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**Final project report**

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# ECOTOXICITY OF HIGHLY FLUORINATED COMPOUNDS

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## *Introduction*

The ubiquitous detection of several perfluorinated surfactants, such as perfluorooctane sulfonates (PFOS) and perfluorooctanoic acid (PFOA), and their adverse environmental and toxicological effects have rendered them one of the most investigated compounds during the last years. The abnormal properties and activities imparted by the presence of the fluorine atoms have been shown to impede prediction of various properties.

However, prediction of environmentally significant properties and activities of highly fluorinated compounds is crucial to understand their fate and their effects on biota. Thus, a comprehensive model covering a wide range of perfluoroalkyl chain lengths and different functional groups should be established. In order to achieve this, ecotoxicological measurements of certain compounds that have been preselected by means of statistical experimental design, must be performed.

Within this scope, the ecotoxicity of several compounds on different species had already been investigated. However, results of perfluorocarboxylic acids have not been as assumed and should therefore be thoroughly reinvestigated. Especially toxicity of perfluorobutanoic acid (PFBA) to the green algae *Pseudokirchneriella subcapitata* and the cladoceran *Daphnia magna* had been much higher than assumed. Taking into account that novel fluorosurfactants which will be released might incorporate shorter perfluoroalkyl chain lengths, it is crucial to understand their toxicological and ecotoxicological potential.

## *Materials and Methods*

Perfluorinated compounds were purchased from different chemical suppliers and had purities of > 95%.

Acute toxicity to algae was carried out with the "PAM test" (pulsed amplitude modulation) developed by the Rijksinstituut voor Volksgezondheid en Milieu (RIVM, National institute for Public Health and the Environment) and the University of Amsterdam [1]. Briefly, photosynthetic activity of the green algae *Pseudokirchneriella subcapitata* is measured by a pulsed-amperometric fluorometer under the influence of the selected chemical at different concentrations. The end-point of this measurement is the fluorescence yield compared with a blank after 4.5 h.

Acute toxicity to *Chydorus sphaericus* was assessed by the help of a test developed at RIVM [1]. Briefly, a minimum number of 20 juvenile (age < 24 h) animals were exposed to a series of at least six concentrations of the test chemical and a blank. The animals were divided into at least four groups and the test was carried out in 2 mL HPLC vials with a test volume of 250 µL. Immobilization was assessed after 24 h and 48 h under a microscope after gently shaking the HPLC vial. $EC_{50}$ and $EC_{10}$ values were calculated with the Probit software provided by the US Environmental Protection Agency.

Concentrations of the compounds in assays with PFBA (with pH adjustment), PFOA (with pH adjustment), perfluorononanoic acid (PFNA) and 1H,1H,8H,8H-perfluorooctane-1,8-diol (THPFOdiol) were confirmed by means of Liquid Chromatography – Electrospray Ionization - Tandem Mass Spectrometry (HPLC-ESI-MS/MS). 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 with water/methanol (1:1; V:V) to fit into the calibration curve. For measurement of PFOA, PFNA and THPFOdiol, internal standard $^{13}C_2$-PFOA was added to the samples and used for quantification.

## Results and Discussion

### *Acute toxicity to green algae Pseudokirchneriella subcapitata*

It was found out that problems associated with previous measurements were related to the acidity of the perfluorinated carboxylic acids. Even though problems in assessing the pKa values of perfluorinated acids exist [2], they are likely to be very low due to the electron-withdrawing effects of the perfluoroalkyl chain.

The ecotoxicity test to algae is carried out in so-called 'Dutch standard water' (DSW), which is milli-Q water containing 100 mg/L $NaHCO_3$, 20 mg/L $KHCO_3$ and 180 mg/L $MgSO_4*7H_2O$. Considering no buffer activity of MgSO4, only the carbonate species have buffering effect, resulting in a buffer capacity of ca. 1.4 mM.

Therefore, concentrations of any perfluorinated acid above 1.4 mM will cause a major change in pH, which in turn will lead to a toxic effect on the algae. To circumvent this problem, the stock solutions used for the assay were adjusted to a pH above 8, just like the DSW, with hydrochloric acid and sodium hydroxide solution.

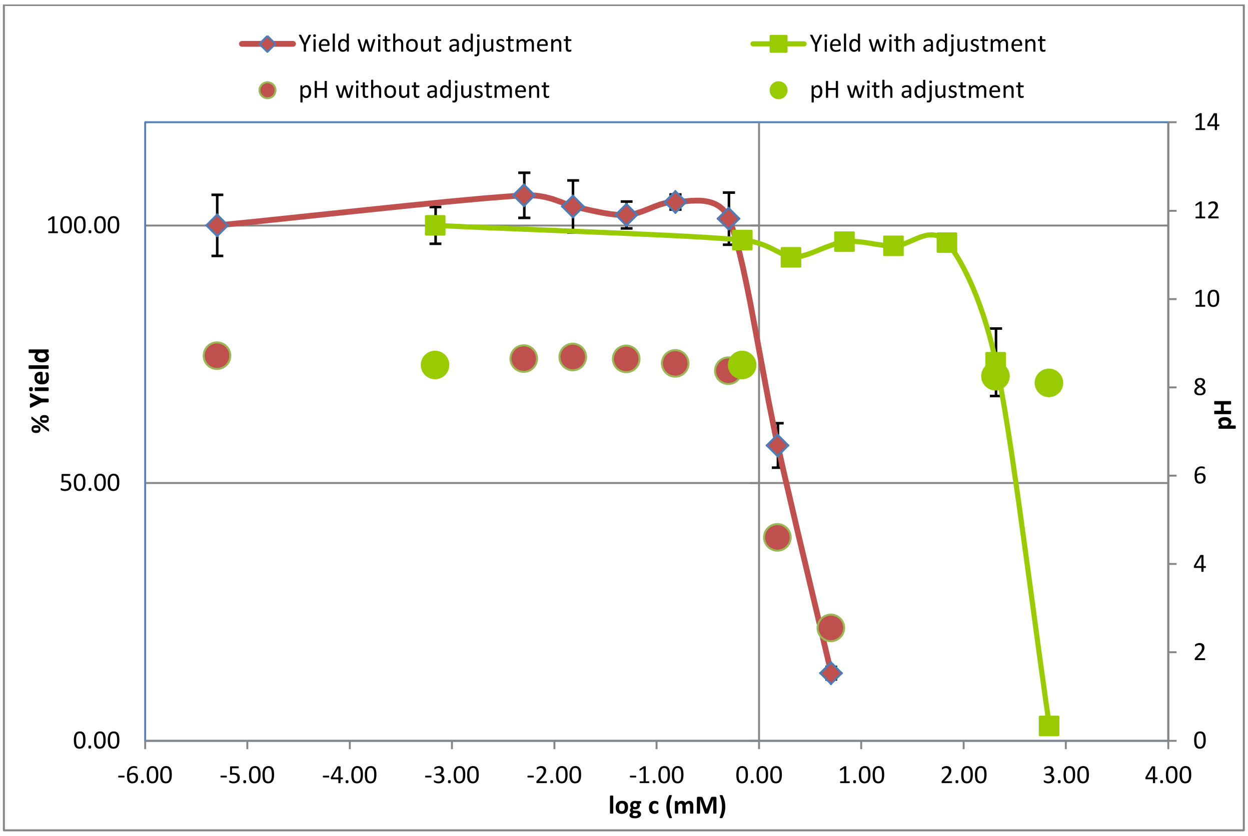



**Figure 1: Logarithmic dose-effect curve for PFBA with and without pH adjustment. The dots represent the pH in the test vials at the respective PFBA concentrations. Red: without pH adjustment; green: with pH adjustment**

Figure 1 shows the logarithmic dose-effect graph for PFBA and contrasts the curves with and without pH adjustment. It is obvious that the pH drop at log c = 0.2 affects an increased toxicity, which is not the case when pH is adjusted. Without pH adjustment, the toxicity is more than two orders of magnitude lower than with non-adjusted stock solution ($EC_{50}$ = 1.76 mM vs. 279 mM). Since perfluorinated acids are supposed to occur in their dissociated form in the environment, the adjustment of pH is actually reasonable.

It could be shown that the previously measured values for other perfluorinated acids were equally biased by a pH effect. Figure 2 demonstrates that also for PFOA, pH adjustment is necessary. Here again, PFOA was measured with and without adjustment. A third assay was performed using the commercially available ammonium perfluorooctanoate (APFO). The dose-response curves for pH-adjusted PFOA and APFO were very similar, which is also expressed by very similar $EC_{50}$ values.

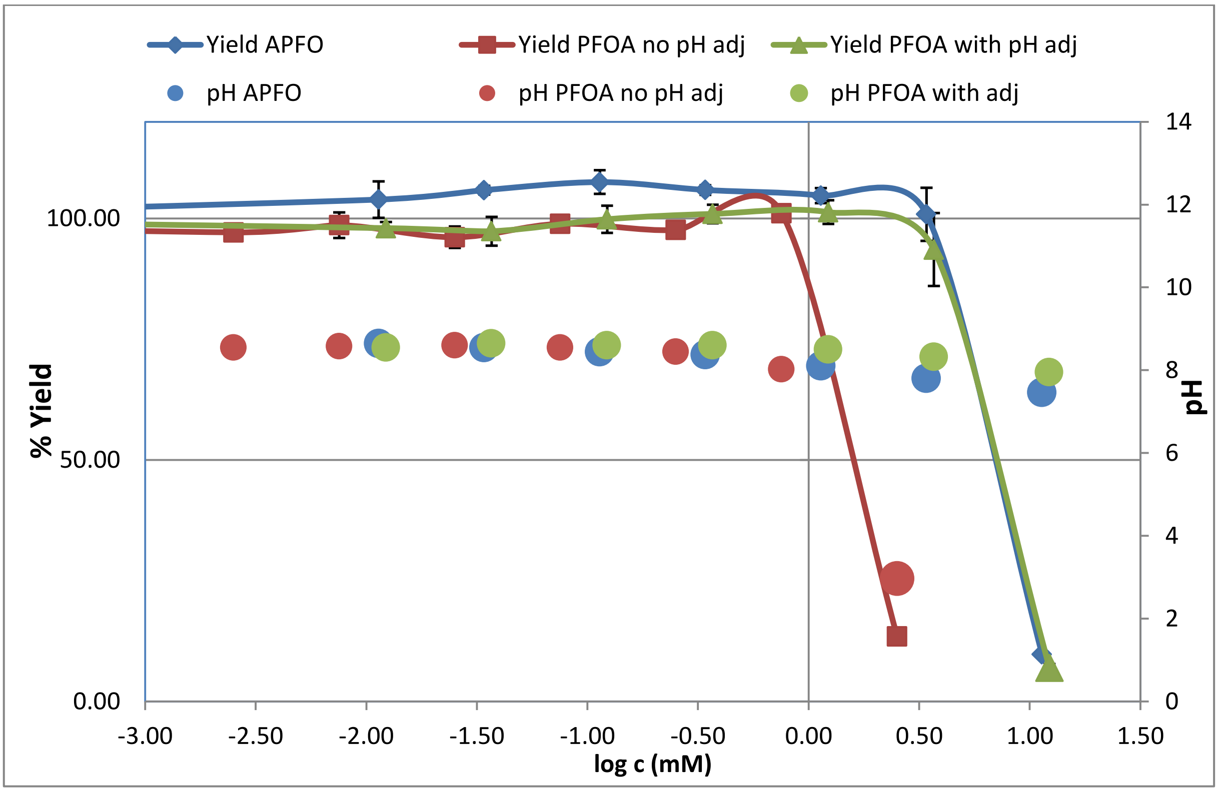
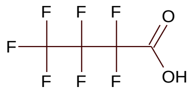
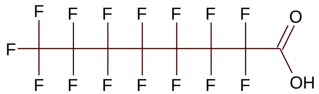
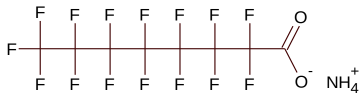
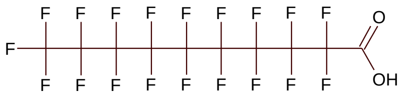
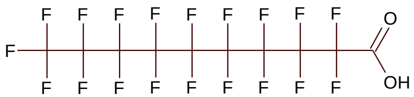
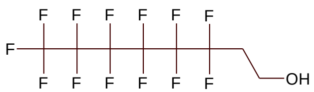
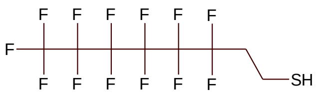


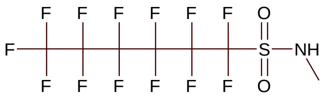
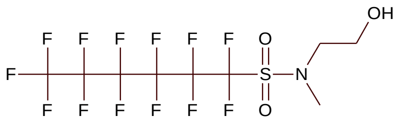
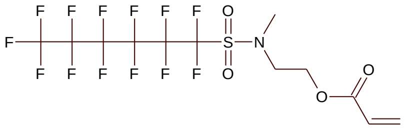
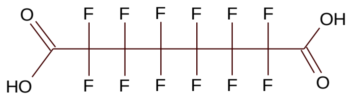
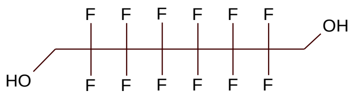
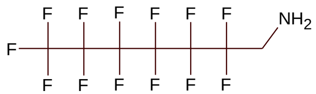

**Figure 2: Dose-response curves for APFO, PFOA without pH adjustment and PFOA with pH adjustment. Dots indicate the pH measured in the vials after the test. Please note that nominal concentrations are given for better comparison of the data between PFOA without pH adjustment and APFO.**

Various other perfluorinated compounds were tested in for acute toxicity on algae. The results are shown in Table 1. For a number of compounds, low water solubility prevented measurements of $EC_{50}$ values, because even at the highest concentrations, no effect was observed.

The comparison of THPFOdiol and PFSubA clearly indicates that toxicity of a compound is not only related to the perfluoroalkyl chain length. In this case, the $EC_{50}$ values will have a difference of at least two orders of magnitude, although both contain six difluoromethylene groups. The toxic effect of the two alcohol groups is much more pronounced than that of the two carboxylic acid functions. However, this may be different for other species, because the PAM test is very specific since an effect will only be observed if the photosynthetic system is affected.

**Table 1: Overview of results for acute toxicity of fluorinated compounds to green algae**

| Structure | Chemical name | Acronym | Concentrations verified | $EC_{50}$ PAM test[a] [mM] | $EC_{10}$ PAM test[a] [mM] |
|---|---|---|---|---|---|
|  | Perfluorobutanoic acid[b] | PFBA | yes | 257 (236 – 279) | 156 (131 – 185) |
|  | Perfluorooctanoic acid[b] | PFOA | yes | 5.96 (5.43 – 6.53) | 3.68 (3.27 – 4.15) |
|  | Ammonium perfluorooctanoate | APFO | no | 6.94 (6.07 – 7.94) | 4.31 (3.60 – 5.16) |
|  | Perfluorononanoic acid[b] | PFNA | yes | 0.732 (0.599 – 0.894) | 0.232 (0.169 – 0.519) |
|  | Perfluorodecanoic acid[b] | PFDA | no | > $S_w$ > 0.438 mM | |
|  | 1H,1H,2H,2H-Perfluorooctanol | 6:2-FTOH | no | > $S_w$ > 0.04 | |
|  | 1H,1H,2H,2H-Perfluorooctanethiol | 6:2-FTSH | no | > $S_w$ > 0.038 | |

| Structure | Chemical name | Acronym | Concentrations verified | $EC_{50}$ PAM test[a] [mM] | $EC_{10}$ PAM test[a] [mM] |
|---|---|---|---|---|---|
|  | N-Methyl perfluorohexanesulfonamide | N-MePFHxSA | no | > $S_w$<br>> 0.02 | |
|  | N-Methyl,N-(2-hydroxyethyl)-perfluorohexanesulfonamide | N-MePFHxSAEt | no | > $S_w$<br>> 0.02 | |
|  | N-Methyl-perfluorohexane sulfonamidoethyl acrylate | N-MePFHxSAEtAc | no | > $S_w$<br>> 0.02 | |
|  | Perfluorooctanedioic acid | PFSubA | no | > 130 | |
|  | 1H,1H,8H,8H-Perfluorooctane-1,8-diol | THPFOdiol | yes | 0.659<br>(0.592 – 0.735) | 0.182<br>(0.143 – 0.231) |
|  | 1H,1H-Perfluoroheptylamine | DHPFHpAm | no | > 0.151 | |

**> $S_w$ : higher than water solubility**

**[a] 95% confidence interval in brackets**  **[b] stock solutions neutralized with sodium hydroxide, therefore toxicity of the anion is assessed**

### *Acute toxicity to Chydorus sphaericus*

The acute toxicity of PFBA was also measured to the cladoceran *Chydorus sphaericus*, which is a common species in Central European waters. Previous measurements yielded an $EC_{50}$ value of 2.51 mM.

However, also in this assay, a medium is used which contains low buffer concentration, in this case 0.77 mM sodium bicarbonate. Therefore, when the PFBA concentration exceeds the bicarbonate concentration, a major pH reduction will occur, although this could not be proven in the test because of the very small volume used.

**Table 2: Results for acute toxicity of PFBA to *Chydorus sphaericus***

| | **24 h** | | **48 h** | |
|---|---|---|---|---|
| | **[mM]** | **95% CI [mM]** | **[mM]** | **95% CI [mM]** |
| **$EC_{50}$** | 30.6 | 25.4 - 35.2 | 22.3 | 16.8 - 26.6 |
| **$EC_{10}$** | 16.6 | 11.0 - 20.9 | 13.1 | 7.5 - 17.2 |

**95% CI: 95% confidence interval**

The test was carried out with a stock solution with pH adjustment. The resulting values are much higher than previous ones with an $EC_{50}$ value of 22.3 mM after 48 h (Table 2). This value is comparatively high and shows that toxic effects of PFBA are not likely to occur in the environment, where concentrations are usually in the pM to nM range.

## References


1. Verweij, W., Durand, A.M., Maas, J.L., and van der Grinten, E., 2010. Protocols belonging to the report 'Toxicity measurements in concentrated water samples' - RIVM Report 607013010/2010,

2. Goss, KU, (2008) The pKa values of PFOA and other highly fluorinated carboxylic acids. *Environ. Sci. Technol.* 42: 456-458.