

ACUTE CONTACT TOXICITY TO TERRESTRIAL INVERTEBRATES (HONEY BEE)

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-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks field: The 3M production lot number was 217. The test substance is a white powder. Sample was stored AT 16-20°C prior to testing. Purity determined to be 86.9% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: USEPA OPPTS 850.3020 (draft), OECD Guideline 214, EPPO Guideline 170

Test type: Acute Contact

GLP: Yes

Year Completed: 2001

Species: *Apis mellifera* L.

Analytical monitoring: None – nominal concentrations

Test honey bee source: Obtained from colony number 32 belonging to the Central Science Laboratory (CSL), Sand Hutton, York, UK, National Bee Unit.

Test honey bee age at study initiation: Young adult

Test honey bee type: Worker honey bees, free of acarine, nosema and amoeba.

Varroacide treatment: None within the 4 weeks prior to test initiation.

Test conditions

Humidity: 65% $\pm$ 5%

Temperature: 25 $\pm$ 2°C

Lighting: Conducted in darkness

Stock and test solutions preparation:

Test substance: Stock solution prepared in analytical grade acetone to a final concentration of 90.4 µg PFOS/µL (nominal concentration). Final test concentrations prepared in acetone from dilutions of this solution.

Reference toxicant: Stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final

concentration of 3.0 µg/µL. Final test concentrations prepared from dilutions of this solution.

Stability of the test chemical solution: A dispersion test was carried out on an 86 µg PFOS/µL acetone solution before the toxicity study was performed. The homogeneity of the mixture was assessed after 2 hours. The test item formed a clear solution on mixing; after 2 hours at room temperature, slight sediment was noted. For the toxicity test, all solutions were re-mixed prior to use. The contract laboratory considered the solutions of the test doses to be homogenous for the purpose of administration.

Exposure vessels: Clean, well-ventilated, inverted petri dishes, measuring approximately 9 cm in diameter.

Feeding: 50% w/v aqueous sucrose solution, continuously available

Number of replicates: Three

Number of bees per replicate: Ten

Negative control: Undosed

Solvent control: Acetone

Reference substance: Dimethoate

Reference substance control: Triton X-100

Number of concentrations: five plus a negative and a solvent control

Dose administration: The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each bee was dosed on the thorax with a 1 µL drop of a given test item concentration or 1 µL acetone before being placed into the test chamber.

Dose frequency: Once

Element basis: Mortality

RESULTS

Nominal concentrations: Negative control, acetone control (1.0 µg/bee), 1.93, 4.24, 9.30, 20.5, 45, µg PFOS/bee

Element value and 95% confidence interval :

24-hour LD₅₀ = 38.9 (28.2 – 71.2) µg/bee

48-hour LD₅₀ = 10.4 (8.2 – 13.0) µg/bee

72-hour LD₅₀ = 6.0 (4.7 – 7.6) µg/bee

96-hour LD₅₀ = 4.78 (3.8 – 5.8) µg/bee

96-hour NOEL = 1.93 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares

regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test ($p < 0.05$)

Biological observations: There was significant mortality at all doses above 1.93 µg/bee with a steep dose response between 4.24 and 9.30 µg/bee

Cumulative percent mortality:

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	3.3	3.3	3.3
Solvent Control	3.3	3.3	3.3	3.3	3.3
1.93	0	6.7	6.7	13	13
4.24	0	0	13	37	37
9.30	0	6.7	40	63	90
20.5	0	40	93	97	100
45.0	0	50	93	100	100

Sub-lethal Effects – Percent Knockdown (K) or Stumbling (S):

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0	0
Solvent Control	0	0	0	0	0
1.93	3.3 (K)	0	0	0	0
4.24	0	0	0	0	0
9.30	0	0	0	0	3.3 (K)
20.5	0	3.3 (K)	0	3.3 (K)	0
45.0	0	3.3 (K)	3.3 (K)	0	0

Control response: satisfactory

Reference toxicant response: satisfactory - dimethoate 96-hour LD₅₀ = 0.19 µg/bee

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour contact LD₅₀ for the honey bee was determined to be 4.78 µg/bee with a 95% confidence interval of 3.8 – 5.8. The 96-hour no observed effect level was 1.93 µg/bee. The dose response was steep between 4.24 and 9.30 µg/bee.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

This study was conducted at Central Science Laboratory, Sand Hutton, York, UK, under contract by Wildlife International, Ltd, Easton, MD at the request of the 3M Company, Lab Request Number U2723, 2001.

OTHER

Last changed: 5/1/01



CENTRAL SCIENCE LABORATORY

SCIENCE SERVING AGRICULTURE, FOOD AND THE ENVIRONMENT

CONTRACT REPORT

STUDY NUMBER HT5601

PERFLUOROOCTANESULFONATE,
POTASSIUM SALT (PFOS):
AN ACUTE CONTACT TOXICITY STUDY
WITH THE HONEY BEE





TITLE PAGE

STUDY NUMBER: HT5601
PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS):
AN ACUTE CONTACT TOXICITY STUDY WITH THE HONEY BEE

REPORT

U.S EPA SERIES 850-Ecological Effects Test Guidelines
OPPTS Number 850.3020 (draft)

EPPO GUIDELINE 170

OECD GUIDELINE 214

AUTHOR:

Paul Wilkins

STUDY INITIATION DATE: 11 September 2000

STUDY REPORT DATE: 23 March 2001

SUBMITTED TO:

3M Corporation
Environmental Laboratory
935 Bush Avenue
St Paul, Minnesota 55106
USA

Performing Laboratory:

Environmental R&D Team
Environmental Biology Group
Central Science Laboratory
Sand Hutton
York YO41 1LZ, UK

Report No 1 of 6

**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

Study number: HT5601

Report title: Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. As stated in the protocol no samples of dosing solutions were collected for chemical analysis. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

This study was conducted in accordance with the UK GLP Regulations SI 1999 (No. 3016). These regulations are in accordance with the Organisation for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (1997 ENV/MC/CHEM (98) 17). It is my understanding that the UK and OECD regulations meet the following standard:

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, (Tokyo, 1984).

The test item was not characterized in accordance with full GLP compliance; however, the characterization was performed according to 3M Standard Operating Procedures and Methods, and all raw data are being maintained in the 3M archives. The Sponsor has stated that the test item is being recharacterized in accordance with GLP. No samples of the dosing solutions were collected for chemical analysis.

Signed: _____

A handwritten signature in dark ink, appearing to read 'P. Wilkins'.

Date: 23/03/01 (dd/mm/yy)

Mr P Wilkins
Study Director
Environmental R&D Team, CSL

**QUALITY ASSURANCE STATEMENTS**

Quality assurance inspections of this study were made on the following dates:

Date	Activity	Date reported
08/09/00	Verification of study plan	08/09/00
Range test		
11/09/00	Dispensing (test item and toxic reference)	15/09/00
12/09/00	Preparation of dilutions	15/09/00
	Dosing bees	
	Assessments	
14/09/00	Assessments	15/09/00
Definitive test		
18/09/00	Dosing bees	21/09/00
20/09/00	Assessments	21/09/00

Signed Clare Walker Date 23/03/01 (dd/mm/yy)

Mrs C V Walker
QA Auditor
Central Science Laboratory

This report has been audited by the Quality Assurance Unit of the Central Science Laboratory.

Signed Clare Walker Date 23/03/01 (dd/mm/yy)

Mrs C V Walker
QA Auditor
Central Science Laboratory



REPORT APPROVAL

SPONSOR: 3M Corporation

TITLE: Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee

CSL STUDY NUMBER: HT5601

STUDY DIRECTOR:

P Wilkins

DATE 23/03/01 (dd/mm/yy)

Mr P Wilkins
Environmental R&D Team, CSL

Approval for Report issue

MANAGEMENT:

M H Bew

DATE 23. 3. 01 (dd/mm/yy)

Mr M H Bew
Head Environmental Biology Group, CSL



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SUMMARY

SPONSOR:	3M Corporation Environmental Laboratory 935 Bush Avenue St Paul, Minnesota 55106 USA
SPONSOR'S REPRESENTATIVE:	Ms Rochelle Robideau
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Central Science Laboratory Sand Hutton, York, YO41 1LZ UK

CSL STUDY NUMBER:	HT5601
TEST ITEM:	Perfluorooctanesulfonate, Potassium Salt (PFOS)
STUDY:	Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee
TEST CONCENTRATIONS:	Negative control, Positive control, 45.0, 20.5, 9.30, 4.24 and 1.93 µg a.i./bee
TEST DATES:	Range test: 12-16 September 2000 Definitive test: 18-22 September 2000
LENGTH OF EXPOSURE:	Single dose
LENGTH OF TEST:	96 hours

TEST ORGANISM:	HONEY BEE (<i>Apis mellifera</i> L.)
SOURCE OF TEST ORGANISMS:	National Bee Unit CSL Sand Hutton, York YO41 1LZ
LIFE STAGE OF ORGANISMS:	Adult

CONTACT LD50 (96 hrs):	4.78 µg a.i./bee (95% CL 3.8 – 5.8 µg a.i./bee)
STATISTICALLY DETERMINED CONTACT NO OBSERVED EFFECT LEVEL:	1.93 µg a.i./bee



INTRODUCTION

This study was carried out by the Environmental R&D Team, Central Science Laboratory (CSL), for 3M Corporation at the CSL facility in Sand Hutton, York, UK. The tests were conducted from 12th September to 22nd September 2000. The protocol, protocol amendment, raw data, all notes to file and the final report associated with this study will be retained in the CSL GLP archives for a minimum of 20 years after submission of the final report. The test item will be disposed of by CSL within two months after issue of the final report.

OBJECTIVE

The objective of this study was to evaluate the acute contact toxicity of Perfluorooctanesulfonate, Potassium Salt (PFOS) administered to the honey bee (*Apis mellifera*).

EXPERIMENTAL DESIGN

Contact exposure evaluated cuticular absorption of the test item following the application of a droplet to the body surface. Three batches of bees in groups of 10 bees were topically dosed on the thorax with 1µl drops containing PFOS in acetone.

To determine an approximate toxicity a range-finding test was performed. Four doses of the test item separated by a factor of ten were administered to groups of 30 young adult worker honey bees. Five geometrically spaced doses of the test item were then administered to groups of 30 young adult worker honey bees to define the LD₅₀. At initiation of the test, each bee received a single topical dose of the test item applied to the thorax with a micro-applicator pipette. The bees were then observed for up to 96 hours for toxicological responses and an LD₅₀ value and 95% confidence limits were calculated. All doses of test item are reported as µg active ingredient (a.i.)/bee. At the end of each test any bees remaining alive were killed by freezing and all bees used in the study were disposed of by incineration. All data are retained on the study file.

The doses administered are shown below. Mortality and sub-lethal effects were assessed at 4, 24, 48, 72 and 96 hours after dosing.

Range finding contact test dose levels

Dose group	PFOS (µg a.i./bee)	Dimethoate (µg ai/bee)
1	103	0.30
2	10.3	0.15
3	1.03	0.075
4	0.103	-
Control 1	Acetone	Water containing 1g/l Triton X-100
Control 2	Undosed	N/A



Definitive contact test dose levels

Dose group	PFOS ($\mu\text{g a.i./bee}$)	Dimethoate ($\mu\text{g ai/bee}$)
1	45.0	0.30
2	20.5	0.15
3	9.30	0.075
4	4.24	-
5	1.93	-
Control 1	Acetone	Water containing 1g/l Triton X-100
Control 2	Undosed	N/A

Estimates of LD₅₀ values were calculated for the definitive contact test. The no observed effect levels were determined by a statistical evaluation of the mortality data.

Negative controls, solvent controls (acetone) and positive control groups (dimethoate as BASF 40 formulation nominally containing 400 g/l) were maintained concurrently to check that the bees were reacting normally to the test item during the studies.

MATERIALS AND METHODS

The methods, species used and route of administration described in this report are based upon procedures specified in U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.3020 *Honey Bee Acute Contact Toxicity* (draft); EPPO Guideline 170, *Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees*; and the OECD Test Guideline 214, *Honeybees, Acute Contact Toxicity Test*. In order to control bias, bees were impartially distributed to treatment and control groups. No other potential sources of bias are expected to affect the results of the study. The study was considered valid if: 1) control mortality is 10% or less and 2) the calculated dimethoate LD₅₀ falls within the OECD standard, i.e. 0.10 - 0.30 $\mu\text{g ai/bee}$.

The droplet application method allows for a precise exposure to the test item that simulates a likely route of exposure to bees and other nontarget insects. Exposure is most likely to occur either through absorption or from grooming.

Test item

The PFOS (IUPAC Name 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-potassium salt, CAS Number 2795-39-3, white powder, lot 217) used in this study was supplied by Wildlife International Ltd. on behalf of 3M Corporation. Each sample of the material tested in this study was uniquely labelled with the Test Item number NBU105. The test item was stored, as recommended by the sponsor, at 16-20 °C prior to use.

PFOS was dispersed in acetone (analytical grade) before use due to a low solubility in water (approximately 500 mg/l). A dispersion test was carried out, before the toxicity tests were performed, in which PFOS was dispersed in acetone at the approximate concentration required to deliver the highest doses. The homogeneity of the mixture was assessed after 2 hours. In this report "solution" is



used to include material which may be suspended or dispersed. Homogeneity of such solutions of the PFOS were checked visually during dispersion tests before the day of the test and immediately before use. All solutions were re-mixed prior to use. Solutions of the test doses were homogenous for the purpose of administration. The test item formed a clear solution on mixing at the highest dose, after two hours there was a slight sediment at room temperature at a concentration of 86.0 µg a.i./µl PFOS (the approximate concentration required to prepare the highest diluted dose in the test). The use of acetone negated the need to perform an application test as acetone solutions can be readily applied to bees in contact dosing tests.

Toxic reference

Dimethoate toxic standard NBU90, BASF 40 lot 37M 9020106 (a blue liquid EC formulation, 37.4% w/w ai, 400 g/l nominal concentration), was purchased from UAP York on 14/06/00 and was stored at 0-11°C (on one occasion the maximum temperature recorded was 14°C - this was after the door to the refrigerator had been opened) suitable to maintain stability for 2 years according to information supplied by the manufacturer. Dose and LD₅₀ calculations are for corrected dimethoate concentration based on data supplied by BASF.

Test bees

The honey bee (*Apis mellifera*) is useful in evaluating the potential hazards of agricultural chemicals to nontarget insects since it is an important pollinator of various agricultural crops. There is also a substantial data base on the effect of agrochemicals upon bees with which to categorize potential hazards.

Worker honey bees (*Apis mellifera*) were obtained from colonies belonging to the CSL National Bee Unit. Bees from the colony 32 were used in the range-finding and definitive test. Bees were examined prior to the start of the test and shown to be free of acarine, nosema and amoeba. The colony from which bees were taken had not been treated with a varroacide within the last 4 weeks. Worker bees were collected from the hive by using a small amount of smoke, gently shaking them from the combs and transferring them (40-50 per cage) into cylindrical mesh cages. In the laboratory the mesh cages were placed into the incubator (25 ± 2°C, 65 ± 5% relative humidity) until needed for the test.

Conditions during the tests

Test bees were housed in test chambers which were clean, well ventilated, inverted petri dishes, measuring approximately 9 cm in diameter. A small inverted petri dish (approximately 3 ml) containing the sucrose solution was affixed in each chamber. Each test chamber contained 10 worker bees and were identified by study number, dosage group and replicate. After dosing, test cages were kept in darkness at 25 ± 2°C and 65% ± 5% humidity, except during observations. Temperature and relative humidity within the environmental chamber were recorded continuously during the test.

Dose preparation

Doses of the test item were made by preparing a solution of 103 µg a.i./µl (range-finding test) and 90.4 µg a.i./µl (definitive test) PFOS and making a series of dilutions from these. All dose solutions were made in acetone. One set of control bees was treated with acetone and a second set of control bees was untreated.



Stock solutions of the positive control, dimethoate, were prepared in deionised water containing 1 g/l Triton X-100. Stock solutions of 2.8 µg ai/µl (range-finding test) and 3.0 µg ai/µl (definitive test) dimethoate in deionised water containing 1 g/l Triton X-100 were prepared. Control bees were treated with 1 g/l Triton X-100 in deionised water. The tests were carried out in parallel with those for PFOS.

Dose administration

The bees were anaesthetised with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each bee was dosed on the thorax with a 1 µl drop of a given test item concentration or 1 µl acetone using an Anachem EDP Plus micropipette. The lid was then placed on the cage, the bees allowed to recover and kept in the incubator with a continuous supply of 50% w/v aqueous sucrose solution as food.

Observations

Mortality and any bees knocked down; i.e. alive but immobile, or stumbling were assessed at intervals of 4, 24, 48, 72 and 96 hours after dosing.

Analysis of data

Analysis of the data comprised plotting probit mortality recorded after 24, 48, 72 and 96 hours against the logarithm of dose (CSL Probit 1 package). A least - squares regression (Finney 1971) was fitted to these. Toxicity was expressed as LD₅₀ in µg of active ingredient per bee (a.i.) with the 95% confidence limits, NOEL and slope of the response curve. The NOELs (no observed effect levels) were estimated using Student's t-test (p<0.05).

The LD₅₀ value was used to classify the toxicity of the test item according to the ICBB (1985). The categories used were:

Highly toxic:	less than 1 µg ai/bee
Moderately toxic:	1-10 µg ai/bee
Slightly toxic:	10-100 µg ai/bee
Virtually non-toxic:	greater than 100 µg ai/bee

Data for the dimethoate 24-, 48-, 72- and 96-hour mortality results were analysed using the CSL probit program (Probit 1, version 4).

RESULTS AND DISCUSSION

The results of the tests are summarised in Tables 1 and 2 and detailed results are listed in Appendix 1. Further details are given below. The contact toxicity of the test item resulted in a 96 hr LD₅₀ value for the honeybee of 4.78 µg a.i./bee.



Range test

Mortality significantly greater than seen in the controls was observed at all doses of 10.3 µg a.i./bee and above with a steep dose response between a dose of 10.3 and 103 µg a.i./bee. No dose related sublethal effects were observed at any dose level.

Definitive test

There was significant mortality at all doses above 1.93 µg a.i./bee with a steep dose response between 4.24 and 9.30 µg a.i./bee. The 96 hr LD₅₀ was calculated as 4.78 µg a.i./bee, with 95% confidence limits of 3.8 – 5.8 µg a.i./bee. The NOEL was 1.93 µg a.i./bee at 96 hours (Student's t-test p<0.05). No dose related sublethal effects were observed at any dose level.

The 24, 48, 72 and 96-hour contact LD₅₀ values for dimethoate in the definitive test are shown in Table 2. Mortality of bees exposed to the toxic reference in the tests allowed calculation of a contact LD₅₀ of 0.19 µg ai dimethoate/bee at 24, 48 and 96-hours and 0.20 µg ai dimethoate/bee at 72 hours. These results show a toxicity level within the ranges reported by the OECD guidelines showing the bees were reacting normally in this test.

CONCLUSIONS

Exposure of honeybees to PFOS by contact dosing resulted in a steep dose response curve with significant mortality above 1.93 µg a.i./bee and a 96-hour LD₅₀ calculated as 4.78 µg a.i./bee. The NOEL based on statistical analysis of the data (Student's t test p<0.05) was 1.93 µg a.i./bee at 96 hrs.

These results demonstrate the toxicity of PFOS as 4.78 µg a.i./bee and according to the ICBB (1985) the test item is classified as moderately toxic by contact.

Table 1. Contact LD₅₀ of PFOS

Time Hrs	LD ₅₀ (µg a.i./bee)	95% Confidence Interval for LD ₅₀	Estimate of slope of response line
24	38.9	28.2 – 71.2	2.2
48	10.4	8.2 – 13.0	3.0
72	6.00	4.7 – 7.6	2.9
96	4.78	3.8 – 5.8	4.0

Table 2. Contact toxicity of dimethoate toxic reference

Time Hrs	LD ₅₀ (µg a.i./bee)	95% Confidence Interval for LD ₅₀	Estimate of slope of response line
24	0.19	0.16 – 0.24	4.2
48	0.19	0.16 – 0.24	4.7
72	0.20	0.16 – 0.24	4.8
96	0.19	0.15 – 0.23	5.1



REFERENCES

- European and Mediterranean Plant Protection Organization.** 1992. *Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees.* EPPO Bulletin, 22, 203-215.
- Finney, D. J.** 1971. *Statistical Methods in Biological Assay*, 2nd ed., Griffin Press, London.
- International Commission for Bee Botany (ICBB).** 1985. *Recommendations for harmonization of methods for testing hazard of pesticides to honeybees.* Third Symposium on harmonization of methods for testing hazard of pesticides to bees, England.
- Organization for Economic Cooperation and Development.** 1997. *Guideline 214 Honeybees, Acute Contact Toxicity Test.*
- U.S. Environmental Protection Agency.** 1996. *Honeybee Acute Contact Toxicity.* Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.3020.

PRINCIPAL WORKERS

STUDY MANAGEMENT

Study Director	Mr P Wilkins
CSL Management Representative	Mr M H Bew

OPERATIONAL SUPERVISORS

Principal Scientist	Dr H M Thompson
Scientist & Test Item Controller	Miss U B Pietsch
Scientist & Deputy Test Item Controller	Miss S C Green
Scientist	Mr A H Battersby
Scientist	Dr R Spinks
Scientist	Mr M J Kelly

QUALITY ASSURANCE

QA Manager	Mrs R M Brookes
QA Auditor	Mrs C V Walker



DISTRIBUTION OF REPORT

Sponsor's Representative	Ms R Robideau	2 copies
Sponsor's Monitor	Mr D Palmer	1 copy
Study Director	Mr P Wilkins	1 copy
CSL Management Representative	Mr M H Bew	1 copy
QA Manager	Mrs R M Brookes	1 copy



APPENDIX 1 (TABLES A1-A8)
DETAILED CONTACT TOXICITY TEST RESULTS

Table A1. Results of range-finding contact dosing test with PFOS - Mortality

Actual dose (µg a.i./bee)	Cage No.	Cumulative number dead (n=10)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
103	13	0	6	10	10	10
103	14	0	7	10	10	10
103	15	0	5	10	10	10
10.3	16	0	0	4	5	7
10.3	17	0	0	3	5	9
10.3	18	0	0	1	6	8
1.03	19	0	0	0	0	0
1.03	20	1	1	1	1	1
1.03	21	0	0	0	0	0
0.103	22	0	0	0	0	0
0.103	23	0	0	0	0	0
0.103	24	0	0	0	0	0
0 ¹	25	0	0	0	0	1
0 ¹	26	1	1	1	1	2
0 ¹	27	0	0	0	0	0
0 ²	28	0	0	0	0	0
0 ²	29	0	0	0	0	0
0 ²	30	0	0	0	0	0

¹ dosed with acetone

² undosed



Table A2. Results of range-finding contact dosing test with PFOS – Sub-lethal effects

Actual dose ($\mu\text{g a.i./bee}$)	Cage No.	Number knockdown (K) or stumbling (S)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
103	13	0	2K	0	0	0
103	14	0	0	0	0	0
103	15	0	2S	0	0	0
10.3	16	0	0	0	0	0
10.3	17	0	0	0	1K	0
10.3	18	0	0	0	0	0
1.03	19	0	0	0	0	0
1.03	20	0	0	0	0	0
1.03	21	0	0	0	0	0
0.103	22	0	0	0	0	0
0.103	23	0	0	0	0	0
0.103	24	0	0	0	0	0
0 ¹	25	0	0	0	0	0
0 ¹	26	0	0	0	0	0
0 ¹	27	0	0	0	0	0
0 ²	28	0	0	0	0	0
0 ²	29	0	0	0	0	0
0 ²	30	0	0	0	0	0

¹ dosed with acetone² undosed



Table A3. Results of range-finding contact dosing test with Dimethoate – Mortality

Actual dose (μg a.i./bee)	Cage No.	Cumulative number dead (n=10)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
0.30	1	2	10	10	10	10
0.30	2	0	10	10	10	10
0.30	3	0	9	9	10	10
0.15	4	0	5	6	6	6
0.15	5	0	5	6	6	6
0.15	6	0	3	4	5	6
0.075	7	0	0	0	0	0
0.075	8	0	0	0	0	0
0.075	9	0	0	0	0	0
0 ¹	10	0	0	0	0	0
0 ¹	11	0	0	0	0	0
0 ¹	12	0	0	0	0	0

¹ dosed with Triton X-100

**Table A4. Results of range-finding contact dosing test with Dimethoate – Sub-lethal effects**

Actual dose ($\mu\text{g a.i./bee}$)	Cage No.	Number knockdown (K) or stumbling (S)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
0.30	1	2K	0	0	0	0
0.30	2	4K	0	0	0	0
0.30	3	3K	0	0	0	0
0.15	4	1K	0	0	0	0
0.15	5	0	0	0	0	0
0.15	6	0	0	0	1K	0
0.075	7	0	0	0	0	0
0.075	8	0	0	0	0	0
0.075	9	0	0	0	0	0
0 ¹	10	0	0	0	0	0
0 ¹	11	0	0	0	0	0
0 ¹	12	0	0	0	0	0

¹ dosed with Triton X-100



Table A5. Results of definitive contact dosing test with PFOS - Mortality

Actual dose ($\mu\text{g a.i./bee}$)	Cage No.	Cumulative number dead (n=10)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
45.0	213	0	7	9	10	10
45.0	214	0	3	9	10	10
45.0	215	0	5	10	10	10
20.5	216	0	4	10	10	10
20.5	217	0	3	8	9	10
20.5	218	0	5	10	10	10
9.30	219	0	1	3	5	9
9.30	220	0	1	5	9	9
9.30	221	0	0	4	5	9
4.24	222	0	0	2	4	4
4.24	223	0	0	2	4	4
4.24	224	0	0	0	3	3
1.93	225	0	0	0	0	0
1.93	226	0	1	1	1	1
1.93	227	0	1	1	3	3
0 ¹	228	0	0	0	0	0
0 ¹	229	0	0	0	0	0
0 ¹	230	1	1	1	1	1
0 ²	231	0	0	0	0	0
0 ²	232	0	0	1	1	1
0 ²	233	0	0	0	0	0

¹ dosed with acetone² undosed



Table A6. Results of definitive contact dosing test with PFOS – Sub-lethal effects

Actual dose (µg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
45.0	213	0	1K	1K	0	0
45.0	214	0	0	0	0	0
45.0	215	0	0	0	0	0
20.5	216	0	0	0	0	0
20.5	217	0	0	0	1K	0
20.5	218	0	1K	0	0	0
9.30	219	0	0	0	0	0
9.30	220	0	0	0	0	1K
9.30	221	0	0	0	0	0
4.24	222	0	0	0	0	0
4.24	223	0	0	0	0	0
4.24	224	0	0	0	0	0
1.93	225	0	0	0	0	0
1.93	226	0	0	0	0	0
1.93	227	1K	0	0	0	0
0 ¹	228	0	0	0	0	0
0 ¹	229	0	0	0	0	0
0 ¹	230	0	0	0	0	0
0 ²	231	0	0	0	0	0
0 ²	232	0	0	0	0	0
0 ²	233	0	0	0	0	0

¹ dosed with acetone² undosed



Table A7. Results of definitive contact dosing test with Dimethoate - Mortality

Actual dose (μg a.i./bee)	Cage No.	Cumulative number dead (n=10)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
0.30	201	2	8	8	8	8
0.30	202	3	6	6	6	7
0.30	203	1	9	10	10	10
0.15	204	1	3	3	3	3
0.15	205	0	5	5	5	5
0.15	206	1	4	4	4	4
0.075	207	0	0	0	0	0
0.075	208	0	1	1	1	1
0.075	209	0	0	0	0	0
0 ¹	210	0	1	2	2	2
0 ¹	211	0	0	0	0	0
0 ¹	212	0	0	0	1	1

¹ dosed with Triton X-100



Table A8. Results of definitive contact dosing test with Dimethoate – Sub-lethal effects

Actual dose (μg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)				
		4 hrs	24 hrs	48 hrs	72 hrs	96 hrs
0.30	201	0	0	0	0	0
0.30	202	0	1K	0	0	0
0.30	203	1K	0	0	0	0
0.15	204	0	0	0	0	0
0.15	205	0	0	0	0	0
0.15	206	0	0	0	0	0
0.075	207	0	0	0	0	0
0.075	208	0	0	0	0	0
0.075	209	0	0	0	0	0
0 ¹	210	1K	0	0	0	0
0 ¹	211	0	0	0	0	0
0 ¹	212	0	0	0	0	0

¹ dosed with Triton X-100



APPENDIX 2
INTERIM CERTIFICATE OF ANALYSIS FOR TEST ITEM

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity ¹		86.9%
Appearance	White Crystalline Powder	Conforms
Identification		
NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.005 wt./wt. %
2. Magnesium		2. 0.001 wt./wt. %
3. Sodium		3. 1.439 wt./wt. %
4. Potassium ²		4. 6.849 wt./wt. %
5. Nickel		5. <0.001 wt./wt. %
6. Iron		6. 0.005 wt./wt. %
7. Manganese		7. <0.001 wt./wt. %
Total % Impurity (NMR)		1.93 wt./wt. %
Total % Impurity (LC/MS)		8.41 wt./wt. %
Total % Impurity (GC/MS)		None Detected
Related Compounds - POAA		0.33 wt./wt. %
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable ³
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt. %
2. Fluoride		2. 0.59 wt./wt. %
3. Bromide		3. <0.040 wt./wt. %
4. Nitrate		4. <0.009 wt./wt. %
5. Nitrite		5. <0.006 wt./wt. %
6. Phosphate		6. <0.007 wt./wt. %
7. Sulfate ⁴		7. 8.76 wt./wt. %
Organic Acids ⁵ (IC)		
1. TFA		1. <0.1 wt./wt. %
2. PFPA		2. <0.1 wt./wt. %
3. HFBA		3. 0.10 wt./wt. %
4. NFPA		4. 0.28 wt./wt. %
Elemental Analysis ⁶ :		
1. Carbon	1. Theoretical Value = 17.8%	1. 12.48 wt./wt. %
2. Hydrogen	2. Theoretical Value = 0%	2. 0.244 wt./wt. %
3. Nitrogen	3. Theoretical Value = 0%	3. 1.74 wt./wt. %
4. Sulfur	4. Theoretical Value = 5.95%	4. 8.84 wt./wt. %
5. Fluorine	5. Theoretical Value = 60%	5. 54.1 wt./wt. %

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12/03/01



INTERIM CERTIFICATE OF ANALYSIS
Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen $\leq -10^{\circ}\text{C}$

Re-assessment Date: 08/31/01

¹Purity = 100% - (sum of metal impurities, 1.45% + LC/MS impurities, 8.41% + Inorganic Fluoride, 0.59% + NMR impurities, 1.93% + organic acid impurities, 0.38% + POAA, 0.33%)

Total impurity from all tests = 13.09%

Purity = 100% - 13.09% = 86.9%

²Potassium is expected in this salt form and is therefore not considered an impurity.

³Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

⁴Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

⁵ TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, $\text{C}_8\text{F}_{17}\text{SO}_3\text{K}^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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INTERIM CERTIFICATE OF ANALYSIS
Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	8.41

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____
David S. Bell
Scientist, Centre Analytical Laboratories

Date

Reviewed By: _____
John Flaherty
Laboratory Manager, Centre Analytical Laboratories

Date

COA023-018A



Study title	Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee
Test facility	Environmental R&D Team, Environmental Biology Group Central Science Laboratory, Sand Hutton, York, YO41 1LZ
Study number	HT5601
Amendment number:	1
Number of pages:	2

**Study Director
Paul Wilkins**

Signature M. K. Singh Date 14/03/01 (dd/mm/yy)

Management Representative
Medwin Bew

Signature M H Baw Date 14.3.07 (dd/mm/yy)



CENTRAL SCIENCE LABORATORY PROTOCOL AMENDMENT

Amendment No 1 to Protocol HT5601

After clarification by the Sponsor, it was requested that the Test Item name be changed from Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS) to Perfluorooctanesulfonate, Potassium Salt (PFOS) and consequently the study title changed from:

Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee

to

Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute contact toxicity study with the honey bee.

This has no impact on the Study

Circulation list

Sponsor's Representative
Sponsor's Monitor
Study Director
CSL Management Representative
CSL, QA Unit

Ms R Robideau, 3M
Mr D Palmer, Wildlife International
Mr P Wilkins, CSL Environmental R&D
Mr M Bew, CSL
Mrs R Brookes, CSL

Environmental Laboratory Project Number U2723