SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 27, No. 10, pp. 2159–2168, 2008
ffi 2008 SETAC
Printed in the USA
0730-7268/08 $12.00 ffi .00

TOXICITY OF PERFLUOROOCTANE SULFONIC ACID AND PERFLUOROOCTANOIC ACID ON FRESHWATER MACROINVERTEBRATES (*DAPHNIA MAGNA* AND *MOINA MACROCOPA*) AND FISH (*ORYZIAS LATIPES*)

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(*Received* 29 *September* 2007; *Accepted* 3 *April* 2008)

Abstract—Because of their global distribution, persistence, and tendency to bioaccumulate, concerns aboutperfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are growing. We determined the toxicity of PFOS and PFOA in several freshwater organisms, including two cladocerans, *Daphnia magna* and *Moina macrocopa*, and the teleost *Oryzias latipes*. In general, PFOS is approximately 10 times more toxic than PFOA in these organisms. In *M. macrocopa*, the median lethal concentration (LC50) was 17.95 mg/L for PFOS and 199.51 mg/L for PFOA. *Moina macrocopa* exhibited greater sensitivity than *D. magna* to both perfluorinated compounds in both acute and chronic exposures. In the 48-h acute toxicity test, *M. macrocopa* was approximately two times more sensitive than *D. magna*. In the 7-d chronic toxicity test, *M. macrocopa* showed significant reproductive changes at 0.31 mg/L for PFOS, which was approximately seven times lower than the effect concentrations observed over the 21-d exposure in *D. magna*. Two-generation fish toxicity tests showed that parental exposure to both compounds affected the performance of offspring. Unexposed progeny-generation (F_1) fish exhibited elevated mortality and histopathological changes that were correlated with exposure in the parental generation (F_0). Continuous exposure from F_0 through F_1 generations increased the extent of adverse effects. Considering the persistent nature of PFOS and PFOA, more research is required to determine potential consequences of long-term exposure to these compounds in aquatic ecosystems.

Keywords—Perfluorinated compounds Thyroid Transgenerational toxicity Water flea Aquatic toxicity

INTRODUCTION

Perfluorinated chemicals are used in many industrial and commercial processes, such as lubricants, fire retardants, polymeradditives,pesticides,andsurfactants[1,2].Concernsabout perfluorinated chemicals, particularly perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are growing because of the global distribution, environmental persistence, and bioaccumulative nature of these compounds [3]. Because of their high-energy carbon–fluorine bonds, PFOS and PFOA resist hydrolysis, photolysis, biodegradation, and metabolism [1–3]. Numerous studies have detected the presence of PFOS and PFOA at the level of parts per trillion to parts per billion in several terrestrial and aquatic species [4–6]. In response to these concerns, the 3M Company (St. Paul, MN, USA) announced it would voluntarily cease manufacturing perfluorooctanyl-related materials by 2003 [7]. A decline in trends of PFOS and PFOA has been reported in human sera [8] but has not yet been clearly demonstrated in the environment.

Perfluorooctane sulfonic acid and PFOA may affect aquatic invertebrates and fish in various ways. The 21-d lethality no-observed-effect concentration (NOEC) of PFOS determined for *D. magna* is reported 5.3 mg/L, while a 48-h immobility NOEC value is 0.8 mg/L [9]. Sanderson et al. determined lowest-observed-effect concentration values for PFOA that ranged between 10 and 70 mg/L from various zooplankton species, including *Daphnia*, *Cyclops*, and *Rotifera* spp., in a 35-d indoor aquatic microcosm study [7]. Also, PFOS and PFOA affect fish reproduction through physiological effects on the hypothalamic–pituitary–gonadal axis [10]. These compounds may alter plasma concentrations of both steroidal androgens and estrogens in rainbow trout and fathead minnows [11,12]. In addition, PFOS and PFOA inhibit thyroid hormone homeostasis. Because thyroid hormone is important for regulating growth, embryo and larval development, metamorphosis, reproduction, and behavior in teleost fish [13], the perturbation of thyroid homeostasis may affect fitness of individual organisms and may cause population- or community-level effects. Little is known about the sublethal consequences of long-term exposure to PFOS and PFOA in aquatic fauna. A zooplankton community-level NOEC of PFOS was reported to be 3.0 mg/L from a 35-d aquatic microcosm study [14]. In a 39-d exposure study employing PFOA levels much higher than those normally observed in the environment, modest changes in fitness and relative liver and gonad size, along with significant declines in circulating plasma steroids, were observed in *Pimephales promelas* [15]. However, there is still paucity of chronic toxicity information for these perfluorinated compounds. In the present study, we performed acute and chronic toxicity tests using water fleas (*Daphnia magna* and *Moina macrocopa*) and a two-generation test using Japanese medaka (*Oryzias latipes*) to provide more information on the adverse effects of long-term exposure to PFOS and PFOA in the aquatic ecosystem.

MATERIALS AND METHODS

Test chemicals

Both acid forms of PFOS ($C_8HF_{17}SO_3$; Chemical Abstract Service registry number [CAS RN] 1763-23-1) and PFOA

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Published on the Web 6/10/2008.

ED_001556_00002765-00001

($C_8HF_{15}O_2$; CAS RN 335-67-1) were obtained from WakoPure Chemical (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Solvent-free stock solutions of PFOS(200 mg/L) and PFOA (2,000 mg/L) were prepared by dissolving the solid in MilliQffl(Millipore Asia, Yonezawa, Japan) water via sonication. Beyond the critical micelle concentration, PFOS may aggregate and confound exposures carried out in an aqueous medium. However, PFOS has critical micelle concentrations of 370 mg/L in fresh water and 570 mg/L in pure water [16], which are much higher than the maximum concentration used in our experiments (200 mg/L). Perfluorooctanoic acid is reported to have nearly complete solubility in water up to 20,000 mg/L [17]; hence, PFOA solubility was not a concern. Chemical measurements were not made, and nominal concentrations were used throughout the present study. Because the hydrolytic half-lives of PFOS (ffl41 years) and PFOA (J 92 years) are very long, chemical degradation during the exposure was not likely. In the present study, we used glass beakers as the test vehicle. Although glass containers have been widely used in studies involving these compounds [11,18,19], and there is a literature supporting the use of glassware [20], it should be noted that the use of glass might not be appropriate for this group of compounds because of potential sorption onto the glass surface [9,21].

Test organisms and maintenance

Three test species were used to evaluate toxicity. *D. magna* and *M. macrocopa* were housed in moderately hard water manufactured according to the U.S. Environmental Protection Agency (U.S. EPA) guidelines [22] under a 16:8-h light:dark photoperiod. Water quality parameters, including hardness, alkalinity, pH, conductivity, temperature, and dissolved oxygen, were routinely monitored using standard methods [23]. Cultures of *D. magna* were maintained at 21 | 1–C in 6-L glass jars, and *M. macrocopa* cultures were maintained at 25 | 1–C in 3-L glass beakers in the Environmental Toxicology Laboratory at Seoul National University (Korea). *Moina macrocopa* and *D. magna* were fed daily with a 1:1:1 mixture of yeast (ACH Food Companies, Memphis, TN, USA), cerophyll (Nutraceutical, Park City, UT, USA), and tetramin (Tetra,Melle, Germany), and algae (*Pseudokirchneriella subcapitata*); the culture water was changed twice weekly.

Japanese medaka was maintained in an incubation room at 25 | 1–C for several years in our laboratory. The fish were maintained under a 16:8-h light:dark photoperiod and fed with *Artemia nauplii* (• 24 h after hatching) twice daily.

D. magna *and* M. macrocopa *toxicity tests*

The 48-h acute toxicity test for *D. magna* was conducted in accordance with the procedure outlined by the U.S. EPA [22]. Definitive test concentrations for each compound were determined via preliminary range-finding tests. Four replicates with five neonates each (• 24 h old) were exposed to various concentrations of PFOS (0, 6.25, 12.5, 25, 50, and 100 mg/L) and PFOA (0, 62.5, 125, 250, 500, and 1,000 mg/L) at 21 | 1–C. The number of organisms rendered immobile was recorded 24 and 48 h after exposure. Test organisms were not fed during this period. Water quality parameters (pH, temperature, conductivity, and dissolved oxygen) were measured 48 h after exposure. Reference tests using sodium chloride as a reference toxicant were carried out monthly to assure comparable sensitivity among cohorts of test organisms over time (data not shown). The 48-h acute toxicity test for *M. macrocopa* was conducted using thesame procedureasthe *D. magna* test at an elevated temperature (25 | 1–C).

The effects of long-term exposure to PFOS and PFOA on survival, reproduction, and growth in *D. magna* were assessed using a standard test protocol [24]. Ten replicates with one neonate each (• 24 h old) were exposed to various concentrations of PFOS (0, 0.3125, 0.625, 1.25, 2.5, and 5 mg/L) and PFOA (0, 3.125, 6.25, 12.5, 25, and 50 mg/L) for 21 d at 21 | 1–C. The medium was renewed at least threetimesperweek, and the neonates produced by each parent animalwerecounted and removed daily. Water quality parameters were measured after changing the medium. Mortality among the parent animals and the number of living offspring were recorded daily. At the end of the test, the body length of each water flea (i.e., from the top of the head capsule to the base of the shell spine) was measured using a stereomicroscope (Dongwon, Bucheon, Korea) as described by Allen and Gerald [25]. The population growth rate (PGR) was calculated using the followingequation [26]:

$$\text{ffi}_x m_x e^{\square rx}$$

where l_x is the proportion of individuals surviving to age x, m_x is the age-specific fecundity (number of females produced per surviving female at age x), e is the base of the natural logarithm, and x is time in days.

We calculated the value of the PGR from the 21-d chronic exposure of *D. magna*. Due to the importance of fecundity during the early life stage, a PGR derived from the 21-d exposure provides a good estimate of the PGR for the entire life spanof *D. magna* [27].Chronic *M. macrocopa* exposureswere conducted according to the protocol described by S.R. Oh (2007, Master's thesis, Seoul National University, Seoul, Korea). The test protocol was similar to the chronic *D. magna* test except for the test duration (7 d), an exposure temperature (25 | 1–C), and the feeding regime (100- 1 yeast:cerophyll: tetramin mixture and 200- 1 algae suspension per day).

O. latipes *two-generation toxicity test*

The fish toxicity tests started with breeding and continued until 100 d after the hatching of offspring. We determined the effects of PFOS and PFOA using various endpoints within the parental (F_0) and progeny (F_1) generations.

F_0 fish exposure study. Breeding medaka pairs (body length ffl2.5 | 1 cm) were maintained at 25 | 1–C for at least 7 d in 1-L beakers filled with dechlorinated tap water, which was prepared by serial filtration through a sediment and two granular activated carbon filters. Thirty-six mating pairs that spawned more than eight eggs per breeding and bred more than five times per week were selected and randomlyseparated into four groups. Nine mating pairs were assigned to each treatment group and the control. Based on the preliminary range-finding results using adult medaka, we selected the following concentrations for definitive tests: PFOS, 0.01, 0.1, and 1 mg/L; PFOA, 0.1, 1, and 10 mg/L. The exposure duration for F_0 fish was limited to 14 d, during which the fish were fed *A. nauplii* (• 24 h after hatching) ad libitum twice daily. The exposure medium was renewed at least three times per week. Dead fish were removed as soon as possible. Eggs were counted every day, and the eggs spawned on the seventh day were saved for the F_1 generation exposure study. On day 14, all surviving fish were euthanized and body length and weight were measured, from which the condition factor (100 total weight/total length^3) was calculated. The gonads and livers

Table 1. Acute toxicity values of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) obtained using *Daphnia magna* and *Moina macrocopa*[ab]

Chemicals	Species name	Exposure (h)	EC50	CI	NOEC	LOEC
PFOS	*D. magna*	24	76.82	62.09–91.56	25	50
		48	37.36	30.72–43.99	12.5	25
	M. macrocopa	24	38.58	30.13–47.04	12.5	25
		48	17.95	14.72–21.18	6.25	12.5
PFOA	*D. magna*	24	675.05	559.62–790.50	500	1,000
		48	476.52	375.32–577.72	250	500
	M. macrocopa	24	348.76	272.65–424.87	125	250
		48	199.51	153.89–245.13	62.5	125

[a] Units are in mg/L unless otherwise noted.
[b] EC50 ♂ median effective concentration; CI ♂ confidence interval; NOEC ♂ no-observed-effect concentration; LOEC ♂ lowest-observed-effect concentration.

were also measured, and the gonadosomatic index (GSI; 100 gonad wt/body wt) and the hepatosomatic index (HSI; 100 liver wt/body wt) were calculated.

F_1 fish exposure study. Fertilized eggs collected from F_0 fish exposed to each concentration of PFOS or PFOA and the control were randomly separated into groups of 25 eggs each and then assigned to varying concentrations of PFOS (0, 0.01, 0.1, or 1 mg/L) or PFOA (0, 0.1, 1, or 10 mg/L) By doing so, we maintained all possible combinations of F_0 F_1 exposure concentrations for a given compound in the F_1 fish exposure study. Exposure was initiated in 50-ml beakers less than 12 h after fertilization. The developing embryos were observed daily under a stereoscopic microscope, and dead embryos were removed. This procedure was repeated until all living embryos had hatched. Hatching was defined as the disruption of the chorion [28].

Newly hatched larvae were then randomly transferred to 100-ml beakers and observed daily for swim-up success and survival for an additional two weeks. Larvae were fed *A. nauplii* (• 24 h after hatching) ad libitum twice daily. After 14 d, replicates with five fry each were randomly selected fromeach treatment group and transferred to 1-L beakers for the 100-d posthatchobservation.Allsurvivorsweresacrificed100dafter hatching, and body length and weight were measured. The gonads and livers were weighed to determine GSI and HSI.

Fish histopathology. The fish were examined for histological changes in the thyroid gland, as described by Bradford et al. [29]. We randomly selected five adult fish from among the survivors, which were then euthanized and fixed in Bouin's solution (75% saturated picric acid solution, 20% formalin, and 5% glacial acetic acid) for 24 h. The tissues were dehydrated in a series of ethyl alcohol and xylene baths and embedded in paraffin. Because thyroid tissues are usually found around the ventral aorta and brachial arteries near the gills and tongue in the lower jaw region of fish, serial sections were made in this region. Longitudinal sections (4 m) were made using a rotary microtome (HM 315; Microm, Heidelberg, Germany), mounted on slides, stained with hematoxylinandeosin, and examined using a light microscope. Five serial sections perfish wereselectedatrandom,andthepercentageoffollicles showing colloid depletion (the reduction or absence of colloid or the presence of pale, lacy, or granular material in the follicular lumen), hypertrophy (elongated cuboidal epithelial cells), and hyperplasia (multiple layers of follicular epithelium) was determined.Thyroiddamagewasevaluatedaccording to the criteria described by the U.S. EPA Pathology Working Group [30]. The degrees of colloid depletion, hypertrophy, and hyperplasia were scored as 0, 1, or 2 in the order of increasingseverity.Thesescoreswereusedtoquantifydamage to the thyroid gland [31].

Statistical analysis

In the 48-h acute toxicity tests for *D. magna* and *M. macrocopa*, the median effective concentrations (EC50) and confidence intervals (CIs) were calculated by probit analysisusing ToxStatfflver 3.5 (West, Cheyenne, WY, USA). No-observed-effect concentrations and lowest-observed-effect concentrations were calculated using Fisher's exact test in ToxStat. To analyze crustacean reproductive data, one-way analysis of variance and *t* tests with Bonferroni adjustment or Dunnett's test were performed using SPSS 12.0K for Windowsffl(SPSS, Chicago, IL, USA). Fisher's exact test was also used to analyze medaka survival data. For other types of toxicity data, one-way analysis of variance, regression analysis, and *t* tests, in combination with Dunnett's test, were performed using SPSS.

RESULTS

Toxicity of PFOS and PFOA in D. magna *and* M. macrocopa

The acute toxicities of PFOS and PFOA in *D. magna* and *M. macrocopa* are summarized in Table 1. In both species of water flea, PFOS was more toxic than PFOA (p • 0.05). For *D. magna*, the 48-h EC50 for PFOS was 37 mg/L, compared to 477 mg/L for PFOA. A similar pattern was observed in *M. macrocopa*, for which the 48-h EC50 for PFOS was 18 mg/L,whereasthatofPFOAwas200mg/L. *Moina macrocopa* was generally much more sensitive to both compounds than *D. magna* was (p • 0.05).

The results of 21-d chronic exposure to PFOS and PFOA in *D. magna* are summarized in Table 2. To *D. magna*, PFOS was more toxic than PFOA. The reproduction NOEC based on the number of young per adult determined for PFOS and PFOA was 1.25 and 12.5 mg/L, respectively. Exposure to PFOA and PFOA delayed the time required for reproduction of the first brood. *Daphnia* growth as determined by body length was also affected by the exposure but in a less sensitive manner.

The effects of the 7-d chronic exposure to PFOS and PFOA in *M. macrocopa* are summarized in Table 3. Again PFOS was more toxic than PFOA to *M. macrocopa*. Survival of *M. macrocopa* was significantly reduced at 2.5 mg/L for PFOS and at 25 mg/L for PFOA (p • 0.05). The number of young per adult decreased even at the lowest concentration of PFOS exposure (0.3125 mg/L). The meannumberofyoungperbrood and the number of broods per adult decreased at 0.625

Table 2. Results of 21-d chronic toxicity test, with *Daphnia magna* exposed to perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)[a]

Chemical	concn. (mg/L)	Adult survival (%)	Days to first brood		No. young per adult		No. young per brood		Growth (mm)		Population growth rate
PFOS	Control	100	7.80	0.92	83.20	6.29	16.33	1.09	3.61	0.10	0.403
	0.3125	100	7.90	0.74	80.70	2.41	16.14	0.48	3.58	0.10	0.388
	0.625	100	8.50	1.08	78.30	3.95	16.01	0.75	3.55	0.11	0.371
	1.25	90	8.50	1.07	78.25	5.95	16.57	1.53	3.41	0.35	0.350
	2.5	70	9.71	1.11*	56.57	9.16*	12.88	1.26*	3.34	0.38	0.291
	5	70	11.80	0.84*	42.40	9.24*	11.08	1.57*	3.19	0.51	0.196
PFOA	Control	100	7.50	0.85	80.70	4.83	15.29	1.01	3.61	0.10	0.418
	3.125	100	7.90	0.99	79.90	7.72	15.76	1.65	3.57	0.09	0.391
	6.25	100	8.40	1.07	77.30	7.66	15.49	1.04	3.54	0.12	0.369
	12.5	90	9.56	1.13*	73.11	6.05	16.23	2.29	3.51	0.08	0.332
	25	90	9.70	0.82*	61.80	9.83*	13.46	1.46	3.49	0.12	0.321
	50	80	10.40	1.58*	61.20	19.67*	12.80	3.13	3.44	0.11*	0.302

[a] Values represent mean | standard deviation of each PFOS and PFOA concentration. Asterisk (*) denotes a significant difference from the control (p • 0.05).

mg/L for PFOS. The NOEC value for reproduction was less than 0.3125 mg/L for PFOS and 3.125 mg/L for PFOA, which was a lower concentration than the equivalent value to *D. magna*.

The PGR was adversely influenced by PFOS and PFOA exposure in a concentration-dependent manner (p • 0.05). However, an overall population decrease was not expected for either *D. magna* or *M. macrocopa* because the calculated growth rates were all greater than zero.

Toxicity of PFOS and PFOA in adult O. latipes

The effects of PFOS and PFOA on survival, GSI, HSI, and condition factors in adult Japanese medaka (F_0 generation) are summarized in Table 4. The GSI was most strongly affected in female medaka. We observed enlargement of the ovary at the lowest exposure concentration for PFOS (0.01 mg/L). The HSI in female medaka was also affected at 1 mg/L for PFOS. However, the exposure range tested in the present study did not result in significant effects on mortality or condition factors.

During the 14-d exposure to PFOS and PFOA, the number of eggs spawned by each female decreased in a concentration-dependent manner (Fig. 1). This difference in fecundity became more evident after day 8 among the fish treated with higher PFOS or PFOA concentrations.

Transgenerational toxicity after exposure to PFOS and PFOA in O. latipes

The PFOS and PFOA exposure affected embryonic development, hatchability, and time to hatch of medaka eggs (F_1 generation). Significant sublethal changes of F_1 fish were observed, especially when the parental generation was exposed to the highest concentrations (Fig. 2): A significant decrease in hatchability was observed in all three PFOS exposure groups (0.01, 0.1, and 1 mg/L) when the parental generation was exposed to 1 mg/L for PFOS (Fig. 2A). Time to hatch was also significantly delayed when both F_0 and F_1 organisms were exposed to the highest concentrations of both compounds (Fig. 2B). Swim-up success decreased significantly with PFOS exposure only when their parental generation was exposed to 1 mg/L for PFOS (Fig. 2C). Some hatched larvae in the PFOS and PFOA treatment groups also showed abnormal symptoms, such as erratic swimming with subsequent immobilization or lying on the bottom of the beaker.

The survival of F_1 fish decreased markedly with parental exposure to PFOS and PFOA (Fig. 2D). When we analyzed cumulative mortality 28 d after hatching, it was evident that parental exposure to both compounds resulted in negative effects on the next generation. The mortality observed among F_1 controls was correlated to the concentrations of exposure

Table 3. Results of 7-d chronic toxicity test with *Moina macrocopa* exposed to perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)[a]

Chemical	concn. (mg/L)	Adult survival (%)	Days to first brood		No. young per adult		No. young per brood		No. broods per adult		Population growth rate
PFOS	Control	100	3.00	0.00	50.60	3.50	12.37	0.75	4.10	0.32	1.104
	0.3125	100	3.00	0.00	43.40	4.79*	11.51	1.41	3.80	0.42	1.036
	0.625	100	3.00	0.00	33.70	6.86*	9.63	1.28*	3.50	0.53*	0.930
	1.25	100	3.10	0.32	29.00	3.56*	8.88	1.20*	3.30	0.48*	0.860
	2.5	50*	3.40	0.55	24.00	6.40*	7.47	1.56*	3.20	0.45*	0.607
	5	40*	3.75	0.50	20.25	5.19*	6.75	1.73	3.00	0.00*	0.482
PFOA	Control	100	3.00	0.00	51.10	7.91	12.79	1.61	4.00	0.47	1.105
	3.125	100	3.00	0.00	43.80	4.29	11.23	0.64	3.90	0.32	1.048
	6.25	100	3.00	0.00	33.30	7.23*	9.53	1.60*	3.50	0.53	0.918
	12.5	100	3.10	0.32	29.10	6.85*	8.59	1.60*	3.40	0.52*	0.896
	25	50*	3.20	0.45	23.60	3.65*	7.87	1.22*	3.00	0.00*	0.592
	50	0*	—		—		—		—		—

[a] Values represent mean | standard deviation of each PFOS and PFOA concentration. — means not available, and asterisk (*) denotes a significant difference from the control (p • 0.05).

Table 4. Survival, gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (K) determined in male and female F_0 generation medaka exposed to varying concentrations of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) for 14 d[a]

Chemical concn. (mg/L)	Survival (%)		GSI (%)				HSI (%)				K (%)			
	Male	Female	Male		Female		Male		Female		Male		Female	
Control	100	100	0.56	0.11	3.45	0.67	1.83	0.55	2.53	0.88	0.98	0.11	0.97	0.09
PFOS 0.01	88.9	88.9	0.62	0.14	7.26	2.12*	2.22	0.83	3.21	0.61	0.99	0.11	0.97	0.11
PFOS 0.1	100	66.7	0.65	0.35	8.12	1.92*	2.52	0.56	3.73	1.09	0.98	0.11	0.99	0.07
PFOS 1.0	100	66.7	0.67	0.36	8.75	2.60*	2.76	0.95	3.86	0.58*	0.95	0.11	0.98	0.09
Control	100	100	0.71	0.15	5.00	1.34	1.96	0.32	2.49	0.38	0.97	0.10	0.92	0.08
PFOA 0.1	100	77.8	1.13	0.30	5.88	0.99	2.26	0.80	3.03	0.99	0.94	0.07	0.94	0.15
PFOA 1.0	77.8	66.7	1.23	0.59	6.03	2.30	2.42	0.84	3.05	0.51	0.95	0.07	0.98	0.12
PFOA 10	77.8	77.8	1.37	0.76	6.80	1.62	2.62	0.96	3.06	0.79	0.99	0.13	0.93	0.10

[a] Values represent mean | standard deviation of each PFOS and PFOA concentration with female and male sample. Asterisk (*) denotes a significant difference from the control (p • 0.05).

in the parental generation. Continuous exposure to the same concentration from F_0 through F_1 generations increased the extent of the damage (Fig. 2D).

When observed for 100 d after hatching, body length and weight of F_1 control fish decreased significantly even at the lowest concentration of their parental PFOS exposure (0.01 mg/L; Table 5). In contrast, this effect was not observed for the concentrations of PFOA used in the present study. The GSI and HSI did not show any meaningful changes.

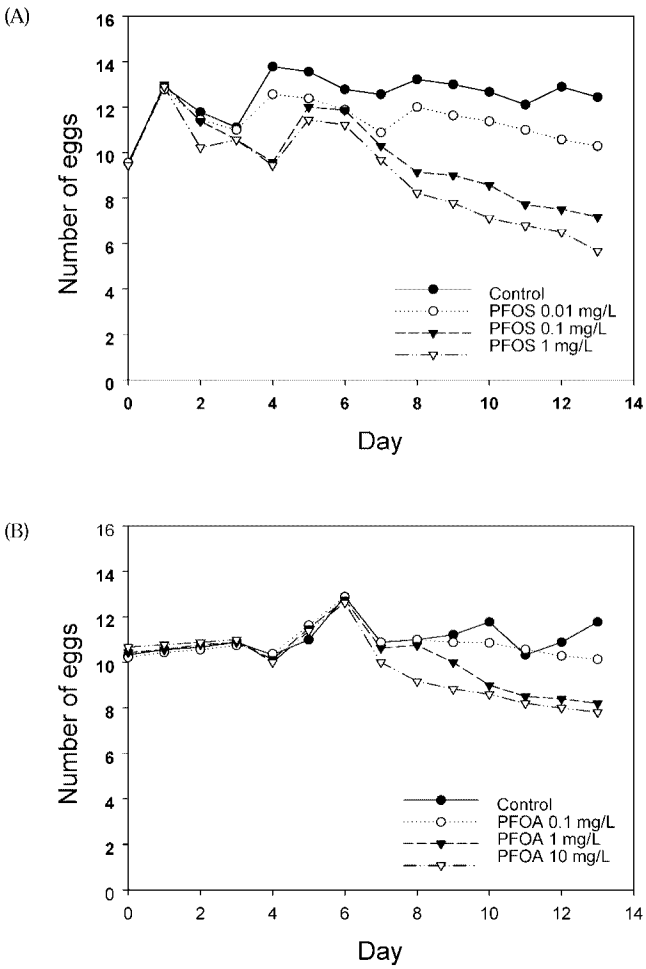

Fig. 1. Concentration-dependent effects of (**A**) perfluorooctane sulfonic acid (PFOS) and (**B**) perfluorooctanoic acid (PFOA) on medaka reproduction as shown in number of eggs per pair.

Parental exposure to PFOS and PFOA also caused histopathological changes to the thyroid gland in offspring. Figure 3 shows hematoxylin and eosin–stained thyroid gland sections from medaka exposed for 100 d after hatching. Compared to thyroid follicles observed in the control (Fig. 3A), the exposed fish showed various histological changes (Fig. 3B to D), such as hyperplasia, hypertrophy, and colloidal depletion. Depending on the concentration of parental exposure, the control F_1 fish generally showed increased frequencies of hyperplasia, hypertrophy, and colloidal depletion of the thyroid gland (Fig. 4).

DISCUSSION

Toxicity of PFOS and PFOA in D. magna *and* M. macrocopa

Our results show that PFOS is more toxic than PFOA in freshwater fleas (Table 1). The 48-h EC50 and NOEC values for PFOS were approximately one order of magnitude lower than the equivalent values for PFOA in both species. These findings are similar to the results of other studies that have evaluated the toxicity of both chemicals to aquatic organisms [32,33]. The 96-h LC50 for PFOA was reported to be 300 mg/L, while that for PFOS was 9.1 mg/L for fathead minnows (*P. promelas*) [32,33]. For *P. subcapitata*, 96-h EC50 for PFOA was more than 1,000 mg/L, while 72-h EC50 for PFOS was 120 mg/L [33].

Comparedto *D. magna*, *M. macrocopa*, anendemicKorean water flea, was much more susceptible to both compounds. For PFOS, the 48-h EC50 for *M. macrocopa* was 18 mg/L (95% CI ♂ 15–21 mg/L), while that for *D. magna* was 37 mg/L (95% CI ♂ 31–44 mg/L). For PFOA, the 48-h EC50 value for *M. macrocopa* was 200 mg/L (95% CI ♂ 154–245 mg/L), while that for *D. magna* was 477 mg/L (95% CI ♂ 375–578 mg/L). This trend is shown by other studies that used potassium salt of PFOS (CAS RN 2795-39-3) even though statistical significanceis notclear.Lee,Kim,andChoireported that the 48-h LC50 of potassium salt of PFOS for *M. macrocopa* was 28 mg/L [19], while Boudreau et al. reported the 48-h EC50 for *D. magna* was 67 mg/L (95% CI ♂ 31–89 mg/L) [9]. The 48-h EC50 (endpoint ♂ immobility) for the potassium salt of PFOS was 134 mg/L in *D. pulicaria* and 67 mg/L in *D. magna* [9]. A freshwater mussel, *Unio complamatus*, showed 96-h LC50 at 57 mg/L (95% CI ♂ 49–65 mg/L) [32]. However, for marine shrimp, *Mysidopsis bahia*, 96-h LC50 was reported to be 3.5 mg/L (95% CI ♂ 2.9–4.4 mg/L), significantly lower than that for *M. macrocopa* [32].

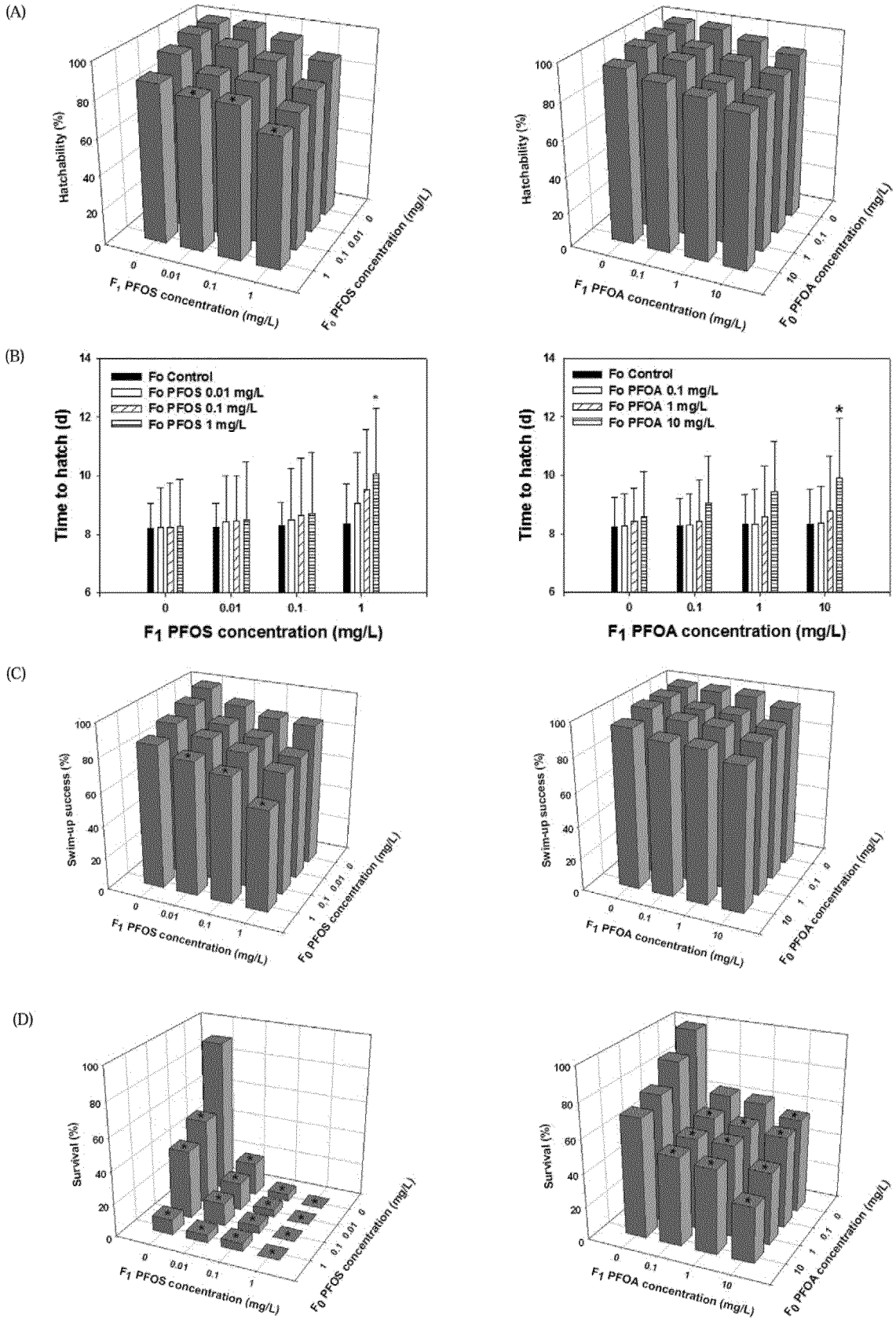

Fig. 2. Effect of transgenerational exposure to perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) on (**A**) hatchability, (**B**) time to hatch, (**C**) swim-up success rates, and (**D**) cumulative mortality 28 d after hatching in F_1 (progeny generation) medaka. The F_1 fish and/or the parental generation (F_0) were exposed to PFOS and PFOA. Exposure concentrations are in mg/L. Asterisk (*)denotesasignificantdifference from the control (p • 0.05).

Table 5. Growth, gonadosomatic index (GSI), and hepatosomatic index (HSI) in male and female F_1 (progeny generation) medaka exposed to varying concentrations of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) for 14 d in F_0 (parental generation) medaka[a]

Chemical	F_0 concn. (mg/L)	Growth				GSI (%)				HSI (%)	
		n	Total length (mm)	Body wt (mg)	*n*	Male	*n*	Female	Male	Female
PFOS	Control	23	2.57 ± 0.13	0.17 ± 0.03	9	0.60 ± 0.03	9	3.43 ± 0.19	1.84 ± 0.11	2.55 ± 0.25
	0.01	13	2.46 ± 0.10*	0.15 ± 0.02*	3	0.75 ± 0.03*	6	3.57 ± 0.36	2.12 ± 0.24	2.67 ± 0.32
	0.1	3	2.37 ± 0.06*	0.13 ± 0.02*	0	—	0	—	—	—
	1	1	2.30 ± 0.00*	0.12 ± 0.00*	0	—	0	—	—	—
PFOA	Control	24	2.56 ± 0.12	0.17 ± 0.03	8	0.60 ± 0.04	11	3.45 ± 0.22	1.84 ± 0.10	2.55 ± 0.18
	0.1	16	2.53 ± 0.11	0.16 ± 0.02	3	0.63 ± 0.03	10	3.55 ± 0.24	1.90 ± 0.07	2.60 ± 0.20
	1	10	2.50 ± 0.11	0.14 ± 0.02	0	—	0	—	—	—
	10	1	2.50 ± 0.00	0.15 ± 0.00	0	—	0	—	—	—

[a] Values represent mean ± standard deviation of each PFOS and PFOA concentration with female and male sample. Asterisk (*) denotes a significant difference from the control ($p \bullet 0.05$), and — denotes not available.

The marine microbe *Photobacterium phosphoreum* showed a 30-min EC50 of 730 mg/L for PFOA, whereas a freshwater fish, the fathead minnow, showed a 48-h EC50 of 720 mg/L for PFOA [34].

Moina macrocopa was also more sensitive to chronic exposure than *D. magna* was in terms of both survival and reproduction, although the exposure duration for *D. magna* (21 d) was three times longer than that for *M. macrocopa*. In *M. macrocopa*, survival decreased significantly at 2.5 mg/L for PFOS and 25 for mg/L for PFOA. However, the survival of *D. magna* was not affected at even the highest exposure concentrations (5 mg/L for PFOS and 50 mg/L for PFOA). These results are consistent with previous report on PFOS ecotoxicity, which showed 21-d NOEC based on adult survival in a range between 5.3 and 12 mg/L for *D. magna* [32]. Among freshwater invertebrates, *Chironomus tentans* is one of the most sensitive species, of which 20-d chronic EC50 based on survival was found at 0.092 mg/L [35].

The reproduction NOEC for PFOS was determined to be 1.25 mg/L from 21-d *D. magna* exposure in the present study, which is 6 to approximately 20 times lower than the NOECs reported for potassium salt of PFOS. With this potassium salt, Boudreau et al. [9] reported a reproduction NOEC at 25 mg/L, while the NOECs based on 21-d (survival) and 28-d (reproduction) exposures were 12 and 7 mg/L, respectively [34]. The difference in sensitivity between reports might be

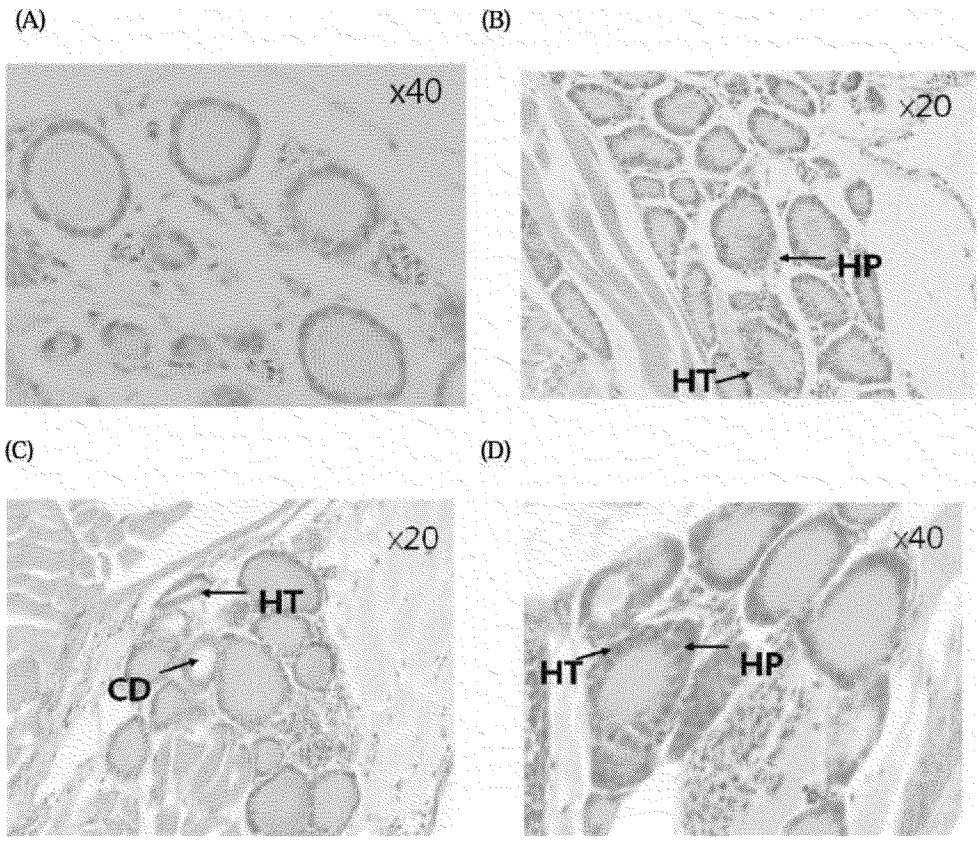

Fig. 3. Photomicrographs of hematoxylin and eosin-stained thyroid gland sections from *Oryzias latipes*. The sections shown here were obtained from control progeny-generation (F_1) fish spawned from parental-generation (F_0) fish exposed to various concentrationsofperfluorooctanesulfonic acid (PFOS) or perfluorooctanoic acid (PFOA). (**A**) No parental exposure, (**B**) 0.01 mg/L of PFOS, (**C**) 0.1 mg/L of PFOA, and (**D**) 1 mg/L of PFOA. Examples of hyperplasia (HP), hypertrophy (HT), and colloidal depletion (CD) are indicated with arrows.

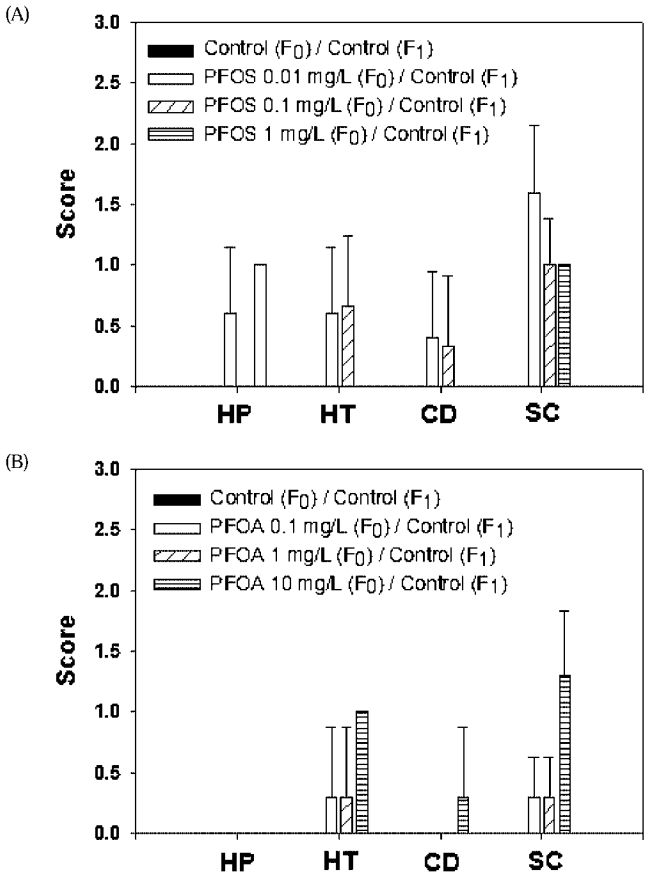

Fig. 4. Histopathological effects observed in F_1 (progeny generation) medaka that spawned from F_0 (parental generation) fish exposed to various concentrations of (**A**) perfluorooctane sulfonic acid (PFOS) and (**B**) perfluorooctanoic acid (PFOA) for 14 d. Scores wereassigned based on the severity of histological changes, such as thyroid follicle hyperplasia (HP), hypertrophy (HT), and colloid depletion (CD). SC ♂ sum score.

explained partly by a difference in culture condition or test protocol. In addition, the type of the test chemical may also partly explain the toxicity difference. For example, Boudreau et al. used clean well water as dilution water and replaced the test medium once a week [9], while we used the U.S. EPA's moderately hard water [22] as dilution water and replaced the test medium three times a week during the 21-d exposure. As shown from the 48-h exposure study with *D. magna*, theacidic form of PFOS that was employed in the present study appeared approximately two times more toxic than the potassium salt.

The PGR can be used in ecological toxicology to describe potential population-level effects of chemical exposure. In *D. magna* and *M. macrocopa*, the PGRs showed strong negative relationships with exposure concentrations (Tables 2 and 3; *p* trend • 0.05). However, the PGRs were greater than zero, indicating that an exposure-related population decrease is not expected under these conditions.

Toxicity of PFOS and PFOA in O. latipes

In the F_0 generation 14-d fish exposure test, PFOS and PFOA did not appear to affect the general fitness of adult medaka, as indicated by the observed condition factors (Table 4). However, the relative weight of the liver (HSI) and ovary (GSI) increased significantly in female medaka after PFOS exposure. An increase in HSI is usually the result of hyperplasia or hypertrophy as an adaptive hepatic response to foreign compounds [36]. Similar effects have been documented for rats [37] and primates [17] treated with PFOA. Rats exposed to PFOA exhibited increased testicular weights [37]. In contrast, no significant changes in male or female GSI were observed after exposing medaka to PFOA.

In general, fish appear to be much more sensitive than invertebrates to chronic PFOS exposure. Chronic data are available for the fathead minnow, *P. promelas*, derived from an early life stage toxicity test that exposed eggs and larvae to PFOS in a flow-through system for 47 d [38]. Minnows exposed to 0.60 mg/L for PFOS showed a significant decrease in hatchability, time to hatch, survival, and growth. In the present study, these effects were not significant among F_1 fish exposed to PFOS unless theirparentswerealsoexposed.When F_0 fish were exposed to PFOS, significant negative effects on hatchability and swim-up success were observed even at the lowest PFOS concentration (0.01 mg/L; Fig. 2A and C).

Medaka exposed to PFOS exhibited growth retardation, as determined by measurements of body length and weight. We observed a decrease in body weight at an exposure of 0.01 mg/L for PFOS, which may reflect interference with the cellular or functional maturation of target organs via the disruption of thyroid hormones. Proper development of the thyroid gland is crucial in regulating growth and development in vertebrates [13]; thus, damage to this gland could cause many adverse effects.

Figure 2 suggests that the exposure to PFOS or PFOA may carry the adverse effects over to the next generation. As shown in Figure 2D, control F_1 fish showed an increased mortality rate that was dependent upon the concentrations to which the parental generation was exposed. Cumulative mortality among these F_1 fish corresponds with the histopathological changes in their thyroid glands. As shown in Figure 3, the thyroid glands of the F_1 controls were histopathologically altered only when their parents were exposed. Histopathological changes in the thyroid gland may affect the thyroid function [18]. In normal fish eggs, enough quantities of maternally derived thyroid hormones are present; therefore, the embryos are thought to rely on this stock of hormones during embryogenesis until endogenous production can begin [13]. Parental exposure to PFOS or PFOA may prevent or limit this transferofthethyroid hormones to the fertilized eggs, thus limiting the available stock during embryogenesis and negatively affecting the development of embryos and the performance of larvae. To our knowledge, the present study is the first that showed transgenerational effects of PFOS and PFOA on both histopathology and survival of medaka.

The annual global production volume of PFOS-related chemicals gradually increased to 4,500 metric tons in 2002. In Korea, PFOS and PFOA concentrations in the waterways of the Shihwa industrial zone are approximately 89 and 19 ng/L, respectively [39]. Considering these real environmental concentrations, an ecological impact due to these compounds is unlikely except for accidental spills [40]. However, the persistent and bioaccumulative nature of these compounds creates the possibility of chronic effects. In addition,transgenerational carryover, as observed here, can take place within a relatively short exposure period.

CONCLUSION

PerfluorooctanesulfonicacidandPFOAaretwoofthemost pervasive perfluorinated compounds, with trace amounts presentinmanyenvironmentalmediaworldwide.Wedemonstrated

that parental exposure in Japanese medaka transferred adverse effects to offspring. Even without F_1 exposure, the exposure for the parental generation only could lead to a concentration-dependent increase of cumulative mortality in F_1 generation. Various histological changes in the thyroid gland may at least partly explain the mechanismof this transgenerationalincrease in mortality. In water fleas, the threshold of PFOS is approximately 10 times higher than that ofPFOA. *Moina macrocopa*, an endemic Korean species, was more susceptible than *D. magna*. The results of the present study should help increase the base of knowledge on risk assessment of perfluorinated compounds, and additional research is required to assess the potential adverse impacts of these compounds on aquatic systems.

Acknowledgement—This work was supported by the Korea Research Foundation grant funded by the Korean government (MOEHRD) (KRF-2006-D00278).

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