AR226_1017

CENTRAL SCIENCE LABORATORY

SCIENCE SERVING AGRICULTURE, FOOD AND THE ENVIRONMENT

CONTRACT REPORT

STUDY NUMBER HT5602

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

MAFF

TITLE PAGE

STUDY NUMBER: HT5602
PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS):
AN ACUTE ORAL TOXICITY STUDY WITH THE HONEY BEE

REPORT AMENDMENT NO 1

EPPO GUIDELINE 170

OECD GUIDELINE 213

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

Environmental Laboratory Project Number U2723

CENTRAL SCIENCE LABORATORY

CONFIDENTIAL

REPORT AMENDMENT 1

Study title	Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute oral toxicity study with the honey bee
Test facility	Environmental R&D Team, CSL, Sand Hutton, York, UK
Study number	HT5602
Number of pages:	5

Signature P Wilkins Date 05/04/01 (dd/mm/yy)

Study Director
P Wilkins
Environmental R&D Team, CSL

Authorisation of release of report amendment

Signature M H Bew Date 5.4.01 (dd/mm/yy)

Management Representative
M H Bew
Head Environmental Biology Group, CSL

This amendment has been noted by Quality Assurance as correction to the report

Signature Clare Walker Date 05/04/01 (dd/mm/yy)

Mrs C V Walker
QA Auditor
Central Science Laboratory

Environmental Laboratory Project Number U2723

Reason for amendment: There was a typographical error on pages 7 and 8 of the final report where the Test Item had been mis-spelt as Perfuorooctanesulfonate, Potassium Salt (PFOS). This has been corrected to Perfluorooctanesulfonate, Potassium Salt (PFOS). A formatting error had occurred on page 7, which had obscured the length of exposure. This has been corrected.

Distribution

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

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:	HT5602
TEST ITEM:	Perfluorooctanesulfonate, Potassium Salt (PFOS)
STUDY:	Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute oral toxicity study with the honey bee
NOMINAL TEST CONCENTRATIONS:	Negative control, Positive control, 4.78, 2.17, 0.991, 0.450 and 0.205 µg a.i./bee
TEST DATES:	Range test: 12-15 September 2000 Definitive test: 21-24 September 2000
LENGTH OF TEST:	72 hours
LENGTH OF EXPOSURE:	4 hours

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

ORAL LD_{50} (72 hrs):	0.40 µg a.i./bee (95% CL 0.33 – 0.48 µg a.i./bee)
STATISTICALLY DETERMINED ORAL NO OBSERVED EFFECT LEVEL (BASED ON MEAN INTAKE):	0.21 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 24th September 2000. The protocol, protocol amendments, 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 oral toxicity of Perfluorooctanesulfonate, Potassium Salt (PFOS) administered to the honey bee (*Apis mellifera*).

EXPERIMENTAL DESIGN

The oral ingestion method with administration of the test item in aqueous sucrose solution allows for precise exposure over the 4-hour dosing period. This reflects one main potential route of exposure for bees and other non-target insects: oral intake of contaminated food (pollen, nectar etc.). At the initiation of the test three batches of bees in groups of 10 bees at each dose level received a single dose of the test item dissolved in sucrose. This was removed after 4 hours and replaced with untreated sucrose.

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 LD_{50}. The toxicity of the test item could not be accurately determined in that trial due to high levels of mortality. Therefore another test (second main test) was carried out with using a lower dose of test item (issued as a protocol amendment). Only the results of the range test and second main test (hereafter referred to as the definitive toxicity test) are reported here. All data are retained on the study file. At initiation of the test, each group of 10 bees was offered 200 µl of the test item in 50% w/v sucrose. After 4 hrs the test feed was removed and the dose taken by each group of bees calculated. Mortality and sub-lethal effects were assessed at 4 hrs then a further 24 and 48 hrs after removal of feeders. In the range test and definitive test an additional assessment was made at a further 72 hrs after removal of the test feeders. All doses of test item are reported as µg a.i. (active ingredient)/bee and have been adjusted for analysed content. At the end of the tests any remaining live bees were killed by freezing and incinerated.

CENTRAL SCIENCE LABORATORY
AMENDMENT TO PROTOCOL

Study title	Perfluorooctane Sulfonic Acid, Potassium Salt (PFOS): An acute oral 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	HT5602
Amendment number:	2
Number of pages:	2

Study Director
Paul Wilkins

Signature _____ **Date** 14/03/01 **(dd/mm/yy)**

Management Representative
Medwin Bew

Signature _____ **Date** 14.3.01 **(dd/mm/yy)**

Environmental Laboratory Project Number U2723

000008

CENTRAL SCIENCE LABORATORY
PROTOCOL AMENDMENT

Amendment No 2 to Protocol HT5602

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 oral toxicity study with the honey bee

to

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

This has no impact on the Study

Circulation list

Sponsor's Representative	Ms R Robideau, 3M
Sponsor's Monitor	Mr D Palmer, Wildlife International
Study Director	Mr P Wilkins, CSL Environmental R&D
CSL Management Representative	Mr M Bew, CSL
CSL, QA Unit	Mrs R Brookes, CSL

Environmental Laboratory Project Number U2723

000009

Wildlife International, Ltd.
ECOTOXICOLOGY & ANALYTICAL TESTING SERVICES

ANALYSIS FOR PFOS IN AQUEOUS AND SUCROSE SOLUTIONS USED BY CENTRAL SCIENCE LABORATORIES FOR CONDUCTING HONEY BEE TESTING

Enclosed please find a data summary for our screen for PFOS in aqueous and sucrose solutions employed by Central Science Laboratory in the honeybee studies (Sample IDs HT5601/02 Water and HT5601/02 Sucrose). Included are the results table, calibration curve, and standard/sample ion chromatograms for the screens performed on our triple quadrupole LC/MS/MS system. As can be seen in the data, the samples were less than the limit of quantitation, 0.0500 mg a.i./L (0.000500 mg a.i./L low standard x sample dilution factor (100)). The screen was conducted on October 26, 2000. These results apply to solutions representative of those used in the following studies.

PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): An Acute Contact Toxicity Study with the Honey Bee (CSL Study Number: HT5601)

PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS): An Acute Oral Toxicity Study with the Honey Bee (CSL Study Number: HT5602)

Raymond J. Van Hoven, Ph.D.
Scientist

4/18/01
DATE

MANAGEMENT:

Willard B. Nixon, Ph.D.
Director, Analytical Chemistry

4/18/01
DATE

8598 Commerce Drive • Easton, Maryland 21601 • Tel. 410-822-8600 • Fax 410-822-0632 • E-mail: ecotox@wildlifeinternational.com
European Office: Bergkampweg 1 • 7231 CL Warnsveld • The Netherlands • Tel. 31(0) 575-573848 • Fax 31(0)575-574813 • E-mail: aleopold@worldonline.nl

MacQuan, version 1.6
Printed: Thu, Oct 26, 2000 12:58
Calibration File: Cal PFOS 102600 MD Path: Macintosh HD:API3000:PROJECT DATA:454C-101:454C101_102600 MD:
Comments: PFOS: 454C-101: Method Development - Screening fish flake, water, sucrose -(LC/MS/MS-MRM)

PFOS
No Internal Standard
499.0->99.1
Weighted (1/x)
Intercept = 323.17
Slope = 8193.32
Correlation Coeff. = 0.99949
Use Area

Filename	Filetype	Sample Name	Conc.	Dil. Factor	Calc. Conc.	Units	Accuracy	Area	Height	Sample Desc.
PFOS_1	Standard	STD 0.500 ug a.i./L	0.5000	1.0	0.4770	ug a.i./L	95.4	4231.52	393.90	4675A-011D-6
PFOS_2	Standard	STD 1.00 ug a.i./L	1.0000	1.0	0.9884	ug a.i./L	98.8	8421.33	746.57	4675A-011D-7
PFOS_3	Standard	STD 2.50 ug a.i./L	2.5000	1.0	2.5376	ug a.i./L	101.5	21116.08	1925.75	4675A-011D-8
PFOS_4	Standard	STD 3.50 ug a.i./L	3.5000	1.0	3.6246	ug a.i./L	103.6	30020.62	2739.86	4675A-011D-9
PFOS_5	Standard	STD 5.00 ug a.i./L	5.0000	1.0	5.0132	ug a.i./L	100.3	41397.56	3819.74	4675A-011D-10
PFOS_6	Blank	MEOH	0.0	1.0	n / a	ug a.i./L	n / a	n / a	n / a	MEOH
PFOS_7	Blank	DIL SOLVENT	0.0	1.0	n / a	ug a.i./L	n / a	n / a	n / a	DIL SOLVENT
PFOS_8	Blank	FISH FLAKE	0.0	100.0	n / a	ug a.i./g	n / a	n / a	n / a	454C-101-
PFOS_9	Blank	WATER	0.0	100.0	n / a	ug a.i./L	n / a	n / a	n / a	454C-101-
PFOS_10	Blank	SUCROSE	0.0	100.0	0.8041	ug a.i./L	n / a	389.06	41.96	454C-101-
PFOS_11	Standard	STD 0.500 ug a.i./L	0.5000	1.0	0.5087	ug a.i./L	101.7	4491.33	367.09	4675A-011D-6
PFOS_12	Standard	STD 1.00 ug a.i./L	1.0000	1.0	1.0267	ug a.i./L	102.7	8735.45	826.57	4675A-011D-7
PFOS_13	Standard	STD 2.50 ug a.i./L	2.5000	1.0	2.4725	ug a.i./L	98.9	20581.27	1836.54	4675A-011D-8
PFOS_14	Standard	STD 3.50 ug a.i./L	3.5000	1.0	3.5098	ug a.i./L	100.3	29079.89	2538.52	4675A-011D-9
PFOS_15	Standard	STD 5.00 ug a.i./L	5.0000	1.0	4.8413	ug a.i./L	96.8	39989.71	3515.79	4675A-011D-10

000011

MacQuan, version 1.6
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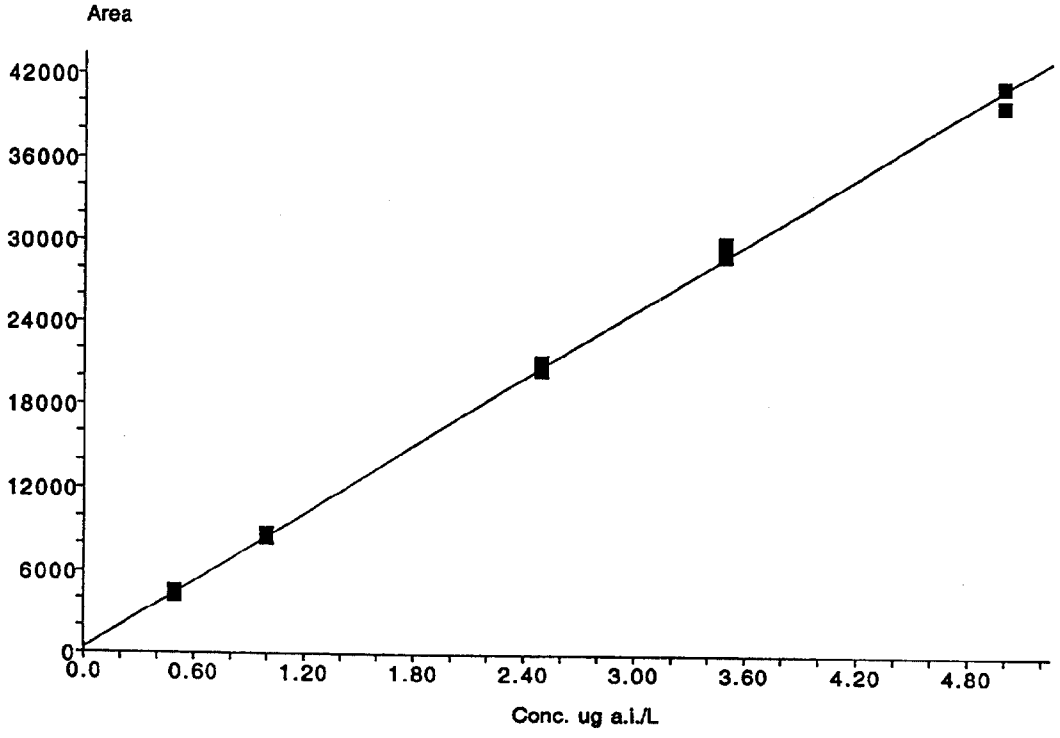
Calibration File: Cal PFOS 102600 MD Path: Macintosh HD:API3000:PROJECT DATA:454C-101:454C101_102600 MD:
Comments: PFOS: 454C-101: Method Development - Screening fish flake, water, sucrose -(LC/MS/MS-MRM)

JMM 10-26-00

PFOS 499.0->99.1 No Internal Standard
Weighted (1/x)
Intercept = 323.17
Slope = 8193.32
Correlation Coeff. = 0.99949



CERTIFIED COPY
OF THE ORIGINAL
INITIALS
10-30-00
DATE

000012

MacQuan, version 1.6
Printed: Thu, Oct 26, 2000 12:59
Calibration File: Cal PFOS 102600 MD Path: Macintosh HD:API3000:PROJECT DATA:454C-101:454C101_102600 MD:
Comments: PFOS: 454C-101: Method Development - Screening fish flake, water, sucrose -(LC/MS/MS-MRM)

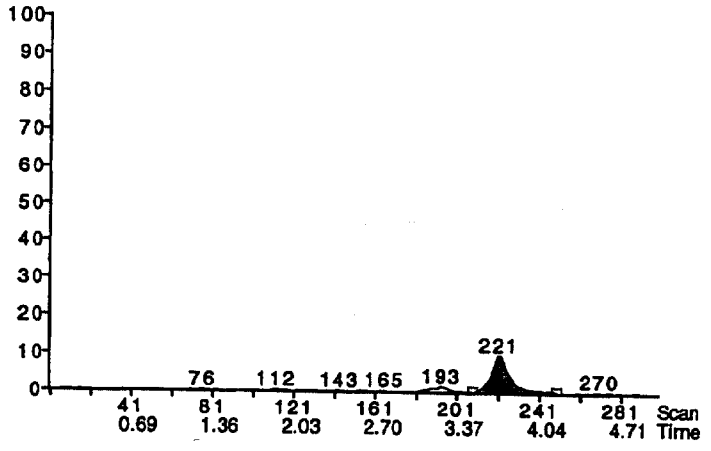
Jam 10-30-00

PFOS_1 STD 0.500 ug a.i./L Thu, Oct 26, 2000 10:34
4675A-011D-6

4.98 in 1 period
PFOS
No Internal Standard
Use Area

1: 4.97 MRM, 298 scans
499.0->99.1

Noise Thres.	2.0
Quant Thres.	1.0
Min. Width	12
Mult. Width	10
Base. Width	40
RT Win. (secs)	10
Smooth	2
Expected RT	3.70
Area	4231.52
Height	393.90
Start Time	3.50
End Time	4.17
Integration Width	0.67
Retention Time	3.70
Integration Type	A - BB

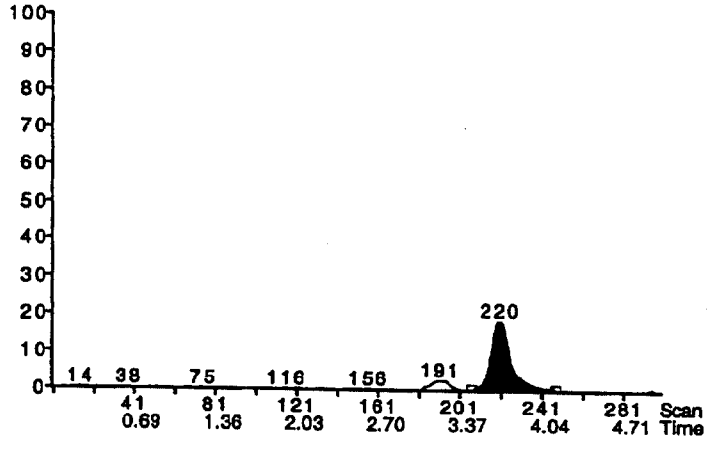


PFOS_2 STD 1.00 ug a.i./L Thu, Oct 26, 2000 10:40
4675A-011D-7

4.98 in 1 period
PFOS
No Internal Standard
Use Area

1: 4.97 MRM, 298 scans
499.0->99.1

Noise Thres.	2.0
Quant Thres.	1.0
Min. Width	12
Mult. Width	10
Base. Width	40
RT Win. (secs)	10
Smooth	2
Expected RT	3.70
Area	8421.33
Height	746.57
Start Time	3.47
End Time	4.14
Integration Width	0.67
Retention Time	3.69
Integration Type	A - BB



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MacQuan, version 1.6
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Comments: PFOS: 454C-101: Method Development - Screening fish flake, water, sucrose -(LC/MS/MS-MRM)

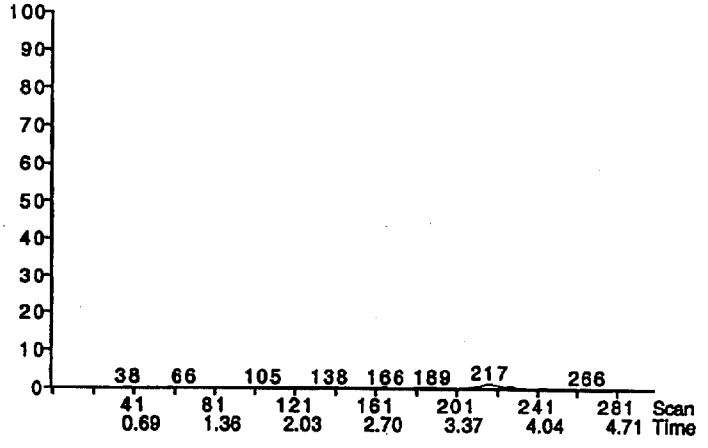
PFOS_9 **WATER** Thu, Oct 26, 2000 11:22
454C-101-

4.98 in 1 period
PFOS
No Internal Standard
Use Area

Intensity: 4000 cps

1: 4.97 MRM, 298 scans
499.0->99.1

Noise Thres.	2.0
Quant Thres.	1.0
Min. Width	12
Mult. Width	10
Base. Width	40
RT Win. (secs)	10
Smooth	2
Expected RT	3.70
Area	0.0
Height	0.0
Start Time	0.00
End Time	0.00
Integration Width	0.00
Retention Time	0.00
Integration Type	



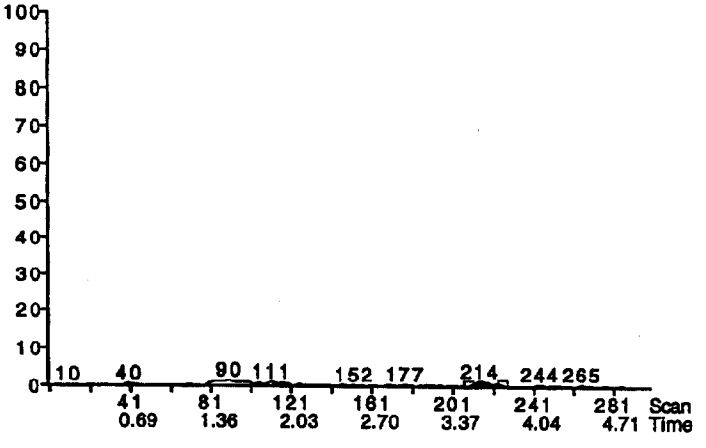
PFOS_10 **SUCROSE** Thu, Oct 26, 2000 11:28
454C-101-

4.98 in 1 period
PFOS
No Internal Standard
Use Area

Intensity: 4000 cps

1: 4.97 MRM, 298 scans
499.0->99.1

Noise Thres.	2.0
Quant Thres.	1.5
Min. Width	12
Mult. Width	10
Base. Width	40
RT Win. (secs)	10
Smooth	2
Expected RT	3.70
Area	389.06
Height	41.96
Start Time	3.50
End Time	3.77
Integration Width	0.27
Retention Time	3.59
Integration Type	A* - BB



000014

TITLE PAGE

STUDY NUMBER: HT5602
PERFLUOROOCTANESULFONATE, POTASSIUM SALT (PFOS):
AN ACUTE ORAL TOXICITY STUDY WITH THE HONEY BEE

REPORT

EPPO GUIDELINE 170

OECD GUIDELINE 213

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

Environmental Laboratory Project Number U2723

000015

MET 0438377

2734.0014

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study number: HT5602

Report title: Perfluorooctanesulfonate, Potassium Salt (PFOS): An acute oral 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: P Wilkins Date: 23/03/01 (dd/mm/yy)

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

Environmental Laboratory Project Number U2723

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
Main test		
18/09/00	Dosing bees	21/09/00
20/09/00	Assessments	21/09/00
Definitive test		
20/09/00	Dispensing (test item and toxic reference)	25/09/00
21/09/00	Preparation of dilutions	25/09/00
	Dosing bees	
	Assessments	
	Weighing feeders	
22/09/00	Assessments	25/09/00

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 oral toxicity study with the honey bee

CSL STUDY NUMBER: HT5602

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

TABLE OF CONTENTS

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:	HT5602
TEST ITEM:	Perfuorooctanesulfonate, Potassium Salt (PFOS)
STUDY:	Perfuorooctanesulfonate, Potassium Salt (PFOS): An acute oral toxicity study with the honey bee
NOMINAL TEST CONCENTRATIONS:	Negative control, Positive control, 4.78, 2.17, 0.991, 0.450 and 0.205 µg a.i./bee
TEST DATES:	Range test: 12-15 September 2000 Definitive test: 21-24 September 2000
LENGTH OF TEST:	72 hours
LENGTH OF EXPOSURE	4 h

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

ORAL LD_{50} (72 hrs):	0.40 µg a.i./bee (95% CL 0.33 – 0.48 µg a.i./bee)
STATISTICALLY DETERMINED ORAL NO OBSERVED EFFECT LEVEL (BASED ON MEAN INTAKE):	0.21 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 24th September 2000. The protocol, protocol amendments, 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 oral toxicity of Perfuorooctanesulfonate, Potassium Salt (PFOS) administered to the honey bee (*Apis mellifera*).

EXPERIMENTAL DESIGN

The oral ingestion method with administration of the test item in aqueous sucrose solution allows for precise exposure over the 4-hour dosing period. This reflects one main potential route of exposure for bees and other non-target insects: oral intake of contaminated food (pollen, nectar etc.). At the initiation of the test three batches of bees in groups of 10 bees at each dose level received a single dose of the test item dissolved in sucrose. This was removed after 4 hours and replaced with untreated sucrose.

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 LD_{50}. The toxicity of the test item could not be accurately determined in that trial due to high levels of mortality. Therefore another test (second main test) was carried out with using a lower dose of test item (issued as a protocol amendment). Only the results of the range test and second main test (hereafter referred to as the definitive toxicity test) are reported here. All data are retained on the study file. At initiation of the test, each group of 10 bees was offered 200 µl of the test item in 50% w/v sucrose. After 4 hrs the test feed was removed and the dose taken by each group of bees calculated. Mortality and sub-lethal effects were assessed at 4 hrs then a further 24 and 48 hrs after removal of feeders. In the range test and definitive test an additional assessment was made at a further 72 hrs after removal of the test feeders. All doses of test item are reported as µg a.i. (active ingredient)/bee and have been adjusted for analysed content. At the end of the tests any remaining live bees were killed by freezing and incinerated.

The nominal doses administered are shown below.

Range finding oral test dose levels

Dose group	PFOS (µg a.i./bee)	Dimethoate (µg ai/bee)
1	103	0.60
2	10.3	0.30
3	1.03	0.15
4	0.103	0.075
Control 1	50% w/v sucrose containing 5% acetone	50% w/v sucrose containing Triton X-100
Control 2	50% w/v sucrose	N/A

Definitive test dose levels

Dose group	PFOS (µg a.i./bee)	Dimethoate (µg ai/bee)
1	4.78	0.60
2	2.17	0.30
3	0.991	0.15
4	0.450	0.075
5	0.205	-
Control 1	50% w/v sucrose containing 5% acetone	50% w/v sucrose containing Triton X-100
Control 2	50% w/v sucrose	N/A

Estimates of LD_{50} values were calculated for the main oral test. The no observed effect levels were determined by a statistical evaluation of the mortality data.

Negative controls, solvent controls (5% 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 protocol are based upon procedures specified in EPPO Guideline 170, *Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees*; and the OECD Test Guideline 213, *Honeybees, Acute Oral 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 with: a) control mortality of 10% or less; and b) the calculated dimethoate LD_{50} fell within the OECD standard, i.e. 0.10 - 0.35 µg ai/bee.

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-heptadecafluoro-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 µg a.i./µl PFOS (the approximate concentration required to prepare the highest diluted dose in the test).

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 LD_{50} calculations are for corrected dimethoate concentration based on data supplied by BASF.

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 throughout the tests. Bees were examined prior to the start of the test and shown to be free of acarine, nosema and amoeba. The colony from which the 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) to starve the bees for 1.5 to 2 hrs before the test.

Conditions during the tests

Test bees were housed in test chambers which are clean, well ventilated, inverted petri dishes, measuring approximately 9 cm in diameter. A small inverted petri dish (approximately 3 ml) to contain the sucrose solution was affixed in each chamber. Each test chamber contained 10 worker bees and was 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 47.8 µg a.i./µl (definitive test) PFOS and making a series of dilutions from this. Doses were prepared in acetone and then further diluted in 50% w/v sucrose for dosing. In the range test and definitive test 0.5 ml of each acetone solution was then diluted to 10 ml with 50% w/v sucrose giving a final acetone concentration of 5%. Although this concentration of acetone was greater than recommended in the OECD guidelines (1%) it was required to ensure the highest concentrations remained in solution for the oral test doses. One set of control bees was treated with the same level of acetone in 50% w/v sucrose (0.5 ml acetone in 10 ml sucrose) and a second set of control bees was treated with 50% w/v sucrose.

Stock solutions of the positive control, dimethoate, were prepared in deionised water containing 1 g/l Triton X-100. Stock solutions of 2.80 µg ai/µl (range-finding test) and 3.00 µg ai/µl (definitive test) dimethoate in deionised water containing 1 g/l Triton X-100 were prepared and a series of dilutions made in 1 g/l Triton X-100. This series of dilutions (0.5 ml) were then further diluted to 10 ml with 50% w/v sucrose. Control bees were dosed with 0.5 ml 1 g/l Triton X-100 in deionised water diluted to 10 ml with 50% w/v sucrose. The tests were carried out in parallel with those for PFOS.

Samples of the sucrose solution used in the study were analysed by Wildlife International Ltd. and no PFOS background levels were found.

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 group of 10 bees was offered 0.2 ml of a given concentration (or controls as above), the dose being measured into a small, pre-weighed, glass feeder within the cage using a variable volume pipette. This is equivalent to 20 µl/bee. At each dose for the test item and dimethoate and for each control there were 3 replicate cages of 10 bees. Doses were administered within 2 hours of preparation.

After 4 hours the glass feeders were removed and weighed and the sucrose feeders filled with approximately 3 ml 50% w/v aqueous sucrose so that bees had continuous access to sucrose for the remainder of the study. The dose consumed was determined by comparison of the weight of the dose remaining in the glass feeders with the weight of a known volume of the test solutions.

Observations

Mortality and any bees knocked down, i.e. alive but immobile, or stumbling were assessed when the feeders were removed and 24, 48 and 72 hours after removal of the test feeders (i.e. 4, 28, 52 and 76 hours after the start of the test).

Analysis of data

Analysis of the data comprised plotting probit mortality recorded after 24, 48 and 72 hours against the logarithm of dose (CSL Probit 1 package). A least - squares regression (Finney 1971) was fitted to these. Toxicity was expressed as LD_{50} in µg a.i. per bee 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 LD_{50} was used to classify the toxicity of the test item according to the ICBB (1985). The categories used were:

Highly toxic:	less than 1µg 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- and 72-hour mortality results were analysed using the CSL probit program (Probit 1, version 4).

RESULTS AND DISCUSSION

The results of the oral tests are summarised in Tables 1 and 2 and detailed results are listed in Appendix 1. Further details are given below. The oral toxicity of the test item resulted in a 72 hr LD_{50} value for the honeybee of 0.40 µg a.i./bee.

Range test

Mortality was observed at all doses from mean intake 1.0 µg a.i./bee with a steep dose response between a mean intake of 1.0 and 10 µg a.i./bee. Sublethal effects were observed as knockdown and stumbling at 4 hrs at the two highest dose levels with these individuals dying by 24 hrs.

Definitive test

There was significant mortality at all doses above a mean intake of 0.21 µg a.i./bee with a steep dose response between a mean intake of 0.45 and 2.2 µg a.i./bee. The 72 hr LD_{50} was calculated as 0.40 µg a.i./bee (95% confidence limits 0.33 – 0.48 µg a.i./bee). The NOEL was 0. 21 µg a.i./bee at 72 hours based on mean intake data (Student's t-test $p<0.05$). Sublethal effects were seen observed as knockdown at the highest dose level at 4 hrs with these individuals dying after 24 hours.

The 24, 48 and 72 -hour contact LD_{50} 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 an oral LD_{50} of 0.12 µg ai dimethoate/bee at 24-hours and 0.11 µg ai dimethoate/bee at 48 and 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 oral dosing resulted in a steep dose response curve with significant mortality above a mean intake of 0.21 µg a.i./bee and a 72 hr LD_{50} calculated as 0.40 µg a.i./bee. The NOEL based on statistical analysis of the data (Student's t test $p<0.05$) was 0.21 µg a.i./bee at 72 hrs.

These results demonstrate the oral toxicity of PFOS as 0.40 µg a.i./bee and according to the ICBB (1985) is classified as highly toxic by ingestion.

Table 1. Oral LD_{50} of PFOS from definitive test data

Time Hrs	LD_{50} (µg a.i./bee)	95% Confidence Interval for LD_{50}	Estimate of slope of response line
24	0.72	0.60 - 0.85	5.0
48	0.46	0.32 - 0.55	4.7
72	0.40	0.33 – 0.48	4.8

Table 2. Oral toxicity of dimethoate toxic reference from definitive test data

Time Hrs	LD_{50} (µg a.i./bee)	95% Confidence Interval for LD_{50}	Estimate of slope of response line
24	0.12	0.10 – 0.14	6.4
48	0.11	0.096 – 0.13	7.3
72	0.11	0.096 – 0.13	8.1

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.

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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 213 Honeybees, *Acute Oral Toxicity Test.*

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Environmental Laboratory Project Number U2723

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

Table A1. Results of range-finding oral dosing tests with PFOS - Mortality

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Cumulative number dead (n=10)			
			4 hrs	24 hrs	48 hrs	72 hrs
103	96	46	5	10	10	10
103	94	47	5	10	10	10
103	101	48	7	10	10	10
10.3	10	49	0	10	10	10
10.3	10	50	0	10	10	10
10.3	10	51	0	10	10	10
1.03	1.0	52	0	0	2	3
1.03	1.0	53	0	1	1	2
1.03	1.0	54	0	0	0	4
0.103	0.10	55	0	0	0	0
0.103	0.10	56	0	0	0	0
0.103	0.10	57	0	0	0	0
0[3]	0	58	0	0	0	0
0[3]	0	59	0	0	0	0
0[3]	0	60	0	0	0	0
0[4]	0	61	0	0	0	0
0[4]	0	62	0	0	0	0
0[4]	0	63	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)
[3] acetone dosed
[4] undosed

Table A2. Results of range-finding oral dosing tests with PFOS – Sub-lethal effects

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)			
			4 hrs	24 hrs	48 hrs	72 hrs
103	96	46	4K	0	0	0
103	94	47	1S,4K	0	0	0
103	101	48	1S,2K	0	0	0
10.3	10	49	2K	0	0	0
10.3	10	50	1S,3K	0	0	0
10.3	10	51	2K	0	0	0
1.03	1.0	52	0	0	0	1K
1.03	1.0	53	0	0	0	0
1.03	1.0	54	0	0	1K	0
0.103	0.10	55	0	0	0	0
0.103	0.10	56	0	0	0	0
0.103	0.10	57	0	0	0	0
0[3]	0	58	0	0	0	0
0[3]	0	59	0	0	0	0
0[3]	0	60	0	0	0	0
0[4]	0	61	0	0	0	0
0[4]	0	62	0	0	0	0
0[4]	0	63	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)
[3] acetone dosed
[4] undosed

Table A3. Results of range-finding oral dosing tests with dimethoate - Mortality

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Cumulative number dead (n=10)			
			4 hrs	24 hrs	48 hrs	72 hrs
0.60	0.56	31	3	10	10	10
0.60	0.54	32	0	10	10	10
0.60	0.60	33	0	10	10	10
0.30	0.25	34	2	10	10	10
0.30	0.30	35	0	10	10	10
0.30	0.30	36	1	10	10	10
0.15	0.15	37	0	8	8	8
0.15	0.15	38	0	4	5	5
0.15	0.15	39	0	5	7	7
0.075	0.075	40	0	0	0	0
0.075	0.074	41	0	0	0	0
0.075	0.074	42	0	1	1	2
0	0	43	0	0	0	0
0	0	44	0	0	0	0
0	0	45	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)

Table A4. Results of range-finding oral dosing tests with dimethoate – Sub-lethal effects

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)			
			4 hrs	24 hrs	48 hrs	72 hrs
0.60	0.56	31	2K	0	0	0
0.60	0.54	32	2K	0	0	0
0.60	0.60	33	7K	0	0	0
0.30	0.25	34	1K	0	0	0
0.30	0.30	35	0	0	0	0
0.30	0.30	36	0	0	0	0
0.15	0.15	37	0	0	0	0
0.15	0.15	38	0	0	0	0
0.15	0.15	39	0	0	0	0
0.075	0.075	40	0	0	0	0
0.075	0.074	41	0	0	0	0
0.075	0.074	42	0	0	0	0
0	0	43	0	0	0	0
0	0	44	0	0	0	0
0	0	45	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)

Table A5. Results of definitive oral dosing tests with PFOS - Mortality

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Cumulative number dead (n=10)			
			4 hrs	24 hrs	48 hrs	72 hrs
4.78	4.8	316	3	10	10	10
4.78	4.8	317	3	10	10	10
4.78	4.8	318	3	10	10	10
2.17	2.2	319	1	10	10	10
2.17	2.2	320	0	10	10	10
2.17	2.2	321	1	10	10	10
0.991	0.96	322	0	7	10	10
0.991	0.96	323	0	8	9	10
0.991	0.96	324	0	6	9	9
0.450	0.45	325	0	1	4	5
0.450	0.45	326	0	3	7	7
0.450	0.45	327	0	2	4	6
0.205	0.21	328	0	0	0	1
0.205	0.21	329	0	0	0	0
0.205	0.21	330	0	0	2	2
0[3]	0	331	0	1	1	1
0[3]	0	332	0	0	0	0
0[3]	0	333	0	0	0	0
0[4]	0	334	0	0	0	0
0[4]	0	335	0	0	0	0
0[4]	0	336	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)
[3] acetone dosed
[4] undosed

Table A6. Results of definitive oral dosing tests with PFOS – Sub-lethal effects

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)			
			4 hrs	24 hrs	48 hrs	72 hrs
4.78	4.8	316	1K	0	0	0
4.78	4.8	317	1K	0	0	0
4.78	4.8	318	1K	0	0	0
2.17	2.2	319	0	0	0	0
2.17	2.2	320	0	0	0	0
2.17	2.2	321	0	0	0	0
0.991	0.96	322	0	0	0	0
0.991	0.96	323	0	0	0	0
0.991	0.96	324	0	0	0	0
0.450	0.45	325	0	1S	0	0
0.450	0.45	326	0	0	0	0
0.450	0.45	327	0	0	1K	1K
0.205	0.21	328	0	0	0	0
0.205	0.21	329	0	0	0	0
0.205	0.21	330	0	0	0	0
0[3]	0	331	0	0	0	0
0[3]	0	332	0	0	0	0
0[3]	0	333	0	0	0	0
0[4]	0	334	0	0	0	0
0[4]	0	335	0	0	0	0
0[4]	0	336	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)
[3] acetone dosed
[4] undosed

Table A7. Results of definitive oral dosing tests with dimethoate - Mortality

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Cumulative number dead (n=10)			
			4 hrs	24 hrs	48 hrs	72 hrs
0.60	0.57	301	8	10	10	10
0.60	0.58	302	2	10	10	10
0.60	0.60	303	5	10	10	10
0.30	0.30	304	4	10	10	10
0.30	0.30	305	2	10	10	10
0.30	0.30	306	5	10	10	10
0.15	0.15	307	1	9	10	10
0.15	0.15	308	0	7	9	9
0.15	0.15	309	2	6	6	7
0.075	0.074	310	0	2	2	2
0.075	0.075	311	0	1	1	1
0.075	0.074	312	0	1	1	1
0	0	313	0	0	0	1
0	0	314	0	0	0	0
0	0	315	0	1	1	1

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)

Table A8. Results of definitive oral dosing tests with dimethoate – Sub-lethal effects

Nominal dose[1] (µg a.i./bee)	Actual dose[2] (µg a.i./bee)	Cage No.	Number knockdown (K) or stumbling (S)			
			4 hrs	24 hrs	48 hrs	72 hrs
0.60	0.57	301	2K	0	0	0
0.60	0.58	302	6K	0	0	0
0.60	0.60	303	5K	0	0	0
0.30	0.30	304	3K	0	0	0
0.30	0.30	305	3K	0	0	0
0.30	0.30	306	2K	0	0	0
0.15	0.15	307	0	0	0	0
0.15	0.15	308	0	0	0	0
0.15	0.15	309	0	0	1K	0
0.075	0.074	310	1K	0	0	0
0.075	0.075	311	0	0	0	0
0.075	0.074	312	0	0	0	0
0	0	313	0	0	0	0
0	0	314	0	0	0	0
0	0	315	0	0	0	0

[1] based on nominal consumption of test solution of 20µl/bee
[2] based on amount of test solution consumed (average of 10 bees)

APPENDIX 2
INTERIM CERTIFICATE OF ANALYSIS FOR TEST SUBSTANCE

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 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 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 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

COA023-018A

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Environmental Laboratory Project Number U2723

CC0037

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 ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 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%

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

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

[4]Sulfur 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

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

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

COA023-018A

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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 Date
Scientist, Centre Analytical Laboratories

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

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