보건학석사학위논문

Effects of acute and chronic exposure to perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (PFTrDA) in *Daphnia magna* and Zebrafish (*Danio rerio*) with endocrine disruption potential

과불화화합물 perfluorodecanoic acid (PFDA)와 perfluorotridecanoic acid (PFTrDA)의 만성노출에 의한 수서생물 *Daphnia magna* 와 *Danio rerio* 의 내분비계 교란독성

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서울대학교 보건대학원

환경보건학과 환경보건 전공

조 아 름

Abstract

Perfluorolalkyl acids (PFAAs) have been frequently detected in both the environment and biota, however the endocrine disruption potentials and underlying mechanism of long-chain PFAAs have not yet been fully understood. In the present study, the effects of perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (PFTrDA) on *Daphnia magna* and sex steroid hormones and expression of mRNA of selected genes in hypothalamus-pituitary-gonad (HPG) axis were evaluated after 120 d exposure of zebrafish. To investigate underlying mechanism of endocrine disruption, production of sex hormones and expression of steroidogenic genes were measured after *in vitro* exposure of H295R cells for 48 h. Exposure to PFTrDA resulted in less production of testosterone (T) along with lesser expression of *CYP17A* mRNA by H295R cells. PFDA and PFTrDA affected growth and delayed the time required for reproduction of the first brood of *D. magna*, respectively. *CYP19A* and *VTG1* genes were up-regulated in male fish by exposure to PFDA and PFTrDA. In male zebrafish, concentrations of 11-ketotestosterone **(**11-KT) and T were significantly decreased at 0.1 mg/L PFTrDA. Significantly increased ratios of 17β-estradiol (E2)/T and E2/11-KT were observed in male zebrafish after exposure to PFDA and PFTrDA, indicating balance of sex hormones were disrupted, which could further result in adverse effects on reproduction in fish. The results of this study showed that long term exposure to PFDA and PFTrDA could affect sex steroid hormones and related gene transcriptions. The predicted no effect concentrations (PNEC) derived based on the toxicity values from the present study and literature were 0.1 mg/L PFDA and 0.2 μg/L PFTrDA respectively, suggesting that

long chain perfluoroalkyl carboxylic acid could pose negligible risk for aquatic organisms.

Keywords

endocrine disruption, gene transcription, perfluoroalkyl acids, sex hormones, HPG axis

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1. Introduction

Perfluoroalkyl acids (PFAAs) have been used in surfactants, lubricants, fire fighting foams, and indoor application such as protective coatings, fabrics, and carpets over the past 50 years (Key et al., 1997). The production of perfluorooctyl sulfonyl fluoride, a major precursor of perfluorooctane sulfonic acid (PFOS), was voluntarily phased out in 2002, however many PFAAs are still produced by manufacturers (Prevedouros et al., 2006). Several PFAAs, in particular perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), have received great attention due to their persistence in the environment (Naile et al., 2010), bioaccumulation potential (Martin et al., 2003), and possible adverse effects on human and wildlife (Lau et al., 2007). Consequently, PFOS, one of the most important PFAAs, has been added to the list of persistent organic pollutants (POPs) of the Stockholm Convention on POPs (2009).

PFAAs have been globally found in aquatic environment, wildlife, and human samples. One of the long chain PFCAs, perfluorodecanoic acid (PFDA, C_{10} PFCA), has been detected at concentrations of 0.23-15.4 ng/L in west coast of Korea (Naile et al., 2010), 3.74-160 ng/L in the Conasauga River in USA nearby carpet industry (Konwick et al., 2008), and 0.13-0.66 ng/L from Baiyangdian lake water in China (Shi et al., 2012). In Australia, the median concentration of 1.2 ng/L of PFDA and <0.1 ng/L of perfluorotridecanoic acid (PFTrDA, C_{13} PFCA) were detected in Parramatta river (Thompson et al., 2011). Previous temporal trend studies have observed a significant decreasing trend of some PFSAs and perfluorooctane sulfonamide, but continuously increasing trend of PFCAs (Ahrens et al., 2009; Hart et al., 2008; Holmström et al., 2010). For example, temporal trends (1974-2007) of PFCAs concentrations in eggs of peregrine falcon

were increased over the entire study period, while PFOS leveled off after the mid 1980s (Holmström et al., 2010).

Most toxicity studies of PFAAs have concentrated on PFOS and perfluorooctanoic acid (PFOA), hence limited information is currently available on the toxicological effects and risk of PFCAs with longer chains than PFOA. In relation to toxicity effects of PFDA, most investigations have been limited to lethal effects during acute exposures (Ding et al., 2012; Hoke et al., 2012). It has been reported that perfluorononanoic acid (PFNA, C_9 PFCA) could induce thyroid-disrupting effects and trans-generational effects by long-term sub-lethal exposure (Liu et al., 2011). Since half-lives and uptake rates increased with increasing perfluoroalkyl chain length until the length reached 13 carbons (Martin et al., 2003), studies on the sub-lethal consequences of long-term exposure to long chain PFCAs in aquatic organisms are needed.

Apart from the general toxic effects of PFAAs, several studies on PFCAs have revealed their endocrine-disrupting properties. For example, studies conducted on fish have revealed that *in vivo* PFOA exposure can alter circulating sex steroid hormone levels, induce expression of estrogen-responsive genes and vitellogenin gene in mature males, and even lead to decrease in overall egg production (Oakes et al., 2004; Wei et al., 2007). In addition, PFOA, PFNA, PFDA, and perfluoroundecanoic acid (PFUnDA, C_{11} PFCA) were all potent inducers of vitellogenin in juvenile rainbow trout, and weakly bound to estrogen receptor *in vitro* (Benninghoff et al., 2011). The fluorotelomer alcohols (FTOHs), which are widely used as precursor compounds in the manufacturing of PFCAs alter plasma sex hormone and gene transcription in the hypothalamic–pituitary–gonadal (HPG) axis and consequently impair reproductive success of zebrafish (Liu et al., 2009, 2010). These studies indicated the estrogenic activities of PFCAs, and thus

it is speculated that exposure to long chain PFCAs may cause endocrine-disrupting effects.

In the present study, we investigated the endocrine disrupting effects of PFDA and PFTrDA and their underlying mechanisms using *in vitro* H295R cell-based bioassay and *in vivo Daphnia* and fish assay. In the *in vitro* assay, production of the steroid hormones (17β-estradiol (E2) and testosterone (T)) and transcription of genes involved in steroidogenic pathways were examined in H295R cells. In the *in vivo* assay, survival, reproduction, and growth effects on *Daphnia magna* after chronic exposure, and effects on levels of sex steroid hormone and mRNA expressions of specific genes in HPG axis in zebrafish (*Danio rerio*) were investigated. Finally, the predicted no effect concentrations (PNECs) of these compounds were estimated using data that were derived from the present study or obtained from available literature. The results of this study will be useful in developing risk management plant for long chain PFCAs in freshwater environment.

2. Materials and methods

2.1. Chemicals

PFDA (CAS No. 335-72-6, 98% purity) and PFTrDA (CAS No. 72629-94-8, 97% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Information on physical and chemical properties is provided in Table 1.

2.2. Daphnia magna culture and toxicity tests

D. magna were cultured in Elendt M4 media according to the U.S. Environmental Protection Agency (U.S. EPA) guidelines (2002). Daphnids were maintained at 21 ± 1^{o}C in 6 L glass jars, and were fed algae (*Pseudokirchneriella subcapitata*) daily. Water quality parameters, i.e., hardness, alkalinity, pH, conductivity, temperature, and dissolved oxygen, were routinely monitored following standard methods (American Public Health Association, 1992).

The 48 h acute toxicity tests were carried out following the method described by U.S. EPA (2002) with minor modification. Test concentrations were prepared by dissolving PFDA or PFTrDA in culture media with 10-fold serial dilution (0, 0.01, 0.1, 1, 10, and 100 mg/L). Four replicates with five neonates each (<24 h old) were exposed for 48 h. The number of immobilized organisms was counted after 24 and 48 h exposure.

The effects of chronic exposure to PFDA and PFTrDA on survival, reproduction, and growth in *D. magna* were assessed according to the OECD test guideline 211 (OECD, 2008) with minor modification. Test solutions were prepared in 4-fold serial dilution (0, 1.95, 7.81, 31.3, 125,

and 500 μg/L of PFDA, and 0, 0.78, 3.13, 12.5, 50, and 200 μg/L of PFTrDA). Each neonate of 10 replicates was exposed for 21 d at 21 ± 1°C. The test solution was renewed 3 times per week and newborn neonates were counted and removed daily. Mortality of parent *Daphnia* and the number of living offspring were recorded every day. At the end of the test, the body length of each *Daphnia* was measured. The population growth rate was calculated using the Euler-Lotka equation (Lotka, 1993).

2.3. Fish culture and exposure

Male and female adult zebrafish (> 3 month old) were acclimated in tanks filled with conditioned water (sodium bicarbonate, calcium sulfate, and sea salt in distilled water) for 2 weeks at 25 ± 1°C under a photoperiod of 16:8 hr light/dark. After the acclimation period, four male and six female fish were placed in a spawning aquarium (20 L filled in 15 L exposure water). *Artemia* nauplii (< 24 h after hatching) or *Chironomus* sp. were fed *ad libitum* twice daily. Water quality parameters, including pH, conductivity, temperature, and dissolved oxygen were measured routinely.

Fertilized eggs produced during the previous 24 h period were obtained from adult zebrafish pairs. Four replicates with 10 eggs each were exposed to various concentrations of PFDA and PFTrDA (0, 0.01, 0.1, 1, and 10 mg/L). Based on the preliminary 96 h acute toxicity test using larvae fish (11 day post fertilization (dpf)), we selected exposure concentrations for definitive tests. Exposure was initiated in 50 mL beakers before hatching, and dead embryos were removed as soon as possible. Newly hatched larvae were then transferred to 250 mL beakers and observed survival for an additional three weeks. On 8 dpf, we began to feed with *Chironomus* sp. four-times per day. Half of test solution was changed three times per week

and water quality parameters were checked at every water change. On 21 dpf, juvenile fish were transferred to 1 L beakers for the additional observation and then transferred to 3 L beakers from 60 dpf to end of experiment. Juvenile and adult fish were fed *Artemia* nauplii *ad libitum* twice per day. Mortality at larvae, juvenile, and adult was observed during the exposure period. All surviving fish were euthanized on 120 dpf, and body length and weight were measured for each fish. For hormone and gene expression analysis, three males and females per each replicate tank were randomly sampled from four replicate tanks of each PFDA or PFTrDA treatment.

2.4. H295R cell culture and exposure

The H295R human adrenocortical carcinoma cell line was obtained from the American Type Culture Collection (ATCC # CRL-2128, ATCC, Manasas, VA, USA) and cultured at 37^{o}C in a 5% CO_2 atmosphere as described by Kim et al. (2012). The effects on the hormone production and expression of mRNAs involved in steroidogenesis were investigated with H295R cell bioassays. To minimize the influence of cytotoxicity on H295R cells, viability was checked with an MTT bioassay (Mosmann, 1983), and non-cytotoxic doses (>80% of survival) were determined for evaluation of effects on hormone production and steroidogenic gene transcription. For compounds that were determined to affect steroid hormone production, the mechanisms of steroidogenic effect were investigated by measuring changes in expression of genes in the steroidogenic pathways in H295R cells. In brief, H295R cells were seeded into 24-well plates at a concentration of 3×10^5 cells/mL in 1 mL of medium per well. After 24 h, cells were exposed to PFDA (0, 0.1, 1, 10, and 100 mg/L) or PFTrDA (0,

0.05, 0.5, 5, and 50 mg/L) for 48 h, and culture medium and remaining cells were used for hormone measurements and gene transcription analysis, respectively.

2.5. Quantification of hormones

Extraction and quantification of hormones in the medium of H295R cells or plasma of zebrafish were performed following the method described elsewhere (Ma et al., 2012) with minor modification. Briefly, 500 μL medium was extracted twice with diethyl ether and solvent was evaporated under a gentle stream of nitrogen. The residue was dissolved in 120 μL enzyme-linked immunosorbent assay (ELISA) buffer and hormones in the medium were determined by ELISA (Cayman Chemical Company; Ann Arbor, MI; E2 (Cat No. 582251) and T (Cat No. 582701), following the manufacturer's instructions.

After exposure for 120 d, the tail of each zebrafish was transected, and blood was collected from caudal vein in a glass capillary tube. For measure hormone, each blood samples were collected in 1.7 mL eppendorf tube filled EIA buffer and stored at -80℃. Whole blood with 250 μL ELISA buffer was used for quantification of sex hormones. Whole blood samples were used due to the lack of sample volume. However, hormone concentrations did not statistically different compare to whole blood and plasma. (Figure S1). Sex steroid hormones (E2, T, and 11-ketotestosterone (11-KT; Cat No. 582751)) were measured by ELISA by use of the methods suggested by the manufacturer (Cayman Chemical Company).

2.6. Quantitative real-time polymerase chain reaction (PCR) assay

Brain and gonad samples were collected from each fish and preserved in RNA later reagent (QIAGEN, Korea Ltd., Seoul, Korea) at -80^{o}C until analysis. Primer sequences that were used for H295R cells and zebrafish are listed in Table S1 with reference gene (*β-actin*), respectively. Total RNAs were extracted using RNeasy mini-kit (QIAGEN, Valencia, CA, USA). The purity of the RNA preparations was checked by the ratio of absorbance at 260 nm and 280 nm (range 1.8 ± 0.2) using Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The complementary DNAs were synthesized from the purified RNA samples using iScript™ cDNA Synthesis kit (BioRad, Hercules, CA, USA). Quantitative real-time PCR was performed using the ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The PCR reaction comprised an initial denaturation step at 95^{o}C for 10 min, followed by 40 cycles at 95^{o}C for 30 s and 60^{o}C for 1 min. The amount of PCR product obtained was quantified by use of the threshold cycle (*C*t) method. The mRNA expression level of each target gene was normalized to the mRNA content of its reference gene (*β-actin*) using $^{\Delta\Delta}$*C*t method (Livak and Schmittgen, 2001). For each selected gene, real-time PCR reactions were performed with three replicate samples.

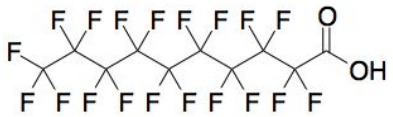
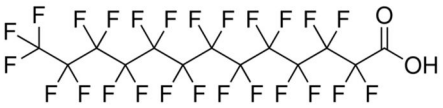
2.7. Statistical analysis

The median effective concentrations (EC_{50}) and 95% confidence interval (Hilscherova et al., 2004) of *D. magna* were calculated by probit analysis using ToxStat (version 3.5; West Inc., Cheyenne, WY, USA). No observed effective concentrations (NOECs) and the lowest observed effective

concentrations (LOECs) were calculated by Fisher's exact test in ToxStat. For other types of toxicity data, t-test, one-way ANOVA with Dunnett's test, and Kruskal-Wallis test were performed using SPSS 18.0K for Windows® (SPSS, Chicago, IL, USA). To evaluate linear trend of gene expression and hormone changes, linear regression analysis was adopted using SPSS 18.0K (SPSS). *P*-values less than 0.05 were considered to be statistically significant.

PNECs were calculated as a conservative estimate of the concentration of each compound in freshwater that is unlikely to produce adverse effects on aquatic biota. Estimation of the PNEC values were followed by the guidance provided in European Commission (2003). PNECs were derived from the present study or obtained from available literature, and only toxicity data that measure ecologically relevant endpoints (e.g., mortality, immobilization, reproduction, or growth inhibition) were used. If a full base set (algae, invertebrate, and fish) of freshwater chronic aquatic toxicity studies is available, the PNEC is determined by dividing the lowest chronic toxicity endpoint by an assessment factor of 10.

Table 1. Physical and chemical properties of PFDA and PFTrDA

Compounds	Chemical formula	Chemical structure	log K_{ow} (L/L)	pKa
PFDA	$C_{10}HF_{19}O_2$		5.40 [a]	-0.17 [b]
PFTrDA	$C_{13}HF_{25}O_2$		-	-

[a] The log K_{ow} data was obtained from Kelly et al. (2009).

[b] The pKa data was obtained from ATSDR, 2009.

-: not applicable

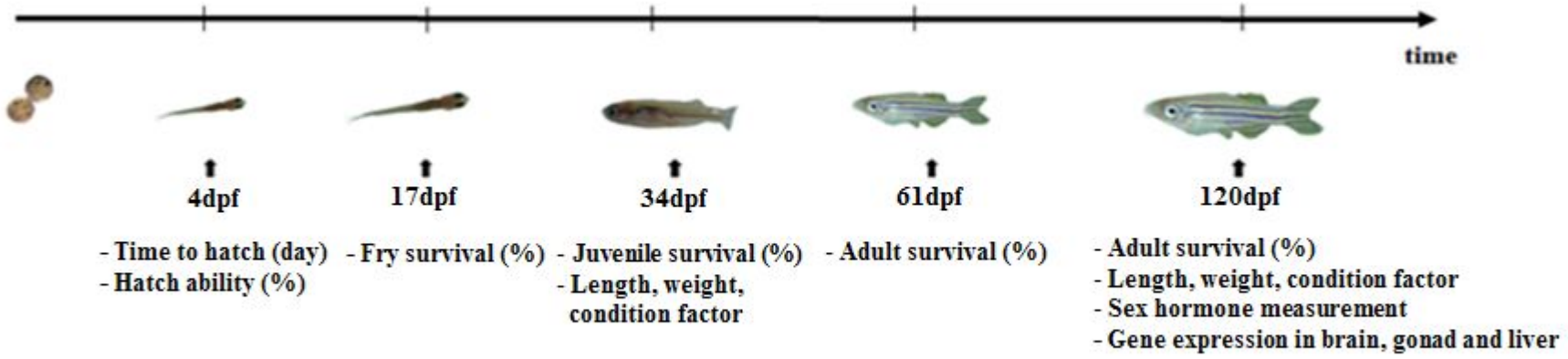


Figure 1. Experimental design of long-term experiment in zebrafish and each endpoint.

3. Results

3.1. D. magna toxicity test

The 48 h EC_{50} of PFDA and PFTrDA were estimated to be 80.0 mg/L (95% CI 62.3-98.5 mg/L) and 8.2 mg/L (95% CI 6.2-10.3 mg/L), respectively (Table 2). Chronic effects of PFDA and PFTrDA on survival, reproduction, and growth of *D. magna* are shown in Figure 2. After 21 d exposure, the survival NOEC for PFDA and PFTrDA was 0.5 mg/L and 0.0125 mg/L, respectively. Exposure to 0.2 mg/L PFTrDA significantly delayed the time required for reproduction of the first brood. *Daphnia* growth as determined by body length was also significantly affected by the PFDA exposure at ≥0.0125 mg/L. The population growth rate decreased in PFTrDA group, but the magnitude of the effect was not expected to result in negative population growth.

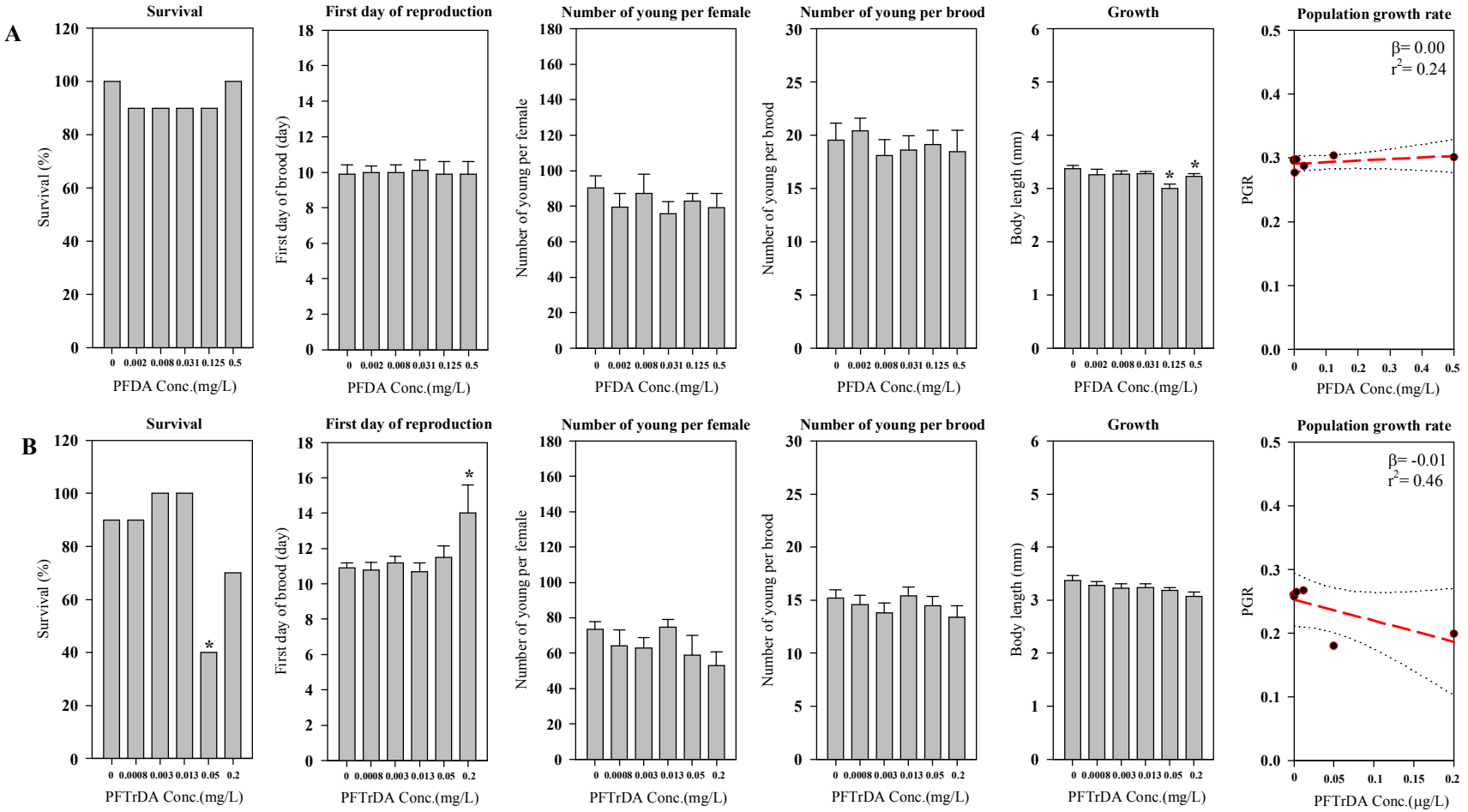


Figure 2. Results of 21 d exposure of *D. magna* to (A) PFDA and (B) PFTrDA. Results show survival (%), first day of reproduction, number of young per female, number of young per brood, growth, and population growth rate of *D. magna*. The results are shown as mean ± standard deviation of ten replicates. Asterisk (*) indicates a significant difference from the control (p<0.05). Conc. = concentration, β = slope, p = p for trend. Bold dotted line shows least-squares linear regression line, and dotted line shows its 95% confidence interval.

Table 2. Acute toxicity values of PFDA and PFTrDA using *D. magna*

Chemicals	Exposure duration (h)	EC_{50} (95% CI) (mg/L)	NOEC (mg/L)	LOEC (mg/L)
PFDA	48	80.00 (62.28-98.45)	10	100
PFTrDA	48	8.23 (6.16-10.30)	1	10

Abbreviation: CI=confidence interval; EC_{50}=median effective concentration; LOEC=lowest observed effective concentration; NOEC=no observed effective concentration.

3.2. Zebrafish long-term exposure

Survivals of larvae (NOEC: 0.01 mg/L of PFDA and 0.1 mg/L PFTrDA), juvenile fish (NOEC: 0.01 mg/L of PFDA and 0.1 mg/L PFTrDA), and adult fish (NOEC: 1 mg/L of PFDA and 0.01 mg/L PFTrDA) exhibited significant decrease in dose-response manner ($p<0.05$, Table 3). Some hatched larvae exposed to PFDA showed malformation, i.e., spine crooked malformation (Figure S2). In juvenile fish (34 dpf) and adult fish (120 dpf), the growth of test organisms including length and wet weight exhibited no significant difference compared to control, whereas condition factor of 120 dpf adult fish showed significant increase by PFTrDA exposure (Table 4).

Concentrations of E2 were significantly increased in male fish exposed to 0.01 mg/L PFTrDA ($p<0.05$, Figure 3A). Concentrations of T and 11-KT were significantly decreased in males exposed to 0.1 mg/L PFTrDA ($p<0.05$, Figures 3B and 3C). The ratios of E2/T and E2/11-KT were significantly increased at 1 mg/L PFDA and ≥0.01 mg/L PFTrDA in male fish ($p<0.05$, Figures 3D and 3E). In the male brain, expressions of *CYP19B*, *ERα*, and *ER2β* mRNA in male fish were also significantly increased by exposure to 1 mg/L PFDA (Figure 4A, 4I and 4J). In addition to, *VTG1* and *CYP19A* were significantly up regulated in 1mg/L male liver and gonad, while *LHβ* was significantly down-regulated at 0.01 mg/L PFDA. In female *FSHβ* were significantly increased with a concentration-dependent manner. Following exposure to PFTrDA, *GnRHR2*, *GnRHR4* and *ERα* caused significant up-regulation in male brain. But in the female, significant up-regulation of *GnRHR2*, *HMGRB*, *3βHSD*, and *CYP11A* were observed after PFTrDA exposure. In contrast, gene transcription of *VTG1* was also significantly decreased with a concentration-dependent manner (Figure 4 and 5).

Table 3. Time to hatch, hatchability, and survival of zebrafish exposed to PFDA and PFTrDA

Chemical	Conc. (mg/L)	Time to hatch (day)	Hatchability (%)	Survival (%)			
				Larvae (17 dpf)	Juvenile (34 dpf)	Juvenile (61 dpf)	Adult (120 dpf)
PFDA	0	2.85 ± 0.20	100.00 ± 0.00	97.50 ± 0.80	94.44 ± 1.10	93.75 ± 1.32	84.38 ± 3.03
	0.01	3.15 ± 0.20	100.00 ± 0.00	97.50 ± 0.80	97.22 ± 0.94	96.88 ± 1.12	87.50 ± 2.73
	0.1	3.08 ± 0.27	100.00 ± 0.00	80.00 ± 1.42 *	77.78 ± 1.71 *	75.00 ± 2.08 *	75.00 ± 2.08
	1	3.03 ± 0.24	100.00 ± 0.00	77.50 ± 1.72 *	72.22 ± 2.81 *	68.75 ± 3.44 *	65.63 ± 4.66
	10	3.15 ± 0.21	100.00 ± 0.00	5.00 ± 4.08 *	0.00 ± 0.00 *	-	-
PFTrDA	0	2.80 ± 0.10	100.00 ± 0.00	92.50 ± 2.47	82.99 ± 2.73	80.80 ± 3.19	78.13 ± 3.15
	0.01	2.92 ± 0.10	95.00 ± 0.94	86.95 ± 2.68	82.64 ± 4.07	80.36 ± 4.86	75.00 ± 4.66
	0.1	3.10 ± 0.15	97.50 ± 0.80	92.22 ± 0.87	79.86 ± 1.16	61.16 ± 2.42	40.63 ± 3.32 *
	1	3.18 ± 0.10	97.50 ± 0.80	12.78 ± 5.60 *	0.00 ± 0.00 *	-	
	10	3.27 ± 0.13	92.50 ± 1.57	0.00 ± 0.00 *	-	-	

Values represent mean ± SEM. Asterisk denotes a significant difference from the control (p<0.05).

- : not available.

Table 4. Growth index exposed to PFDA and PFTrDA in zebrafish

Chemical	Conc.	N		Length (cm)		Weight (g)		Condition factor		
	(mg/L)	Juvenile (34 dpf)	Adult (120 dpf)	Juvenile (34 dpf)	Adult (120 dpf)	Juvenile (34 dpf)	Adult (120 dpf)	Juvenile (34 dpf)	Adult (120 dpf)	
PFDA	0	4	20	1.18 ± 0.05	3.27 ± 0.03	0.013 ± 0.003	0.258 ± 0.007	0.80 ± 0.09	0.74 ± 0.01	
	0.01	4	13	1.23 ± 0.05	3.18 ± 0.10	0.012 ± 0.002	0.242 ± 0.019	0.67 ± 0.07	0.72 ± 0.02	
	0.1	4	16	1.25 ± 0.06	3.33 ± 0.04	0.013 ± 0.001	0.279 ± 0.009	0.70 ± 0.06	0.76 ± 0.02	
	1	4	-	1.18 ± 0.02	-	0.012 ± 0.001	-	0.76 ± 0.05	-	
PFTrDA	0	4	17	1.18 ± 0.09	3.17 ± 0.07	0.011 ± 0.003	0.244 ± 0.016	0.64 ± 0.02	0.74 ± 0.02	
	0.01	4	16	1.23 ± 0.05	3.08 ± 0.04	0.015 ± 0.004	0.200 ± 0.010	0.80 ± 0.13	0.68 ± 0.02	
	0.1	4	9	1.01 ± 0.07	2.95 ± 0.13	0.008 ± 0.001	0.244 ± 0.034	0.81 ± 0.13	0.89 ± 0.04	*

Values represent mean ± SEM. Asterisk denotes a significant difference from the control (p<0.05).

- : not available.

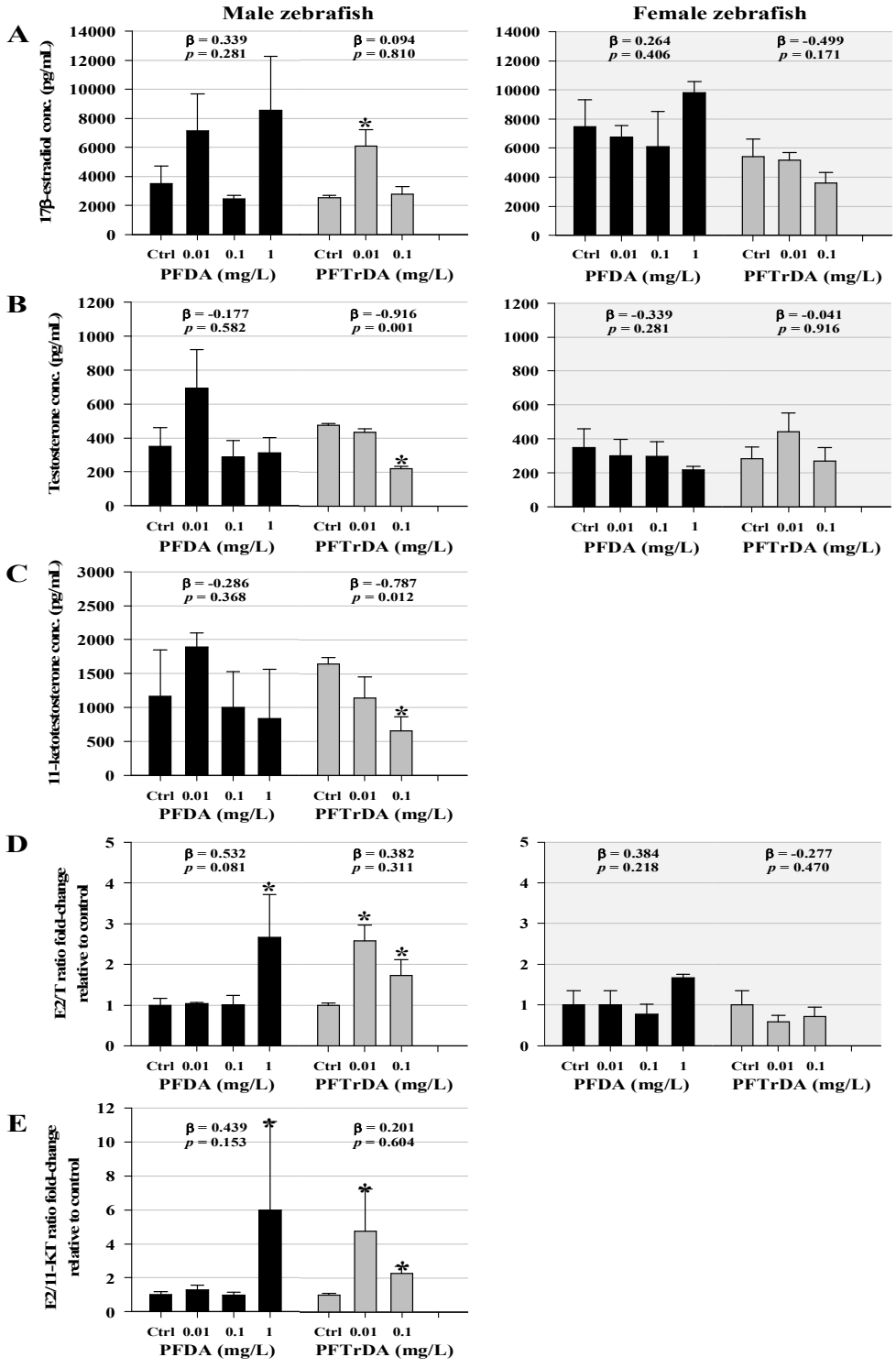


Figure 3. Effects of PFDA and PFTrDA on (A) concentrations of E2, (B) concentrations of T, (C) concentrations of 11-KT, (D) E2/T ratio, and (E) E2/11-KT ratio in male and female zebrafish by the exposure to PFDA and PFTrDA for 120 d (n=3). The results are shown as mean ± standard error of three replicates for four tanks. Asterisk (*) indicates a significant difference from the control (p<0.05). β = slope, p = p for trend.

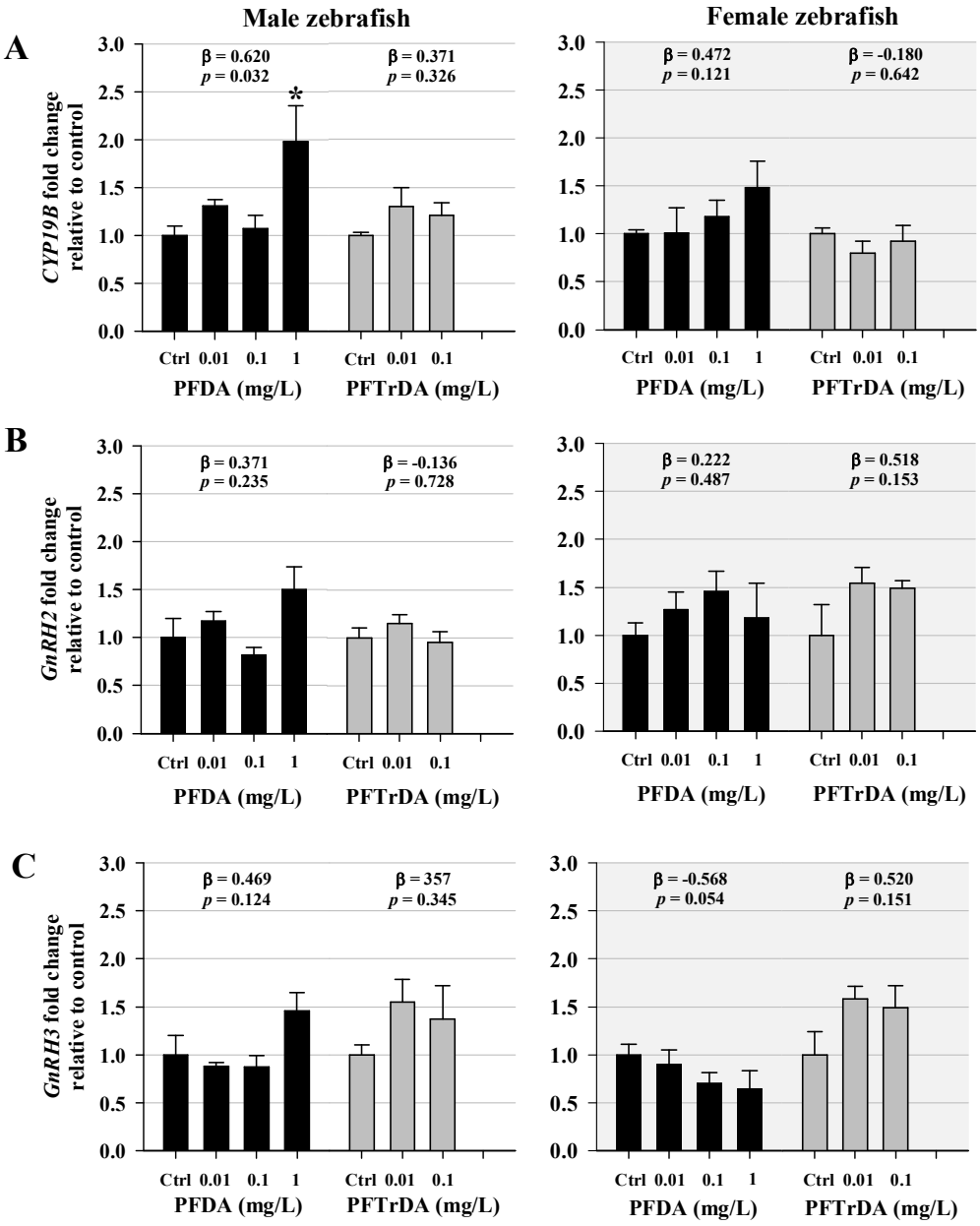
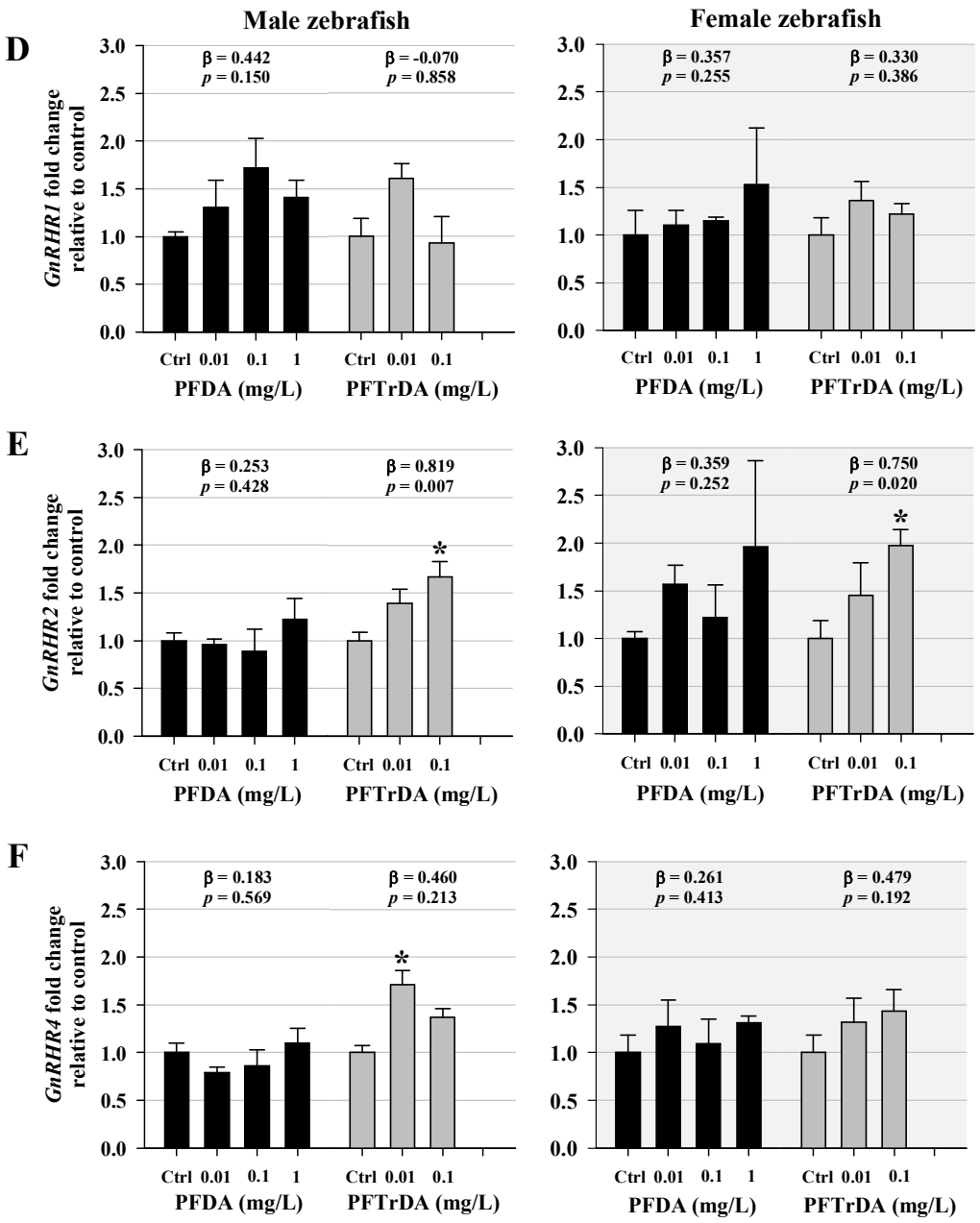


Figure 4. Gene expression profiles of (A) *CYP19B*, (B) *GnRH2*, (C) *GnRH3*, (D) *GnRHR1*, (E) *GnRHR2*, (F) *GnRHR4*, (G) *FSHβ*, (H) *LHβ*, (I) *AR*, (J) *ERα*, and (K) *ER2β* in male and female zebrafish brain after exposure to PFDA and PFTrDA for 120 d (*n=3*). Values represent the mean ± standard error of three replicates. Asterisk (*) indicates a significant difference between exposure groups and the corresponding control ($p<0.05$). β = slope, $p = p$ for trend.



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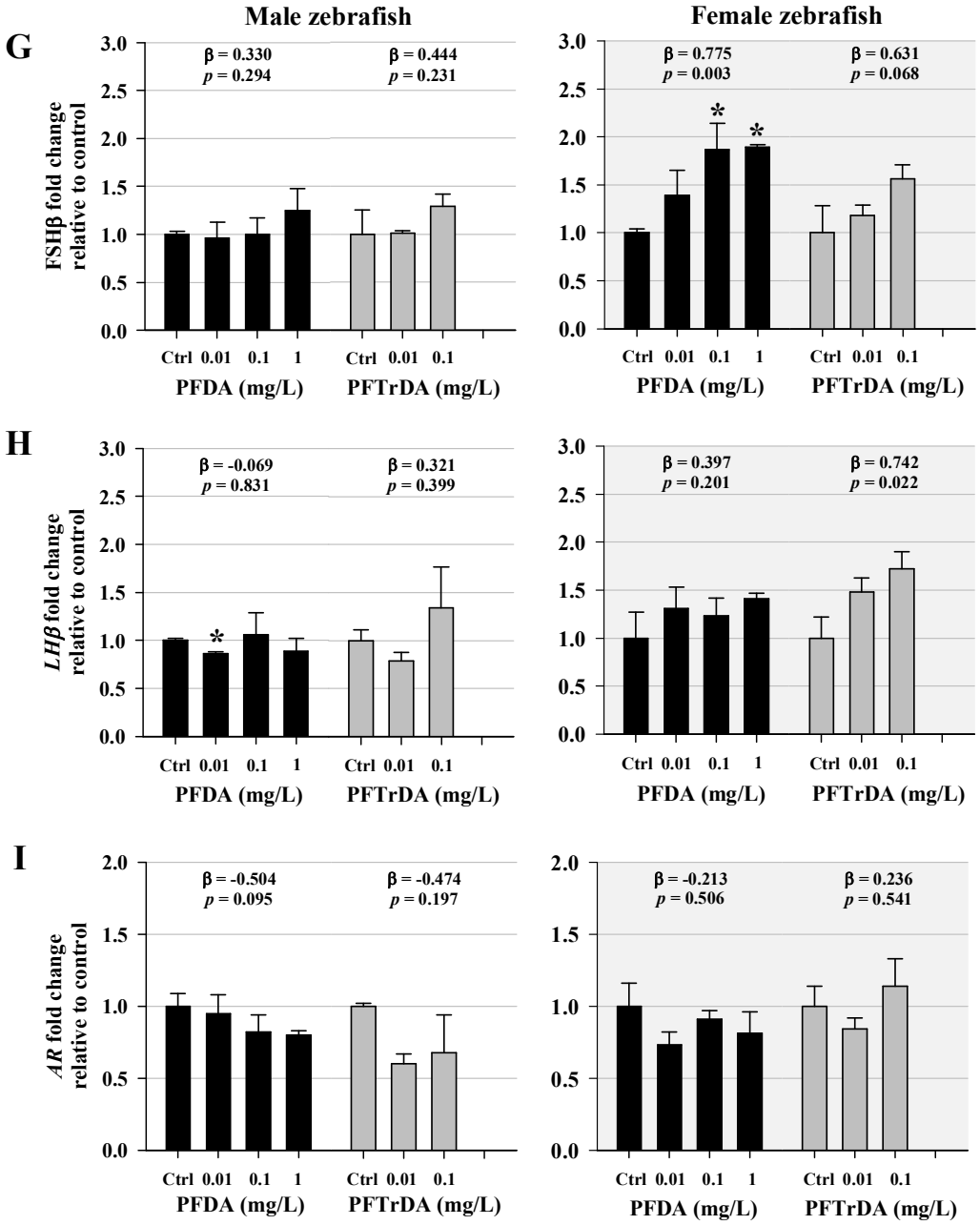

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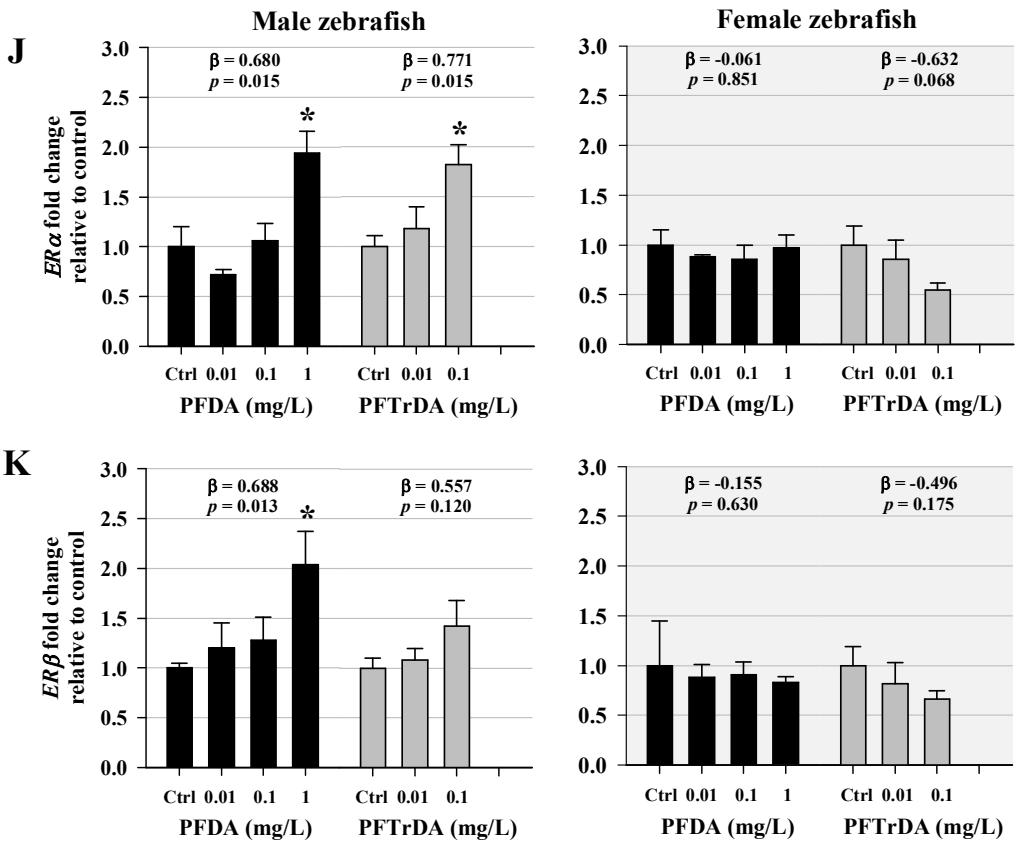


Figure 4 continued.

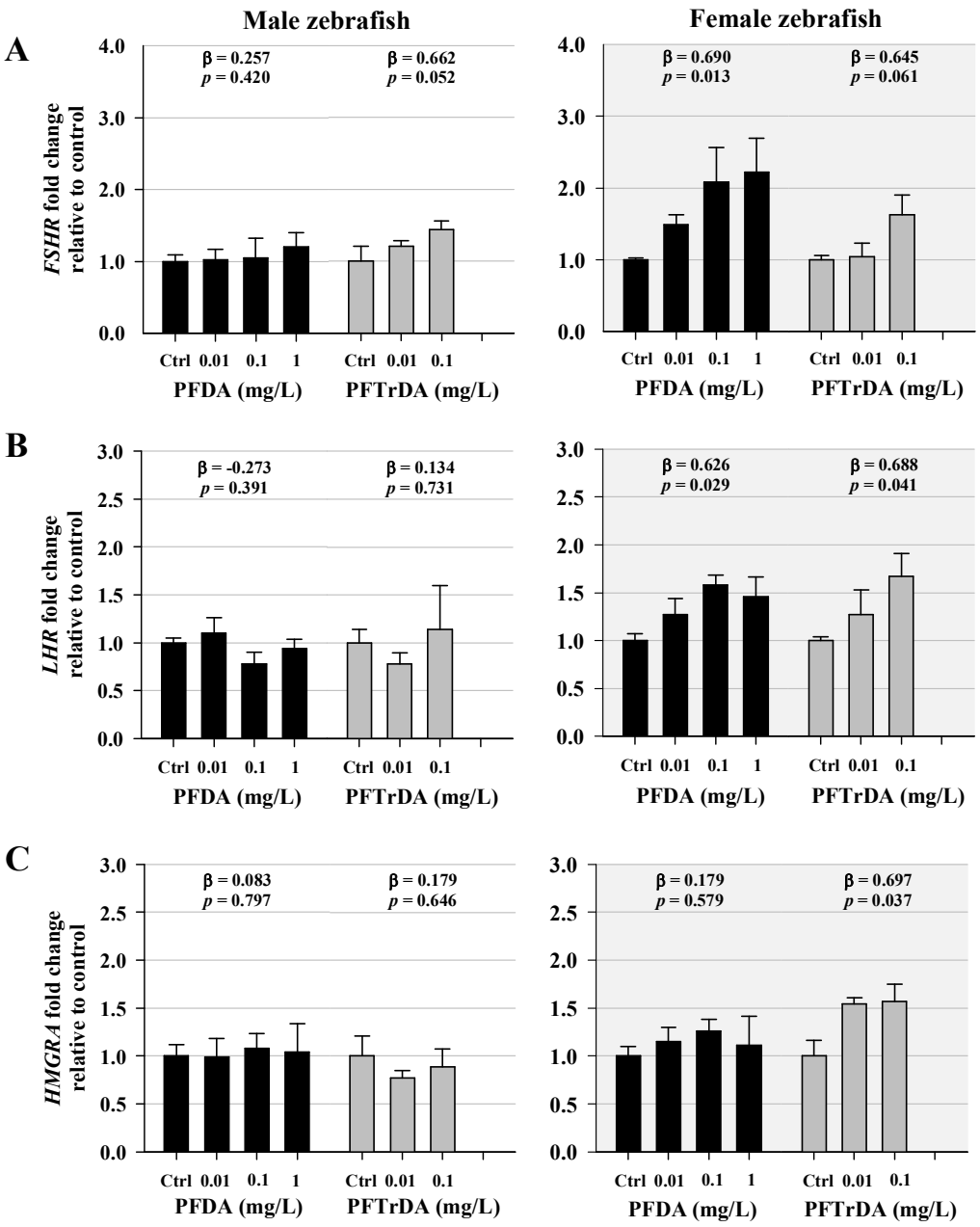
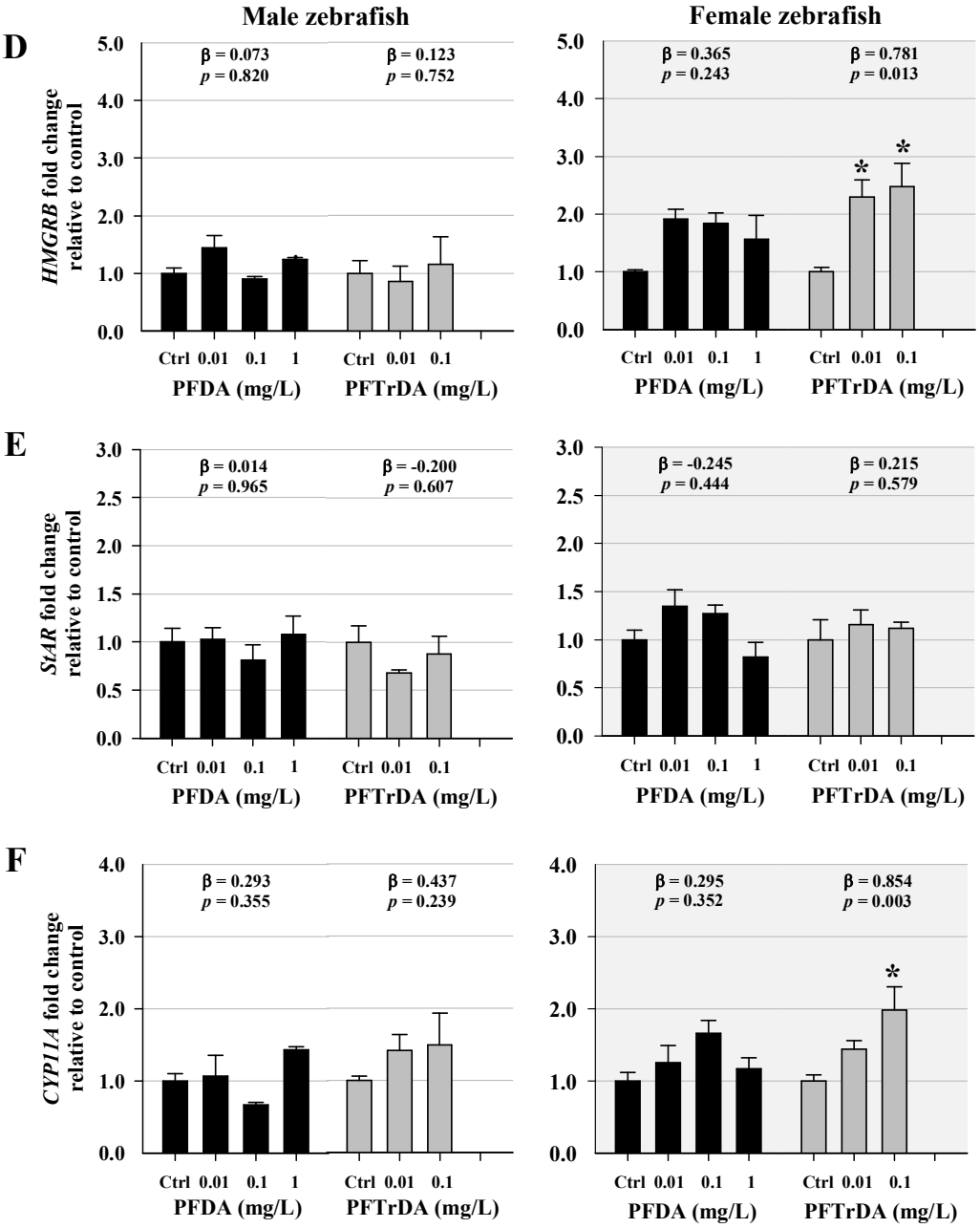


Figure 5. Gene expression profiles of (A*) FSHR*, (B) *LHR*, (C) *HMGRA*, (D) *HMGRB*, (E) *StAR*, (F) *CYP11A*, (G) *3βHSD*, (H) *CYP17*, (I) *17βHSD*, and (J) *CYP19A* in male and female gonad and (K) *VTG1* in male and female liver after exposure to PFDA and PFTrDA for 120 d (n=3). Values represent the mean ± standard error of three replicates. Asterisk (*) indicates a significant difference between exposure groups and the corresponding control (p<0.05). β = slope, $p = p$ for trend.



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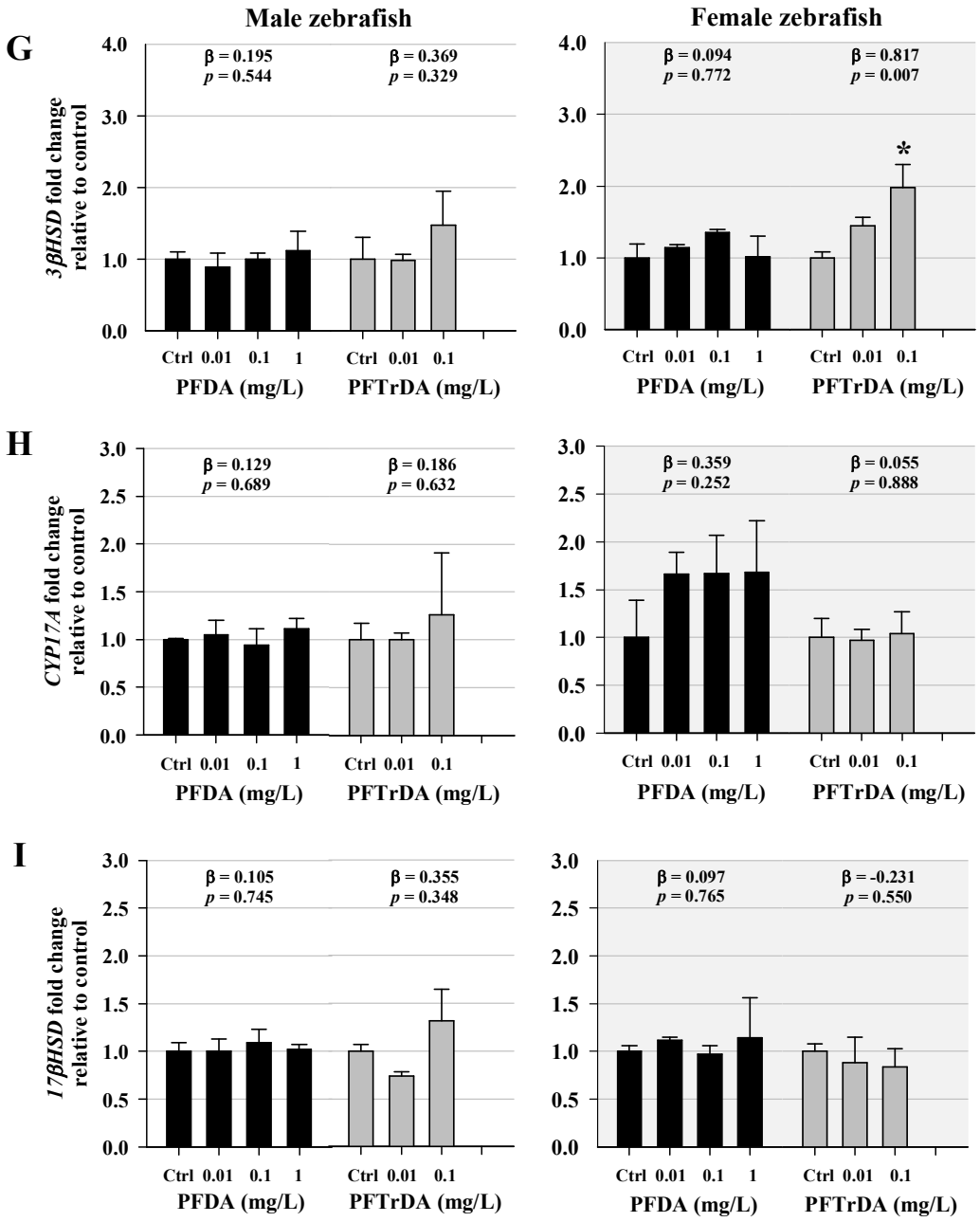

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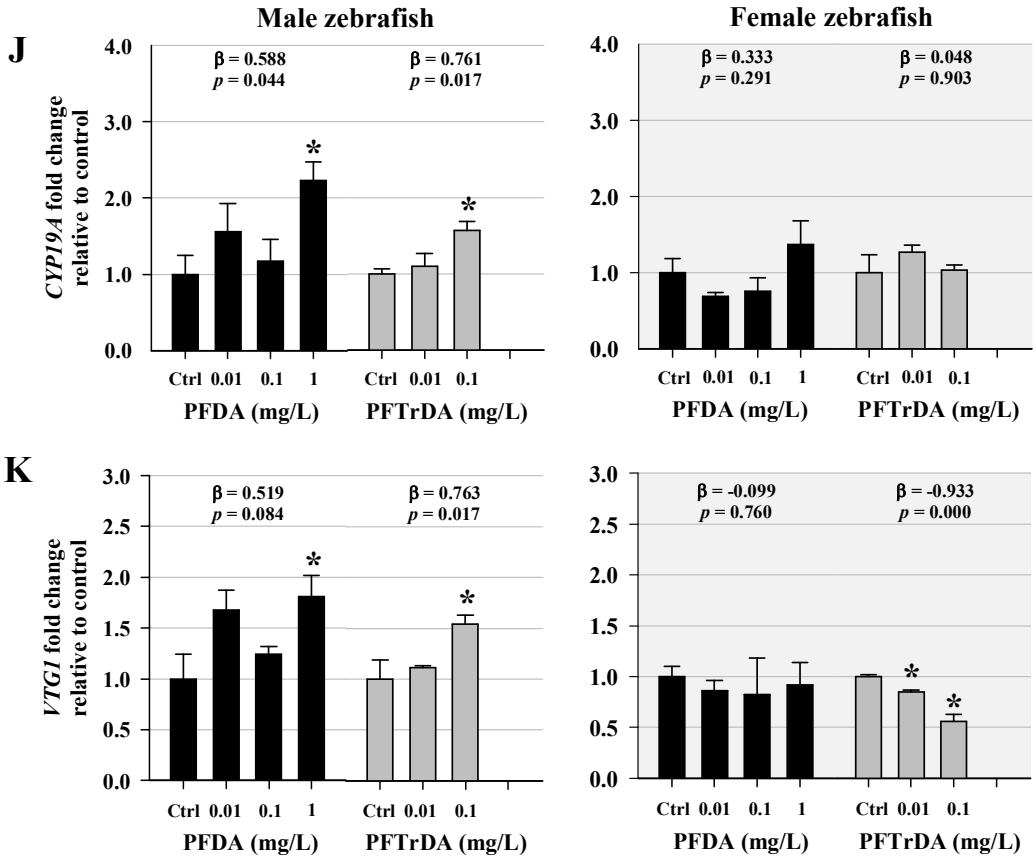

Figure 5 continued.

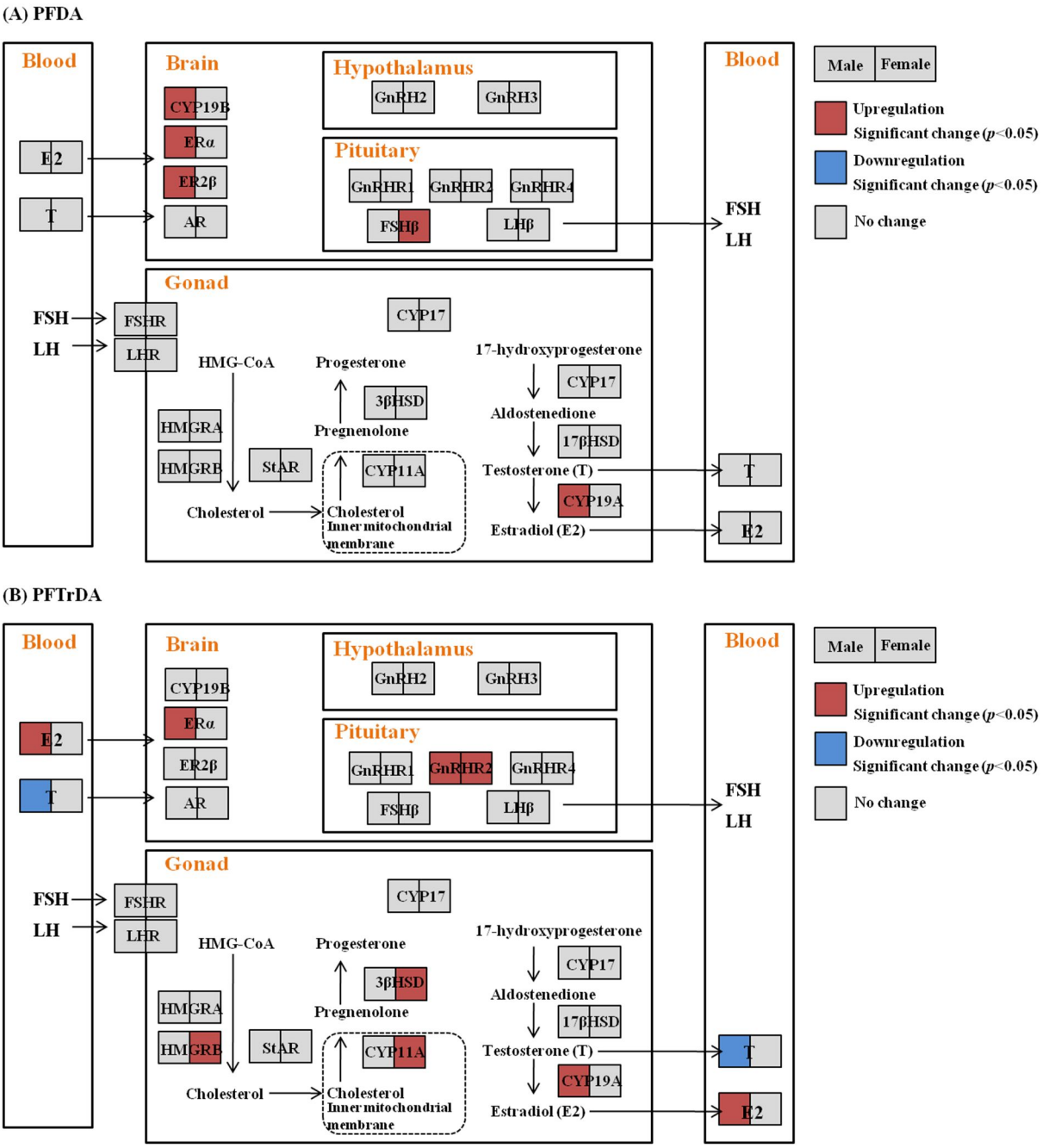

Figure 6. Sex-dependent effects in adult zebrafish after 120 days of exposure to (A) PFDA and (B) PFTrDA. Colors represent statistically significant changed transcription patterns of *p* for trend in hypothalamic-pituitary-gonad (HPG) axis from different tissues ($p<0.05$). Red: statistically significant up-regulation; blue: statistically significant down-regulation; gray: no statistically significant change.

3.3. Hormone production and mRNA expression in H295R cells

Concentrations of E2 were slightly increased in cells exposed to PFTrDA, however statistical significances were not observed (Figure 7A). Concentrations of T were significantly decreased in cells exposed to 50 mg/L PFTrDA (Figure 7B), and the ratio of E2 and T was significantly greater after exposure to 50 mg/L PFTrDA (Figure 7C). However, exposure range tested of PFDA in the present study did not result in significant effects on E2, T, and E2/T ratio (Figure 7).

Exposure to PFTrDA affected expression of mRNA for genes involved in steroidogenesis (Figure 8). After 48 h exposure, mRNA expression of *CYP11A1* and *CYP17A* genes were significantly down-regulated in H295R cells exposed to ≥0.5 mg/L PFTrDA (Figures 8B and 8C). A small up-regulation of *CYP19A* mRNA and down-regulation of *CYP11B2* mRNA were observed (Figures 8A and 8D), but the effect was not statistically significant.

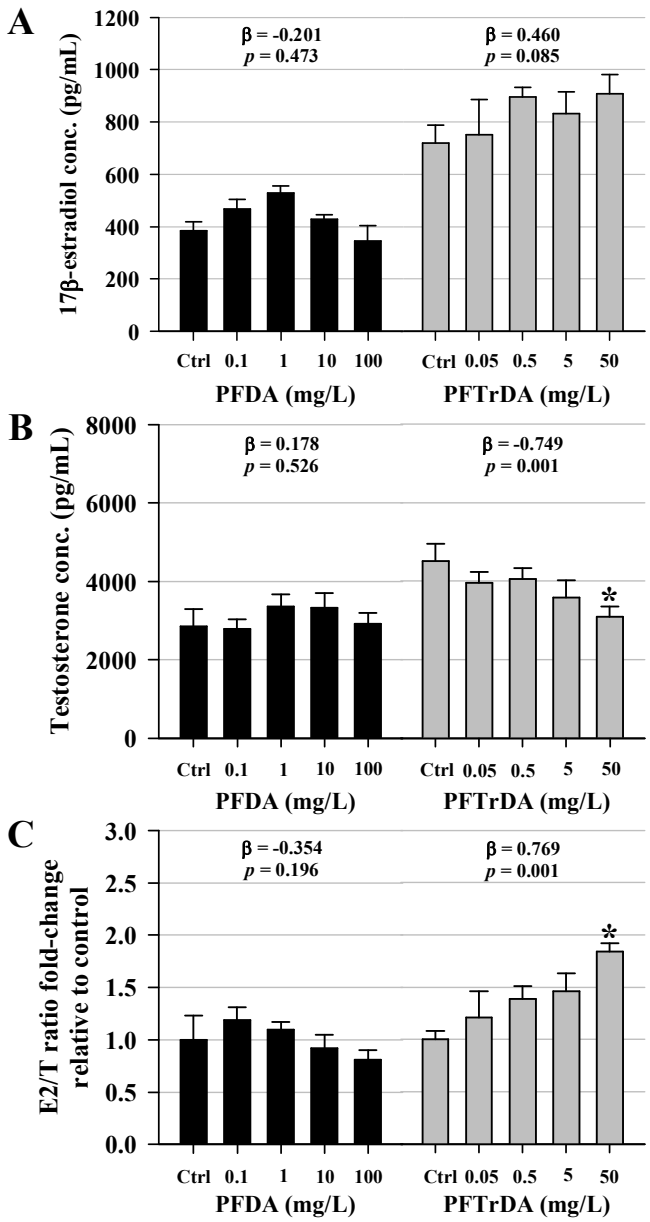

Figure 7. Effects of PFDA and PFTrDA on (A) 17β-estradiol (E2) hormone concentration, (B) testosterone (T) hormone concentration, and (C) E2/T ratio in H295R cells (n=3). The results are shown as mean ± standard error of three replicates. Asterisk (*) indicates a significant difference from the control (p<0.05). β = slope, $p = p$ for trend.

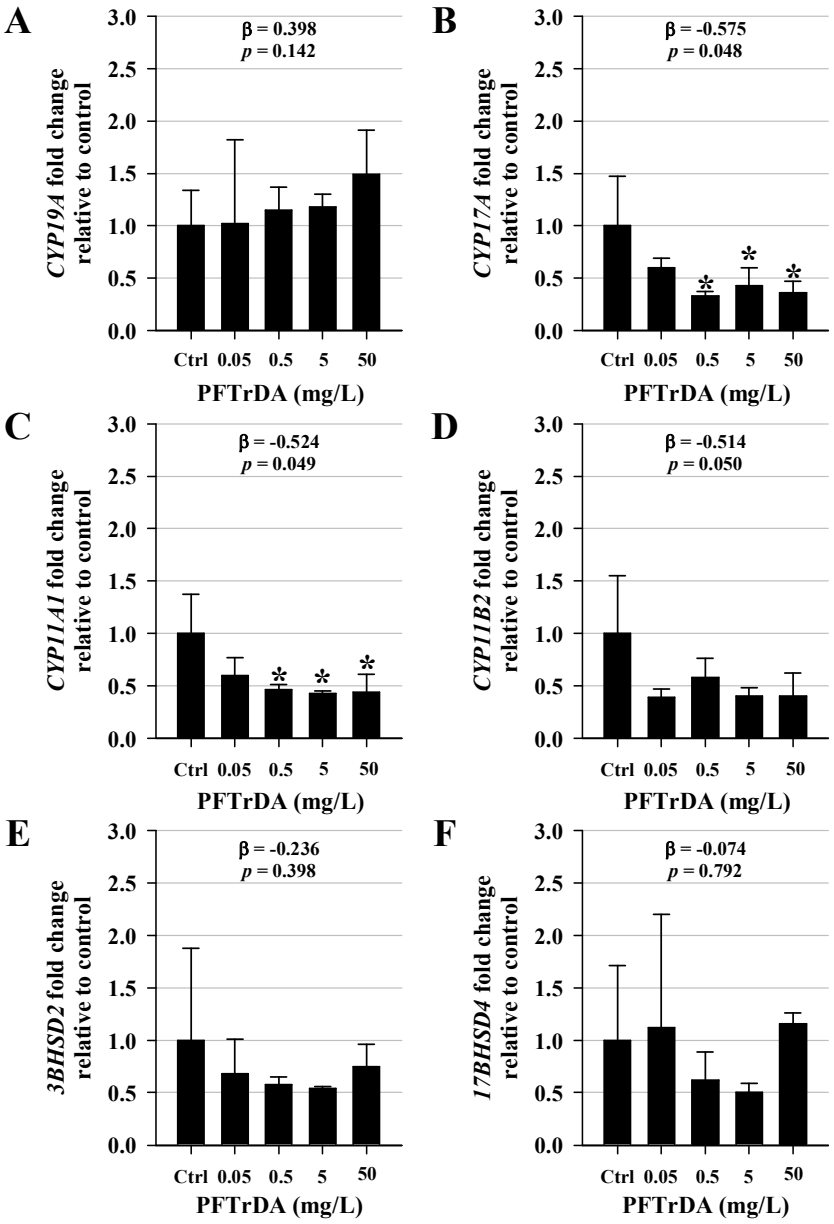

Figure 8. Effects of PFDA and PFTrDA on expressions of (A) *CYP19A*, (B) *CYP17A*, (C) *CYP11A1*, (D) *CYP11B2*, (E) *3βHSD2*, and (F) *17βHSD4* gene in H295R cells (n=3). Values represent the mean ± standard error of three replicates. Asterisk (*) indicates a significant difference between exposure groups and the corresponding control (p<0.05). β = slope, $p = p$ for trend.

4. Discussion

4.1. Toxicities of PFDA and PFTrDA

In the present study, the order of potency based on the 48 h EC_{50} values in *D. magna* was PFTrDA (8.23 mg/L) > PFDA (80 mg/L), which also were lower values than PFCAs with less than ten carbons (Ding et al., 2012; Hoke et al., 2012). In previous studies were observed that PFCA with ten carbons was more toxic than the shorter one based on the 72 h EC_{50} values in algae (Hoke et al., 2012; Latala et al., 2009). The 48 h EC_{50} and NOEC values of the PFCAs in *D. magna* decreased with increasing fluorinated carbon chain length up to 12 (Ding et al., 2012). PFCA with longer chain length (C8) was more toxic to zebrafish embryos than PFCA with shorter chain length (C4) (Hagenaars et al., 2011). The 96 h LC_{50} value for fathead minnow were 32 mg/L PFDA and >99.2 mg/L perfluorohexanoic acid (PFHxA, C_6 PFCA) (Hoke et al., 2012). The increased toxicity could be explained by the increased hydrophobicity that higher bioaccumulation potential for organic chemicals with longer carbon chains. The length of the perfluorinated tail of PFCA molecules might be an important factor in determining toxicity. PFAAs structures have both sided aspects of hydrophobic and oleophobic characteristics. In general chlorinated and brominated hydrophobic organic chemicals more are accumulated in bile, liver, intestine, blubber than in blood. But some monitoring studies have been reported that longer chain PFCAs (>C8) bioaccumulate and persist in protein-rich compartments such as blood and liver than lipid-rich part in fish, birds, and marine mammals (Conder et al., 2008; Jeon et al., 2010). As for the toxicity, also the bioconcentration potential of PFAAs in fish has been correlated with the length of the fluorinated carbon chain and the

functional group (Kannan et al., 2005 and Martin et al., 2003).

PNEC values derived in this study were great to the measured environmental concentrations in surface water. Based on the data that were derived from the present study or obtained from available literature, PNECs of PFDA and PFTrDA were estimated. Because chronic toxicity data are available from test organisms of three trophic levels, i.e., algae, daphnids, and fish, an assessment factor of 10 was used for PFDA, while an assessment factor of 50 was used for PFTrDA. The calculated PNECs for PFDA and PFTrDA were 0.1 mg/L and 0.2 μg/L, respectively (Table 6). Hoke et al. (2012) proposed a freshwater PNEC of 11 μg/L for PFDA based on the lowest freshwater acute toxicity endpoint with an assessment factor of 1000, which is 10-times lower than value derived in the present study because it was based on chronic study. Furthermore the concentrations of long chain PFCAs in surface water mostly ranged from several nanograms per liter to several tens of nanograms per liter (Table S2). These results suggest indirect ecological risks of PFDA and PFTrDA. But the environmental implication of this observation are negligible in ambient water environment.

Table 5. Acute and chronic effects of the tested perfluoroalkyl acids on aquatic organisms

Chemical	Exposure type	Species	Duration	Endpoints	Conc. (mg/L)	Reference
PFDA	Acute	*Daphnia magna*	48 h	immobilization EC_{50}	163.48	Ding et al., 2012
		Daphnia magna	48 h	immobilization NOEC	77.11	Ding et al., 2012
		Daphnia magna	48 h	immobilization EC_{50}	> 100	Hoke et al., 2012
		Daphnia magna	48 h	immobilization EC_{50}	80	This study
		Daphnia magna	48 h	immobilization NOEC	10	This study
		Chydorus sphaericus	48 h	immobilization EC_{50}	45.24	Ding et al., 2012
		Chydorus sphaericus	48 h	immobilization NOEC	5.14	Ding et al., 2012
		Oncorhynchus mykiss	96 h	juvenile survival LC_{50}	32	Hoke et al., 2012
		Pimephales promelas	96 h	survival NOEC	10	Hoke et al., 2012
	Chronic	*Pseudokirchneriella subcapitata*	72 h	growth EC_{50}	10.6	Hoke et al., 2012
		Daphnia magna	21 d	survival NOEC	> 0.5	This study
		Daphnia magna	21 d	reproduction NOEC	> 0.5	This study
		Danio rerio	120 dpf	survival NOEC	1	This study
PFTrDA	Acute	*Daphnia magna*	48 h	immobilization EC_{50}	8.23	This study
		Daphnia magna	48 h	Immobilization NOEC	1	This study
	Chronic	*Daphnia magna*	21 d	survival NOEC	0.01	This study
		Daphnia magna	21 d	reproduction NOEC	0.05	This study
		Danio rerio	120 dpf	survival NOEC	0.01	This study

PFDA = perfluorodecanoic acid, PFTrDA = perfluorotridecanoic acid.

Table 6. Derivation of predicted no effect concentration (PNEC) of perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (PFTrDA)

Compounds	Lowest NOEC (mg/L)	AF	PNEC (mg/L)
PFDA	1 [a]	10	0.1
PFTrDA	0.01 [b]	50	0.2 [c]

[a] Based on the lowest *Danio rerio* 17, 34, and 61 dpf survival NOEC in the present study.

[b] Based on the lowest *Daphnia magna* 21 d survival NOEC, *Danio rerio* 120 dpf survival NOEC in the present study.

[c] The unit of concentration was indicated as μg/L.

NOEC: no observed effective concentration. AF: assessment factor.

4.2. Endocrine disruption potential and its mechanisms

The present study demonstrates that long chain PFCAs altered plasma sex hormone levels as well as the gene transcription in zebrafish. Measurement of sex steroid hormones has been suggested to be one of the most integrative and functional endpoints for reproduction in zebrafish (Ma et al., 2012). In the present study, significantly greater production of E2 and lesser production of T and 11-KT were observed after exposure to PFTrDA in male zebrafish. Since T and 11-KT regulate spermatogenesis and reproduction in male fish (Weltzien et al., 2002), decrease of production of these hormones indicates delay in spermatogenesis, testicular development as well as reproductive cycle. Significant increase of E2 and decrease of T has also been previously reported in zebrafish exposed to 8:2 FTOH (Liu et al., 2010; Rosenmai et al., 2013). Exposure to PFNA was significantly increased concentrations of E2 and decreased concentrations of T in male rats (Feng et al., 2009). These results suggest that long chain PFCAs can induce endocrine disruption in fish.

The ratio of E2/T or E2/11-KT is a sensitive biomarker of abnormal sex hormones in fish. In some fish species, disturbing the balance of sex hormones could affect reproduction, sex development, gametogenesis and sex differentiation (Folmar et al., 1996; Shang et al., 2006). We observed significantly increased ratios of E2/T and E2/11-KT in male zebrafish after exposure to PFDA and PFTrDA. The findings of significantly greater E2/T ratio in H295R cells exposed to PFTrDA support the results of *in vivo* exposure. The current results are consistent with a previous study where increased E2/T ratio was observed in male fish exposed to 8:2 FTOH (Liu et al., 2010). The E2/T ratio is indicative of P450 aromatase activity and hence the conversion of T to E2. Polyfluoroalkyl phosphate surfactants

(PAPS) and 8:2 FTOH showed that inhibition of steroidogenesis pathway (decreased androgen, increased estrogen levels) through increased *CYP19* gene expression (Rosenmai et al., 2013).

In the present study, increased concentrations of E2 and increased concentrations of were accompanied by significant up-regulation of *CYP19A* gene in male fish as well as significant change of *CYP19B* gene in the brain. *CYP19B* is primarily regulated by E2 through the estrogen responsive element on its promoter sequence (Pellegrini et al., 2005). In previous study where up-regulation of *CYP19B* has been reported following increased concentration of E2 in zebrafish by exposed to 6:2 FTOH (Liu et al., 2009). In addition to transcription of *CYP11A*, *3βHSD*, and *HMGRB* were significantly changed by treated with PFTrDA. The results of the study indicate that balance of sex hormones were disrupted by PFDA and PFTrDA exposure, which could further result in adverse effects on gametogenesis, sexual development or reproduction in fish.

Up regulation of hepatic *VTG1* gene transcription were observed in male fish with the exception of down regulation in female fish. A recent study reported elevated *VTG1* and *ERα* expression levels in male medaka after 6:2 FTOH and 8:2 FTOH exposure (Ishibashi et al., 2008). *VTG1* is one of yolk precursor genes (Zhang et al., 2008a) and it is produced by estrogenic chemicals (e.g., E2, xenoestrogen) binding to specific ERs which activates VTG gene expression. Therefore increased *VTG1* gene expression could be demonstrate increased in E2 levels and common response as responsible for E2-induced VTG synthesis in the liver.

In the present study *GnRHR2* and *GnRHR4* gene transcription were increased both male and female fish. GnRH receptor acts gonadotrophin regulator that responsiveness of the pituitary to GnRH stimulation in female fish (Zhang et al., 2008b). The stimulation of *GnRHR* could lead to

more secreation of gonadotrophins in female fish. *FSHβ* were significantly up regulated in female brain. In female fish, the developmental stage is controlled under the gonadotropin FSH, while the oocyte maturation stages were effected by LH. In this study, increased FSH gene in female and decreased LH gene transcription in male were observed after PFDA exposure. It is conceivable that may lead to the stimulation of E2 and inhibition of T synthesis.

In vitro study showed that PFTrDA can be a potential endocrine disruption chemical. Greater production of E2 and significantly lesser production of T were observed after exposure of H295R cells to PFTrDA, and this was accompanied by up-regulation pattern of *CYP19A* gene. The effect on production of sex hormones in male fish was similar to H295R cells and expression of *CYP19A* mRNA increased in males exposed to PFTrDA. In the present study, H295R cells were used to evaluate effects of PFDA and PFTrDA on hormones and genes involved in steroidogenesis, and to understand the underlying mechanisms of effects on endocrine system of zebrafish. In few studies have compared the results of the *in vitro* H295R assay with the results of *in vivo* fish investigations (Han et al., 2010; Ji et al., 2010; Kim et al., 2012; Liu et al., 2012; Ma et al., 2012). Liu et al. (2010) reported on the similar results when compared to H295R and zebrafish. The analogue trends of steroid production were observed by exposure to some organophosphate flame retardants (OPFR). Although these results differ by species, sex, age, and their metabolism, however some report have been provide as supplement information. In this study, we showed PFTrDA might also lead to endocrine disruption by inhibiting steroidogenic processes in cell lines. But, their mechanisms were unclear and further study on the effects on endocrine regulation systems may provide more understanding of mechanisms exposure to long-chain PFAAs.

5. Conclusion

In summary, the present study provides the first observation that toxicity of long-chain PFAAs for long-term exposure in multiple aquatic organisms that *D.magna* and zebrafish. Our results demonstrated that waterborne exposure of long term exposure to PFTrDA (and PFDA to lesser extent) to zebrafish could affects survival of fish, and alters synthesis of sex steroid hormone and related gene transcription in fish. In addition, there was the mechanism of inhibiting steroidogenesis pathway by exposure to PFTrDA in H295R cells. Although this study showed that potential effects of endocrine disruption following chronic exposure, but the environmental implication of this observation is not clear because the effect level is much greater than the environmental levels. Therefore, further studies are needed to understand the more detailed mechanism of endocrine disruption effects considering environmental relevance.

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Supporting Information

Quantitative real-time PCR array

Table S1. Sequences of primers for the genes measured

Organism	Gene name	Accession number	Description/ Assay ID[a]	Sequence (5'-3')
Homo sapiens	*β-actin*	NM_001101.3	Hs99999903_m1	
	CYP19A	NM_031226	Hs00903413_m1	
	CYP17A	NM_000102	Hs01124136_m1	
	CYP11A1	NM_001099773	Hs00167984_m1	
	CYP11B2	NM_000498	Hs01597732_m1	
	3βHSD2	NM_001166120	Hs00605123_m1	
	17βHSD4	NM_000414	Hs00264973_m1	
Danio rerio	*β-actin*	NM_131031	Forward primer	TGCTGTTTTCCCCTCCATTG
			Reverse primer	TCCCATGCCAACCATCACT
			TaqMan probe	FAM-CCCGAGGCTCTCTTC-NFQ
	gnrh2	AY657018	Forward primer	CTGAGACCGCAGGGAAGAAA
			Reverse primer	TCACGAATGAGGGCATCCA
	gnrh3	NM_182887	Forward primer	TTGCCAGCACTGGTCATACG
			Reverse primer	TCCATTTCACCAACGCTTCTT
	gnrhr1	NM_001144980	Forward primer	ACCCGAATCCTCGTGGAAA
			Reverse primer	TCCACCCTTGCCCTTACCA
	gnrhr2	NM_001144979	Forward primer	CAACCTGGCCGTGCTTTACT
			Reverse primer	GGACGTGGGAGCGTTTTCT
	gnrhr4	NM_001098193	Forward primer	CACCAACAACAAGCGCAAGT
			Reverse primer	GGCAACGGTGAGGTTCATG
	fshβ	NM_205624	Forward primer	GCTGTCGACTCACCAACATCTC
			Reverse primer	GTGACGCAGCTCCCACATT
	lhβ	NM_205622	Forward primer	GGCTGCTCAGAGCTTGGTTT
			Reverse primer	TCCACCGATACCGTCTCATTTA
	cyp19b	AF183908	Forward primer	GTCGTTACTTCCAGCCATTCG
			Reverse primer	GCAATGTGCTTCCCAACACA
	erα	NM_152959	Forward primer	CAGACTGCGCAAGTGTTATGAAG

		Reverse primer	CGCCCTCCGCGATCTT
er2β	NM_174862	Forward primer	TTCACCCCTGACCTCAAGCT
		Reverse primer	TCCATGATGCCTTCAACACAA
ar	NM_001083123	Forward primer	TCTGGGTTGGAGGTCCTACAA
		Reverse primer	GGTCTGGAGCGAAGTACAGCAT
fshr	NM_001001812	Forward primer	CGTAATCCCGCTTTTGTTCCT
		Reverse primer	CCATGCGCTTGGCGATA
lhr	AY424302	Forward primer	GGCCATCGCCGGAAA
		Reverse primer	GGTTAATTTGCAGCGGCTAGTG
hmgra	BC155135	Forward primer	GAATCCACGGCCTCTTCGT
		Reverse primer	GGGTTACGGTAGCCACAATGA
hmgrb	NM_001014292	Forward primer	TGGCCGGACCGCTTCTA
		Reverse primer	GTTGTTGCCATAGGAACATGGA
star	NM_131663	Forward primer	GGTCTGAGGAAGAATGCAATGAT
		Reverse primer	CCAGGTCCGGAGAGCTTGT
cyp11a	NM_152953	Forward primer	GGCAGAGCACCGCAAAA
		Reverse primer	CCATCGTCCAGGGATCTTATTG
3βhsd	AY279108	Forward primer	AGGCACGCAGGAGCACTACT
		Reverse primer	CCAATCGTCTTTCAGCTGGTAA
cyp17	AY281362	Forward primer	TCTTTGACCCAGGACGCTTT
		Reverse primer	CCGACGGGCAGCACAA
17βhsd	AY306005	Forward primer	TGCATCTCGCATCAAATCCA
		Reverse primer	GTCCAAGTTCCGCATAGTAGCA
cyp19a	AF226620	Forward primer	GCTGACGGATGCTCAAGGA
		Reverse primer	CCACGATGCACCGCAGTA
vtgI	NM_001044897	Forward primer	GGAGGAATCCATGAAGCTCTTCTAAA
		Reverse primer	TGCTCTCAGTGTACGCTTAATCTTT
		TaqMan probe	FAM-ACGGTCAACACTTTCATCTG-NFQ

[a] Sequence is not available. Primers for all genes in H295R cells and *β-actin* and *vtgI* genes in zebrafish were commercially designed by Applied Biosystems.

Concentration of PFDA and PFTrDA in surface water

Table S2. Concentration of perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (PFTrDA) reported in surface water worldwide

Chemicals	Location	N	LOQ (ng/L)	Concentration (ng/L)			Reference
				Mean	Min.	Max.	
PFDA	Australia, Parramatta river	20	0.2	1.2	0.8	1.6	Thompson et al., 2011
	Canada, Welland river	1	0.25	<LOQ	<LOQ	<LOQ	De Solla et al., 2012
	China, Baiyangdian lake	18	-	0.31	0.13	0.66	Shi et al., 2012
	China, East and South China sea	21	0.035	<LOQ	<LOQ	<LOQ	Cai et al., 2012
	China, Baiyangdian lake	26	0.1	0.4	0.1	0.6	Zhou et al., 2012
	China, Pearl river	6	0.005	0.35[a]	<LOQ	0.57	So et al., 2007
	China, Yangtze river	11	0.005	0.39[a]	<LOQ	3.8	So et al., 2007
	Germany, Elbe river	15	0.11	-	0.2	0.7	Ahrens et al., 2009b
	India, Southern river	10	0.083	-	<LOQ	0.36	Yeung et al., 2009
	Japan, Tsurumi river	9	-	-	2.1	4.3	Zushi et al., 2008
	Korea, surface water near Yellow sea	15	0.2	2.3	<LOQ	9.3	Naile et al., 2013
	Korea, estuarine, Nakdong river	7	0.3[b]	3.5	2.7	4.9	Hong et al., 2013
	Korea, inland creek, Youngsan river	7	0.3[b]	1.2	0.59	2.4	Hong et al., 2013
	Spain, Catalonia drinking water	40	0.12	0.52	<0.12	10	Ericson et al., 2009
	USA, Conasauga river	20	0.1	-	3.74	160	Konwick et al., 2008
PFTrDA	Australia, Parramatta river	20	0.1	<LOQ	<LOQ	<LOQ	Thompson et al., 2011
	Canada, Welland river	1	0.25	<LOQ	<LOQ	<LOQ	De Solla et al., 2012
	China, East and South China sea	21	0.025	-	0.028	0.028	Cai et al., 2012
	Germany, WWTP effluents	9	0.08	-	<LOQ	0.4	Ahrens et al., 2009a

[a] For concentrations below limit of quantification (LOQ), a proxy value equal to an LOQ divided by square root of 2 was used for calculating mean concentration.

[b] Method detection limits.

-: not available.

WWTP : waste water treatment plant.

Zebrafish long-term exposure

Comparison analysis of hormone concentration between whole blood and plasma

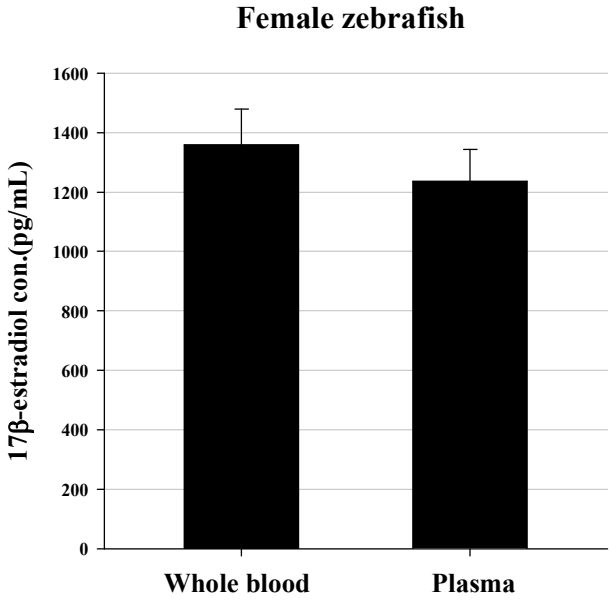

Figure S1. 17β-estradiol (E2) concentrations of whole blood and plasma in female zebrafish (*n*=3).

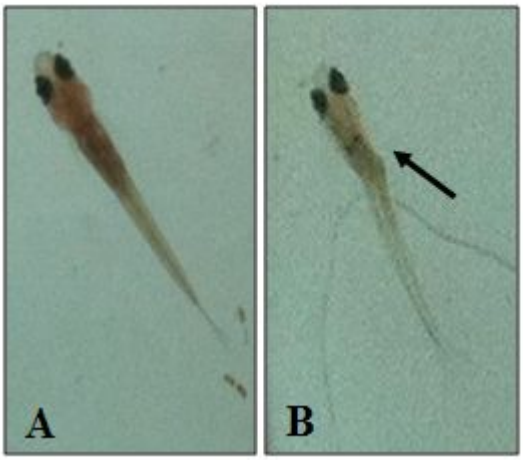

Figure S2. Phenotypic changes in larvae (A) control and (B) 10 mg/L PFDA at 20 day post-fertilization. Arrow indicates bent spine and tail.

국문 초록

과불화화합물 perfluorodecanoic acid (PFDA)와 perfluorotridecanoic acid (PFTrDA)의 만성노출에 의한 수서생물 *Daphnia magna* 와 *Danio rerio* 의 내분비계 교란독성

조 아 름

서울대학교 보건대학원

환경보건학과 환경보건 전공

지도교수 최 경 호

연구 배경: 프라이팬, 일회용기의 코팅제 등으로 널리 이용되고 있는 과불화화합물은 환경 중에 배출될 가능성이 큰 화학물질의 하나로 생태계 영향이 우려된다. 그러나 현재까지 수행된 대부분의 생태독성 연구는 perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) 등 주요 과불화화합물의 급성 영향에 대한 것이다. 그 외의 과불화화합물에 장기간 노출되었을 경우 나타날 수 있는 생물학적 영향에 대한 이해가 부족한 형편이다.

연구 목적: perfluorodecanoic acid (PFDA)와 perfluorotridecanoic acid (PFTrDA)가 물 환경에 만성 노출되었을 때 수서생물에 미치는 영향을 살펴보고 세포를 통한 기전연구를 통해 내분비계 교란독성을 밝힌다.

연구 방법: 성어의 짝짓기를 통해 얻은 400 개의 알을 대조군을 포함한 5 개의 농도군에 zebrafish (*Danio rerio*)를 120 일간 노출시킨 후, 성 호르몬 농도 및 성 호르몬 합성에 관여하는 주요 mRNA 발현 변화 수준을 측정하고 수서 무

척추 동물인 *D.magna* 의 급·만성 독성시험을 실시하여 1 일 1 회 생존여부와 산자수를 측정하고 시험 종결 일에는 신장을 관찰하여 생존능력, 번식능력, 성장능력의 변화를 조사한다. 마지막으로 세포를 이용한 기전 연구인 H295R cell bioassay 를 통해 testosterone 과 17β-estradiol 의 농도를 측정하고, 스테로이드 생합성 과정에 관여하는 유전자인 aromatase (*CYP19A*), *CYP17, CYP11A1, CYP11B2, 3βHSD, 17βHSD* 의 발현 정도를 측정한다.

연구 결과: 물벼룩 만성독성 시험에서는 PFTrDA 50 μg/L 에 노출된 그룹에서 생존수와 새끼를 낳는 시기에 있어 대조군과 유의한 차이가 있었고 0.1 mg/L PFTrDA 에 노출시킨 수컷 zebrafish 의 testosterone 과 11-ketotestosterone 의 농도가 유의하게 감소하였으며, 17β-estradiol 의 농도가 유의하게 증가하였다. 또한 성 호르몬 합성에 관여하는 유전자인 *CYP19A* 와 *VTG1* 의 발현 정도가 유의하게 증가하였으며 세포를 이용한 시험에서도 유사한 결과를 나타내었다.

결론: PFDA 와 PFTrDA 의 만성노출은 수서생물 zebrafish 와 *D.magna* 의 생존 및 번식능력에 악영향을 미치며 성호르몬의 불균형을 초래하는 것으로 보인다. 하지만 노출농도가 환경중의 농도보다 높게 설정되었으며, 따라서 실제 환경에서의 PFDA 와 PFTrDA 에 대한 독성영향은 무시할만한 수준이다.

주요어: endocrine disruption, gene transcription, perfluoroalkyl acids, sex hormones, HPG axis

학번: 2011-22058