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Toxicity of Polyfluorinated and Perfluorinated Compounds to Lettuce (*Lactuca sativa*) and Green Algae (*Pseudokirchneriella subcapitata*)

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Abstract Recently, polyfluorinated and perfluorinated compounds (PFCs) have been detected in most surface waters around the world. Because some PFCs are persistent and tend to accumulate in surface waters, their potential adverse effects to aquatic organisms have received increasing attention. Nevertheless, currently available toxicity information is limited. The aim of this study was to evaluate the toxicity effects of seven PFCs on root elongation of lettuce (*Lactuca sativa*) and photosynthesis of green algae (*Pseudokirchneriella subcapitata*). It was found that the toxicity profiles of both species tested were similar and had good relations with the fluorinated carbon-chain length of the PFCs investigated. One of the compounds tested, perfluorobutanoic acid, was found to be more toxic than expected in the algae test, which may be related with acidification of the test solution. It was concluded that because short-chained PFCs are becoming the predominant PFC pollutants in surface waters, their long-term toxicity and mixture toxicity with other PFCs should be studied in greater detail.

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Polyfluorinated and perfluorinated compounds (PFCs) are a class of anthropogenic substances characterized by a partially or fully fluorinated alkyl chain and a terminal functional group. The unique structures of PFCs enable them to repel both water and oil, to decrease surface tension better than other surfactants, and to work well under harsh conditions; thus, PFCs have been manufactured for a large number of technical and consumer applications for decades (Kissa 2001; Schultz et al. 2003; Lehmler 2005; Prevedouros et al. 2006; Paul et al. 2009). Because they are widely used and persistent, PFCs have been detected in nearly all environmental media, humans, and wildlife throughout the world (Giesy and Kannan 2002; Yamashita et al. 2005; Houde et al. 2006; Lau et al. 2007; Dreyer et al. 2009; Fromme et al. 2009). The widespread distribution, extended residence times, and bioaccumulation of some PFCs have led to increased attention on their potential health and ecological risks (Giesy and Kannan 2002; Renner 2003; Kannan et al. 2005; Lau et al. 2007; Fromme et al. 2009). Because PFCs tend to accumulate in surface waters, their toxicity to aquatic organisms is of particular interest. However, there is limited information on the aquatic toxicity of the thousands of PFCs, and most of the studies focus simply on perfluorooctane sulfonate acid (PFOS) and perfluorooctanoic acid (PFOA) or their salts.

Regarding the toxicity to aquatic plants, Boudreau et al. (2003) tested the toxicity of the potassium salt of PFOS (PFOSK) on the floating macrophyte *Lemna gibba*. Values for 50% inhibition of growth (IC_{50}) after 7 days of exposure were measured as 0.110 mM for frond number and 0.058 mM for wet weight. The no observed–effect concentrations (NOECs) from frond number and wet weight were 0.054 and 0.012 mM, respectively. Phillips et al. (2007) performed a 7-day acute toxicity test of saturated and unsaturated fluorotelomer carboxylic acids on *L. gibba*.

Results showed that toxicity increased with increasing fluorinated carbon-chain length between 4 and 8, and the saturated forms of the telomer acids were usually more toxic than their unsaturated counterparts. Li (2009) evaluated the toxicity of PFOSK and pentadecafluorooctanoic acid ammonium salt (APFO) on three plant species: lettuce (*L. sativa*), cucumber (*Cucumis sativus*), and pakchoi (*Brassica rapa* chinensis). It was found that root elongation (5-day EC_{50} value) was more sensitive than seed germination (5-day LC_{50} value) and that PFOSK was more toxic than APFO for all species tested. Based on EC_{10}, EC_{50}, and NOEC values, the 5-day root-elongation sensitivity of test plants to both chemicals was in the order of lettuce (*L. sativa*) >pakchoi (*B. rapa* chinensis) >cucumber (*C. sativus*).

For the toxicity to algae, the Organisation for Economic Co-operation and Development (OECD) (2002) listed some toxicity PFOSK data on the freshwater algae. The 96-h EC_{50} values on growth rate and cell density of *P. subcapitata* were 0.234 and 0.132 mM, respectively. The 96-h EC_{50} values were 0.327 mM for *Anabaena flos-aquae* and 0.567 mM for *Navicula pelliculosa* based on growth rate. Boudreau et al. (2003) reported 96-h IC_{50} values for *P. subcapitata* to be 0.090 and 0.110 mM from cell density and chlorophyll (*a*) concentration, respectively. However, *Chlorella vulgaris* was slightly less sensitive than *P. subcapitata*, with IC_{50} valiues of 0.152 mM for cell density and 0.164 mM for chlorophyll (*a*). Colombo et al. (2008) tested the 48- and 96-h EC_{50} values of APFO on *P. subcapitata*, which were > 0.928 mM. Liu et al. (2008) evaluated the toxicity of six PFCs on the freshwater green alga *Scenedesmus obliquus*. It was reported that the IC_{50} values of PFOS, perfluorododecanoic acid (PFDoA), and perfluorotetradecanoic acid (PFTeA) were in the range of 0.134 to 0.261 mM based on cell density and chlorophyll fluorescence and that toxicity increased with increasing carbon-chain length. Latała et al. (2009) tested the 72-h toxicity of four perfluoroalkyl carboxylic acids (PFCAs) to three representative marine algae in the Baltic Sea: the green alga *C. vulgaris*, the diatom *Skeletonema marinoi,* and the blue-green alga *Geitlerinema amphibium*. The EC_{50} values obtained range from 0.28 to 12.84 mM, and the toxicity was also shown to be well correlated with the perfluoroalkyl chain length.

Chlorophyll fluorescence analysis can be applied in assessing the impact of pollutants or other environmental stresses on in vivo photosynthesis of plants and algae (Schreiber et al. 1994). A pulse-amplitude modulation (PAM) fluorometer has been developed for this purpose and has been applied to measure the inhibition of photosynthetic efficiency under various stressors (Van Beusekom 1999; Juneau et al. 2002; Frankart et al. 2003; Schreiber et al. 2007; Van der Grinten et al. 2010). The PAM fluorometer records changes in the fluorescence signal after stimulation with a light impulse. Photosynthesis efficiency can be calculated from the recorded signals. Compared with the 96-h algae growth-inhibition test, the PAM test is relatively rapid because it takes just 5 h to obtain EC_{50} values of the inhibition of photosynthetic efficiency, making the test less time consuming. In addition, the PAM test requires less test solution than the 96-h algae growth-inhibition test. Therefore, the PAM test was used here to evaluate the acute toxicity of PFCs on the green algae (*P. subcapitata*) instead of the commonly used 96-h growth-inhibition test.

Because experimental determination of toxicity is costly and time consuming, it will take much money and time to perform the toxicity tests on thousands of kinds of PFCs. Therefore, it is desirable to develop mathematical predictive models to theoretically quantify toxicity. Quantitative structure–activity relations (QSARs) provide a convenient tool for toxicity evaluation and prediction and are based on the statistical relationship between the molecular structure of the compound and the corresponding biological activities (Ding et al. 2009; Bhhatarai and Gramatica 2010). Therefore, in this study, the aquatic toxicity of seven PFCs to lettuce (*L. sativa*) seeds and green algae (*P. subcapitata*) was first tested to provide more toxicity information for PFCs. Subsequently, QSAR models for the toxicity were developed and toxicity profiles across these two species 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 included the following: (1) perfluorobutanoic acid (PFBA; CAS no. 375-22-4, purity 98%), (2) 2,2,3,3,4,4,5,5-Octafluoro-1-pentanol (5H 4:1 FTOH; CAS no. 355-80-6, purity 98%), (3) PFOA (CAS no. 335-67-1, purity 96%), (4) perfluorononanoic acid (PFNA; CAS no. 375-95-1, purity 97%), (5) perfluorodecanoic acid (PFDA; CAS no. 335-76-2, purity 98%), (6) perfluoroundecanoic acid (PFUnA; CAS no. 2058-94-8, purity 95%), and (7) PFDoA (CAS no. 307-55-1, purity 97%). All of these chemicals were purchased from Sigma-Aldrich. Because the water solubility of some PFCs was low, dimethyl sulfoxide (DMSO) was used for preparing stock solutions. However, the concentration of DMSO in test solutions did not exceed 0.2%, and a solvent control was tested simultaneously.

Root Elongation Test on Lettuce (*L. sativa*)

The 5-day root-elongation test was performed according to test guideline OPPTS 850.4200 (United States

Environmental Protection Agency [USEPA] 1996) and test guideline 208 (OECD 2006) with slight modifications. A 90 × 15-mm plastic Petri dish with an appropriate filter article placed inside was used to avoid sorption of PFCs to the wall of the container. For each test, six exposure concentrations and a blank were conducted with three replicates. Because PFCAs are relatively stable in aquatic environments (Kissa 2001; Frömel and Knepper; 2010), the concentrations of test solutions were not analyzed, and nominal concentrations were used. Nominal concentrations of final test solutions are listed in Table 1. In those cases for which it was necessary to use DMSO for the preparation of stock solutions, a solvent control was also tested simultaneously. To each Petri dish, 3 ml either test solution, solvent control, or nutrient solution (blank control) was added. Five lettuce (*L. sativa*) seeds were placed on the wet filter paper, and then the Petri dishes were sealed with parafilm to prevent evaporation. Petri dishes containing lettuce seeds were placed in a plant test chamber with a constant room temperature of 18°C ± 2°C. The photoperiod was set as 16 h light to 8 h darkness. After 5 days of incubation, the number of germinated seeds was determined, and the length of the roots was measured with a ruler to the closest millimeter.

PAM Test on Algae (*P. subcapitata*)

The test follows the protocol of *PAM Test: Acute Effects on Photosynthesis in Algae* developed in the RIVM (Verweij et al. 2009). The unicellular green alga *P. subcapitata* was used for the PAM test. The alga was taken from a continuous culture. The algal culture was first diluted with Dutch standard water to an initial cell density of 3×10^6 cells ml^{-1}. Then the algal suspension was exposed to a concentration series of chemicals and placed in an incubator for 4.5 h with 650-nm pulsed excitation light. Light energy was absorbed by the alga's photosystems and was then transformed into photoproducts, heat, and fluorescence. Blocking of the photosynthesis by chemicals changes the intensity of the fluorescence signal. After 4.5 h of incubation, changes in the fluorescence signal were recorded and the effects of the toxicant on the photosynthetic efficiency derived. For each test, seven exposure concentrations and a blank were conducted with two replicates. Nominal concentrations of final test solutions are listed in Table 1.

Data Analysis

The concentrations that caused 50% inhibition effect (EC_{50}) with 95% confidence limits (CLs) were calculated by a nonlinear curve-fitting procedure in GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA, http://www.graphpad.com). The fitted model between the logarithm of nominal concentration C and the effect E is in the following equation (Eq. 1):

$$E = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10 \wedge ((\text{LogEC50} - C)*\text{HillSlope})). \quad (1)$$

Here it is hypothesized that the effect starts at the bottom of the effect curve and goes to the top with a sigmoid shape. HillSlope is the variable slope of the dose-response curve. NOECs were determined by Dunnett Software version 1.5 obtained from the United States Environmental Protection Agency.

Table 1 Bioassay methods and nominal concentration of seven PFCs used in the toxicity tests

Chemicals	Species	Test method	Test duration	Nominal concentration (mM)
PFBA	Lettuce (*L. sativa*)	US EPA OPPTS 850.4200 and OECD test guideline 208	5 days	0, 1, 2, 4, 6, 8, and 10
5H 4:1 FTOH				0, 0.5, 1, 2, 3, 4, and 6
PFOA				0, 1, 1.5, 2, 2.5, 3, and 4
PFNA				0, 0.3, 0.5, 0.8, 1, 2, and 3
PFDA				0, 0.1, 0.2, 0.4, 0.8, 1, and 2
PFUnA				0, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2
PFDoA				0, 0.005, 0.01, 0.02, 0.05, 0.1, and 0.2
PFBA	Green algae (*P. subcapitata*)	PAM test (Verweij et al. 2009)	4.5 h	0, 1, 1.5, 2, 2.5, 3, 4, and 5
5H 4:1 FTOH				0, 1, 2, 3, 4, 5, 7, and 9
PFOA				0, 1, 1.5, 1.8, 2, 2.2, 2.5, and 3
PFNA				0, 0.6, 0.8, 1, 1.3, 1.6, 2, and 2.5
PFDA				0, 0.6, 0.8, 1, 1.3, 1.6, 2, and 2.5
PFUnA				0, 0.025, 0.04, 0.06, 0.09, 0.12, 0.15, and 0.2
PFDoA				0, 0.025, 0.04, 0.06, 0.09, 0.12, 0.15, and 0.2

Results and Discussion

Toxicity to Root Elongation of Lettuce (*L. sativa*)

The EC_{50}s and NOECs derived for each of the PFCs studied are listed in Table 2. The end point of interest, root elongation of lettuce (*L. sativa*) after a total exposure time of 5 days, includes possible effects of PFCs on germination. Limited by the solubility and aggregation of PFUnA and PFDoA, the EC_{50} values for these two chemicals could not be experimentally determined. In addition, enhanced aggregation and possible binding to filter article might affect their bioavailability and subsequently the extent of toxicity. The measured EC_{50} values of the additional PFCs studied were in the range of 0.266 to 4.186 mM. Li (2009) evaluated and reported 5-day EC_{50} values of APFO and PFOSK on root elongation of lettuce (*L. sativa*) as 0.394 and 0.184 mM, respectively. The reported EC_{50} value of APFO, the ammonium salt of PFOA, is approximately 4.57 times lower than the EC_{50} value of PFOA determined in this study. PFOSK has a fluorinated carbon-chain length of 8, which is the same as that of PFNA. The EC_{50} value of PFOSK obtained by Li (2009) is approximately 4.6 times lower than the value obtained for PFNA in our study, which is similar to the ratio of the EC_{50} values between APFO and PFOA. The deviation may be caused by the different gegenions and experimental conditions. The differing gegenions can induce variation between the data because the different physicochemical properties of PFCs and their salts lead to different environmental behavior and, subsequently, aquatic toxicity. In addition, the different experimental conditions, such as temperature, photoperiod and the amount of test solution, can also lead to some deviation.

Table 2 also lists the fluorinated carbon-chain length of PFCs, which was represented as nC. It can be seen that the EC_{50} values decrease with increasing nC. This means that toxicity increased with fluorinated carbon-chain length for the structurally similar compounds investigated here. A good relationship between log-transformed EC_{50} values and nC was obtained (Fig. 1). From this relationship, the EC_{50} values of PFUnA and PFDoA could be predicted as being 0.210 and 0.142 mM. This is indeed well above the water solubility of 3.19×10^{-5} and 8.87×10^{-7} mM of these two chemicals at 25°C as calculated by the online software SPARC version 4.5 in the absence of experimental data on water solubility. From these data it can be concluded that acute toxicity of PFUnA and PFDoA to lettuce seeds will not be observed in the real environment because the water solubility is far lower than the predicted EC_{50} values.

Compared with the EC_{50} values, the NOEC values also decreased with nC, except that the NOEC of 5H 4:1 FTOH was an outlier compared with the NOECs of the PFCAs. The NOEC of 5H 4:1 FTOH was found to be just 0.1 mM. This is similar to the values obtained for PFNA and PFDA, both being PFCs with a greater fluorinated carbon-chain length. Because 5H 4:1 FTOH has a lower NOEC value, we recommended paying more attention to the long-term chronic toxicity and mixture toxicity of FTOH in future research.

Toxic Effects on Photosynthesis of Algae (*P. subcapitata*)

The EC_{50} values of PFBA, 5H 4:1 FTOH, PFOA, PFNA, and PFDA were experimentally determined, whereas the EC_{50} values of PFUnA and PFDoA could again not be experimentally determined due to their limited water solubility and aggregation. The toxicity data obtained for *P. subcapitata* are also listed in Table 2. Except for PFBA, the EC_{50} values decreased with increasing nC. The log-transformed EC_{50} values had a good linear relationship with nC (Fig. 1). As expected on the basis of nC, PFDA has the lowest experimental EC_{50} value (0.851 mM), whereas the greatest EC_{50} value was obtained for 5H 4:1 (4.853 mM). The relationship between log-EC_{50} value and nC could be used to predict the EC_{50} values of PFUnA and

Table 2 Effects of seven PFCs on 5-day root elongation of lettuce (*L. sativa*) and photosynthesis of green algae (*P. subcapitata*)

Chemicals	CAS no.	nC	Lettuce (*L. sativa*)		Green algae (*P. subcapitata*)	
			EC_{50} (95% CL [mM])	NOEC (mM)	EC_{50} (95% CL [mM])	NOEC (mM)
PFBA	375-22-4	3	4.186 (3.90–4.50)	3	1.225 (1.00–1.50)	<1
5H 4:1 FTOH	355-80-6	4	2.976 (2.50–3.50)	0.1	4.853 (4.10–5.80)	2
PFOA	335-67-1	7	1.801 (1.60–2.00)	1.5	1.807 (1.76–1.86)	1
PFNA	375-95-1	8	0.846 (0.60–1.30)	0.1	1.038 (0.98–1.10)	<1
PFDA	335-76-2	9	0.266 (0.19–0.38)	0.1	0.851 (0.64–1.12)	<1
PFUnA	2058-94-8	10	0.210[a]	–	0.565[a]	–
PFDoA	307-55-1	11	0.142[a]	–	0.394[a]	–

[a] Predicted by the relationship found between log EC_{50} value and nC

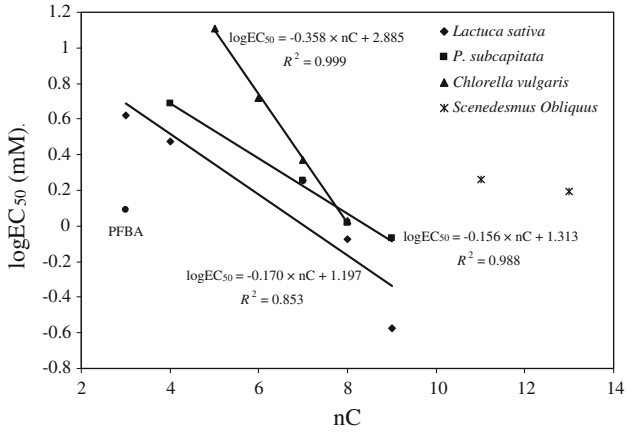

Fig. 1 Relations between log-EC_{50} values of PFCs and the fluorinated carbon-chain length nC

PFDoA to *P. subcapitata*, and the predicted EC_{50} values are listed in Table 2.

It is interesting to observe that the log EC_{50} values of PFBA deviates from the relationship obtained between log EC_{50} value and nC. According to this relationship, the EC_{50} value of PFBA should be approximately 7 mM. However, the actual EC_{50} value of PFBA is 1.225 mM, which is 5.7 times lower than that predicted from the relationship obtained. The pKa value of PFBA is approximately 0.39, which is lower than those of other PFCs investigated (Moroi et al. 2001); therefore, the PFBA solution is more acid than that of other PFCAs or 5H 4:1 FTOH (Hagenaars et al. 2011). Because the unicellular *P. subcapitata* is sensitive to the pH value of the test solution, the actual EC_{50} value of PFBA is lower and deviates from the relationship between log EC_{50} value and nC. Although PFBA did not exhibit a large acute toxicity, its toxicity and mixture toxicity with other PFCs in local water bodies warrants additional evaluation. Furthermore, taking into account the current strict regulation on PFOS and related chemicals, the short-chained compounds, such as PFBS and PFBA, are becoming the predominant PFC pollutants in surface waters (Taniyasu et al. 2008; Ahrens et al. 2009; Möller et al. 2010). Therefore, toxicity of the short-chained PFCs and their mixture toxicity should be investigated in greater detail as well. Similar to the observation regarding the EC_{50} value of PFBA, the NOEC of PFBA was lower than expected based on the number of carbon atoms in the molecule: The NOEC was<1 mM and lower than that of 5H 4:1 FTOH.

NOECs were used in a hazard-quotient (HQ) approach to assess the risk to lettuce and algae plants from PFCs investigated under field conditions. The HQ was calculated as HQ = EEC/TBC. Here TBC is the toxicological benchmark concentration (*i.e.*, the toxicity data for the most sensitive species tested in this study), and the EEC is the highest expected environmental concentration. Values >1 indicate a potential for toxic effects to occur, and values <1 indicate that toxicity is not likely to occur (Hanson et al. 2005a, b). The lowest NOEC values for PFBA, 5H 4:1 FTOH, PFOA, PFNA, and PFDA obtained in this study are<241.4 (<1 mM), 23.2 (0.1 mM), 414.06 (1 mM), 46.4 (0.1 mM), and 51.4 mg l^{-1} (0.1 mM), respectively. In contrast, it has been reported that PFCs are widely present in surface, ground, marine, and drinking waters at concentrations that generally vary from pg l^{-1} to μg l^{-1} (Yamashita et al. 2005; Rayne and Forest 2009; Houde et al. 2006; Lau et al. 2007). The highest concentration reported in river water are 500 ng l^{-1} for PFBA, 87.1 μg l^{-1} for PFOA, 54 ng l^{-1} for PFNA, and 11 ng l^{-1} for PFDA (Rayne and Forest 2009). The calculated HQ values were all<0.01. This indicates that based on current environmental concentrations of PFCs, acute harmful effects to at least green algae are not expected to occur.

Variation in Toxicity Profiles Across Species

Figure 1 shows the relations between the log-EC_{50} value and nC for lettuce and some algae species. For the toxic effect of PFCs on root elongation of lettuce (*L. sativa*), a simple linear regression model was obtained (Eq. 2):

$$\begin{aligned} \text{Log } EC_{50,\text{lettuce}} &= -0.170(\pm 0.0409) \times \text{nC} \\ &\quad + 1.197(\pm 0.271) \\ &\quad n = 5, R^2 = 0.853, p = 0.0252. \end{aligned} \tag{2}$$

Excluding the EC_{50} value of PFBA, the regression model for the acute toxic effects of PFCs on the photosynthesis of green algae (*P. subcapitata*) was as follows (Eq. 3):

$$\begin{aligned} \text{Log } EC_{50,\text{algae}} &= -0.156(\pm 0.0122) \times \text{nC} \\ &\quad + 1.313(\pm 0.0881) \\ &\quad n = 4, R^2 = 0.988, p = 0.006. \end{aligned} \tag{3}$$

The R^2 values of these two models are>0.85, and the confidence level is>95%, so they can be used to reliably predict the corresponding toxicity effect of PFCs with similar structures. Because the QSAR models were developed based on linear PFCAs and 5H 4:1 FTOH, they are suitable to predict the EC_{50} values of PFCAs with similar structure but not for branched PFCAs and perfluoroalkyl sulfonate acids. PFUnA and PFDoA are two similar PFCAs with longer fluorinated carbon-chain lengths, so the QSARs were used to extrapolate their EC_{50} values. In the future, if more toxicity data could be obtained for other PFCs with different structures, a general QSAR model may be developed for PFCs with more molecular structural descriptors involved.

From parameters of these two models, it can be seen that they are similar (parallel slopes); thus, a relationship for the toxicity effects across lettuce and green algae can be developed as follows (Eq. 4):

$$\begin{aligned} \text{Log } EC_{50,\text{lettuce}} &= 1.196(\pm 0.437) \\ &\times \log EC_{50,\text{algae}} - 0.245(\pm 0.161) \\ n &= 4, R^2 = 0.789, p = 0.112. \end{aligned} \quad (4)$$

As pointed by Liu et al. (2008), perfluoroalkyl acids are likely to be incorporated into the lipid bilayer of the cell membrane, thus increasing membrane permeability and subsequently causing toxic effects on *Scenedesmus obliquus*. The PFCs investigated here are likely to have similar modes of action for lettuce seeds and green algae in the sense that they might disrupt the membrane properties first and then induce harmful impacts later. In an absolute sense, due to differences in inherent sensitivity of test species, and differences in test procedures and end points, the toxic effects will be different. For instance, the test on lettuce seeds took 5 days, whereas the PAM test required only 4.5 h. Given the shorter duration of the PAM test and the observed parallel response in this study, the PAM test might be used to replace the 5-day root-elongation test on lettuce seeds for acute-toxicity assessment of corresponding PFCs based on further evaluation of PAM as an assay tool.

Liu et al. (2008) tested the 72-h toxicity of PFHxA, PFOA, PFDoA, and PFTeA on growth of the freshwater green alga *S. obliquus* using optical density and in vitro chlorophyll fluorescence. It was found that PFDoA and PFTeA inhibited algal growth in a concentration-dependent manner, whereas PFHxA and PFOA did not inhibit algal growth within the tested concentration ranges. Based on in vitro chlorophyll fluorescence, IC_{50} values were 0.261 and 0.192 mM for PFDoA and PFTeA, respectively. The log-transformed IC_{50} values are also shown in Fig. 1,and were found to be higher than the regression line of the PAM test. The PAM test thus is more sensitive than the 72-h algal growth-inhibition test on *S. obliquus* based on these data. Latała et al. (2009) tested the 72-h toxicity of PFHxA, PFHpA, PFOA, and PFNA to *C. vulgaris*. The EC_{50} values obtained ranged from 1.07 to 12.84 mM, which were also log-transformed and presented in Fig. 1. It can be seen in this case that the log EC_{50} values were well correlated with nC. However, the regression line is significantly steeper than that of the PAM test given the slope of -0.358 ± 0.008. These two lines intersect at a fluorinated carbon-chain length of approximately 8. This implies that the freshwater algae *P. subcapitata* in the PAM test is more sensitive than the marine algae *C. vulgaris* in the 72-h algal growth-inhibition test for PFCs with $nC < 8$. From this comparison it can be seen that the PAM test is not only time-efficient but also has a relatively high sensitivity. The PAM test can therefore be used to evaluate the acute toxicity effects of PFCs to algae and plant seeds.

Conclusion

The aquatic toxicity of seven polyfluorinated and perfluorinated compounds was investigated on the root elongation of lettuce (*L. sativa*) seeds and photosynthesis of green algae (*P. subcapitata*). The toxic effects on lettuce seeds and green algae were found to be similar in a relative sense and were shown to have a good relationship with the fluorinated carbon-chain length. The toxicity of these chemicals increased with increasing fluorinated carbon-chain length. It is interesting to observe that PFBA is more toxic than expected, which may be related with acidification of the test solution. Because short-chained PFCs are becoming the predominant PFCs pollutants in the surface waters, their long-term toxicity and mixture toxicity with other PFCs should be studied in greater detail. Compared with other tests, the PAM test is more time efficient and relatively sensitive to the toxicity of PFCs, so it could be used for acute toxicity evaluation of PFCs.

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