

TOXICITY TO AQUATIC INVERTABRATES (E.G., DAPHNIDS)

EE-15
AR226-0110

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks field: The test substance is a white powder of uncharacterized purity.

The following summary is abbreviated due to the fact that this study has been superceded by a more recent test.

METHOD

Methods: ASTM, 1981, Proposed Standard Practice for Conduction Renewal Life-Cycle Toxicity Tests with the *Daphnia magna*; OECD, 1981, *Daphnia* sp., 14-day Reproduction Test.

Type: Acute static and Chronic renewal

GLP: No

Year completed: 1984

Species: *Daphnia magna*

RESULTS

48-hour EC ₅₀ (immobilization):	27 (25-28) mg/L
14-day EC ₅₀ (reproduction)*:	14.7 (12-18) mg/L
21-day EC ₅₀ (reproduction)*:	12.4 (11-14) mg/L
28-day EC ₅₀ (reproduction)*:	11.4 (10-13) mg/L
28-day NOEC**:	7 mg/L
14, 21, and 28-day MATC:	11.2 mg/L

* Cumulative (Young/Adult)

** This concentration applies to no observed effects on Cumulative results of Young/Adult and Broods/Adult and on the total number of Young/Adult.

DATA QUALITY

Reliability: Klimisch ranking = 2. This study satisfied all criteria for quality testing at the time performed, but actual concentrations were not measured. Results were based on nominal concentrations. Additionally, sample purity was not adequately characterized.

REFERENCES

001512

This study was conducted by the 3M Company, Environmental Laboratory, 1984.

OTHER

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331,
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TECHNICAL REPORT SUMMARY

File TDB
FC-95

TO: TECHNICAL COMMUNICATIONS CENTER - 201-2C-12

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Division Environmental Laboratory (EE & PC)		Dept. Number 0535
Project New Methods Development		Project Number 9970030000
Report Title Effect of Potassium Perfluorooctanesulfonate on Survival, etc.		Report Number # 011
To R. L. Bohon		Period Covered or Date 2/13/84
Author(s) A. N. Welter		Employee Number(s) 09362
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SECURITY ▶	☒ Open Report & Summary (Company Confidential) ☐ Closed Report - Open Summary (Special Authorization)	3M CHEMICAL REGISTRY ▶ ☐ Check box if new chemicals are reported. U cal Registry Form 6092 to report all new su

KEYWORDS: Lab Code Other Keywords <u>EE & PC</u> <u>ENV. LAB.</u>	CURRENT OBJECTIVE: Technology Transfer: To develop a daphnid chronic life cycle capability.
REPORT ABSTRACT: This abstract information is distributed by the Technical Communications Center to alert 3M's to Company R&D.	Abstract - Acute static and chronic renewal <u>Daphnia magna</u> tests were conducted to evaluate this organisms response to varying concentrations of potassium perfluorooctanesulfonate. The acute 48-h EC₅₀ was 27 mg/L, with 95% C.L. of 25 to 28 mg/L. In 14, 21, 28-day chronic exposure studies the NOEC was 7 mg/L. At the highest concentration tested (18 mg/L) reproductive indices; viz: cumulative live young per adult, average number of young per brood, and average number of broods per adult, were inhibited to a significant degree $p < 0.05$. This concentration of potassium perfluorooctanesulfonate was several orders of magnitude greater than levels anticipated under normal use conditions. The survival, growth, and reproduction of this species will not be impacted negatively by chronic 14, 21, or 28-day exposure to potassium perfluorooctanesulfonate. This study should be repeated since the two highest concentrations tested, 7 and 18 mg/L represent the NOEC and significant effect levels respectively. There is a need to develop information on concentrations lying between these two extremes.

Key Words: Toxicity, acute, chronic, Daphnia magna

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Initials: AM

001514

INTRODUCTION

Ubel et al⁽¹⁾ reported that perfluorinated chemicals had been found in the serum of manufacturing plant workers. One of these materials has been the subject of animal toxicity studies⁽²⁾. Data on a similar compound, potassium perfluorooctanesulfonate, a off-white solid, indicates that this material is water soluble and preliminary information suggests that it will neither readily biodegrade nor bioconcentrate. Preliminary aquatic testing of potassium perfluorooctanesulfonate indicated that toxic signs could be elicited during acute exposure conditions.

Of the aquatic species available for use as test organisms, the crustacean, Daphnia magna is being utilized widely in acute and chronic effects testing. Advantages associated with the use of daphnids are their size, comparatively short life cycle, ecological importance, ease of laboratory culturing, sensitivity, etc.^(3,4,5) Guidelines for conducting acute 48-h tests have been published⁽⁶⁾, and chronic exposure protocols are being developed or have been adopted⁽⁷⁾. A broad database consisting of both acute and chronic studies utilizing D. magna presently exists. Daphnids were used, therefore, to evaluate the effect of acute (48-h) and 14, 21, or 28-day chronic exposures to varying concentrations of potassium perfluorooctanesulfonate.

METHODS AND MATERIALS

Water Diluent

The dilution water used in all tests was an unchlorinated, carbon filtered well water, aerated to saturation before use. Mean values for some water quality parameters were: pH 7.6 (+0.1), total hardness as mg/L CaCO₃ of 256 (247-265), alkalinity as mg/L CaCO₃ of 224 (219-229) and conductivity as umhos/cm at 25°C of 349 (325-373).

Test Species

The laboratory stock culture of D. magna was maintained under intermittent illumination, 16h light and 8h dark, at a temperature of 20±2°C. Stock and organisms used during chronic exposure tests were fed blended fish food of known nutritional quality and yeast at the rate of 30 mg solids/L of test solution.

Acute Tests

During these tests the dilution water temperature averaged 22±1°C, while a photoperiod of 16-h light and 8-h darkness was maintained throughout the test. For the 48-h tests, 20 daphnids 12 ± 12-h old were placed in duplicate⁽⁶⁾ test beakers, 250 ml capacity, each containing 200 ml of test solution. All acute tests were run without food additions. Five concentrations of potassium perfluorooctanesulfonate, and an untreated control group were run in duplicate. The endpoint used in these tests was daphnid immobilization as characterized by a lack of coordinated movement. The calculated EC₅₀ value was used to define the range of concentrations to be used in the life-cycle renewal test.

Chronic Tests

The chronic daphnid studies using first instars 12 + 12-h old were conducted in a temperature controlled growth chamber at $23 \pm 1^\circ\text{C}$ with photoperiod automatically fixed to a 16-h light, and 8-h dark cycle. This static renewal chronic test was conducted using ten 250 ml beakers containing 200 ml test solution at each of five concentrations of the test material plus an untreated control set of 20 beakers. Each test concentration was replicated three times with five Daphnia magna first instars per beaker for survival data and seven times with but one daphnid per beaker for reproductive measurements. Stock solutions were prepared in deionized water. Fresh solutions were made up thrice weekly (MWF) with food also being replenished at this time. Disposable glass pipettes having an I.D. of 5 mm were used to transfer the adult daphnids to fresh solution. The young daphnids were then counted and discarded.

During the test the following data were tabulated: day of initial brood release, number of young per adult per day, number of broods per adult, mortality of young or adult daphnids. All data are based on nominal concentrations of potassium perfluorooctanesulfonate.

Statistics

The 48-h, acute and 14, 21, and 28-day chronic EC_{50} values and their associated 95% confidence limits were determined⁽⁸⁾ by either probit analysis or the moving angle average method⁽⁹⁾. Reproductive and survival data were analyzed by analysis of variance. Throughout the text "significant" will refer to statistical significance, having a p value of .05.

RESULTS

Acute Test

The calculated 48-h EC_{50} value was 27 mg/L of potassium perfluorooctanesulfonate with a 95% C.L. ranging from 25 to 28 mg/l. Based on this value the range of test concentrations selected for the daphnid chronic test were 0.26, 1.0, 2.6, 7.0, and 18 mg/l of potassium perfluorooctanesulfonate. Temperature and pH varied minimally while the dissolved oxygen level did not fall below 70% saturation.

Chronic Test - 14, 21, and 28-Day Duration

Indices of reproductive performance and survival of Daphnia magna exposed to the above listed concentrations of the test material are tabulated in Tables 1-4.

Untreated control reproductive and survival indices, as shown in Table 1 are in agreement with reported literature values⁽¹⁰⁾. Literature values cited for the cumulative number of young per adult ranged from 30-122 at temperatures of $20-25^\circ\text{C}$ while at temperatures of $18-20^\circ\text{C}$ Daphnia magna survival varied from 80-92%.

The effect on reproductive indices elicited by chronic exposure to potassium perfluorooctanesulfonate are illustrated (Table 1). Over the 28-day exposure period and at chemical concentrations varying from 0.26 to 7.0 mg/L the mean values of the above cited parameters varied minimally when compared to control data. Thus, the absence of a concentration dependent response up to 7.0 mg/L is apparent.

Interference with the normal reproductive process did occur at a toxicant concentration between 7.0 and 18.0 mg/L. At this latter concentration, the cumulative number of live young per adult were reduced by 80%, average number of live young per brood was inhibited by 55%, and the number of broods per adult was reduced by 62%.

It has been recommended⁽⁷⁾ that three brood studies replace 21 or 28 day chronic exposure tests (Table 2). At potassium perfluorooctanesulfonate concentrations up to and including 7.0 mg/L the time to three brood release was either 14 or 15 days after initiation of the study. At 18 mg/L it required approximately 17 days for release of the three broods, a statistically significant difference when compared to control values.

Calculated values for 50% reproductive impairment at 14, 21, and 28 days of exposure to potassium perfluorooctanesulfonate are shown (Table 3). Adult mortality EC_{50} values could not be determined as they exceeded the highest test concentration used in this study. The 28-day EC_{50} values calculated for all reproductive parameters were similar, ranging from 11.4 to 16.1 mg/l. Overlap of the 95% C.L. is indicative of the absence of statistically significant differences in these values.

In this study statistically significant changes in all reproductive indices did occur at a concentration of 18 mg/L potassium perfluorooctanesulfonate (Table 4). Exposure to concentrations of 0.26, 1.0, 2.6, and 7.0 mg/L of the test substance did not affect these indices or mortality to a significant degree. Thus, a concentration of 7.0 mg/L of potassium perfluorooctanesulfonate is considered to be the chronic NOEC or no observable effect concentration.

Table 5 illustrates the derived acute/chronic ratios for those reproductive indices evaluated throughout the potassium perfluorooctanesulfonate exposure period. The 28-day acute/chronic ratios for all parameters studied were identical and were equivalent to the 14 and 21-day acute/chronic ratios derived from cumulative number of young per adult data. This may indicate a concentration dependent process which is not affected by increasing the exposure duration.

Table 6 shows the chronic renewal toxicity of potassium perfluorooctanesulfonate expressed as the NOEC, MATC, and EC_{50} which values were derived from reproductive indices. As in the preceding table, the derived values are chemical dependent and time independent. Thus, the NOEC and MATC are identical for all parameters measured at all exposure periods. The EC_{50} values differ slightly however there is no statistical difference in these values based on variable exposure durations.

DISCUSSION

Reproductive measurements have been used as indicators of toxicant effects following exposure to sublethal concentrations of PCB, p,p'DDT, or Atrazin®. Furthermore, Parkhurst et al⁽³⁾ investigated the reproducibility of the Daphnia magna chronic test and concluded that, of the toxicity criteria studied, the number of young produced per brood or per adult were sensitive indicators of a chronic toxicant effect.

Our results are in agreement with those reported by Maki and Johnson⁽¹³⁾ and Parkhurst et al⁽³⁾ that changes in indices of reproduction are sensitive parameters of a toxicant effect. Changes occurring at concentration levels of 18 mg/L of potassium perfluorooctanesulfonate included reductions in: 1) the average number of broods per parent, 7 to 3; 2) number of live young per parent, 190 to 37; 3) average number of young per brood 27 to 13. An increase in the number (to 54) of dead young per parent was also noted. When combining the number of live and dead young a 52% reduction in total number of young released was observed. The most noteworthy result of daphnid exposure to high levels of potassium perfluorooctanesulfonate has been the attenuation of all reproductive indices. On the other hand, at this concentration maturation was not delayed nor was longevity of the parent affected.

Water quality guidelines have specified the use of two protective concentrations, namely a 24-h average equivalent to the no observable effect concentration (NOEC) and either a 24-h maximum or 48-h EC₅₀ level. Neither of these protective concentrations should be exceeded over the indicated time period. Utilizing this concept, protective concentrations of 7.0 and 27 mg/L would be established for potassium perfluorooctanesulfonate. Over the 28-day exposure period no observable effects occurred using either mortality or reproductive indices as the response criteria. A NOEC of 7 mg/L and the toxicant level which elicited an effect, 18 mg/L, give us a gross approximation of the GM-MATC, equal to 11 mg/L. Conversely, during a 28-day chronic exposure to 18 mg/L of potassium perfluorooctanesulfonate, reproductive impairment was noted. This phenomenon was observed as early as the second week and remained at the same EC₅₀ value for the duration of the study.

Based on these findings and considering the concentrations of test chemical necessary to elicit a chronic effect, it is concluded that potassium perfluorooctanesulfonate will not impact negatively on the survival, growth and reproduction of Daphnia magna under present environmental release conditions.

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TABLE 1

Chronic Exposure of Daphnia magna to
Potassium Perfluorooctanesulfonate:
Young Reproduction^a

Treatment mg/L	n	Cumulative Live Young/Adult Number	Average Number Young/Brood	Avg. Number Broods/Adult
Control	11	190 <u>+36</u>	27 <u>+4</u>	7 <u>+1</u>
0.26	11	169 <u>+33</u>	26 <u>+4</u>	7 <u>+1</u>
1.0	7	183 <u>+40</u>	25 <u>+4</u>	7 <u>+1</u>
2.6	7	186 <u>+29</u>	26 <u>+4</u>	7 <u>+1</u>
7.0	6	199 <u>+19</u>	27 <u>+3</u>	7 <u>+1</u>
18.0	7	37 ^b <u>+28</u>	12 ^b <u>+8</u>	3 ^b <u>+1</u>

^a 28-day exposure data. Mean $\pm$ S.D.
^b $p \leq 0.05$.

TABLE 2

Chronic Exposure of Daphnia magna to Potassium
Perfluorooctanesulfonate: Comparison of 1st and
3rd Brood Release Days

Treatment mg/L		Initial Brood Release Day	Third Brood Release Day
Control	n ^a	9 <u>+1</u>	15 <u>+1</u>
0.26	17	10 <u>+2</u>	15 <u>+2</u>
1.0	10	9 <u>+1</u>	15 <u>+2</u>
2.6	10	9 <u>+0.3</u>	15 <u>+2</u>
7.0	9	9 <u>+0.3</u>	14 <u>+1</u>
18.0	10	10 <u>+2</u>	17 ^b <u>+2</u>

^a Beakers in test.
^b $p \leq 0.05$.

Table 3

Acute and Chronic Toxicity of Potassium Perfluorooctanesulfonate
to Daphnia magna: Acute and Chronic EC₅₀

<u>Exposure Period</u>	<u>Immobilization</u>	<u>Reproduction EC₅₀ mg/L (95% C.L.)</u>		
		<u>Cumulative Young/Adult</u>	<u>Live Young Per Brood</u>	<u>Avg. No. Broods/Adult</u>
48-hr	26.6 (25-28)			
14 Day		14.7 (12-18)	a	a
21 day		12.4 (11-14)		
28 day		11.4 (10-13)	16.1 (13-20)	14.7 (13-18)

a Not calculated.

TABLE 4

Comparison of the Chronic Effects of 7 and 18 mg/L
Potassium Perfluorooctanesulfonate to Daphnia magna

	<u>14-Day</u>	<u>21-Day</u>	<u>28-Day</u>
A. 7.0 mg/L:			
Number Live Young /Adult	59	129	199
Number Dead/Adult	3	3	4
Total Number Young	369	793	1220
Adult Mortality %	0	4.5	4.5
B. 18.0 mg/L:			
Number Live Young /Adult ^a	24	33	37
Number Dead/Adult ^a	12	51	54
Total Number Young ^a	249	591	641
Adult Mortality %	4.5	9.1	18.2

^a Comparison of 7.0 and 18.0 mg/L $p \leq 0.05$.

TABLE 5

Acute/Chronic EC₅₀ Ratios Following Exposure of
Daphnia magna to Potassium Perfluorooctanesulfonate^a

<u>Day of Exposure</u>	<u>Cumulative Young/Adult</u>	<u>Cumulative Broods/Adult</u>	<u>Number of Young/Adult</u>
14	1.5		
21	1.5		
28	1.5	1.5	1.5

^a Acute/Chronic EC₅₀ Ratio =
$$\frac{48\text{h Concentration Eliciting an Acute Effect in } 50\% \text{ of Organisms}}{(\text{Exposure Duration}) \text{ Concentration Eliciting a Chronic Effect in } 50\% \text{ of Organisms}}$$

TABLE 6

Chronic Renewal Toxicity of Potassium
Perfluorooctanesulfonate to *Daphnia magna*^a

Days	14	Cumulative Young/Adult 21	28	Cumulative Broods/Adult 28	Number of Young/Adult 28
NOEC ^b	7	7	7	7	7
MATC ^{c,d}	11.2	11.2	11.2	11.2	11.2
EC ₅₀ ^e	14.7 (12-18)	12.4 (11-14)	11.4 (10-13)	14.7 (13-18)	16.1 (13-20)

^a Data in mg/L.

^b No observable effect concentration.

^c Maximum acceptable toxicant concentration.

^d MATC = Geometric mean between highest test concentration having no significant ($p > 0.05$) effect and the lowest test concentration having a significant ($p \leq 0.05$) effect.

^e Dose at which 50% of organisms are affected by the toxicant. Expressed as Mean (95% C.L.).