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Prefertilization Exposure of Rainbow Trout Eggs to Per- and Polyfluoroalkyl Substances to Simulate Accumulation During Oogenesis

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Abstract: Aqueous film–forming foams (AFFFs) are used in firefighting and are sources of per- and polyfluoroalkyl substances (PFAS) to the environment through surface runoff and groundwater contamination at defense and transportation sites. Little is known regarding the toxicity and bioaccumulation of newer AFFF formulations containing novel PFAS. To mimic maternal transfer of PFAS, prefertilization rainbow trout eggs were exposed to three PFAS using novel methodologies. Batches of unfertilized oocytes were exposed for 3 h to 0, 0.01, 0.1, 1, or 10 μg/ml separately to perfluorooctanoic acid, perfluorohexanoic acid, or perfluorooctanesulfonic acid in either coelomic fluid or Cortland's solution. After exposure, the gametes were fertilized and rinsed with dechlorinated water. Egg yolk was aspirated from a subset of fertilized eggs for PFAS quantification. Each PFAS was detected in yolks of eggs exposed to the respective PFAS, and yolk concentrations were directly proportional to concentrations in aqueous media to which they were exposed. Exposure in coelomic fluid or Cortland's solution resulted in similar concentrations of PFAS in egg yolks. Ratios of PFAS concentrations in oocytes to concentrations in exposure media (oocyte fluid ratios) were <0.99 when exposed from 0.01 to 10 μg/ml and <0.45 when exposed from 0.1 to 10 μg/ml for both media and all three PFAS, demonstrating that the water solubility of the chemicals was relatively great. Prefertilization exposure of eggs effectively introduced PFAS into unfertilized egg yolk. This method provided a means of mimicking maternal transfer to evaluate toxicity to developing embryos from an early stage. This method is more rapid and efficient than injection of individual fertilized eggs and avoids trauma from inserting needles into eggs. *Environ Toxicol Chem* 2021;40:3159–3165. © 2021 SETAC

Keywords: Aqueous film–forming foams; Oocytes; Fish; Salmonid; Yolk; Development

INTRODUCTION

Per- and poly-fluorinated substances (PFAS) are synthetic chemicals that were first produced in the 1940s and became recognized contaminants of concern at the turn of the 20th century (Lindstrom et al., 2011). Thousands of chemicals are included in this class, of which perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the best known. Mainly because of the strength of the carbon–fluorine bond, PFAS are thermally and chemically stable and resistant to environmental degradation (Post et al., 2012; Renner, 2001; Sungur, 2018). The physical and chemical characteristics of this class of compounds have made it difficult to interpret their environmental distribution, based on historical knowledge about neutral organochlorine chemicals (Lindstrom et al., 2011). They are not considered volatile, and exposure is expected to be orally via food or water (Sungur, 2018). Abundances of PFAS in the environment, especially aquatic ecosystems, combined with their persistence and toxic potencies have led to increased awareness and public concern (Giesy & Kannan, 2002; Renner, 2001). Decreased production and releases to the environment of some of the most significant PFAS, such as PFOS and PFOA, have resulted from more stringent national and international regulations, with PFOS being listed for virtual elimination on Appendix B of the Stockholm Convention on Persistent Organic Pollutants (United Nations Environmental Programme, 2001). Presently,

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87 chemicals that can degrade to PFOS are banned in Canada (Government of Canada, 2016).

Aqueous film–forming foams (AFFFs) are used in firefighting and are a major source of PFAS to the environment. This is particularly so in the habitat of Arctic char (*Salvelinus alpinus*), a salmonid of cultural and economic significance in Canada's north, where military and other aviation facilities are the most significant sources of PFAS. These products are commonly found associated with transportation and defense facilities or airports. Fluorotelomerization is most commonly used to create PFAS found in AFFFs that are ostensibly less toxic, less persistent, and less likely to bioaccumulate than their sulfonic acid analogues (Seow, 2013). However, there is very little known about the potential effects and bioaccumulation of newer PFAS and their AFFF formulations, which can contain mixtures of many PFAS. It is thought that AFFFs likely enter the environment through runoff at airports and military facilities following firefighting or training exercises (Seow, 2013). Groundwater contamination has been regularly described at military bases throughout the world, including the United States (McGuire et al., 2014; Moody & Field, 2000) and Sweden (Filipovic et al., 2015); but information about PFAS concentrations at sites in Canada is scarce. Accumulation of PFAS in biota of northern Canada is of particular concern because there is the potential for contamination of sensitive aquatic ecosystems in proximity to airports and defense facilities. The potential for PFAS to impact populations of Arctic char is the main focus of the current studies.

Early-life stages of fish are considered the most sensitive to adverse effects caused by exposure to chemicals (Embry et al., 2010; Lillicrap et al., 2016) compared to other life stages and are most likely to be affected by PFAS contamination. The chorion of fish eggs becomes a barrier to many chemicals once fertilization and water hardening have taken place, and in ovo exposure of embryos might not occur until after hatch (Lillicrap et al., 2016). Maternal contributions to egg yolk would be expected from fish exposed to PFAS passing those PFAS to the egg yolk. Therefore, embryonic exposure to PFAS would occur mainly during early developmental stages, while the embryo is in the egg and utilizing these yolk reserves. Some of these stages of development might be sensitive to effects of PFAS if exposed. For these reasons, the most appropriate vector of exposure is through maternal deposition during oogenesis. However, this can be logistically difficult to achieve. One method of exposure is long-term exposures during which adults exposed to PFAS could accumulate PFAS over the gills or through the diet. Such studies are cost- and resource-intensive and especially difficult to do with some species that reproduce only once per year such as salmonids and that need to be retained in the laboratory for long periods of time before reaching maturity. An alternative for exposure of early-life stages to known graded doses of contaminants such as PFAS is nano-injection (Y. B. Li et al., 2015; Villalobos et al., 2003). However, injecting individual eggs after fertilization generally requires removing the chorion and allows for only small numbers of eggs to be injected. Thus, this is a very time-consuming process. Furthermore, the insertion of the needle can cause significant trauma to the egg.

To address these limitations, a method of exposure was applied in the present study that mimicked maternal deposition of PFAS to larger numbers of eggs and could be used to determine effects of PFAS during early in ovo development. The method was designed to deliver predictable amounts of PFAS into the yolk of eggs prior to fertilization and water hardening, to provide a biologically relevant exposure route without the need for long-term exposure of adults. To validate the exposure method, gametes of a representative salmonid, rainbow trout (*Oncorhynchus mykiss*), were exposed to three representative PFAS, including perfluorohexanoic acid (PFHxA), PFOA, and PFOS in a proof-of-concept study, to better examine the methodology, make the comparison with other references, and guide the exposure of novel PFAS in future studies. The objective of the study was to expose rainbow trout eggs to PFAS prior to fertilization using an existing method of elevating yolk hormone concentrations (Raine et al., 2004; Raine & Leatherland, 2003).

MATERIALS AND METHODS

Rainbow trout gametes, pooled from three males and four females, were obtained from Troutlodge (Sumner, WA, USA). Nominal concentrations of 0.01, 0.1, 1, and 10 µg/ml PFOA, perfluorohexanoic acid (PFHxA), and PFOS were dissolved separately, in either the coelomic fluid associated with the oocytes or in Cortland's solution. These chosen concentrations were meant not to simulate concentrations in the environment to which eggs might be exposed but rather to facilitate accumulation of PFAS to concentrations that might be accumulated into eggs via dietary exposure of females during oogenesis. Coelomic fluid was separated from oocytes using a plastic strainer. Cortland's solution contains 124.1 mM NaCl, 5.1 mM KCl, 1.0 mM $MgSO_4 \cdot 7H_2O$, 1.6 mM $CaCl_2 \cdot 2H_2O$, 5.6 mM glucose, and 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid with pH adjusted to 8.5. The standards of PFHxA, PFOA, and PFOS used for exposure were all from SynQuest Laboratories with purity >97%, while other chemicals were all from Sigma. Each exposure was replicated three times, and each replicate was held in a 6-well plate containing 60 unfertilized oocytes. Exposed as well as unexposed eggs were incubated for 3 h at 6 °C. This temperature was chosen because subsequent studies were to include Arctic char and it is between the optimal rearing temperatures of Arctic char and rainbow trout. At the conclusion of the 3-h incubation period, oocytes from each treatment were fertilized with pooled milt and then rinsed thoroughly with 4 ml of dechlorinated facility water at 6 °C. Twenty fertilized eggs from each triplicate treatment were placed in a Petrie dish, and the yolk was aspirated to a microcentrifuge tube, using previously described methods (Raine & Leatherland, 2003). Quantifying PFAS in yolk minimized interference from PFAS bound externally to the egg and allowed the internal dose to be determined as a more realistic estimate of what developing embryos would be exposed to in ovo. Furthermore, PFAS, such as PFOS and PFOA, are known to preferentially bind to proteins, such as those in yolk, rather than

partition into lipids (Jones et al., 2003). The remaining exposure solutions and yolk samples were stored at –20 °C until concentrations of PFAS could be determined.

Methods for identifying and quantifying perfluorinated compounds followed previous methods (Naile et al., 2010) with a few modifications. In brief, 200 µl of freeze-dried yolk samples were fortified with internal standards (Wellington Laboratories) and then extracted at room temperature using 2 ml 0.01 N KOH in methanol for 16 h. One milliliter of supernatant was diluted with 100 ml of H_2O before being loaded onto a preconditioned solid-phase extraction cartridge (Waters WAX; 6 cc, 150 mg, preconditioned with 4 ml 0.1% NH_4OH in methanol, methanol, and water, sequentially). The cartridges were then washed with 4 ml of 25 mM ammonium acetate (pH 4) buffer, and target PFAS were eluted by using 4 ml of 0.1% NH_4OH in methanol. Eluents were concentrated to 1 ml under high-purity nitrogen and filtered through a polypropylene filter before quantification by liquid chromatography (LC)–mass spectrometry. As previously described, 50–100 µl of exposure solutions prepared in coelomic fluid were freeze-dried and then extracted, and 40–100 µl of exposure solutions prepared in Cortland's solution were directly diluted with 100 ml H_2O and then extracted.

The LC (Vanquish UHPLC; Thermo Scientific) equipped with the Betasil C18 column (2.1 × 100 mm, 5 µm; Thermo Scientific) and a solvent trapping Betasil C18 column (2.1 × 10 mm, 3 µm; Thermo Scientific) was run at 40 °C with a flow rate of 0.3 ml/min, starting with 90% A (2 mM ammonium acetate) and 10% B (methanol); ramping from the initial state at 2 min to 30% B at 2.1 min, then to 75% B at 9 min, and to 100% B at 12 min; keeping for 2 min; returning to the initial state at 22 min; and keeping for another 5 min. The LC was interfaced to an ultrahigh-resolution mass spectrometer (Q Exactive HF; Thermo-Scientific). The electrospray ionization source was run in negative ionization mode. Parallel reaction monitoring was used for qualitative analysis, and simultaneously a full-scan analysis was used for quantitative analysis.

Limits of detection were defined as three times the standard deviation (SD), which is derived from the variance (2, 10, 50 ng/ml quantified five times, respectively) extrapolated to a concentration of zero (σ^2_0; Taylor, 1987). Method detection limits (MDL) for PFAS in egg yolk and Cortland's solution were 0.747 ng PFHxA/ml, 0.282 ng PFOA/ml, and 0.888 ng PFOS/ml, while they were 1.49 ng PFHxA/ml, 0.564 ng PFOA/ml, and 1.78 ng PFOS/ml in coelomic fluid. Procedural blanks, run every 10 samples, were subtracted from the results. Recoveries of PFOA, PFOS, and PFHxA spiked into sample matrices ranged from 80 to 120%. Recoveries of isotopically labeled surrogates ranged from 70 to 120%. Ratios of concentrations of individual PFAS in egg yolk to that in the exposure media, coelomic fluid (oocyte fluid ratio [OFR_{CF}]), or Cortland's solution (OFR_{CS}) were determined (Equation 1).

$$\text{OFR} = \text{PFAS conc. in yolk (ng/ml)}/\text{actual PFAS conc. in exposure media (ng/ml)} \quad (1)$$

All statistical analyses were performed using IBM SPSS Statistics. Data are expressed as means ± SD. Any probability (p) for a type I error (α) <0.05 was considered to be statistically significant. Concentrations of PFAS in yolk were tested to determine if they met assumptions of normality and homogeneity of variance using the Shapiro-Wilks test and Levene's test, respectively. If data met these assumptions, one-way analysis of variance (ANOVA) was used to test for significant differences among concentrations of PFAS in various treatments, followed by Tukey's test for equal sample size or Scheffe's test for unequal sample size to check for differences between pairs of treatments or between a treatment and a control. If concentrations of PFAS in yolk did not meet the assumptions of normality or homogeneity of variance, individual values were transformed by ln (X + 1) to more closely meet requirements for assumptions of parametric tests. Before transformation, PFHxA in the yolk of oocytes exposed via Cortland's solution met assumptions of normality and homogeneity of variance, while after transformation, PFHxA, PFOA, and PFOS in the yolk of oocytes exposed using coelomic fluid all met assumptions of normality and homogeneity of variance, so one-way ANOVA (α = 0.05) with Tukey's or Scheffe's test was conducted. Even after transformation, PFOA and PFOS in yolk of oocytes exposed using Cortland's solution did not meet the assumptions of a parametric test, so the Kruskal-Wallis test with all pairwise comparisons was conducted on these data.

RESULTS

Each of the three PFAS—PFOS, PFOA, and PFHxA—was detected in the exposure solutions and the yolk of eggs exposed to the corresponding PFAS (Table 1 and Figures 1 and 2). Concentrations of the three PFAS in exposure solutions were similar for both coelomic fluid and Cortland's solution, and most were consistent with expected nominal concentrations (Table 1).

The three PFAS were accumulated into yolk in a concentration-dependent manner using either of the two media (Figures 1 and 2). Concentrations of PFHxA and PFOA in yolk were comparable using both coelomic fluid and Cortland's solution. When exposed to 10 µg PFHxA/ml or 10 µg PFOA/ml in coelomic fluid, concentrations in egg yolk were 2.12 ± 0.68 µg PFHxA/ml and 2.81 ± 0.28 µg PFOA/ml (Figure 2A). Similarly, exposure to 10 µg/ml in Cortland's solution resulted in concentrations of 2.54 ± 0.72 µg PFHxA/ml and 4.59 ± 4.51 µg PFOA/ml in yolk (Figure 2B). Because of the lower actual PFOS concentrations in exposure media, the mean concentration accumulated into egg yolk from coelomic fluid was 0.28 ± 0.21 µg PFOS/ml, while that accumulated from Cortland's solution was 0.41 ± 0.35 µg PFOS/ml (Figure 2) at the nominal 10 µg/ml exposure concentration.

Slopes determined from linear regressions of measured concentrations of PFOS, PFOA, and PFHxA in yolk of rainbow trout oocytes (log-transformed) exposed to measured concentrations of the PFAS in coelomic fluid (log-transformed) were 0.808, 1.513, and 0.983, respectively (Figure 1A). Similarly, exposure of trout oocytes to the same treatment concentrations in Cortland's solution also resulted in elevated yolk levels of PFOS, PFOA, and PFHxA (Figure 1B). Slopes of accumulation of PFOS, PFOA, and PFHxA were 0.566, 1.224,

TABLE 1: Measured concentrations of the three per- and polyfluoroalkyl substances in the two different media used to prepare the exposure solutions

Medium	Treatment chemical	Nominal concentration (μg/ml)	Measured concentration (μg/ml)
Coelomic fluid	PFHxA	0	ND
		0.01	0.01
		0.1	0.12
		1.0	2.79
		10	10.63
	PFOA	0	ND
		0.01	0.08
		0.1	0.67
		1.0	2.12
		10	10.58
	PFOS	0	ND
		0.1	0.07
		1.0	1.20
		10	5.76
Cortland's solution	PFHxA	0	<MDL
		0.01	0.01
		0.1	0.10
		1.0	0.96
		10	10.83
	PFOA	0	<MDL
		0.01	0.01
		0.1	0.11
		1.0	1.11
		10	10.11
	PFOS	0	<MDL
		0.01	0.01
		0.1	0.06
		1.0	0.87
		10	7.47

PFHxA = perfluorohexanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; ND = not done; MDL = method detection limit.

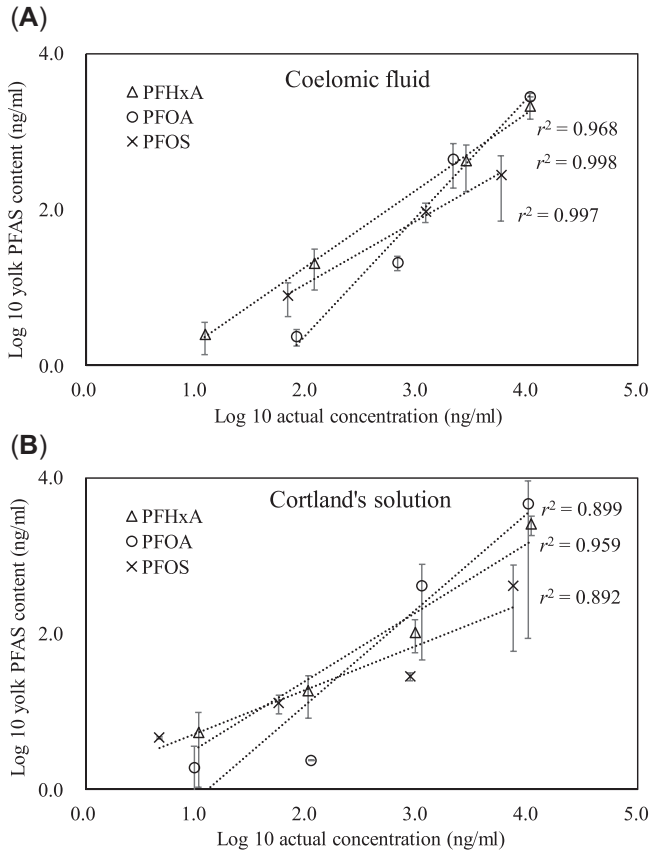

FIGURE 1: Linear relationship between perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexanoic acid (PFHxA) in (**A**) coelomic fluid or (**B**) Cortland's solution and in the egg yolk. When exposed in coelomic fluid, equations for PFHxA, PFOA, and PFOS were $y = 0.983x - 0.694$ ($R^2 = 0.998$, $p < 0.05$), $y = 1.513x - 2.623$ ($R^2 = 0.968$, $p < 0.05$), and $y = 0.808x - 0.561$ ($R^2 = 0.997$, $p < 0.05$), respectively; and when exposed in Cortland's solution, equations for PFHxA, PFOA, and PFOS were $y = 0.879x - 0.354$ ($R^2 = 0.959$, $p < 0.05$), $y = 1.224x - 1.351$ ($R^2 = 0.899$, $p = 0.052$), and $y = 0.566x + 0.154$ ($R^2 = 0.892$, $p = 0.055$), respectively. PFAS = per- and polyfluoroalkyl substances.

and 0.879, respectively. Linear relationships between concentrations of PFAS in yolk and in exposure media were similar between exposures to coelomic fluid and Cortland's solution for all three PFAS (Figure 1; $p > 0.05$).

Concentrations of PFAS in yolk varied among treatments. Concentrations of PFHxA and PFOA in yolks of controls were less than the respective MDLs for both exposure media. Concentrations of PFHxA and PFOA in yolk of oocytes exposed in coelomic fluid were all significantly different among treatments ($p < 0.05$; Figure 2A). Concentrations of PFOS in yolk of oocytes exposed to 1 or 10 μg/ml in coelomic fluid were not significantly different from one another but were significantly different from concentrations in yolks of unexposed control oocytes and those exposed to 0.1 μg/ml ($p < 0.05$; Figure 2A). Concentrations of PFHxA in yolk of oocytes exposed using Cortland's solution exhibited no significant differences between those exposed to 0.01, 0.1, or 1 μg/ml (Figure 2B), while all these concentrations were significantly ($p < 0.05$) different from those exposed to 10 μg/ml (Figure 2B). There were no significant differences among concentrations of PFOA in oocytes exposed to any concentrations of PFOA via Cortland's solution (Figure 2B). Concentrations of PFOS in yolk of oocytes exposed using Cortland's solution exhibited a significant ($p < 0.05$) difference between control and those exposed to 10 μg/ml treatment (Figure 2B). The SDs were derived from the three replicates of exposure, causing the large coefficient of variation.

The OFRs for all three PFAS and both coelomic fluid and Cortland's solution, with all OFR values <0.99 when exposed from 0.01 to 10 μg/ml and <0.45 when exposed from 0.1 to 10 μg/ml (Table 2), were small compared to what would be expected for neutral organochlorine compounds. The OFRs calculated for PFOS were generally less than those for PFOA and PFHxA accumulated from either coelomic fluid or Cortland's solution (Table 2). Exceptions to this were observed for the lesser concentrations of PFAS.

Because of impurities in the commercial PFAS used in the present study, PFHxA was detected in yolk samples of eggs that were not exposed to PFHxA. When eggs were exposed to coelomic fluid containing 10 μg PFOA/ml, 2.65 ± 0.41 ng PFHxA/ml was detected in the yolk. When eggs were exposed to Cortland's solution containing 10 μg PFOA/ml, 2.00 ± 2.11 ng PFHxA/ml was detected in the yolk. Also, PFHxA and PFOA were

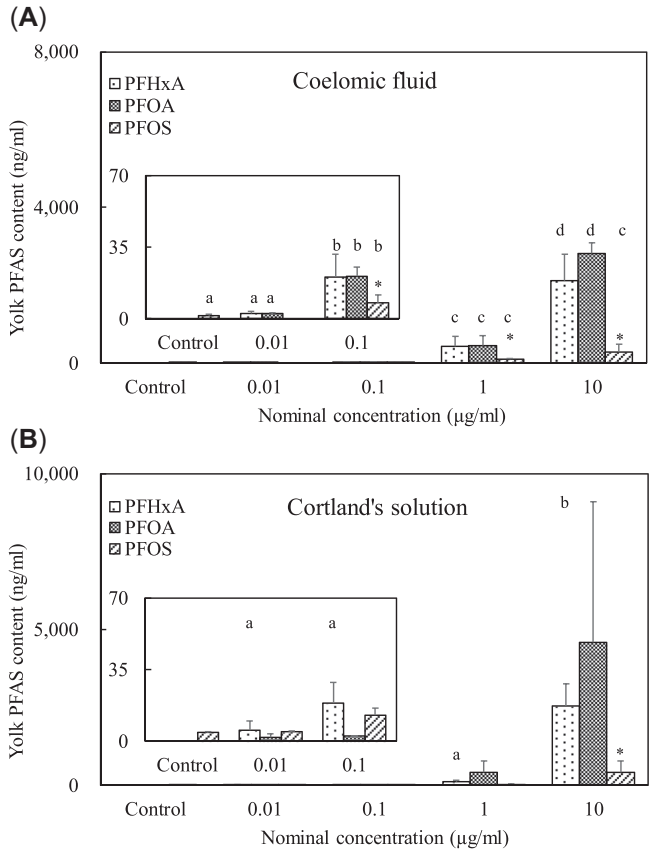

FIGURE 2: Elevation of yolk concentrations of three different per- and polyfluoroalkyl substances (PFAS) using two different exposure media: (**A**) coelomic fluid and (**B**) Cortland's solution. Inset graphs provide increased magnification of low yolk PFAS concentrations. Mean ± standard deviation. Significant groups listed as a, b, c, and d mean that the yolk PFAS content in treatments of the same significant group was not significantly different ($p > 0.05$) while being significantly different ($p < 0.05$) from the PFAS content in treatments of other significant groups. *Significantly different ($p < 0.05$) from the control group. Perfluorooctanoic acid and perfluorohexanoic acid in the yolk of controls were less than the method detection limits for both exposure media. PFHxA = perfluorohexanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid.

TABLE 2: Oocyte fluid ratio for each of the three per- and polyfluoroalkyl substances using coelomic fluid or Cortland's solution for oocyte exposures

Medium	Nominal treatment concentration (μg/ml)	Oocyte fluid ratio		
		PFHxA	PFOA	PFOS
Coelomic fluid	0.01	0.21	0.03	ND
	0.1	0.17	0.03	0.12
	1	0.15	0.21	0.08
	10	0.20	0.27	0.05
Cortland's solution	0.01	0.51	0.20	0.99
	0.1	0.18	0.02	0.23
	1	0.11	0.37	0.03
	10	0.23	0.45	0.06

PFHxA = perfluorohexanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctanesulfonic acid; ND = not done.

detected in exposure media that were not supposed to contain such chemicals (Supporting Information, Table S1).

DISCUSSION

The results of the present study suggest that PFAS can be introduced into the yolk of unfertilized salmonid eggs prior to fertilization to mimic maternal transfer of these contaminants. This suggests that this method of exposure is a viable and practical procedure to study in ovo developmental effects of PFAS on large-bodied species of fish. This exposure method has been used previously to deliver thyroid hormones, cortisol, and bisphenol A into prefertilization rainbow trout oocytes using coelomic fluid as the exposure medium (Aluru et al., 2010; Birceanu et al., 2015; Coffin et al., 2012; M. Li et al., 2010; Raine et al., 2004, 2011; Raine & Leatherland, 2003). Coelomic fluid is difficult to obtain in quantity, and its association with the oocytes makes it difficult to prepare treatment solutions in advance of an experiment. Because they bind to circulating plasma proteins, PFAS tend to accumulate in the blood and liver (Giesy & Kannan, 2001; Jones et al., 2003; Martin et al., 2003). Teleost blood has a similar inorganic composition to salmonid coelomic fluid and Cortland's solution, and the latter has been used in tissue culture and for storage of salmonid oocytes (Goetz & Coffman, 2000; Lahnsteiner et al., 1995; Wolf & Quimby, 1969). Fertility of rainbow trout oocytes and survival to the eyed stage of development were the same or better following storage in Cortland's solution when compared to storage in coelomic fluid or fertilization prior to storage (Goetz & Coffman, 2000). Coelomic fluid has been shown to be an effective delivery medium in this and other studies (Raine et al., 2004, 2011; Raine & Leatherland, 2003), but Cortland's solution provides a logistically simpler medium to use and appears to work quite successfully in this application.

Although all three PFAS were successfully introduced into eggs using this exposure method, lower concentrations of PFOS were found in the eggs compared to PFHxA and PFOA. Considering that the realistic exposure concentrations of PFOS were lower than the nominal ones and that the linear regression slope of PFOS was similar to the other two PFAS, the lower PFOS yolk concentrations are possibly due to lower exposure concentrations; but the accumulation is possibly similar to that of PFHxA and PFOA. Further studies including a wider range of exposure concentrations are needed to demonstrate this. Low OFR values determined for each of the PFAS using the two different exposure media are consistent with water solubilities of these chemicals and likely their relatively low levels of accumulation in the lipid-rich yolk of the oocytes.

Evidence in the literature suggests that PFAS enter eggs primarily through maternal transfer during oogenesis. Adult zebrafish exposed to PFOS exhibited lesser survival of larvae, embryonic growth, and development in a transgenerational study (M. Y. Wang et al., 2011). Wild fish contained PFOS in eggs that likely resulted from maternal transfer (Kannan et al., 2005). Radiolabeled PFOA showed high adsorption to the oocyte chorion and none in the yolk of immature previtellogenic

oocytes, while mature vitellogenic oocytes contained the radiolabeled chemical throughout (Ulhaq et al., 2015). It is likely that PFAS are incorporated into the maturing oocytes during vitellogenesis and bound to vitellogenin and other yolk proteins. The PFOA observed on the chorion in that study was likely binding to eggshell proteins (Ulhaq et al., 2015). The prefertilization method used in the present study is not the natural means by which PFAS would be expected to enter the yolk of developing oocytes. However, this method does allow accumulation of PFAS early in development without the need for long-term exposure of adult females to accumulate PFAS and deposit them into the yolk of eggs. In birds, PFOS accumulates mainly in the yolk of the eggs, and it was anticipated that PFAS in salmonid eggs would most likely be present bound to proteins in the yolk (Custer et al., 2010; Y. Wang et al., 2008).

Relatively large concentrations of PFAS in solution were needed to drive the chemicals into the egg, as found previously with thyroid hormone oocyte experiments (Raine & Leatherland, 2003). Three hours was chosen as the optimal exposure time and appears appropriate for the current exposure, although the duration of exposure could be increased to try to further elevate PFAS concentrations in the yolk. At all times it must be remembered that when dealing with unfertilized gametes, lengthening the time before fertilization and water hardening decreases fertilization success. Concentrations of individual PFAS reported in the present study represent those in yolk after fertilization, water hardening, and rinsing of the oocytes with dechlorinated water. During water hardening the egg absorbs water, and the chorion hardens (Blaxter, 1988). It is possible that there is loss of PFAS during this process, but certainly there will be some dilution. Artificially elevated concentrations of thyroid hormone in yolk were observed to decrease after fertilization and water hardening (Raine et al., 2004; Raine & Leatherland, 2003). However, the goal of the present study was to mimic the maternal contribution of PFAS to the yolk by accumulating PFAS into yolk, so this method was considered the most biologically relevant means of doing so. It would be expected that if this is the case, there would likely be a similar loss of PFAS during fertilization and water hardening in wild fish as well. Furthermore, it was anticipated that PFAS could potentially adhere to the outside of the chorion during the exposure and not enter the yolk of the egg. In this case, measurement of PFAS in whole eggs could provide positively biased concentrations of PFAS, which would represent extraneous and biologically unavailable values that would not be representative of what the embryos would be exposed to in the egg. Thus, the eggs were thoroughly rinsed and the yolk was removed from the eggs and analyzed separately. Similarly, it might be that PFAS bind directly to the chorion of the oocyte (Ulhaq et al., 2015). This component of egg PFAS content would likely not be biologically available to the developing embryo either, and this should be considered if egg PFAS content is used for monitoring or risk assessment of PFAS contamination.

The use of biologically relevant exposure methods for evaluation of environmental contamination is critical to understanding potential toxicity and adverse effects on aquatic species. This is especially important when determining the sensitivity of early developmental stages of fish to chemicals that can accumulate in reproductively mature fish and be transferred to their offspring in the yolk. The results of the present study demonstrated that the method of exposure provides an effective means of delivery of PFOS, PFOA, and PFHxA in particular, and likely other PFAS, into the yolk of prefertilization oocytes. Use of an artificial coelomic fluid provides a much more consistent and logistically simpler procedure for chemical delivery and oocyte incubation. This technique can be an effective means of providing a biologically relevant exposure to early developmental stages of salmonid fishes for evaluation of toxicity and adverse outcomes to predict the effects of environmental exposure.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5200.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (canons_sue@126.com).

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