-	-	-	-	-	-
25 ng/mL	1/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50 ng/mL	1/24	-	-	-	-	-	-	1/23	-	-	-	-	-	-	-	1/23
100 ng/mL	2/24	-	-	-	-	-	-	3/22	-	-	-	-	-	-	-	1/22
200 ng/mL		1/24	-	3/24	-	-	-	1/24	-	-	1/24	-	1/24	-	-	1/24

Table C. 3. FSN waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	4/48	1/44	1/44	2/44	-	1/44	-	1/44	1/44	-	1/44	1/44	1/44	1/44	-	1/44
25 ng/mL	3/24	-	-	-	-	-	-	2/21	-	-	-	-	-	-	-	1/21
50 ng/mL	3/24	1/21	-	2/21	-	1/21	-	2/21	-	-	-	-	-	-	-	-
100 ng/mL	3/24	-	-	-	-	-	-	1/21	-	-	-	-	-	-	-	-

Table C. 4. PFPA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/48	1/46	-	2/46	-	1/46	-	2/46	-	-	1/46	-	-	-	-	-
25 ng/mL	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50 ng/mL	1/24	2/23	1/23	1/23	1/7	1/23	-	2/23	2/23	-	2/23	1/23	1/23	-	-	2/23
100 ng/mL	1/24	3/23	-	1/23	-	1/23	1/23	1/23	-	-	3/23	1/23	1/23	1/23	-	1/23
200 ng/mL	3/24	2/21	-	2/21	-	-	-	1/21	-	-	-	-	-	-	-	1/21

Table C. 5. PFHxS waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	5/48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 ng/mL	1/24	-	-	-	-	-	-	1/24	-	-	-	-	-	-	-	1/24
50 ng/mL	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 ng/mL	1/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200 ng/mL	1/24	-	-	2/23	-	-	-	3/23	1/23	-	1/23	-	1/23	1/23	-	2/23

Table C. 6. PFBS waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	4/24	3/20	-	-	-	-	-	3/20	3/20	-	3/20	-	3/20	2/20	-	1/20
25 ng/mL	1/24	-	-	-	-	-	-	9/23	1/23	-	1/23	-	1/23	1/23	-	1/23
50 ng/mL	-	4/24	-	2/24	-	-	-	11/24	4/24	-	4/24	1/24	4/24	3/24	-	3/24
100 ng/mL	3/24	4/21	-	3/21	-	-	-	12/21	4/21	-	5/21	3/21	4/21	4/21	-	3/21
200 ng/mL	3/24	2/21	1/21	2/21	-	-	-	5/21	2/21	-	2/21	2/21	2/21	2/21	-	2/21

Table C. 7. FSE waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	1/24	1/23	-	-	-	-	-	3/23	1/23	-	1/23	-	1/23	-	-	2/23
25 ng/mL	1/24	5/23	-	-	-	-	-	5/23	4/23	-	3/23	2/23	3/23	3/23	-	3/23
50 ng/mL	2/24	1/22	-	1/22	-	-	-	6/22	1/22	-	1/22	-	1/22	1/22	-	1/22
100 ng/mL	-	5/24	-	-	-	-	-	9/24	5/24	-	5/24	2/24	5/24	5/24	-	5/24
200 ng/mL	1/24	3/23	-	1/23	-	1/23	-	7/23	3/23	-	3/23	3/23	3/23	3/23	-	2/23

Table C. 8. MeFOSE waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/24	3/24	-	-	-	-	-	4/24	1/24	-	1/24	1/24	-	-	-	2/24
25 ng/mL	-	3/24	-	-	-	-	-	6/24	3/24	-	3/24	1/24	2/24	1/24	-	1/24
50 ng/mL	3/24	1/21	-	-	-	-	-	7/21	1/21	-	1/21	-	1/21	-	-	1/21
100 ng/mL	3/24	5/21	-	3/21	-	-	-	7/21	5/21	-	5/21	5/21	5/21	3/21	-	3/21

Table C. 9. MeFOSEA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	2/24	1/22	-	1/22	-	1/22	-	2/22	2/22	-	2/22	1/22	-	1/22	-	-
25 ng/mL	2/24	6/22	1/22	2/22	-	1/22	-	10/22	6/22	-	6/22	2/22	5/22	5/22	-	3/22
50 ng/mL	4/24	2/20	1/20	2/20	-	1/20	-	15/20	2/20	-	2/20	1/20	1/20	2/20	-	1/20
100 ng/mL	2/24	3/22	-	-	-	-	-	16/22	4/22	-	4/22	2/22	4/22	2/22	-	-
200 ng/mL	7/24	1/17	-	1/17	-	1/17	-	14/17	1/17	-	1/17	-	1/17	1/17	-	-

Table C. 10. MeFOSEMA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 ng/mL	-	-	2/24	-	-	-	-	2/24	-	-	-	-	-	-	-	-
50 ng/mL	-	-	-	-	-	-	-	1/24	-	-	-	-	-	-	-	-
100 ng/mL	1/24	2/23	1/23	-	-	-	1/23	10/23	4/23	-	4/23	4/23	4/23	3/23	-	3/23
200 ng/mL	-	-	-	1/24	-	-	-	5/24	-	-	-	-	-	-	-	1/24

Table C. 11. EtFOSE waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	-	2/24	-	1/24	-	-	-	2/24	1/24	-	2/24	-	1/24	-	-	-
25 ng/mL	1/24	1/23	-	1/23	-	-	-	4/23	1/23	-	1/23	1/23	1/23	1/23	-	-
50 ng/mL	-	2/24	-	-	-	-	-	6/24	2/24	-	2/24	2/24	2/24	-	-	1/24
100 ng/mL	-	6/24	1/24	1/24	-	-	-	14/24	5/24	-	6/24	6/24	6/24	5/24	-	6/24

Table C. 12. FOSA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (1% DMSO)	1/24	2/23	-	-	-	-	-	3/23	3/23	-	3/23	-	2/23	-	-	-
25 ng/mL	2/24	3/22	-	2/22	-	-	-	9/22	3/22	-	3/22	-	3/22	-	-	-
50 ng/mL	2/24	3/22	1/22	1/22	1/22	1/22	1/22	9/22	3/22	1/22	3/22	1/22	1/22	1/22	1/22	3/22
100 ng/mL	1/24	2/23	-	-	-	-	-	12/23	2/23	-	2/23	1/23	2/23	1/23	-	2/23
150 ng/mL	1/24	2/23	1/23	1/23	-	-	-	14/23	2/23	-	2/23	-	2/23	2/23	-	3/23

Table C. 13. PFOA waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 ng/mL	2/24	1/23	-	-	-	-	1/23	1/23	1/23	-	1/23	1/23	1/23	1/23	-	2/23
50 ng/mL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/24
100 ng/mL	2/24	1/22	1/22	1/22	-	-	1/22	1/22	1/22	-	1/22	1/22	1/22	1/22	-	1/22
200 ng/mL	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table C. 14. PFOS waterborne exposure at higher concentration raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25 ng/mL	1/24	-	-	-	-	-	-	1/23	-	-	-	-	-	-	-	-
50 ng/mL	1/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 ng/mL	1/24	3/23	-	-	-	-	-	10/23	3/23	-	3/23	3/23	2/23	-	-	3/23
200 ng/mL	2/24	2/22	2/22	2/22	-	1/22	2/22	3/22	1/22	-	1/22	1/22	1/22	1/22	-	-

Appendix D. Six FCs initial microinjection raw malformation data tables.

Abbreviation: **YSE** = Yolk Sac Edema, **NC** = Notochord, **C-Fin** = Caudal Fin, **P-Fin** = Pectoral Fin, **PE** = Pericardial Edema, **TR** = Touch Response

Table D. 1. PFDA initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1% DMSO)	3/24	2/21	-	-	-	-	-	2/21	-	-	-	-	2/21	2/21	-	-
10 ng/mL (0.0.0002 mg/kg)	-	5/24	-	-	-	-	-	5/24	-	-	5/24	3/24	5/24	5/24	-	1/24
100 ng/mL (0.00024 mg/kg)	9/24	3/15	1/15	2/15	2/15	-	1/15	-	2/15	-	3/15	-	3/15	3/15	-	2/15
1000 ng/mL (0.00245 mg/kg)	1/24	1/23	1/23	1/23	1/23	1/23	1/23	2/23	1/23	-	1/23	1/23	1/23	1/23	-	1/23

Table D. 2. PFDS initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1% DMSO)	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL (0.0.0002 mg/kg)	6/24	1/18	1/18	1/18	1/18	1/18	1/18	1/18	1/18	-	1/18	-	1/18	1/18	-	1/18
100 ng/mL (0.00024 mg/kg)	4/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1000 ng/mL (0.00245 mg/kg)	5/24	1/19	1/19	1/19	1/19	1/19	1/19	1/19	1/19	-	1/19	1/19	1/19	1/19	-	1/19

Table D. 3. PFHxA initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1%DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL (0.0.0002 mg/kg)	6/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 ng/mL (0.00024 mg/kg)	4/24	1/20	-	-	-	-	-	1/20	1/20	-	1/20	-	1/20	-	-	-
1000 ng/mL (0.00245 mg/kg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table D. 4. MeFOSEA initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1%DMSO)	4/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL (0.0.0002 mg/kg)	8/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/16
100 ng/mL (0.00024 mg/kg)	3/24	1/21	1/21	1/21	-	1/21	-	1/21	1/21	-	1/21	-	1/21	1/21	-	1/21
1000 ng/mL (0.00245 mg/kg)	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table D. 5. PFOA initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1%DMSO)	2/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1/22
10 ng/mL (0.0.0002 mg/kg)	7/24	-	-	-	-	1/17	-	-	-	-	1/17	-	1/17	-	-	-
100 ng/mL (0.00024 mg/kg)	9/24	4/15	-	4/15	-	-	-	4/15	4/15	-	3/15	2/15	3/15	2/15	-	3/15
1000 ng/mL (0.00245 mg/kg)	10/24	1/14	-	-	-	-	-	4/14	-	-	1/14	1/14	1/14	-	-	1/14

Table D. 6. PFOS initial microinjection raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR
0 ng/mL (0.1%DMSO)	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 ng/mL (0.0.0002 mg/kg)	8/24	1/16	-	1/16	-	-	-	1/16	-	-	1/16	-	-	-	-	-
100 ng/mL (0.00024 mg/kg)	9/24	1/15	-	1/15	-	-	-	1/15	-	-	1/15	-	-	-	-	1/15
1000 ng/mL (0.00245 mg/kg)	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix E. Four FCs microinjected at higher doses raw malformation data tables.

Abbreviation: **YSE** = Yolk Sac Edema, **NC** = Notochord, **C-Fin** = Caudal Fin, **P-Fin** = Pectoral Fin, **PE** = Pericardial Edema, **TR** = Touch Response **HD** = Hatch Delay

Table E. 1. PFDA microinjected at higher doses raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR	HD
0 ng/mL (0.1% DMSO)	6/24	1/18	-	-	-	-	-	1/18	-	-	-	-	-	-	-	1/18	-
12.5 ng/mL (0.03 mg/kg)	5/24	3/19	1/19	1/19	-	1/19	1/19	2/19	1/19	-	1/19	1/19	1/19	1/19	-	4/19	-
25.0 ng/mL (0.06 mg/kg)	3/24	5/21	1/21	1/21	-	1/21	1/21	3/21	1/21	-	1/21	-	-	-	-	-	-
50.0 ng/mL (0.12 mg/kg)	5/24	3/19	-	-	-	-	-	4/19	-	-	-	-	-	-	-	-	-

Table E. 2. PFHxA microinjected at higher doses raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR	HD
0 ng/mL (0.1%DMSO)	4/24	2/20	-	-	-	-	2/20	2/20	2/20	-	2/20	2/20	2/20	2/20	-	2/20	-
7.5 ng/mL (0.018 mg/kg)	10/24	3/14	1/14	2/14	-	1/14	-	2/14	-	-	-	-	3/14	1/14	-	1/14	-
15.0 ng/mL (0.036 mg/kg)	8/24	6/16	2/16	2/16	-	1/16	2/16	2/16	1/16	-	1/16	2/16	2/16	2/16	-	3/16	-
30.0 ng/mL (0.073 mg/kg)	4/24	6/20	-	-	-	-	-	-	-	-	-	-	-	-	-	1/20	-

Table E. 3. PFOA microinjected at higher doses raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR	HD
0 ng/mL (0.1% DMSO)	3/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0 ng/mL (0.024 mg/kg)	5/24	3/19	-	-	-	-	-	3/19	-	-	-	-	-	-	-	-	-
20.0 ng/mL (0.049 mg/kg)	9/24	4/15	-	4/15	-	-	-	4/15	4/15	-	3/15	2/15	3/15	2/15	-	3/15	-
40.0 ng/mL (0.098 mg/kg)	10/24	1/14	-	-	-	-	-	4/14	-	-	1/14	1/14	1/14	-	-	1/14	-

Table E. 4. PFOS microinjected at higher doses raw malformation data.

Concentration	Mortality	YSE	Trunk	Axis	NC	C-Fin	P-Fin	PE	Brain	Somite	Head	Eye	Snout	Jaw	Otic	TR	HD
0 ng/mL (0.1% DMSO)	2/24	3/22	-	-	-	-	-	-	-	-	-	-	-	-	-	1/22	-
12.5 ng/mL (0.03 mg/kg)	19/24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25.0 ng/mL (0.06 mg/kg)	11/24	1/13	1/13	1/13	-	1/13	1/13	3/13	1/13	-	1/13	1/13	1/13	1/13	-	1/13	-
50.0 ng/mL (0.12 mg/kg)	13/24	2/11	-	-	-	-	-	6/11	-	-	-	-	-	-	-	-	-