# Evaluation of the toxicity and risk to plant from perfluorooctane sulfonate

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**Abstract**
The toxicity of perfluorooctane sulfonate (PFOS) to higher plants (*Brassica chinensis*, *Lactuca sativa*, *Medicago sativa* and *Raphanus sativus*) was investigated. With the concentration of up to 400 mg $L^{-1}$, PFOS had almost no effect on the seed germination, but affected the root elongation of the four plant species. Based on $EC_{10}$, $EC_{50}$ and NOECs, the 96 h root elongation sensitivity of the four plants to PFOS was in the order of *Brassica chinensis* > *Lactuca sativa* > *Medicago sativa* > *Raphanus sativus*. The influence of soil properties on the bioavailability and toxicity of PFOS to *Brassica chinensis* root elongation was also studied. The results showed that soil properties could influence the expression of PFOS toxicity. $EC_{50}$ values for PFOS ranged from 95 to >200 mg $kg^{-1}$.

**Introduction**
Recently perfluorooctane sulfonate(PFOS) have raised scientific interest because of their wide-spread occurrence in the environment[1]. Because of the unique chemical properties of perfluorinated compounds, PFOS are resistant to both chemical and biological degradation under normal environmental conditions and are expected to be highly persistent in the environment[2,3]. At present, they have been found in a wide range of environmental and biological samples including birds, edible marine organisms, marine mammals and humanbeings[4-9]. Therefore, the potential ecological and health effects of these chemicals have attracted much research attention.
This study aims to provide new information about toxicology of PFOS on seed germination and root growth of four higher plant species. The effects of soil properties on PFOS bioavailability to plants, for the first time, have been investigated. We hope that this study can provide some useful information about toxicity of PFOS and help to assess potential effects of PFOS in terrestrial ecosystems.

**Materials and Methods**
Plants
*Brassica chinensis*, *Lactuca sativa*, *Medicago sativa* and *Raphanus sativus* were used as test species. The seeds were commercially available from Dalian Seeds Co. Ltd. China. Two of the plant species (*Lactuca sativa* and *Raphanus sativus*) are among the 10 recommended species by USEPA[10] for the determination of ecological effects of pesticides and toxic substances. The seeds were sterilized with 0.1% sodium hypochlorite (NaClO) solution for 20 min, then soaked with deionized water for 10 min and washed with deionized water for three times prior to use.

Chemicals
All reagents used in this study were analytical grade. PFOS (> 98% purity) was obtained from Fluka (Steinheim, Switzerland). To prevent the adsorption of PFOS onto glass surface, the stock solution of testing chemical was prepared in the deionized water from a Milli-Q water purification system (Millipore, Milford, MA) using polymethyl pentene containers.

Soils
Six soils were sampled from different locations in China. These soils are representative of the major soil types in the region and cover a wide range in soil properties expected to affect the bioavailability of PFOS. Soil samples were air dried at room temperature and then passed through a 2-mm sieve. The physicochemical properities of the six soils were determined. Table 1 shows the selected soil properties.

Table 1 The selected properties of the soils used in this test

| Soil No. | Soil Name | pH | OM(g $kg^{-1}$) | CEC(cmol $kg^{-1}$) | Clay(%) | Sand(%) | Silt(%) |
|---|---|---|---|---|---|---|---|
| 1 | Yujiang | 4.73 | 5.03 | 10.81 | 28.12 | 53.85 | 18.04 |
| 2 | Qiongshan | 5.43 | 6.98 | 9.58 | 34.88 | 38.20 | 26.92 |
| 3 | Zizhong | 7.69 | 10.54 | 11.14 | 14.92 | 47.64 | 37.44 |
| 4 | Suihua | 6.12 | 79.54 | 36.41 | 20.32 | 47.20 | 32.48 |
| 5 | Jiangning | 6.60 | 9.74 | 14.73 | 14.56 | 57.44 | 28.00 |
| 6 | Dalian | 7.40 | 20.02 | 16.76 | 13.60 | 61.36 | 25.04 |

Toxicity experiment

Seed germination and root length assays were performed to assess PFOS toxicity[10]. Seeds were tested on filter papers placed in culture dishes (90 mm × 10 mm). The tested concentrations of PFOS were 0.1, 1, 10, 100, 200, 300 and 400 mg $L^{-1}$. Fifteen sterilized seeds for each test plant were scattered the filter papers moistened with 10 mL toxicants solution. Culture dishes were placed in an incubator. After 96 h in the dark at 25 ±2℃, more than 90% of the control seeds had germinated and developed roots. Then the germination in solution was halted, seed germination rate was calculated, and root length was measured. The root elongations in soil were performed on six types of soils. 30g of each test soil amended with various concentrations of PFOS(0.1, 1, 10, 50, 100, 150 and 200 mg $kg^{-1}$) was put into the culture dish and then 15 of seeds were planted on the soil with 60% of the water holding capacity. After 120 h at 25±2℃, soil was removed carefully in order to avoid broken roots, and the length of the longest root on each plant was recorded. All the treatments for the above tests of germination and root elongation were replicated three times to minimize experimental errors.

Data analysis

Data analysis including regression analysis, variance analysis, calculation of average values and standard deviation (S.D.) was completed with the Microsoft Excel and Spss12.0. The dose-response data were fitted to a log-logistic curve based on Eq.1 for each test using Sigmaplot 10.0[11,12]

$$y = \frac{a-b}{1+\left(x/c\right)^{p}} + b \qquad (1)$$

where y = Plant root length or seed germination; x = $\log_{10}$(actual concentration of added PFOS), and a, b, p are parameters to be fitted, c = $\log_{10}(EC_{50})$ or ($LC_{50}$). No observed effect concentration (NOECs; the highest doses of added PFOS that did not reduce the measured endpoint significantly, $p < 0.05$) were determined by ANOVA and Duncan's multiple range test using Minitab15.

**Results and Discussion**

Toxicity of PFOS to Plants in solutions

$LC_{50}$ values of seed germination and $EC_{50}$ values of root elongation on the four test plants after 96 h exposure to PFOS were calculated, as shown in Table 2. $LC_{50}$ values of PFOS to seed germination were higher than 400 mg $L^{-1}$ for the four plant species. Compared with seed germination, root growth was more sensitive to PFOS When the concentrations were below 10 mg $L^{-1}$, the root elongations of the four plants were not different from the control, and any stimulation on the root growth at these low concentration ranges was not found either. However, from these two concentrations onwards, the root elongation decreased with the increasing concentration of PFOS. At the maximum of the tested concentration, PFOS inhibited the root growth of the four plants by 75% for *Brassica chinensis*, 73% for *Lactuca sativa*, 67% for *Medicago sativa* and 53% for *Raphanus sativus*, respectively. $EC_{50}$ values for PFOS to the four species ranged from 161 to 363 mg $L^{-1}$. The corresponding $EC_{10}$ and NOEC values were also calculated and listed in Table 2. Based on the NOEC, $EC_{10}$ and $EC_{50}$ values, the 96 h root elongation sensitivity of the four plants to both PFOS was in the order of *Brassica chinensis* > *Lactuca sativa* > *Medicago sativa* > *Raphanus sativus*.

**Table 2** NOEC and $LC_{50}$ (mg $L^{-1}$) of seed germination and NOEC, $EC_{10}$ and $EC_{50}$ (mg $L^{-1}$) of root elongation for four tested plants in solutions

| Plant | Seed germination | | | | Root elongation | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NOEC[a] | $LC_{50}$ | 95%CI | $R^2$ | NOEC | $EC_{10}$ | 95%CI | $EC_{50}$ | 95%CI | $R^2$ |
| *Brassica chinensis* | 200 | >400 | - | - | 100 | 91 | 79-103 | 161 | 129-193 | 0.98 |
| *Lactuca sativa* | 200 | >400 | - | - | 100 | 105 | 80-129 | 170 | 141-210 | 0.99 |
| *Medicago sativa* | 200 | >400 | - | - | 100 | 110 | 93-127 | 267 | 239-284 | 0.98 |
| *Raphanus sativus* | >400 | >400 | - | - | 150 | 133 | 122-144 | 363 | 351-375 | 0.97 |

Toxicity of PFOS to *Brassica chinensis* in soils

Compared with the unamended control of the soils, the inhibition of *Brassica chinensis* root length by the maximum tested concentration of PFOS ranged from 33% in the Suihua soil to 92% in the Yujiang soil.Toxicity threshold values based on added PFOS for all the six soils are shown in Table 3. A range of thresholds were found among soils, indicating that PFOS toxicity to root growth of *Brassica chinensis* was affected by soil properties. The $EC_{50}$ and $EC_{10}$ of PFOS in the five test soils (Dalian, Jiangning, Qiongshan, Zizhong and Yujiang) were calculated, based on the fitted log-logistic model, which were 178, 122, 107, 119, 95 mg $kg^{-1}$ and 90, 72, 58, 83, 40 mg $kg^{-1}$, respectively. The $EC_{10}$ in the Suihua soil was estimated by the obtained data model, listed in Table 3. The NOECs of PFOS ranged from 50 mg $kg^{-1}$ in the Yujiang, Qiongshan and Jiangning soils to 150 mg $kg^{-1}$ in the Suihua and Dalian soils. These findings suggest that risk assessments of PFOS need to consider different soil types and particularly, soil properties.

Table 3 NOEC, $EC_{10}$ and $EC_{50}$ (mg $kg^{-1}$) of *Brassica chinensis* root elongation for PFOS added in the six soils

| Soil | NOEC | $EC_{10}$ | 95%confidence interval | $EC_{50}$ | 95%confidence interval | $R^2$ |
|---|---|---|---|---|---|---|
| Suihua | 150 | 115 | 98-133 | >200 | -- | 0.91 |
| Jiangning | 50 | 72 | 60-84 | 122 | 107-137 | 0.98 |
| Qiongshan | 50 | 58 | 50-66 | 107 | 96-118 | 0.97 |
| Zizhong | 100 | 83 | 58-108 | 119 | 91-148 | 0.99 |
| Yujiang | 50 | 40 | 29-51 | 95 | 81-110 | 0.94 |
| Dalian | 150 | 90 | 67-113 | 178 | 166-190 | 0.99 |

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