Comparative Toxicity of Two Fluorotelomer-based Surfactants to the Green Alga, *Pseudokirchneriella subcapitata*

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ABSTRACT

Perfluorinated compounds have unique properties and their assessment in various environmental matrices has received increased attention. Their unique properties and use as surfactants, with accompanying potential for environmental release, make aquatic hazard assessment an integral part of product stewardship and risk assessment.

The green alga, *Pseudokirchneriella subcapitata*, (aka *Selenastrum capricornutum*) was exposed for 72 hours to each of two fluorotelomer-based surfactants: one anionic and the other nonionic, in regulatory guideline studies conducted using Good Laboratory Practices to assess the potential aquatic hazard of each compound to algae.

The 72-hour EC50s based on inhibition of healthy cell count, area under the growth curve, and growth rate were greater than 100 mg/L for both compounds. The lowest 72-hour NOEC for the anionic surfactant was 25 mg/L while the 72-hour NOEC for the nonionic surfactant was 100 mg/L. While the anionic surfactant was slightly more toxic, test results suggest both substances are of low concern (i.e., EC50 > 100 mg/L, NOEC > 10 mg/L) for acute and chronic hazard to algae.

INTRODUCTION

Perfluoroalkylated substances (PFAS) are compounds containing a C_nF_{2n+1} group, or mixtures or formulations that contain such compounds. A wide range of chemical structures fall within this category including perfluorinated surfactants that may ultimately reach the aquatic environment. While environmental persistence and potential environmental degradation of these compounds are important areas of research, the development of aquatic hazard data for use in environment risk assessment is also important for those compounds that may reach aquatic ecosystems.

REFERENCES

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European Economic Communities (EEC) (1992). Directive 92/69/EEC Annex V, Part C.3, Algal Inhibition Test. *Methods for the Determination of Toxicity.*

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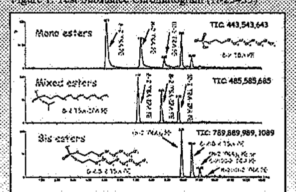
METHODS AND MATERIALS

- Test Species - *Pseudokirchneriella subcapitata*, a freshwater, green alga
- Source - Culture Collection of Algae - The University of Texas at Austin
- Culture Method
  - sterilized 250 mL Erlenmeyer flasks w/ foam stoppers
  - 50 mL of filter-sterilized synthetic (AAP) nutrient medium
  - transferred to fresh medium every 3 to 7 days
  - Miller et al., 1978. EPA-600-78-018; ASTM, 1990. E1218-90
- Test Method
  - OECD TG 201, EEC C.3
  - 50 mL test solution in 250 mL Erlenmeyers w/ foam stoppers
  - nominal test substance concentrations, 6.25, 12.5, 25, 50, and 100 mg/L
  - nutrient medium blank and abiotic control blank
  - 3 replicates per treatment (1 rep for abiotic control); 10,000 cells/mL
  - 72-hour exposure without test medium renewal
  - env. chamber - 24 ± 2°C, 24-hr illumination - 6000-10000 lux, 100 rpm
  - hemacytometer counts of cells at 24, 48, and 72 hours
- Analytical verification of the fluorotelomer phosphate and ethoxylate surfactant concentrations was conducted on test solutions sampled on days 0 and 3.
- Fluorotelomer phosphate surfactant (H-25435) is a mixture of fluoroalkyl phosphate esters derived from fluorotelomer alcohols (Figure 1). The fluoroalkyl phosphate esters present in the test substance can be subdivided into three distinct groups: mono-, bis-, and mixed-esters. One representative ester was selected from each group and analyzed by LC/MS.
- The concentrations of representative esters were represented as the total concentration of the test substance. The selected fluoroalkyl phosphate esters monitored by LC/MS were as follows

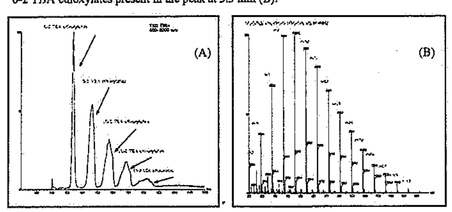
Mono ester: 1-Octanol, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, dihydrogen phosphate ion (abbr. C6-P)
Bis ester: 1-Octanol, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, hydrogen phosphate ion (abbr. C6-P-C6)
Mixed ester: 1-Octanol, 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, 2-propanol, hydrogen phosphate ion (abbr. C6-P-IPA)

Figure 1. Test Substance Chromatogram (H-25435)



- Fluorotelomer ethoxylate surfactant (H-24768) is a mixture of ethoxylates derived from Telomer B Alcohols (TBA) of the following structure: CF_3-$(CF_2)_n$-CH_2-CH_2-O-$(CH_2$-CH_2-$O)_x$-H, where n=3, 5, 7, 9, 11, 13, … and $_x$= 0-25 (Figure 2).
- The major components of the mixture are derived for three TBAs (6-2 TBA, n=5; 8-2 TBA, n=7; 10-2 TBA, n=9). Five of the most intense individual ethoxylates were selected for analysis for each of the TBAs. For 6-2 TBA, 8-2 TBA, and 10-2 TBA, the most intense five ethoxylates represented $_x$=7-11.
- Quantitation was based on the total signal obtained for the five selected ethoxylates for each TBA. Separate calibration curves were made for each group of TBA ethoxylates. The content of each of the selected ethoxylates in the test substance was not known. The total concentration of the test substance was assigned to each group of ethoxylates that were monitored.

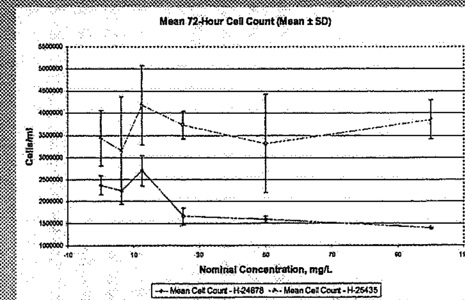
Figure 2. Test Substance (H-24768) Chromatogram (A) and example spectrum of the 6-2 TBA ethoxylates present in the peak at 3.3 min (B).

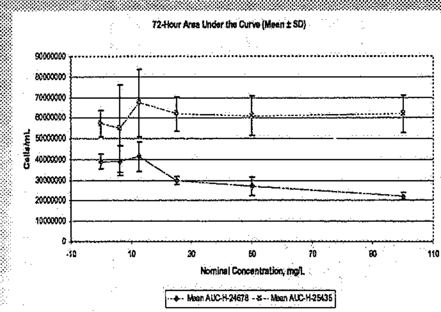


RESULTS

Mean Percent Recoveries

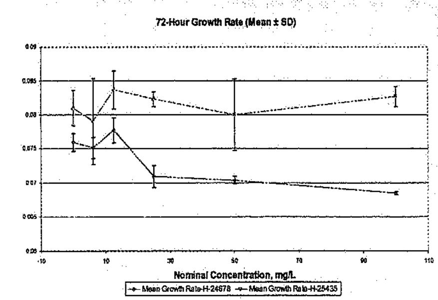
Analyte	Control	6.25 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L
H-25435						
C6-P-C6	ND	90	88	86	84	85
C6-P-IPA	ND	95	106	102	104	97
C6-P mono ester	ND	94	82	90	95	94
H-24768						
6-2 TBA Ethoxy.	ND	85	84	88	87	92
8-2 TBA Ethoxy.	ND	69	73	78	79	82
10-2 TBA Ethoxy.	ND	68	73	80	81	88





EC50 and NOEC Values (mg/L)

Compound	Cell count	AUC	Growth Rate
H-25435			
EC50	> 100	> 100	> 100
NOEC	> 100	> 100	> 100
H-24768			
EC50	> 100	> 100	> 100
NOEC	25	25	25



CONCLUSIONS

- The acute and chronic hazard to algae of two fluorotelomer surfactants was assessed during 72-hour regulatory guideline tests conducted using Good Laboratory Practices.
- EC50s based on inhibition of healthy cell count, area under the growth curve, and growth rate were greater than 100 mg/L for both compounds.
- The lowest 72-hour NOEC for the anionic surfactant was 25 mg/L while the 72-hour NOEC for the nonionic surfactant was 100 mg/L.
- Although the anionic surfactant was slightly more toxic, test results suggest both substances are of low concern (i.e., EC50 > 100 mg/L, NOEC > 10 mg/L) for acute and chronic hazard to algae (Smrchek et al. 1995).