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ACUTE TOXICITY OF POLY- AND PERFLUORINATED COMPOUNDS TO TWO CLADOCERANS, *DAPHNIA MAGNA* AND *CHYDORUS SPHAERICUS*GUANG-HUI DING,^{†‡} TOBIAS FRÖMEL,[§] || EVERT-JAN VAN DEN BRANDHOF,[‡] ROB BAERSELMAN,[‡]
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Abstract—With their global distribution, environmental persistence, and potential risk to human beings and ecosystems, poly- and perfluorinated compounds (PFCs) are of particular concern for research and regulatory communities. However, insufficient toxicity data are available for most poly- and perfluorinated compounds to assess their possible environmental hazards accurately. Therefore, the acute toxicity of seven poly- and perfluorinated carboxylic acids and alcohols on two cladocerans, *Daphnia magna* and *Chydorus sphaericus*, was evaluated in the present study. The adverse effects of these PFCs on these two cladocerans decreased with increasing fluorinated carbon chain length (nC) and quantitative structure–activity relationships were developed to quantify this observation. Because the 50% inhibition effects (EC50) values obtained are far above concentrations typically found in surface water, acute harmful effects of these chemicals to *D. magna* and *C. sphaericus* are not expected in the real environment. Environ. Toxicol. Chem. 2012;31:605–610. © 2011 SETAC

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INTRODUCTION

With their unique surfactant properties, poly- and perfluorinated compounds (PFCs) have been manufactured and used during the past 50 years in a variety of industrial and commercial products, such as household surface finishes, food packaging, water- and stain-resistant materials, and fire-fighting foams [1]. Poly- and perfluorinated compounds are released to the environment during production, usage, and disposition. Recently, PFCs have been detected in nearly all environmental matrices and in wildlife and humans around the world [2–7]. Even in remote areas, such as the Arctic, PFCs are found in the atmosphere and in biotic samples, thus providing evidence that PFCs could be transported over long ranges through the atmosphere and possibly by ocean water [5,8–12]. Their global distribution, together with their environmental persistence and potential risk to human beings and ecosystems, has led to increased attention by research and regulatory communities on PFCs.

Poly- and perfluorinated compounds tend to persist in surface waters. Therefore, their toxicity to aquatic organisms is of particular concern. Serving as a food source for amphibians, fish, and other aquatic organisms, cladocerans are one of the key trophic elements of aquatic ecosystems. In addition to their ecological significance, cladocerans have the advantages of being useful as test organisms because of their short life cycle, their ease of laboratory culturing, their limited space and water volume requirements, and their sensitivity to chemicals [13,14]. Therefore, cladoceran species are widely used in aquatic toxicology. Among freshwater cladocerans, *Daphnia magna* is probably the most commonly used test organism in ecotoxicological studies.

Some toxicity tests have been performed on cladocerans for perfluoro-octane sulfonic acid and perfluoro-octanoic acid (PFOA) or their salts [15–19].

A document published by the Organisation for Economic Co-operation and Development (OECD) [20] lists more than 1,000 kinds of PFCs. Compared with so many kinds of PFCs, the data for the acute aquatic toxicity of PFCs on cladocerans is limited, and the available studies mainly focused on perfluoro-octane sulfonic acid and PFOA or their salts. To better assess their environment risks, more aquatic toxicity data for other PFC compounds are needed. Because the experimental determination of toxicity is costly and time-consuming, predictive models need to be developed to theoretically quantify toxicity. This can be realized by using quantitative structure–activity relationships, which are based on the statistical relationship between the molecular structure of the compound and the corresponding biological activities [21–25].

Chydorus sphaericus is a benthic cladoceran species that may behave differently from the pelagic *D. magna* [26–28]. In addition, *C. sphaericus* is one of the most common cladocerans in The Netherlands, which makes it a more representative test species for the Dutch situation [27,28]. Therefore, in the present study, the acute aquatic toxicity of seven PFCs to two cladocerans, *D. magna* and *C. sphaericus*, was tested to provide additional information for their possible ecological risk. Based on these data, the effect of the fluorinated carbon chain length of PFCs on toxicity was investigated, and quantitative structure–activity relationships were developed. Finally, toxicity profiles across these two species were investigated.

MATERIALS AND METHODS

Test compounds

Seven PFCs were selected for the toxicity assessment on the basis of their chemical structural resemblance. The test set

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included perfluorobutanoic acid (PFBA; CAS number: 375-22-4, 98%); 2,2,3,3,4,4,5,5-octafluoro-1-pentanol (CAS number: 355-80-6, 98%); PFOA (CAS number: 335-67-1, 96%); perfluorononanoic acid (PFNA; CAS number: 375-95-1, 97%); perfluorodecanoic acid (CAS number: 335-76-2, 98%); perfluoroundecanoic acid (PFUnA; CAS number: 2058-94-8, 95%); and perfluorododecanoic acid (CAS number: 307-55-1, 97%). All of these chemicals were purchased from Sigma-Aldrich. Because the water solubility of PFNA, perfluorodecanoic acid, PFUnA, and perfluorododecanoic acid was very low, dimethylsulfoxide was used for preparing stock solutions. However, the concentration of dimethylsulfoxide in test solutions did not exceed 0.2%, and a solvent control was tested simultaneously.

Test organisms

Daphnia magna were purchased from local suppliers and cultured in the laboratory for more than two months before the experiments. Cultures of *D. magna* were maintained at $20 \pm 1^\circ\text{C}$ in 6-L glass jars under a 16:8-h light:dark photoperiod. *Daphnia magna* were fed green algae (*Pseudokirchneriella subcapitata*) every day, and half of the medium was renewed with M4 solution once a week. The M4 solution was prepared following the OECD test guideline [29].

Chydorus sphaericus used in the present study was reared from one gravid female collected in the summer of 1998 from Lake Drontermeer in The Netherlands. The animals were kept in plastic containers filled with 100 ml Dutch standard water and approximately 1 g precombusted quartz sand (Sibelco M32; grain size $100 \sim 400 \mu\text{m}$). *Chydorus sphaericus* were fed 2 ml of a food suspension consisting of dried ground nettle powder (*Urtica dioica*) (0.5% w/v) and $3 \times 10^7 \mu\text{m}^3$ *Nitzschia perminuta*/ml medium three times per week. Every week, approximately 70% of the culture medium was renewed by decanting most of the medium from the container. Cultures of *C. sphaericus* were also maintained at $20 \pm 1^\circ\text{C}$, and a 16:8-h light:dark photoperiod.

Acute immobilization tests on *D. magna*

Daphnia acute immobilization tests were performed according to the OECD test guideline 202 [29] with slight modifications. A total of 50 ml polypropylene disposable tubes (Fisher Scientific) were used as test vessels with 20 ml test solutions inside. Neonates of *D. magna* of less than 24 h were collected

and then exposed to six concentrations of the tested chemicals and the control. Nominal concentrations of definitive test solutions are listed in Table 1. In cases in which using dimethylsulfoxide to prepare stock solutions was necessary, a solvent control was also tested simultaneously. Four replicate test vessels, each with five neonates, were used for each control or test concentration treatment. The animals were not fed during the test period and were inspected at 24 and 48 h. Temperature and photoperiod were kept the same during exposure and culturing. An organism was considered immobile when it was not able to swim within 15 s after gentle stirring. Testing and culturing conditions were similar with reconstituted M4 water used for culturing and testing. Preliminary range-finding tests were performed first to determine the definitive test concentrations for each compound. In the first test, only PFBA could markedly acidify the test solutions with pH as low as 2.35 for a 10-mM test solution. Thus, a second test was performed for PFBA with pH of the stock solution adjusted to 8.0 with sodium hydroxide solution and diluted hydrochloric acid solution.

Acute immobilization tests on *C. sphaericus*

The 48-h acute immobilization tests with *C. sphaericus* followed the protocol of the Chydotox toxicity test developed in the Dutch National Institute for Public Health and the Environment [30]. The tests were carried out in 2-ml high-performance liquid chromatography vials to which 250 μl test solution was added. Neonates (<24 h) of *C. sphaericus* were collected by a mesh filter with a diameter of 250 μm and then exposed to six concentrations of the tested chemicals and the control. Nominal concentrations of definitive test solutions are also shown in Table 1. If dimethylsulfoxide was used in stock solutions, a solvent control was also tested simultaneously. Four replicate test vials, each with five neonates, were used for each control or test concentration. The vials were covered with a lid to prevent evaporation and incubated for 48 h under the same conditions as the culture. The animals were not fed during the test period and were checked under a reverse dissecting microscope at 24 and 48 h. Immobilization was determined based on inactivation of the animals after slightly tapping with a finger to the vial and monitoring them for 30 s. For PFBA, toxicity tests without and with pH adjustment were carried out, because PFBA can acidify the test solutions at the test concentrations used in the present study.

Table 1. Bioassay methods and nominal concentrations of seven poly- and perfluorinated compounds used in the final toxicity tests

Chemicals	Species	Test method	Nominal concentration (mM)
PFBA 5H 4:1 FTOH	<i>Daphnia magna</i>	OECD test guideline 202 [29]	0, 0.7, 0.75, 0.8, 0.83, 0.85, and 0.9
PFOA			0, 0.8, 1, 1.1, 1.3, 1.5, and 1.8
PFNA			0, 0.35, 0.4, 0.45, 0.5, 0.55, and 0.6
PFDA			0, 0.1, 0.2, 0.3, 0.35, 0.5, and 0.6
PFUnA			0, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4
PFDoA			0, 0.1, 0.2, 0.25, 0.3, 0.35, and 0.4
PFBA 5H 4:1 FTOH	<i>Chydorus sphaericus</i>	Chydotox toxicity test [30]	0, 0.08, 0.1, 0.12, 0.15, 0.18, and 0.2
PFOA			0, 1.5, 2, 2.2, 2.5, 3, and 3.5
PFNA			0, 0.5, 1, 1.5, 2, 2.5, and 3
PFDA			0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6
PFUnA			0, 0.05, 0.07, 0.1, 0.15, 0.2, and 0.25
PFDoA			0, 0.01, 0.03, 0.08, 0.1, 0.2, and 0.25
PFBA	<i>Daphnia magna</i>	OECD test guideline 202 [29]	0, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1
5H 4:1 FTOH			0, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06
PFOA			0, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06
PFNA			0, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06

PFBA = perfluorobutanoic acid; 5H 4:1 FTOH = 2,2,3,3,4,4,5,5-octafluoro-1-pentanol; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUnA = perfluoroundecanoic acid; PFDoA = perfluorododecanoic acid.

Chemical analysis

Concentrations of PFBA, PFOA, and PFNA in assays were partly confirmed by liquid chromatography-electrospray ionization-tandem mass spectrometry. Samples containing the highest and the lowest test concentrations were analyzed in all cases to confirm the nominal concentrations. In the case of PFBA, all samples were analyzed. Samples were drawn right after the test and immediately diluted 1:10 (v:v) with methanol to prevent adsorption. For further measurement, these samples were subsequently diluted 1:1 with water/methanol (v:v) to fit into the calibration curve. For measurement of PFOA and PFNA, the internal standard $^{13}\text{C}_2$ -PFOA was added to the samples and used for quantification. Because all concentrations in the tests that were verified by chemical analyses were well in line with the nominal concentrations, the nominal concentrations were used in the present study.

The following devices were used: Applied Biosystems QTrap 3200, 2× PerkinElmer Series 200 Pump, PerkinElmer Series 200 Degasser, and PerkinElmer Series 200 Autosampler. The columns used were: MZ Aqua C18, with a length and diameter of 50×2.0 mm, a sorbent thickness of $5 \mu\text{m}$, and a pore size of 120 \AA ; and MZ Aqua C18, with a length and diameter of 10×2.0 mm, a sorbent thickness of $5 \mu\text{m}$, and a pore size of 120 \AA . Eluents were: A: $\text{H}_2\text{O}/\text{MeOH}$ (95/5; v/v) + 5 mM ammonium acetate and B: $\text{H}_2\text{O}/\text{MeOH}$ (10/90; v/v) + 5 mM Ammonium acetate. Flow rates were $300 \mu\text{l}/\text{min}$, gradient: 0–2 min: 95% A; 2–4 min: linear gradient to 0% A; 4–6 min: 0% A; 6–8 min: linear gradient to 95% A; 8–15 min: 95% A. Injection volume was $5 \mu\text{l}$.

The mass spectrometry was operated in the negative electrospray ionization mode and multiple reaction monitoring of transition m/z 213 to 169. Linear calibration was performed at 0, 250, 500, 750, and 1,000 ng/ml. Repeatability at 250 ng/ml was 4.4% ($n=4$) and limit of detection was estimated to be approximately 0.5 ng/ml (for signal-to-noise ratio = 10).

One sample at each concentration was taken and analyzed. To assess the deviations, all samples at a nominal concentration of 33 mM were analyzed. Relative standard deviation was 6.5%. The measured concentrations were between 82 and 91% of the nominal concentrations. This makes sense when taking into account the dilution by adding the animals to the test solutions.

Data analysis

The concentrations that caused 50% inhibition effects (EC50) with 95% confidence limits were calculated by the Probit program Version 1.5 from the U.S. Environmental Protection Agency (U.S. EPA). The significance of differences in the average percentage immobility per concentration relative to the blank was assessed by the Dunnett program Version 1.5 obtained from the U.S. EPA. No observed effect concentrations (NOECs) were determined based on results of the Dunnett's analyses.

RESULTS AND DISCUSSION

Toxicity to *D. magna*

The acute effects of seven PFCs to *D. magna* are shown in Table 2. Because PFBA caused marked acidification of the test solutions, tests without and with pH adjustment to 8.0 were carried out, and the corresponding EC50 values were obtained. For PFBA, the EC50s changed greatly, which shows that the change of pH values had a significant effect on *D. magna*. The other PFCs did not show obvious acidification of test solutions in the experiments. Limited by the solubility and aggregation of PFUnA as concentrations approached the solubility limit, the 24-h EC50 and NOEC of PFUnA could not be obtained. The 24-h and 48-h immobility EC50 values of PFOA for *D. magna* were found to be 0.531 and 0.511 mM, respectively. These values agree well with values of 0.691 and 0.420 mM for the toxicity of ammonium perfluoro-octanoate on *D. magna* reported by Li [19]. Colombo et al. [17] gave 24-h and 48-h EC50s of ammonium perfluoro-octanoate on *D. magna* as 1.389 and 1.113 mM, respectively, and Ji et al. [18] reported similar values for PFOA of 1.630 and 1.151 mM. Different culture conditions and test procedures may partly account for the differences between the reported data.

The EC50 and NOEC values of the PFCs studied decreased with increasing fluorinated carbon chain length (nC; Table 2). The 24-h EC50 values were in the range of 0.162 to greater than 20 mM, and the 48-h EC50 values were in the range of 0.129 to greater than 20 mM. Because the EC50 value of PFBA with pH adjustment was greater than 20 mM and could not be determined experimentally due to the high amounts of PFBA needed and subsequent impacts on the texture of the aquatic solutions, it

Table 2. Fifty percent inhibition effects (EC50) and no observed effect concentrations (NOECs) (mM) of seven poly- and perfluorinated compounds for *Daphnia magna*

Chemicals	CAS number	nC	24 h		48 h	
			EC50 (95% CL)	NOEC	EC50 (95% CL)	NOEC
PFBA	375-22-4	3	0.865 (0.858–0.871) ^a > 20 ^b	0.85 ^a - _b	0.848 (0.841–0.856) ^a > 20 ^b	0.83 ^a - _b
5H 4:1 FTOH	355-80-6	4	1.332 (1.143–1.476)	1.10	1.222 (1.016–1.395)	1.00
PFOA	335-67-1	7	0.531 (0.506–0.555)	0.40	0.511 (0.446–0.617)	0.50
PFNA	375-95-1	8	0.481 (0.435–0.601)	0.35	0.326 (0.281–0.390)	0.20
PFDA	335-76-2	9	0.339 (0.310–0.366)	0.15	0.318 (0.278–0.345)	0.15
PFUnA	2058-94-8	10	0.238 ^c	-	0.236 (0.163–0.327)	0.10
PFDoA	307-55-1	11	0.162 (0.152–0.174)	0.15	0.129 (0.098–0.160)	0.12

^a Data for the test without pH adjustment.

^b Data for the test with pH adjustment.

^c Predicted by the relationship found between log EC50 and nC.

nC = the fluorinated carbon chain length; CL = confidence limit; PFBA = perfluorobutanoic acid; 5H 4:1 FTOH = 2,2,3,3,4,4,5,5-octafluoro-1-pentanol; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUnA = perfluoroundecanoic acid; PFDoA = perfluorododecanoic acid.

was not used to develop the quantitative structure–activity relationships. For the toxicity of the other six PFCs on *D. magna*, good relationships between the experimental log EC50 and nC could be obtained.

$$\begin{aligned} \text{Log EC50}_{24\text{h}} &= -0.127 (\pm 0.009) \times \text{nC} + 0.646 (\pm 0.071) \\ n &= 5, R^2 = 0.986, p = 7.090 \times 10^{-4} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Log EC50}_{48\text{h}} &= -0.131 (\pm 0.011) \times \text{nC} + 0.615 (\pm 0.096) \\ n &= 6, R^2 = 0.971, p = 3.265 \times 10^{-4} \end{aligned} \quad (2)$$

The two equations are comparable and almost parallel. The statistics of Equation 1 show that this is a significant relationship that can be used to reliably predict the toxicity of nontested PFCs. Among others it is suited to predict the 24-h EC50 of PFUnA. The value was estimated to be 0.238 mM using Equation 1, which is indeed well above its water solubility limit. In surface waters, the concentrations of the PFCs are usually found to be in the range of pM to nM. Because the EC50 values obtained here are far above concentrations typically found in surface water, acute harmful effects of these chemicals to *D. magna* are not expected in the real environment.

From Equations 1 and 2, the 24-h and 48-h EC50s of PFBA were predicted to be 1.84 and 1.66 mM. However, the experimental values obtained were just 0.865 and 0.848 mM without pH adjustment, which indicates that PFBA is more toxic than expected in this test. In another toxicity test on the photosynthesis of green algae (*P. subcapitata*), PFBA was also found to be more toxic than expected [31]. At environmental conditions, PFBA will be present as the deprotonated species and will thus not have this high toxicity. However, environmental monitoring has recently shown that the short-chained compounds such as PFBA and perfluorobutane sulfonate are becoming the predominant PFC pollutants in surface waters [32–34]. Thus, more attention should be paid to the assessment of the mixture toxicity of PFBA with other chemicals or with its derivatives in local surface waters.

Toxicity to *C. sphaericus*

The acute effects of seven PFCs on *C. sphaericus* are shown in Table 3. The 24-h EC50s for the seven PFCs tested were in the range of 0.054 to greater than 20 mM, whereas the 48-h EC50s were lower and ranged from 0.034 mM to greater than

20 mM. The 24-h and 48-h EC50s of PFBA on *C. sphaericus* were determined to be 2.509 mM and 2.160 mM without pH adjustment. However, the EC50s were greater than 20 mM after pH adjustment. This showed that the change of pH values of test solutions also had a significant effect on *C. sphaericus*. Because the EC50 values obtained in the present study are again far above concentrations typically found in surface waters, the PFCs tested here are not expected to have acute harmful effects to *C. sphaericus* in the real environment.

In general, the EC50 and NOEC values decreased with increasing nC. However, PFNA with nC of 8 performed differently from the other chemicals. The 24-h and 48-h EC50s of PFNA were 0.121 and 0.060 mM, respectively. These values were slightly lower than those of perfluorodecanoic acid (nC = 9), which were 0.141 and 0.088 mM, respectively. Although 2,2,3,3,4,4,5,5-octafluoro-1-pentanol has different functional groups compared with the other chemicals investigated, it also conformed to the relationship between log EC50 and nC, which may indicate that the fluorinated chain length is the main factor for the toxicity of poly- and perfluorinated carboxylic acids and alcohols. Therefore, the experimental EC50 values of the six PFCs, except PFBA, were used for modeling the relationships with nC.

$$\begin{aligned} \text{Log EC50}_{24\text{h}} &= -0.209 (\pm 0.024) \times \text{nC} + 0.970 (\pm 0.202) \\ n &= 6, R^2 = 0.950, p = 9.359 \times 10^{-4} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Log EC50}_{48\text{h}} &= -0.201 (\pm 0.039) \times \text{nC} + 0.689 (\pm 0.327) \\ n &= 6, R^2 = 0.871, p = 6.48 \times 10^{-3} \end{aligned} \quad (4)$$

The equations have high statistical significance so they can be used to reliably predict the adverse effects of similar, nontested PFCs. For PFBA, the 24-h and 48-h EC50s to *C. sphaericus* were predicted to be 2.20 and 1.222 mM using Equations 3 and 4. These predicted values are lower than the experimental values obtained without pH adjustment and confirm the limited acute toxicity of short-chained PFCs.

Interspecies relationships

The relationships between log EC50 and nC for the two cladocerans are shown in Figure 1. Interspecies analyses between these two cladocerans were performed based on

Table 3. Fifty percent inhibition effects (EC50) and no observed effect concentrations (NOECs) (mM) of seven poly- and perfluorinated compounds for *Chydorus sphaericus*

Chemicals	CAS number	nC	24 h		48 h	
			EC50 (95% CL)	NOEC	EC50 (95% CL)	NOEC
PFBA	375-22-4	3	2.509 (2.409–2.607) ^a > 20 ^b	2.2 ^a - ^b	2.160 (2.070–2.249) ^a > 20 ^b	2.0 ^a - ^b
5H 4:1 FTOH	355-80-6	4	1.393 (0.829–1.877)	1.0	0.842 (0.239–1.292)	<0.5
PFOA	335-67-1	7	0.426 (0.223–0.537)	<0.2	0.282 (0.122–0.345)	<0.1
PFNA	375-95-1	8	0.121 (0.106–0.136)	0.07	0.060 (0.047–0.070)	<0.05
PFDA	335-76-2	9	0.141 (0.110–0.173)	0.08	0.088 (0.051–0.124)	0.01
PFUnA	2058-94-8	10	0.069 (0.056–0.082)	0.04	0.034 (0.022–0.042)	0.01
PFDoA	307-55-1	11	0.054 (0.044–0.086)	0.02	0.046 (0.034–0.081)	0.02

^aData for the test without pH adjustment.

^bData for the test with pH adjustment.

nC = the fluorinated carbon chain length; CL = confidence limit; PFBA = perfluorobutanoic acid; 5H 4:1 FTOH = 2,2,3,3,4,4,5,5-octafluoro-1-pentanol; PFOA = perfluorooctanoic acid; PFNA = perfluorononanoic acid; PFDA = perfluorodecanoic acid; PFUnA = perfluoroundecanoic acid; PFDoA = perfluorododecanoic acid.

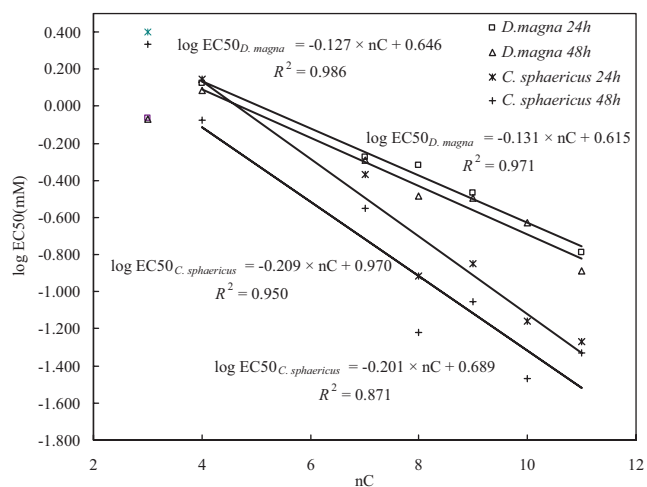


Fig. 1. Relationships between log 50% inhibition effects (EC50) and the fluorinated carbon chain length (nC) for two cladocerans.

24-h and 48-h experimental EC50 values, and two relationships were obtained.

For 24-h toxicity

$$\text{Log EC50}_{C. \text{sphaericus}} = 1.6 (\pm 0.3) \times \text{log EC50}_{D. \text{magna}} - 0.1 (\pm 0.1) \\ n = 5, R^2 = 0.888, p = 0.016 \quad (5)$$

For 48-h toxicity

$$\text{Log EC50}_{C. \text{sphaericus}} = 1.5 (\pm 0.3) \times \text{log EC50}_{D. \text{magna}} - 0.3 (\pm 0.17) \\ n = 6, R^2 = 0.846, p = 0.009 \quad (6)$$

The relationships between the log-transformed EC50 values of the two cladocerans are significant; therefore, the toxicity of a certain PFC for one cladoceran species can be used to predict the toxicity for the other using the equations. *D. magna* is a pelagic species that inhabits the upper water column, whereas *C. sphaericus* is a benthic species that lives on the sediments. Therefore, their EC50s represent the aqueous and sediment toxicity of a chemical via exposure to the water phase, respectively. With these interspecies relationships, one could calculate aqueous or sediment toxicity of a similar PFC with known sediment or aqueous toxicity data. Furthermore, the Chydotox toxicity test needs fewer chemicals and materials, so it may be a promising test method for collection of toxicity data that are needed for environmental risk assessment.

CONCLUSIONS

In the present study, the acute toxicity of seven PFCs to *D. magna* and *C. sphaericus* was evaluated. In general, the measured EC50s and NOECs of the two cladocerans decreased with increasing fluorinated carbon chain length. Because PFBA acidifies the test solutions, its toxicity was tested without and with pH adjustment. The EC50s changed greatly after pH adjustment, which may suggest that acidification has a significant effect on its toxicity. Because the short-chained compounds such as PFBA and PFBS are becoming the predominant PFC pollutants in surface waters, their long-term toxicity and mixture toxicity with other pollutants in local aquatic environment needs more attention. The EC50 values obtained here are far above concentrations typically found

in surface waters. Acute harmful effects of these PFCs to *D. magna* and *C. sphaericus* are therefore not expected in the real environment.

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