

TOXICITY TO TERRESTRIAL PLANTS

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: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 86.9% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: The study was conducted using a protocol based on procedures outlined in U.S. EPA OPPTS Guidelines 850.4100 and 850.4225 and the Organization for Economic Cooperation and Development (OECD) proposed revision of Guideline No. 208.

Test type: Artificial soil

GLP: Yes

Year Completed: 2003

Species Monocots:

Allium cepa (onion – 'Texas Grano')

Lolium perenne (ryegrass – 'Manhattan 3')

Dicots:

Medicago sativa (alfalfa)

Linum usitatissimum (flax)

Lactuca sativa (lettuce – 'Summertime')

Glycine max (soybean – 'Green Envy')

Lycopersicon esculentum (tomato – 'Rutgers')

Analytical monitoring: Test substance concentrations were measured by LCMS in soil at Days 0 and test termination and in plant and (when possible) fruit tissues at test termination.

Statistical methods: EC values calculated, when possible, by non-linear regression analysis of Bruce and Versteeg, or with non-linear interpolation using the ICp approach of Norberg-King. Treatment group means were compared to control means with Dunnett's test ($\alpha = 0.05$) using SAS Version 8 to determine if significant differences in emergence, survival, weight or height from the control occurred.

Test organism source:

Onion: Territorial Seed Co., Cottage Grove, OR

Ryegrass: Meyer Seed Co., Baltimore, MD

Alfalfa: Frontier Natural Products, Norway, IA

Flax: Arrowhead Mills, Hereford, TX

Lettuce: Territorial Seed Co., Cottage Grove, OR
Soybean: Johnny's Selected Seeds, Albion, ME
Tomato: Meyer Seed Co., Baltimore, MD

Test organism age at study initiation: seed

Test conditions

Test media: Artificial loam soil of the following composition:

Kaolin clay, industrial quartz sand and peat in a ratio of 2:25:1 by weight. Crushed limestone (to buffer the pH) and a slow-release fertilizer were also added. Final mix consisted of 49% sand, 30% silt, 21% clay, and 2.1% organic matter. Soil pH was 7.79.

Environmental conditions: Study conducted in a temperature-controlled greenhouse. Artificial lighting supplemented natural sunlight to provide a uniform 14-hour photoperiod.

Stock and test solutions preparation: Approximately 70 kg of each test concentration and the negative control was prepared separately. The test substance was first mixed with a 1000 g aliquot of the 70 kg of test soil (soil moisture of 15%). The test substance-containing aliquot was then added back to the 70 kg of bulk soil and mixed for 10 minutes. The negative control soil was prepared in the same manner, but no test substance was added.

Exposure vessels: Plastic pots, approximately 16 cm wide and 12 cm deep.

Number of replicates: four

Number of seeds per replicate: ten

Number of concentrations: five plus a negative control

Watering procedure: subirrigation

Test conditions parameters:

Temperature range: 14.1 – 32.0°C

Relative humidity range: 8 – 91%

Light intensity range: 12.8 – 26.6 moles PAR

Exposure period (germination and growth test): 21-Days

Exposure period (extended test): up to 205-days (variable by species)

After observations on day 21, all but one plant was clipped from each pot. The remaining plant was maintained in the extended test, until (if possible) fruit formation. At test termination, the one plant was harvested and the fruit and vegetative tissues were analyzed separately for PFOS.

Element Basis: EC₂₅, EC₅₀, NOEC – emergence, survival, shoot height, shoot weight

Other observations: seedling condition

Method of reporting test concentrations: nominal

RESULTS

Nominal concentrations: Bk control, 3.91, 15.6, 62.5, 250, 1000 mg/kg

Initial measured concentrations: <LOQ, 3.61, 11.1, 50.8, 276, 998 mg/kg

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Element values:

21-Day Emergence, mg/kg

Species	LOEC	NOEC	EC₂₅	EC₅₀
Onion	250	62.5	51	210
Ryegrass	250	62.5	200	340
Alfalfa	1000	250	370	745
Flax	1000	250	400	600
Lettuce	1000	250	390	560
Soybean	>1000	1000	>1000	>1000
Tomato	1000	250	310	470

21-Day Survival, mg/kg

Species	LOEC	NOEC	EC₂₅	EC₅₀
Onion	62.5	15.6	47	57
Ryegrass	250	62.5	170	310
Alfalfa	250	62.5	250	450
Flax	250	62.5	140	230
Lettuce	250	62.5	260	390
Soybean	>1000	1000	>1000	>1000
Tomato	62.5	15.6	69	105

21-Day Shoot Height, mg/kg

Species	LOEC	NOEC	EC₂₅	EC₅₀
Onion	62.5	15.6	29	47
Ryegrass	15.6	3.91	46	130
Alfalfa	250	62.5	100	250
Flax	250	62.5	98	140
Lettuce	3.91	<3.91	6.8	40
Soybean	250	62.5	280	460
Tomato	62.5	15.6	22	94

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21-Day Shoot Weight, mg/kg

Species	LOEC	NOEC	EC ₂₅	EC ₅₀
Onion	15.6	3.91	13	28
Ryegrass	15.6	3.91	7.5	54
Alfalfa	250	62.5	53	150
Flax	250	62.5	82	120
Lettuce	3.91	<3.91	8.9	20
Soybean	250	62.5	160	330
Tomato	62.5	15.6	12	29

All element values were calculated using nominal concentrations.

Statistical Evaluation Variables:

Emergence: Number of emerged seedlings per 10 planted seeds per pot.

Survival: Number of living emerged seedlings per 10 seeds per pot.

Weight: Mean shoot wet weight of living emerged seedlings in each pot

Height: Mean height of living emerged seedlings in each pot.

Biological observations:

Onion – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	88	85	0.10	8.1	2.9	None
3.91	93	90	0.09	7.3	3.8	Chlorosis [1]
15.6	83	80	0.07*	7.3	4.8	Necrosis [1]
62.5	63	33*	0.02*	2.6*	48	None
250	40*	0*	-	-	100	-
1000	0*	0*	-	-	-	-

⁽¹⁾ A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

Ryegrass – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	80	80	0.12	16.9	0	None
3.91	93	93	0.11	15.4	0	None
15.6	90	90	0.07*	13.7*	0	None
62.5	85	83	0.08*	13.6*	2.9	None
250	58*	53*	0.01*	3.7*	22	Necrosis [11]
1000	7.5*	7.5*	0.03*	2.0*	13	Necrosis [2]

⁽¹⁾ A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

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Alfalfa – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	90	88	0.12	6.5	4.2	Necrosis [1]
3.91	85	83	0.11	6.4	2.9	None
15.6	80	80	0.10	5.4	5.9	Necrosis [3]
62.5	80	80	0.10	6.1	0.9	Necrosis [1]
250	73	63*	0.03*	2.8*	17	Necrosis [1]
1000	33*	15*	0.02*	1.0*	75	Necrosis [4]

⁽¹⁾A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

Flax – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	73	73	0.19	8.9	1.4	Unshed seed coat [1]
3.91	73	68	0.18	8.7	9.7	Leaf curl [1]
15.6	80	80	0.18	8.8	2.5	Unshed seed coat [1]
62.5	85	85	0.16	8.2	1.2	Necrosis [1]
250	73	33*	0.02*	1.3*	91	Unshed seed coat [13]
1000	0*	0*	-	-	-	-

⁽¹⁾A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

Lettuce – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	88	88	0.38	6.4	0	None
3.91	90	90	0.25*	5.0*	0	None
15.6	90	90	0.24*	5.0*	1.1	Necrosis [1]
62.5	85	85	0.05*	2.6*	33	Necrosis [29]
250	83	68*	0.01*	1.0*	42	Necrosis [17] Leaf curl [1]
1000	13*	5.0*	Insufficient sample*	1.0*	94	Necrosis [1] Unshed seed coat [1]

⁽¹⁾A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

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Soybean – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	100	100	3.63	28.3	0	None
3.91	98	98	3.64	26.8	0.8	Leaf curl [1]
15.6	95	95	3.63	26.9	0	None
62.5	100	100	4.00	30.7	1.8	Leaf curl [2]
250	100	98	2.07*	22.5*	7.0	Chlorosis [2] Leaf curl [3] Necrosis [1]
1000	100	100	0.57*	4.1*	51	Unshed seed coat [2] Stem curl [7] Leaf curl [11] Necrosis [25]

⁽¹⁾A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

Tomato – Day 21

Nominal Soil Concentration, mg/kg	Mean Emergence %	Mean Survival %	Mean Seedling Fresh Weight, g	Mean Seedling Height cm	Mean Seedling Condition Score ⁽¹⁾	Sublethal Effects Noted [# plants with effect]
Neg. Control	93	93	0.35	5.2	0	None
3.91	83	83	0.40	5.6	0	None
15.6	83	80	0.28	4.8	6.7	Leaf curl [1] Necrosis [3]
62.5	85	68	0.08*	2.6*	40	Necrosis [19]
250	73	7.5	0.03*	2.0*	97	Necrosis [3]
1000	10*	0*	-	-	100	-

⁽¹⁾A score of 0 = normal seedling; 100 = dead seedling

*Statistically significantly different from the control (p<0.05)

Control response: Satisfactory for all species

Extended test observations: There were no additional findings of phytotoxicity at the termination of the extended growth portion of the test.

Analytical Methodology: Analyses of test soils and plants were performed at Wildlife International Ltd. using high performance liquid chromatography with triple quadrupole mass spectrometric detection (HPLC/MS/MS). When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) in soil was 1.18 mg/kg. For vegetative tissue, the LOQ **002186**

ranged from 2.4 – 7.6 mg/kg. For fruit, the LOQ ranged from 0.12 – 6.4 mg/kg.

The recovery of matrix fortifications analyzed concurrently during sample analysis ranged from 83.9% – 89.0% in the soil, 86.3% – 117% in the fruit and 88.0% – 110% in the vegetative tissue. Soil samples collected at test initiation had measured values from 71.2% to 110% of nominal. Measured values for samples taken at test termination ranged from 22.8% to 62.8% of nominal.

Values reported as <LOQ were excluded from all calculations of mean concentration.

Summaries of Analytical Chemistry Data (All results reported as dry weight):

Soil, Day 0:

Nominal Test Concentration, mg/kg	Measured Replicate Values, mg/kg	Mean Concentration, mg/kg	Percent of Nominal
Negative Control	All < LOQ ⁽¹⁾	<LOQ	-
3.91	3.43, 2.83, 4.56	3.61	92.3
15.6	11.1, 11.2	11.1	71.2
62.5	50.0, 44.7, 57.6	50.8	81.3
250	415, 244, 170	276	110
1000	1070, 983, 942	998	99.8

⁽¹⁾LOQ = 1.18 mg/kg

Soil, termination (Day 205):

Nominal Test Concentration, mg/kg	Measured Replicate Values, mg/kg	Mean Concentration, mg/kg	Percent of Nominal
Negative Control	All < LOQ ⁽¹⁾	<LOQ	-
3.91	1.21, 1.36	1.29	33.0
15.6	4.91, 2.21	3.56	22.8
62.5	16.3, 16.1	16.2	25.9
250	153, 161	157	62.8
1000	432, 515	474	47.4

⁽¹⁾LOQ = 1.18 mg/kg

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Onion Vegetative Tissue, Termination (67-days):

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All < LOQ ⁽¹⁾	<LOQ
3.91	All < LOQ ⁽¹⁾	<LOQ
15.6	5.86, 15.4, <LOQ	10.6
62.5	Insufficient sample	-
250	No plants living	-
1000	No plants living	-

⁽¹⁾LOQ = 4.7 mg/kg**Onion Fruit, Termination (67-days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	0.517, 0.487, insufficient sample	0.502
3.91	5.81, 0.453, insufficient sample	3.13
15.6	<LOQ ⁽¹⁾ , 19.7, 24.6	22.2
62.5	Insufficient sample	-
250	Insufficient sample	-
1000	No plants living	-

⁽¹⁾LOQ = 0.42 mg/kg**Ryegrass Vegetative Tissue, Termination (205 days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	7.13, 8.70, 8.70	8.18
15.6	13.8, 49.0, <LOQ	31.4
62.5	42.5, 67.0, 36.7	48.7
250	31.9, 37.5, 129	66.1
1000	Insufficient sample	-

⁽¹⁾LOQ = 3.3 mg/kg**Ryegrass fruit – No sample (did not mature by 205 days)****002188**

Alfalfa Vegetative Tissue, Termination (141 days)

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	<LOQ, <LOQ, 6.16	6.16
15.6	5.66, 2.74, <LOQ	4.2
62.5	12.9, 9.66, 11.1	11.2
250	14.2, 15.8, 17.3	15.8
1000	Insufficient sample	-

⁽¹⁾LOQ = 2.7 mg/kg**Alfalfa Fruit, Termination (141 days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	Insufficient sample, insufficient sample, <LOQ	<LOQ
15.6	<LOQ (composite)	<LOQ
62.5	<LOQ (composite)	<LOQ
250	<LOQ (composite)	<LOQ
1000	Insufficient sample	-

⁽¹⁾LOQ = 6.4 mg/kg**Flax Vegetative Tissue, Termination (94 days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	4.56, 6.43, 3.85	4.95
15.6	13.1, 26.4, 17.0	18.8
62.5	54.7, 72.7, 37.5	55.0
250	Insufficient sample	-
1000	No plants living	-

⁽¹⁾LOQ = 2.4 mg/kg

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Flax Fruit, Termination (94 days)

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	0.217, 0.277, 0.195	0.230
15.6	1.07, 0.622, 2.41	1.36
62.5	1.26, 2.20, 4.23	2.56
250	Insufficient sample	-
1000	No plants living	-

⁽¹⁾LOQ = 0.12 mg/kg**Lettuce Vegetative Tissue, Termination (67 days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	12.2, 6.16, 7.52	8.63
15.6	15.5, 7.06, 9.26	10.6
62.5	39.8, 58.9, 27.0	41.9
250	Insufficient sample	-
1000	Insufficient sample	-

⁽¹⁾LOQ = 5.5 mg/kg**Soybean Vegetative Tissue, Termination (67 days)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	17.7, 10.0, 19.1	15.6
15.6	29.3, 48.9, 29.3	35.8
62.5	63.9, 70.7, 55.4	63.3
250	64.2, 175, 103	114
1000	Insufficient sample	-

⁽¹⁾LOQ = 6.0 mg/kg

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Soybean Fruit, Termination (67 days)

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	0.947, 0.339, 2.91	1.40
15.6	0.464, 1.63, 0.528	0.874
62.5	1.14, 1.14, 1.33	1.20
250	1.57, 5.56, 2.49	3.21
1000	Insufficient sample	-

⁽¹⁾LOQ = 0.18 mg/kg**Tomato Vegetation, Termination (day 94)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	All <LOQ	-
15.6	12.5, 27.9, 61.1	33.8
62.5	70.9, 44.5, 34.9	50.1
250	Insufficient sample	-
1000	No plants living	-

⁽¹⁾LOQ = 7.6 mg/kg**Tomato Fruit, Termination (day 94)**

Nominal Test Concentration, mg/kg	Measured Replicate Tissue Values, mg/kg	Mean tissue Concentration, mg/kg
Negative Control	All <LOQ ⁽¹⁾	<LOQ
3.91	<LOQ, <LOQ, insufficient sample	< LOQ
15.6	<LOQ, 1.51, 0.58	1.05
62.5	0.946, <LOQ, <LOQ	0.946
250	Insufficient sample	-
1000	No plants living	-

⁽¹⁾LOQ = 0.47 mg/kg**002191**

Samples of soil and plant tissues from all species were also obtained at Day 21 and analyzed for PFOS. However, plant tissues and soil obtained on Day 21 had considerably higher than expected PFOS concentrations. Day 21 soil samples were collected by scraping the surface of the soil,

whereas a soil core sampler was used for samples collected at test termination. The laboratory concluded that PFOS concentrated in the top layer of the soil as a result of watering by subirrigation. Plant tissues collected at Day 21 were very small in size, and likely contaminated with surface soil due to improper collection techniques. Therefore, Day 21 values were considered to be not representative, and were not reported.

CONCLUSIONS

The potassium perfluorooctanesulfonate 21-day EC₅₀ range for seven species of terrestrial plants for the four study endpoints were found to be:

Emergence: 210 - > 1000 mg/kg

Survival: 57 - >1000 mg/kg

Height: 40 – 460 mg/kg

Shoot Weight: 20 – 330 mg/kg

The 21-day no observed effect concentration ranges were found to be:

Emergence: 62.5 – 1000 mg/kg

Survival: 15.6 – 1000 mg/kg

Height: <3.91 – 62.5 mg/kg

Shoot Weight: <3.91 – 62.5 mg/kg

Submitter: 3M Corporation, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

This study was conducted at Wildlife International, Ltd. Easton, MD at the request of the 3M Company.

OTHER

Last changed: 6/3/03

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