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Toxicity of perfluorononanoic acid and perfluorooctane sulfonate to *Daphnia magna*

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Abstract

In order to study toxicological effects of perfluorononanoic acid (PFNA), perfluorooctane sulfonate (PFOS), and their mixtures (PFNA/PFOS) on *Daphnia magna* (*D. magna*), a suite of comprehensive toxicity tests were conducted, including a 48-h acute toxicity test, a 21-day chronic test, a feeding experiment, and a biomarker assay. *D. magna* were exposed to aqueous solutions of PFNA and PFOS (alone and in combination) at concentrations ranging from 0.008 to 5 mg/L. The survival, growth, and reproduction of *D. magna* were monitored over a 21-day life cycle. The biomarkers, including acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT) activities, were determined after seven days of exposure. PFOS was more toxic than PFNA based on the results of the acute toxicity test. Perfluorinated compounds (PFCs) inhibited both growth and reproduction of *D. magna* during the testing period. The ingestion rates and the biomarkers, including AChE, SOD, and CAT activities, were significantly inhibited by PFCs in most cases. Moreover, the combined effects related to the growth and reproduction showed the antagonistic effects of PFCs.

Keywords: Toxicity effect; Perfluorononanoic acid; Perfluorooctane sulfonate; *Daphnia magna*; Reproduction; Ingestion rate; Biomarker

1. Introduction

Perfluorinated compounds (PFCs) with different functions have been extensively used in many technologies, either individually or as composites in manufactured products (Giesy and Kannan, 2002). For examples, PFCs were used as water-, oil- and grease-repellents or as surfactants (Renner, 2001). In recent years, PFCs have become a focus of public health concern due to their ubiquitous presence in environmental media (air, water, soil, sediment, and house dust), food and drinking water, and in wildlife, including fish, birds, and mammals (Mommaerts et al., 2011; Murakami et al., 2011; Rudel et al., 2011; Zhang et al., 2011; Tatum-Gibbs et al., 2011). PFCs have been detected in urban and remote areas around the world, and some researchers have indicated that PFCs accumulate in animals, including humans (Powley et al., 2008; Cai et al., 2010; Leondiadis et al., 2010).

Of the PFCs, perfluorooctane sulfonate (PFOS) and perfluorononanoic acid (PFNA) have been found to be the representative PFCs in the environment and in biota (Guruge et al., 2005; Kannan et al., 2004), and PFOS is regarded as the final metabolite of many PFCs by degradation or metabolization (Olsen et al., 2004; Calafat et al., 2006). The ionic nature, high solubility, and negligible vapor pressure when PFOS and PFNA are dissolved in water, make them highly mobile, and they exist in groundwater (Plumlee et al., 2008), surface water (Eschauzier et al., 2010), drinking water (Flores et al., 2013), and ocean water (Sánchez-Avila et al., 2010) with a concentration ranging from ng/L to several μg/L. A higher concentration of PFOS (about 43.5 μg/L) has been detected in

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the Langat River, Malaysia (Zainuddin et al., 2012). A composition profile of total PFC concentrations showed that the composition was dominated by PFOS and PFNA in the Yodo River system of Japan (Shivakoti et al., 2011). In sediment, the contents of PFOS were shown to be 28−145 pg/g of the dry weight at Kogaigawa and Sakuragawa cities in Ibaraki Prefecture, in Japan (Ahrens et al., 2011). Kratzer et al. (2011) found that PFOS was the predominant compound (9.57−1 444 ng/g of wet weight), followed by PFNA (0.47−109 ng/g of wet weight), in liver tissue of grey seals (*Halichoerus grypus*) collected from the Baltic Sea. In addition, a study revealed that insect larvae, fish, and crabs contained levels ranging from 0.23 to 144 ng/g of wet weight of PFOS and from 0.07 to 7.5 ng/g of wet weight of PFNA (Fernandez-Sanjuan et al., 2010). PFOS is also listed as a chemical contaminant on the Final Third Drinking Water Contaminant Candidate List (CCL3) considered for future regulation (US EPA, 2009). Clearly, more information is required to guide decisions about regulations of PFOS and PFNA.

Recent studies have indicated unintended biological activity of PFCs toward non-target organisms. The no-observed effect concentration (NOEC) of PFOS determined for *Daphnia magna* (*D. magna*) is reported to be 5.3 mg/L (Boudreau et al., 2003). PFOS has also been found to decrease the heart rate of zebrafish embryos (Ding et al., 2012a). Huang et al. (2012) determined the transcription profiling of *Oryzias melastigma* embryo exposed to PFOS, and indicated that the differentially expressed genes were related to neurobehavioral defects, mitochondrial dysfunction, and the metabolism of proteins and fats. Thyroid-disrupting effects of long-term PFNA exposure have been observed on the levels of genes and proteins in zebrafish (Liu et al., 2011). However, toxicity information regarding PFNA, and especially information on its toxicity when combined with PFOS, is still lacking with regard to *D. magna*. *D. magna*, designated by the U.S. Environmental Protection Agency (U.S. EPA) as a model organism, is widely accepted as an indicator for assessing the toxicity of environmental contaminants because of its high rate of reproduction and sensitivity to different conditions (Ventura et al., 2010). Acute and chronic toxicity tests are simple, cost-effective, and sensitive, and are a widely accepted method for toxicity determination, providing a fast yet accurate estimate of toxicity of a compound (Ventura et al., 2010). The feeding behavior of aquatic organisms has been studied as a physiological response to toxic effects of chemicals (Barata et al., 2008; de Schamphelaere et al., 2007). In addition, antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) have also been widely used for both characterization of the defense mechanisms and evaluation of the toxicity induced by oxidative stressors (Kim et al., 2012).

In order to investigate the biological effects of PFCs on *D. magna* after different exposure periods, we conducted a comprehensive toxicity test of PFOS, PFNA, and their mixtures (PFNA/PFOS), including a 48-h acute toxicity test and a 21-day chronic toxicity test. A feeding experiment was conducted to explore whether the two target compounds could interfere with food intake and ultimately cause toxicity to *D. magna*. Finally, the activities of acetylcholinesterase (AChE), SOD, and CAT were synchronously determined in order to investigate single and combined effects of the two PFCs on multiple biomarkers during a seven-day exposure period. All of the above are important for ecotoxicological risk assessments of PFOS and PFNA.

2. Materials and methods

2.1. Chemicals

PFNA (98% purity) was purchased from the Alfa Aesar Co., Ltd. (Los Angeles, USA). PFOS (98% purity) was purchased from the Matrix Scientific Co., Ltd. (Basel, Switzerland).

The test solution was prepared immediately prior to use by diluting the stock solution with a daphnia culture medium (consisting of 64.75 mg/L of $NaHCO_3$, 5.75 mg/L of KCl, 123.25 mg/L of $MgSO_4 \cdot 7H_2O$, and 294 mg/L of $CaCl_2 \cdot 2H_2O$) reconstituted according to the guideline formulated by the Organization for Economic Cooperation and Development (OECD, 2004).

Acetylthiocholine iodide (ATChI) and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin was purchased from the Shanghai Huixing Biochemistry Reagent Co., Ltd. (Shanghai, China) with a purity of more than 98%. All other chemicals were of analytical grade and were obtained from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Animals

D. magna, a common zooplankton found in freshwater lakes and ponds, is one of the most sensitive organisms used in toxicity tests (Alberdi et al., 1996). In this study, *D. magna* were originally obtained from the Chinese Center for Disease Control and Prevention (Beijing, China). It was cultured in dilution water according to the International Organization for Standardization (ISO, 1996). The culture medium was renewed three times weekly. *D. magna* were fed daily with the green algae, *Scenedesmus obliquus*, which was supplied by the Wuhan Institute of Hydrobiology of the Chinese Academy of Sciences. Exposure water quality was checked daily and maintained at conditions suitable for *D. magna* (temperature of (20 ± 1)°C, pH value of 7.2 ± 0.3, and dissolved oxygen (DO) of (6.5 ± 0.5) mg/L). A daily 16/8-h light/dark photoperiod cycle was used throughout the experiment.

Offspring of *D. magna* were separated at regular intervals from culture dishes that were 3−5 weeks old and the test animals were less than 24 h old.

2.3. Acute and chronic toxicity tests

The 48-h acute toxicity test of *D. magna* was performed according to OECD (2004). Six concentrations of PFC (1, 3,

10, 30, 100, and 300 mg/L) were selected. Ten neonates less than 24 h old from a designated brood were placed in a 100 mL glass beaker containing 45 mL of PFC solution for each test concentration and control group. No PFC was added in the control group. Test *D. magna* were not fed during the testing period. Each treatment was replicated three times simultaneously. Water quality parameters (pH value, temperature, conductivity, and DO) were measured at the very beginning and end of the test. The statuses of immobilization and mortality were checked at 48 h, and results were recorded. *D. magna* that were unable to swim within 15 s after gentle agitation of the test container was considered to be immobile. Those animals whose heartbeats had stopped were considered dead. The heartbeats were observed with a stereomicroscope with a magnification of four.

The effects of two kinds PFCs on the reproductive output were assessed with a semi-static test according to the standard procedure for *D. magna* reproduction testing (OECD, 2012). Based on the results of acute toxicity tests, neonates less than 24 h old were exposed to various concentrations of PFOS and PFNA (0.008, 0.04, 0.2, 1, and 5 mg/L). The solutions of PFNA and PFOS were also mixed with the same concentrations at 0.004, 0.02, 0.1, 0.5, and 2.5 mg/L. Single *D. magna* was cultured in 100 mL glass beakers filled with 45 mL of solution for a 21-day period at (20 ± 1)°C. The test was performed in 20 replicates for each exposure concentration. *D. magna* were fed with 1×10^6 cells/mL of *Scenedesmus obliquus* per day for each animal. The test solution was renewed every other day. Water quality parameters were measured after changing the test solution. Survival, growth, and reproduction (fecundity) of *D. magna* were monitored for each of the 20 replicates. Growth of the surviving adults under each treatment was determined after 21 days of exposure. The body length of each surviving animal was measured from the apex of the helmet to the base of the tail spine. Days to the first pregnancy, days to the first brood, the quantity of the first brood per female, and the average number of offspring in each brood were the criteria used to evaluate the fecundity. Neonates were counted daily and were removed from the beakers.

This study also examined the intrinsic rate of population growth (r) in order to estimate the effect of toxicant exposures on population growth (Pestana et al., 2010; Zhao et al., 2012). It was calculated using the following formula (Lotka, 1913):

$$\sum l_x m_x e^{-rx} = 1 \tag{1}$$

where l_x is the ratio of individuals surviving to age x, m_x is the age-specific fecundity (number of neonates produced per surviving female at age x), and x is in days. As r calculated based on the number of *D. magna* after 21 days is indistinguishable from r estimated for the entire lifespan, due to the great importance of early reproduction (van Leeuwen et al., 1985), all calculations were based on the 21-day experiment.

2.4. Feeding experiment

The feeding experiment was run according to a method described by Zhu et al. (2010). Filtration and ingestion rates were used as measures of the feeding experiment. Ten neonates less than 24 h old were placed in a 100 mL glass beakers containing 45 mL of test solution in a dark time period for 5 h at (20 ± 1)°C. During the exposed period, *D. magna* were fed with 1×10^6 cells/mL *Scenedesmus obliquus* per day for each animal. Then final food concentration was measured with a hemocytometer under an electron microscope with a magnification of 400. The filtration rate (F) was defined as the volume of medium swept clearly by a *D. magna* in a unit of time, and the ingestion rate (I) as the number of cells consumed by a *D. magna* organism during a specific time interval. Eqs. (2) through (4) from Gauld (1951) were used to calculate the average F in μL/(ind·h) and I in cells/(ind·h):

$$F = \frac{V}{n}\frac{\ln C_0 - \ln C_t}{t} - A \tag{2}$$

$$A = \frac{\ln C_0 - \ln C_t'}{t} \tag{3}$$

$$I = F\sqrt{C_0 C_t} \tag{4}$$

where C_0 and C_t are the initial and final food concentrations (cells/μL) respectively, t is time (duration of the experiment in hours), n is the number of *D. magna* in volume V (μL), and A is a correction factor varying with the final concentration C_t' after time t. The expression $\sqrt{C_0 C_t}$ represents the geometric mean of food concentration during time t.

2.5. Biomarker assays

Sixty neonates less than 24 h old from a designated brood were placed in a 1 L glass beaker for each test concentration of PFCs and control group, and the test solution was renewed daily. All experiments were performed in triplicate. Cultured *D. magna* aged seven days were used for determination of AChE, SOD, and CAT activities.

Antioxidant enzymes analyses were conducted according to the procedure of Barata et al. (2005). After exposure, *D. magna* were homogenized in 100 mmol/L of phosphate buffer at a pH value of 7.4, containing 100 mmol/L of KCl and 1 mmol/L of ethylene diamine tetraacetic acid (EDTA) with a 1:4 volume ratio, using a glass homogenizer (Kimble Kontes, Vineland, NJ, USA). After centrifugation at 10 000g for 10 min at 4 °C, the supernatants were used as the enzyme extract for AChE activity determination. AChE activity was determined at a wavelength of 405 nm using a microplate reader (Molecular Device VersaMax, USA) with the method of Guilhermino et al. (1996), by adding 50 μL of homogenate and 250 μL of the reaction solution (1.0 mL of DTNB solution with the concentration of 10 mmol/L, 0.2 mL of ATChI solution with the concentration of 0.075 mol/L, and 30 mL of phosphate buffer) in micro plates, and this activity was

measured at 30 °C for 3 min. AChE activity was expressed in nmol/(mg pro·min). SOD activity was determined at a wavelength of 420 nm by the method of Marklund and Marklund (1974). Three hundred microlitres of Tris-HCl buffer were added to the micro plates, which were warmed at 25 °C for 10 min, then 10 μL of homogenate and 6 μL of preheated pyrogallol were added, and the rate of pyrogallol auto-oxidation was measured for 3 min. SOD activity was expressed in U/mg pro. U was defined as the amount of enzyme required to cause 50% of inhibition of pyrogallol auto-oxidation. CAT activity was determined using ammonium molybdate (Góth, 1991). Two hundred microlitres of the homogenate were incubated with 1 mL of substrate (65 mmol/L of hydrogen peroxide in 60 mmol/L of potassium phosphate buffer, pH value of 7.4) at 37 °C for 60 s. The enzymatic reaction was terminated by adding 1 mL of ammonium molybdate with the concentration of 32.4 mmol/L and the yellow complex of molybdate and hydrogen peroxide was measured at a wavelength of 405 nm. CAT activity was expressed in μmol/(mg pro·min). Protein concentrations were determined at a wavelength of 595 nm using a method developed by Bradford (1976), with bovine serum albumin as the standard.

2.6. Statistical analysis

All data were tested for the normal distribution with Shapiro-Wilk's test and for homogeneity of variances with Levene's test. The results were expressed as the mean with the standard deviation (SD). In the 48-h acute toxicity test for *D. magna*, the median effective concentration (EC50) and median lethal concentration (LC50) were calculated with probit analysis using SigmaPlot 12.5. To analyze reproduction, ingestion, and biomarker data, one-way analysis of variance (ANOVA) and *t*-test with Dunnett's test were performed with the SPSS statistical package (SPSS Co., Chicago, IL, USA). Tests for other types of toxicity data were also performed using SPSS. All differences were considered significant at $P < 0.05$.

3. Results

3.1. Acute toxicity of PFOS and PFNA

Potassium dichromate was used as a reference chemical. The EC50 for *D. magna* at 24 h of 0.96 mg/L was obtained for potassium dichromate, which conforms to the ISO standard (ISO, 1996). Throughout the testing process, the pH values of test media ranged from 7.7 to 8.4, and the DO was 5.3 mg/L. No mortality occurred in the control group in the acute toxicity test.

The immobilization and mortality of *D. magna* increased continuously with elevated PFC concentrations. The EC50 and LC50 values of PFCs were calculated with one-dimensional linear regression analysis of the negative logarithm of exposed PFC concentrations against the immobilization and mortality rates. The EC50 values of PFNA and PFOS for *D. magna* were 43.42 and 23.41 mg/L, and their LC50 values were 80.93 and 49.27 mg/L, respectively.

3.2. Chronic toxicity of PFOS, PFNA, and PFNA/PFOS

The survival, body length, reproduction, and population parameters of adult *D. magna* were assessed after 21 days of exposure and the results are shown in Table 1. The mortality of *D. magna* for all the concentrations of PFOS, PFNA, and PFNA/PFOS never exceeded 20%. No mortality occurred in the control group during the period of experiment. The body length and *r* of *D. magna* decreased significantly at the concentrations greater than or equal to 0.04 mg/L of PFNA, greater than or equal to 0.008 mg/L of PFOS, and greater than or equal to 0.2 mg/L of PFNA/PFOS ($P < 0.05$). Higher

Table 1
Size and fecundity of *D. magna* exposed to PFOS, PFNA, and PFNA/PFOS in 21-day life study.

PFCs	Concentration (mg/L)	Body length (mm)	Time to first pregnancy (d)	Time to first brood (d)	Number of first brood per female (ind)	Number of offspring per brood per female (ind)	*r*
PFNA	Control	2.78 ± 0.09	6.56 ± 0.73	9.17 ± 1.34	7.10 ± 1.10	7.46 ± 1.01	0.288 ± 0.010
	0.008	2.69 ± 0.24	6.93 ± 1.38	9.94 ± 2.02	5.60 ± 2.50	7.46 ± 1.02	0.275 ± 0.028
	0.04	2.49 ± 0.25*	7.13 ± 1.31	10.50 ± 2.07	5.33 ± 3.75	7.41 ± 1.30	0.231 ± 0.030*
	0.2	2.47 ± 0.27*	7.24 ± 0.83	10.63 ± 1.5*	4.94 ± 1.91*	7.21 ± 1.08	0.227 ± 0.036*
	1	2.41 ± 0.13*	7.47 ± 2.13	10.67 ± 2.02*	4.88 ± 2.16*	6.78 ± 2.76	0.222 ± 0.035*
	5	2.35 ± 0.36*	7.69 ± 1.35*	10.72 ± 1.90*	4.33 ± 2.17*	5.52 ± 1.31*	0.212 ± 0.043*
PFOS	Control	2.72 ± 0.11	7.29 ± 1.57	10.00 ± 1.37	6.53 ± 1.99	6.91 ± 1.24	0.233 ± 0.026
	0.008	2.57 ± 0.22*	7.77 ± 2.22	10.35 ± 1.73	6.11 ± 2.08	6.22 ± 1.35	0.204 ± 0.028*
	0.04	2.55 ± 0.14*	7.82 ± 1.07	10.88 ± 2.29	4.67 ± 2.11*	5.00 ± 1.57*	0.198 ± 0.048*
	0.2	2.48 ± 0.36*	8.06 ± 1.59	11.06 ± 1.83	4.65 ± 2.69*	4.92 ± 1.23*	0.195 ± 0.047*
	1	2.47 ± 0.21*	9.00 ± 3.00*	12.06 ± 1.51*	3.77 ± 1.86*	4.55 ± 1.16*	0.183 ± 0.045*
	5	2.45 ± 0.22*	9.06 ± 1.51*	12.24 ± 3.32*	3.47 ± 1.63*	4.37 ± 1.60*	0.166 ± 0.049*
PFNA/PFOS	Control	2.81 ± 0.31	7.51 ± 1.37	10.30 ± 1.73	5.93 ± 1.53	6.44 ± 1.30	0.243 ± 0.036
	0.008	2.57 ± 0.17	7.91 ± 3.33	10.46 ± 3.24	6.13 ± 2.68	6.44 ± 2.14	0.226 ± 0.041
	0.04	2.48 ± 0.41	8.31 ± 1.20	10.63 ± 1.67	6.08 ± 2.23	6.40 ± 1.95	0.216 ± 0.030
	0.2	2.42 ± 0.37*	8.50 ± 2.24	10.75 ± 2.18	5.41 ± 2.34	6.26 ± 2.05	0.213 ± 0.043*
	1	2.39 ± 0.45*	8.94 ± 1.75*	11.24 ± 1.95	5.07 ± 1.58	5.55 ± 1.57*	0.200 ± 0.053*
	5	2.26 ± 0.47*	9.06 ± 1.06*	11.33 ± 1.40	4.09 ± 2.63*	4.58 ± 1.69*	0.178 ± 0.055*

Note: * indicates significant difference from the control group ($P < 0.05$).

Table 2
NOEC, LOEC, and MATC values of PFOS, PFNA and PFNA/PFOS to *D. magna* (mg/L).

PFC	NOEC	LOEC	MATC
PFOS	<0.008	0.008	<0.008
PFNA	0.008	0.04	0.018
PFNA/PFOS	0.04	0.2	0.09

concentrations of PFCs significantly prolonged the time to first pregnancy and the time to first brood of *D. magna* and decreased the number of offspring of first brood. Compared with PFNA, the reproductive toxicity of PFOS seemed to be stronger in *D. magna*. PFNA/PFOS significantly decreased the body length and *r* of *D. magna* at higher concentrations which were greater than or equal to 0.2 mg/L ($P < 0.05$). However, PFNA/PFOS did not change significantly the time to first brood at all the test concentrations.

The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and the lowest observed effect concentration (LOEC). The NOEC, LOEC, and MATC values of PFNA, PFOS, and PFNA/PFOS to *D. magna* are shown in Table 2.

3.3. Ingestion effect

The effects of PFCs on the feeding behavior of *D. magna* are shown in Fig. 1. The ingestion rates were significantly inhibited by PFOS and PFNA/PFOS at concentrations greater than or equal to 0.2 mg/L, while even at the lowest concentration of 0.008 mg/L, the ingestion rate was significantly inhibited by PFNA ($P < 0.05$). The highest concentration of PFNA, PFOS, and PFNA/PFOS exposures led to maximal reduction of ingestion rates, and the inhibition rates were 59%, 55%, and 72% compared with control group values, respectively. The median inhibitory concentrations of ingestion (IC50) were estimated through one variable linear regression analysis, and were 10.78, 14.86, and 7.48 mg/L for PFNA, PFOS, and PFNA/PFOS, respectively. The IC50 values were much lower than the corresponding EC50 for immobilization after 48 h of exposure.

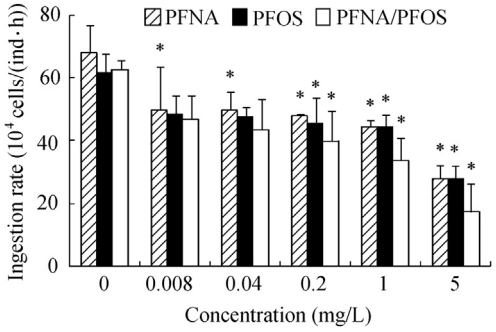

Fig. 1. Ingestion rate of *D. magna* after exposure to PFNA, PFOS, and PFNA/PFOS (* indicates significant difference from control group with $P < 0.05$).

3.4. Biomarker response

AChE, SOD, and CAT activities in *D. magna* are shown in Fig. 2. No mortality occurred during experiments. AChE activity was significantly inhibited by all the test concentrations of PFOS and PFNA/PFOS and concentrations of PFNA greater than or equal to 0.04 mg/L ($P < 0.05$). The inhibition rate of AChE activity matches the concentration increase, and the maximum inhibition rates were 87%, 84%, and 67% respectively, produced by the highest concentrations of PFNA, PFOS, and PFNA/PFOS. SOD activity decreased significantly at concentrations greater than or equal to 0.2 mg/L ($P < 0.05$), and the maximum inhibition rates were 51%, 44%, and 32% for PFNA, PFOS, and PFNA/PFOS, respectively. However, the two lowest concentrations of PFCs increased SOD activity.

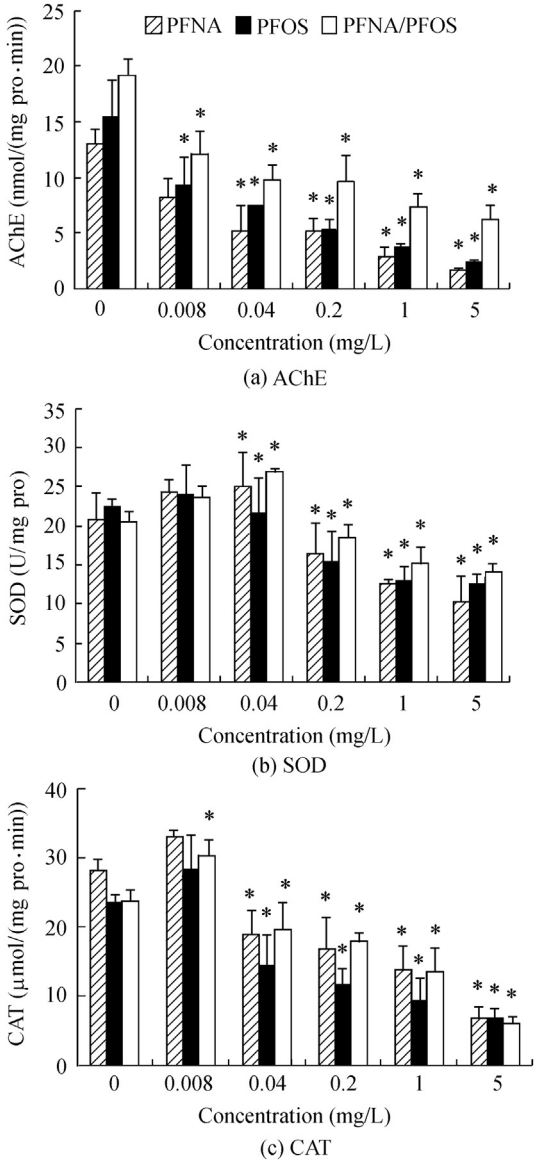

Fig. 2. Biomarker responses of *D. magna* exposed to PFNA, PFOS, and PFNA/PFOS (* indicates significant difference from control group with $P < 0.05$).

The response pattern of CAT activity was similar to that of SOD activity. CAT activity was significantly inhibited by all test concentrations except for the lowest concentration. The highest concentrations of PFNA, PFOS, and PFNA/PFOS induced maximal CAT activity reduction values, which were 76%, 72%, and 75%, respectively. In general, the two PFCs (alone or in combination) induced similar biological responses. Furthermore, the change of enzyme activities exhibited obvious concentration dependence.

4. Discussion

In the acute toxicity test, our results showed that PFOS is more toxic than PFNA to *D. magna*. These results are comparable with those of a previous study by Zheng et al. (2011), which reported that the values of 48-h EC50 of PFNA and PFOS for *D. magna* were 42.84 and 33.76 mg/L, respectively. Ji et al. (2008) also reported that the value of 48-h EC50 of PFOS was 17.95 mg/L for *Moina macrocopa*, whereas *M. macrocopa* was generally much more sensitive to PFOS than *D. magna*. The value of 48-h EC50 of PFNA was 151 mg/L for *D. magna*, as reported by Ding et al. (2012a). The value of 96-h EC50 of PFOS was reported to be 78.13 mg/L, while that for 120 h was 76.53 mg/L for zebrafish embryo (Ding et al., 2012b). However, for marine shrimp, *Mysidopsis bahia*, the value of 96-h LC50 of PFOS was reported to be 3.5 mg/L (Beach et al., 2006), significantly lower than that for the freshwater organisms mentioned above. Different culture conditions and test procedures may partly account for the differences between the reported data in the same species (Ding et al., 2012a).

The use of chronic toxicity data, especially the chronic toxicity of PFNA/PFOS, in environmental protection is an important part of an integrated environmental monitoring and assessment strategy (Dao et al., 2010; Yi et al., 2010). As far as we know, till now there have been no published data on the response patterns of *D. magna* exposed to PFC mixtures, although there are some data on single PFC. Boudreau et al. (2003) investigated the acute and chronic toxicity of PFOS to *D. magna*, and found that PFOS exposure for 21 days could cause significant inhibition of growth and reproduction, and even cause mortality.

Reproduction, and in particular *r*, has been recommended as a superior laboratory toxicological endpoint compared to the acute mortality, because it combines lethal and sublethal effects into one meaningful measure (Zhao et al., 2012; Pestana et al., 2010). In this study, parameter *r* and body length of *D. magna* were confirmed to be more sensitive endpoints in the 21-day chronic toxicity test. Pane et al. (2004) recommended the use of *r* to estimate the chronic toxic effect due to the sensitivity of *r* in chronic toxicity tests. Thus, *r* values were used to estimate the MATC values for PFCs. The reproduction LOEC value of PFNA was approximately five times higher than that of PFOS. The fact that PFOS was found to be more toxic than PFNA in this study is not surprising, because this finding is similar to the results of other studies that have evaluated the toxicity of PFOS and other PFCs in aquatic organisms (Ji et al., 2008; Ding et al., 2012a). However, the reproduction NOEC and LOEC values of PFNA/PFOS were all approximately five times higher than those of a single dose of PFNA or PFOS. A similar result was also found in the MATC. The MATC value of PFNA/PFOS was the largest, followed by the values of PFNA and PFOS, which suggested that the inhibition effects of PFNA/PFOS on growth were lower than those of the corresponding individual exposures, suggesting that an antagonistic effect appeared to exist. Wei et al. (2009) investigated the combined effects of six PFCs on primary cultured hepatocytes from a rare minnow (*Gobiocypris rarus*), and found that the gene expression related to fatty acid biosynthesis and transport showed the antagonistic effects of PFCs. Although the possible mechanisms of combined effects of PFCs have not been previously reported, the present study indicates that the PFOS and PFNA mixture can elicit effects differing from exposure to a single PFC. Therefore, the effects of PFC combination in the assessment of PFC exposure with regard to *D. magna* require further study.

Inhibition of the ingestion rate by PFNA, PFOS, and PFNA/PFOS was observed, and showed obvious concentration dependence in the present study. Toxicants that affect the movement of appendages and the coordination of the nervous system may reduce ingestion rates (Yi et al., 2010). In addition, PFCs could accumulate within the gastrointestinal tract (Kim et al., 2010), which may interfere with normal food intake and cause the toxicity observed in *D. magna*. These findings suggest that a sensitive feeding response is required to evaluate the effect of low-level PFCs.

AChE activity plays an important role in many physiological functions, when the activity of the enzyme decreases, neuronal and muscle injury may occur (Dettbarn et al., 2006). A significant AChE activity decrease by 20% or more can be considered a clear toxicological effect of xenobiotic exposure, and cause reductions in the feeding activity and swimming rate (US EPA, 2000). Xuereb et al. (2009) also found that the feeding rate and locomotive impairment in *Gammarus fossarum* were directly correlated to AChE inhibitions with the correlation coefficient of higher than 50% for chlorpyrifos and methomyl. In the present study, the AChE activity and the ingestion rate of *D. magna* were both obviously inhibited in a concentration-dependent manner. Thus, the inhibition of AChE activity that resulted from exposure to the two PFCs may cause disruption of the nervous system.

CAT plays a critical role in dismutation of the hydrogen peroxide, whereas SOD dismutates the superoxide anion radical (Kim et al., 2012). The SOD-CAT system provides the first defense line against oxygen toxicity and is usually used as a biomarker to indicate reactive oxygen species (ROS) production (Li et al., 2011). However, information on the response of the antioxidant defense system in *D. magna* exposed to PFCs is still lacking. The changes in the SOD and CAT activities were approximately the same as those observed by Qu et al. (2010), where the toxic effects of different concentrations (0.1 mg/L to 200 mg/L) of PFOS on wheat were investigated. Their results showed that low concentrations of PFOS (0.1 mg/L to 10 mg/L) induced SOD and peroxidase (POD) activities in wheat roots and leaves, and high

concentrations of PFOS (200 mg/L) inhibited SOD and POD activities. Pollution induces the expression of antioxidant enzymes that allow organisms to partially or totally overcome stress resulting from exposure to an unsafe environment. However, the decrease of SOD and CAT activities may have been due to excessive ROS production (Xu et al., 2013). These results indicate that treatment with PFOS, PFNA, and PFNA/PFOS resulted in an increase of ROS. ROS, in turn, stimulated the response of antioxidant defenses and resulted in impaired physiological functions.

5. Conclusions

Our study demonstrated the acute and chronic toxicities of two PFCs (single or in combination), and PFOS was found to be more toxic than PFNA. Parental exposure of *D. magna* transferred adverse effects to offspring. The body length and *r* of *D. magna* were confirmed to be more sensitive growth and reproduction parameters for PFCs. In addition, an inhibition of the feeding rate was found in *D. magna*. AChE, SOD, and CAT activities in *D. magna* were also significantly inhibited by the two PFCs in a concentration-dependent manner. The inhibition action attributed to the two PFCs showed positive correlations between the biomarker responses and the effects on growth and feeding rates, which suggested that physiological and biochemical endpoints need to be incorporated into the current PFC regulations. The results of the present study should help increase the base of knowledge on risk assessment of PFCs, and additional research is required to assess the potential adverse impacts of these compounds on aquatic systems.

References

Ahrens, L., Yeung, L.W.Y., Taniyasu, S., Lam, P.K.S., Yamashita, N., 2011. Partitioning of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonamide (PFOSA) between water and sediment. Chemosphere 85 (5), 731−737. http://dx.doi.org/10.1016/j.chemosphere.2011.06.046.

Alberdi, J.L., Saenz, M.E., Di Marzio, W.D., Tortorelli, M.C., 1996. Comparative acute toxicity of two herbicides, paraquat and glyphosate, to *Daphnia magna* and *D. spinulata*. Bull. Environ. Contam. Toxicol. 57 (2), 229−235. http://dx.doi.org/10.1007/s001289900180.

Barata, C., Varo, I., Navarro, J.C., Arum, S., Porte, C., 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 140 (2), 175−186. http://dx.doi.org/10.1016/j.cca.2005.01.013.

Barata, C., Alanon, P., Gutierrez-Alonso, S., Riva, M.C., Fernandez, C., Tarazona, J.V., 2008. A *Daphnia magna* feeding bioassay as a cost effective and ecological relevant sublethal toxicity test for environmental risk assessment of toxic effluents. Sci. Total Environ. 405 (1−3), 78−86. http://dx.doi.org/10.1016/j.scitotenv.2008.06.028.

Beach, S.A., Newsted, J.L., Coady, K., Giesy, J.P., 2006. Ecotoxicological evaluation of perfluorooctanesulfonate (PFOS). Rev. Environ. Contam. Toxicol. 186, 133−174. http://dx.doi.org/10.1007/0-387-32883-1_5.

Boudreau, T.M., Sibley, P.K., Mabury, S.A., 2003. Laboratory evaluation of the toxicity of perfluorooctane sulfonate on *Selenastrum capricornutum, Chlorella vulgaris, Lemna gibba, Daphnia magna*, and *Daphnia pulicaria*. Arch. Environ. Contam. Toxicol. 44 (3), 307−313. http://dx.doi.org/10.1007/s00244-002-2102-6.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1−2), 248−254. http://dx.doi.org/10.1016/0003-2697(76)90527-3.

Cai, Y.Q., Wang, J.M., Shi, Y.L., Pan, Y.Y., Cai, Y.Q., 2010. Perfluorooctane sulfonate (PFOS) and other fluorochemicals in viscera and muscle of farmed pigs and chickens in Beijing. Chin. Sci. Bull. 55 (31), 3550−3555. http://dx.doi.org/10.1007/s11434-010-4098-y.

Calafat, A.M., Needham, L.L., Kuklenyik, Z., Reidy, J.A., Tully, J.S., Aguilar-Villalobos, M., Naeher, L.P., 2006. Perfluorinated chemicals in selected residents of the American continent. Chemosphere 63 (3), 490−496. http://dx.doi.org/10.1016/j.chemosphere.2005.08.028.

Dao, T.S., Do-Hong, L.C., Wiegand, C., 2010. Chronic effects of cyanobacterial toxins on *Daphnia magna* and their offspring. Toxicon 55 (7), 1244−1254. http://dx.doi.org/10.1016/j.toxicon.2010.01.014.

de Schamphelaere, K.A.C., Forrez, I., Dierckens, K., Sorgeloos, P., Janssen, C.R., 2007. Chronic toxicity of dietary copper to *Daphnia magna*. Aquat. Toxicol. 81 (4), 409−418. http://dx.doi.org/10.1016/j.aquatox.2007.01.002.

Dettbarn, W.D., Milatovic, D., Gupta, R.C., 2006. Oxidative stress in anticholinesterase-induced excitotoxicity. In: Gupta, R.C. (Ed.), Toxicology of Organophosphate and Carbamate Compounds. Academic Press, Burlington, pp. 511−532.

Ding, G.H., Fromel, T., van den Brandhof, E.J., Baerselman, R., Peijnenburg, W.J.G.M., 2012a. Acute toxicity of poly- and perfluorinated compounds to two cladocerans, *Daphnia magna* and *Chydorus sphaericus*. Environ. Toxicol. Chem. 31 (3), 605−610. http://dx.doi.org/10.1002/Etc.1713.

Ding, G.H., Zhang, J., Chen, Y.H., Luo, G.Y., Mao, C.H., 2012b. Acute toxicity effect of PFOS on zebrafish embryo. Adv. Mater. Res. 356−360 (2012), 603−606. http://dx.doi.org/10.4028/www.scientific.net/AMR.356-360.603.

Eschauzier, C., Haftka, J., Stuyfzand, P.J., de Voogt, P., 2010. Perfluorinated compounds in infiltrated River Rhine water and infiltrated rainwater in coastal dunes. Environ. Sci. Technol. 44 (19), 7450−7455. http://dx.doi.org/10.1021/es100471z.

Fernandez-Sanjuan, M., Meyer, J., Damasio, J., Faria, M., Barata, C., Lacorte, S., 2010. Screening of perfluorinated chemicals (PFCs) in various aquatic organisms. Anal. Bioanal. Chem. 398 (3), 1447−1456. http://dx.doi.org/10.1007/s00216-010-4024-x.

Flores, C., Ventura, F., Martin-Alonso, J., Caixach, J., 2013. Occurrence of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in NE Spanish surface waters and their removal in a drinking water treatment plant that combines conventional and advanced treatments in parallel lines. Sci. Total Environ. 461, 618−626. http://dx.doi.org/10.1016/j.scitotenv.2013.05.026.

Gauld, T., 1951. The grazing rate of marine copepods. J. Mar. Biol. Assoc. U. K. 29 (3), 695−706. http://dx.doi.org/10.1017/S0025315400052875.

Giesy, J.P., Kannan, K., 2002. Peer reviewed: perfluorochemical surfactants in the environment. Environ. Sci. Technol. 36 (7), 146−152. http://dx.doi.org/10.1021/es022253t.

Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta 196 (2−3), 143−151. http://dx.doi.org/10.1016/0009-8981(91)90067-M.

Guilhermino, L., Lopes, M.C., Carvalho, A.P., Soared, A.M.V.M., 1996. Inhibition of acetylcholinesterase activity as effect criterion in acute tests with juvenile *Daphnia magna*. Chemosphere 32 (4), 727−738. http://dx.doi.org/10.1016/0045-6535(95)00360-6.

Guruge, K.S., Taniyasu, S., Yamashita, N., Wijeratna, S., Mohotti, K.M., Seneviratne, H.R., Kannan, K., Yamanaka, N., Miyazaki, S., 2005. Perfluorinated organic compounds in human blood serum and seminal plasma: a study of urban and rural tea worker populations in Sri Lanka. J. Environ. Monit. 7 (4), 371−377. http://dx.doi.org/10.1039/B412532k.

Huang, Q.S., Dong, S.J., Fang, C., Wu, X.L., Ye, T., Lin, Y., 2012. Deep sequencing-based transcriptome profiling analysis of *Oryzias melastigma* exposed to PFOS. Aquat. Toxicol. 120−121, 54−58. http://dx.doi.org/10.1016/j.aquatox.2012.04.013.

International Organisation for Standardisation (ISO), 1996. Water Quality−Determination of the Inhibition to the Mobility of *Daphnia magna* Straus

(Cladocera, Crustacea): Acute Toxicity Test (ISO 6341). British Standards Institute, London.

Ji, K., Kim, Y., Oh, S., Ahn, B., Jo, H., Choi, K., 2008. Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (*Daphnia magna* and *Moina macrocopa*) and fish (*Oryzias latipes*). Environ. Toxicol. Chem. 27 (10), 2159−2168. http://dx.doi.org/10.1897/07-523.1.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldous, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38 (17), 4489−4495. http://dx.doi.org/10.1021/Es0493446.

Kim, K.T., Klaine, S.J., Cho, J., Kim, S.H., Kim, S.D., 2010. Oxidative stress responses of *Daphnia magna* exposed to TiO_2 nanoparticles according to size fraction. Sci. Total Environ. 408 (10), 2268−2272. http://dx.doi.org/10.1016/j.scitotenv.2010.01.041.

Kim, S., Kim, W., Chounlamany, V., Seo, J., Yoo, J., Jo, H.-J., Jung, J., 2012. Identification of multi-level toxicity of liquid crystal display wastewater toward *Daphnia magna* and *Moina macrocopa*. J. Hazard. Mater. 227−228, 327−333. http://dx.doi.org/10.1016/j.jhazmat.2012.05.059.

Kratzer, J., Ahrens, L., Roos, A., Backlin, B.M., Ebinghaus, R., 2011. Temporal trends of polyfluoroalkyl compounds (PFCs) in liver tissue of grey seals (*Halichoerus grypus*) from the Baltic Sea, 1974-2008. Chemosphere 84 (11), 1592−1600. http://dx.doi.org/10.1016/j.chemosphere.2011.09.001.

Leondiadis, L., Vassiliadou, I., Costopoulou, D., Ferderigou, A., 2010. Levels of perfluorooctane sufonate (PFOS) and perfluorooctanoate (PFOA) in blood samplesfrom different groups of adults living in Greece. Chemosphere 8 (1), 1199−1206. http://dx.doi.org/10.1016/j.chemosphere.2010.06.014.

Li, Z.H., Zlabek, V., Velisek, J., Grabic, R., Machova, J., Kolarova, J., Li, P., Randak, T., 2011. Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): effects on antioxidant responses, hematological parameters and hepatic EROD. Ecotoxicol. Environ. Safe 74 (3), 319−327. http://dx.doi.org/10.1016/j.ecoenv.2010.09.008.

Liu, Y., Wang, J., Fang, X., Zhang, H., Dai, J., 2011. The thyroid-disrupting effects of long-term perfluorononanoate exposure on zebrafish (*Danio rerio*). Ecotoxicology 20 (1), 47−55. http://dx.doi.org/10.1007/s10646-010-0555-3.

Lotka, A.J., 1913. A natural population norm. J. Wash. Acad. Sci. 3, 241−248, 289-293.

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47 (3), 469−474. http://dx.doi.org/10.1111/j.1432-1033.1974.tb03714.x.

Mommaerts, V., Hagenaars, A., Meyer, J., De Coen, W., Swevers, L., Mosallanejad, H., Smagghe, G., 2011. Impact of a perfluorinated organic compound PFOS on the terrestrial pollinator *Bombus terrestris* (Insecta, Hymenoptera). Ecotoxicology 20 (2), 447−456. http://dx.doi.org/10.1007/s10646-011-0596-2.

Murakami, M., Adachi, N., Saha, M., Morita, C., Takada, H., 2011. Levels, temporal trends, and tissue distribution of perfluorinated surfactants in freshwater fish from Asian countries. Arch. Environ. Contam. Toxicol. 61 (4), 631−641. http://dx.doi.org/10.1007/s00244-011-9660-4.

Olsen, G.W., Church, T.R., Larson, E.B., Van Belle, G., Lundberg, J.K., Hansen, K.J., Burris, J.M., Mandel, J.H., Zobel, L.R., 2004. Serum concentrations of perfluorooctanesulfonate and other fluorochemicals in an elderly population from Seattle, Washington. Chemosphere 54 (11), 1599−1611. http://dx.doi.org/10.1016/j.chemosphere.2003.09.025.

Organization for Economic Cooperation and Development (OECD), 2004. Test no. 202: *Daphnia sp.* acute immobilisation test. In: OECD Guidelines for the Testing of Chemicals, Section 2: Effect on Biotic System. OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264069947-en.

Organization for Economic Cooperation and Development (OECD), 2012. Test no. 211: *Daphnia magna* reproduction test. In: OECD Guidelines for the Testing Chemicals, Section 2: Effect on Biotic System. OECD Publishing, Paris. http://dx.doi.org/10.1787/9789264185203-en.

Pane, E.F., McGeer, J.C., Wood, C.M., 2004. Effects of chronic waterborne nickel exposure on two successive generations of *Daphnia magna*. Environ. Toxicol. Chem. 23 (4), 1051−1056. http://dx.doi.org/10.1897/03-208.

Pestana, J.L.T., Loureiro, S., Baird, D.J., Soares, A.M.M., 2010. Pesticide exposure and inducible antipredator responses in the zooplankton grazer, *Daphnia magna* Straus. Chemosphere 78, 41−48. http://dx.doi.org/10.1016/j.chemosphere.2009.10.066.

Plumlee, M.H., Larabee, J., Reinhard, M., 2008. Perfluorochemicals in water reuse. Chemosphere 72 (10), 1541−1547. http://dx.doi.org/10.1016/j.chemosphere.2008.04.057.

Powley, C.R., George, S.W., Russell, M.H., Hoke, R.A., Buck, R.C., 2008. Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere 70 (4), 664−672. http://dx.doi.org/10.1016/j. chemosphere.2007.06.067.

Qu, B., Zhao, H., Zhou, J., 2010. Toxic effects of perfluorooctane sulfonate (PFOS) on wheat (*Triticum aestivum* L.) plant. Chemosphere 79 (5), 555−560. http://dx.doi.org/10.1016/j.chemosphere.2010.02.012.

Renner, R., 2001. Growing concern over perfluorinated chemicals. Environ. Sci. Technol. 35 (7), 154A−160A. http://dx.doi.org/10.1021/es012317k.

Rudel, H., Muller, J., Jurling, H., Bartel-Steinbach, M., Koschorreck, J., 2011. Survey of patterns, levels, and trends of perfluorinated compounds in aquatic organisms and bird eggs from representative German ecosystems. Environ. Sci. Pollut. Res. Int. 18 (9), 1457−1470. http://dx.doi.org/10.1007/s11356-011-0501-9.

Sánchez-Avila, J., Meyer, J., Lacorte, S., 2010. Spatial distribution and sources of perfluorochemicals in the NW Mediterranean coastal waters (Catalonia, Spain). Environ. Pollut. 158 (9), 2833−2840. http://dx.doi.org/10.1016/j.envpol.2010.06.022.

Shivakoti, B.R., Tanaka, S., Fujii, S., Nguyen, P.H.L., Nozoe, M., Kunacheva, C., Okamoto, R., Seneviratne, S.T.M.L.D., Tanaka, H., 2011. Perfluorinated compounds (PFCs) in Yodo River system, Japan. Water Sci. Technol. 63 (1), 115−123. http://dx.doi.org/10.2166/Wst.2011.020.

Tatum-Gibbs, K., Wambaugh, J.F., Das, K.P., Zehr, R.D., Strynar, M.J., Lindstrom, A.B., Delinsky, A., Lau, C., 2011. Comparative pharmacokinetics of perfluorononanoic acid in rat and mouse. Toxicology 281 (1−3), 48−55. http://dx.doi.org/10.1016/j.tox.2011.01.003.

U.S. Environmental Protection Agency (US EPA), 2000. The Use of Cholinesterase Inhibition for Risk Assessment of Organophosphate and Carbamate Pesticides. US EPA, Washington, D.C. http://www.epa.gov/oppfead1/trac/science/cholin.pdf [Retrieved February 22, 2013].

U.S. Environmental Protection Agency (US EPA), 2009. Final Third Drinking Water Contaminant Candidate List (CCL 3). US EPA, Washington, D.C.. http://www.epa.gov/ogwdw/ccl/pdfs/ccl3_docs/fs_cc3_final.pdf [Retrieved February 22, 2013].

van Leeuwen, C.J., Luttmer, W.J., Griffieon, P.S., 1985. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. Ecotoxicol. Environ. Saf. 9 (1), 26−39. http://dx.doi.org/10.1016/0147-6513(85)90031-4.

Ventura, S.P., Gonçalves, A.M., Gonçalves, F., Coutinho, J.A., 2010. Assessing the toxicity on $[C_3mim][Tf_2N]$ to aquatic organisms of different trophic levels. Aquat. Toxicol. 96 (4), 290−297. http://dx.doi.org/10.1016/j.aquatox.2009.11.008.

Wei, Y., Shi, X., Zhang, H., Wang, J., Zhou, B., Dai, J., 2009. Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris raru*) using toxicogenomic analysis. Aquat. Toxicol. 95 (1), 27−36. http://dx.doi.org/10.1016/j.aquatox.2009.07.020.

Xu, D., Li, C., Wen, Y., Liu, W., 2013. Antioxidant defense system responses and DNA damage of earthworms exposed to perfluorooctane sulfonate (PFOS). Environ. Pollut. 174, 121−127. http://dx.doi.org/10.1016/j.envpol.2012.10.030.

Xuereb, B., Lefe'vre, E., Garric, J., Geffard, O., 2009. Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. Aquat. Toxicol. 94, 114−122. http://dx.doi.org/10.1016/j.aquatox.2009.06.010.

Yi, X., Kang, S.W., Jung, J., 2010. Long-term evaluation of lethal and sublethal toxicity of industrial effluents using *Daphnia magna* and *Moina*

macrocopa. J. Hazard. Mater. 178 (1−3), 982−987. http://dx.doi.org/10.1016/j.jhazmat.2010.02.034.

Zainuddin, K., Zakaria, M.P., Al-Odaini, N.A., Bakhtiari, A.R., Latif, P.A., 2012. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in surface water from the Langat River, Peninsular Malaysia. Environ. Forensics 13 (1), 82−92. http://dx.doi.org/10.1080/15275922.2011.643335.

Zhang, X.M., Song, J.L., Jin, Y.H., 2011. Study on reproductive toxicity of perfluorooctane sulfonate (PFOS) in male quail. Asian J. Ecotoxicol. 6 (2), 143−148 (in Chinese).

Zhao, H.Z., Lu, G.H., Xia, J., Jin, S.G., 2012. Toxicity of nanoscale CuO and ZnO to *Daphnia magna*. Chem. Res. Chin. Univ. 28 (2), 209−213.

Zheng, X.M., Feng, Z., Liu, H.L., 2011. Toxicity of typical perfluorinated compounds to *Daphnia magna* and zebrafish (*Brachydanio rerio*) embryos. In: Persistent Organic Pollutants Forum 2010 and the Fifth Session National Academic Symposium of Persistent Organic Pollutants. Chinese Chemical Society, Nanjing, pp. 246−247 (in Chinese).

Zhu, X.S., Chang, Y., Chen, Y.S., 2010. Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*. Chemosphere 78 (3), 209−215. http://dx.doi.org/10.1016/j.chemosphere.2009.11.013.