Existing Chemical Hazard Assessment Report

Australian Government
Department of Health and Ageing
NICNAS

POTASSIUM PERFLUOROBUTANE SULFONATE

November 2005

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

GPO Box 58, Sydney NSW 2001, Australia www.nicnas.gov.au

ISBN 0 9758470 0 7

Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with the Department of Environment and Heritage, which carry out the environmental assessment for NICNAS. NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia.

For the purposes of Section 78(1) of the Act, copies of assessment reports for New and Existing Chemical assessments are freely available from the web (www.nicnas.gov.au) and may be inspected by the public at the library of the Office of Australian Safety and Compensation Council (ASCC). Summary Reports are published in the *Commonwealth Chemical Gazette* (http://www.nicnas.gov.au/publications/#gazette), and are available to the public at the ASCC library and on line at www.nicnas.gov.au.

Copies of this report and other NICNAS reports are available on the NICNAS website. Hardcopies are available from NICNAS at the following address:

GPO Box 58
Sydney NSW 2001
AUSTRALIA
Attention: Office Manager
Tel: +61 (02) 8577 8800
Freecall: 1800 638 528
Fax: +61 (02) 8577 8888
Email: info@nicnas.gov.au

Other information about NICNAS (also available on request) includes:

- NICNAS Annual Reports.
- NICNAS Service Charter.
- Brochure on NICNAS Registration.

More information on NICNAS can be found at the NICNAS web site:

http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the following website:

http://www.nohsc.gov.au

Overview

NICNAS called for information on perfluoroalkyl sulfonate (PFAS) chemicals in November 2002 because of increasing interest in the potential hazards of this group of chemicals.

As a result of the call for information, the 3M Corporation provided information concerning the carbon four PFAS chemical known as potassium perfluorobutane sulphonate (PFBS). The information provided by the 3M Corporation, combined with other relevant publications, was evaluated in the course of the hazard assessment of potassium PFBS.

Potassium PFBS is listed on the Australian Inventory of Chemical Substances (AICS) and is the basis of a specialty range of consumer and industrial fluorochemical products in use in Australia.

To date, no manufacture of potassium PFBS, PFBS derivatives or fluoropolymers that may degrade to PFBS is reported in Australia. Additionally, to date no use in Australia of potassium PFBS as such is reported, however, use of certain fluoropolymers that include monomers based on PFBS are reported. These PFBS-based chemicals have applications in industrial and consumer carpet protection treatments, industrially applied corrosion resistant paints and coatings and high performance industrial chemical applications in the metal processing industry. In Australia, the major use identified to date of PFBS-based chemicals is in the high performance industrial chemical category.

Based on the current use pattern in Australia, potential public exposure to PFBS is expected to be predominantly by secondary exposure via the environment as a result of the degradation of certain fluoropolymers to PFBS.

PFBS is a highly water soluble surfactant substance that exists in a completely dissociated state in an aqueous medium. It is poorly adsorbed to soils and sediments and is expected to remain in the water compartment on release into the environment. In water, PFBS is expected to be persistent as the chemical will not hydrolyse, photolyse or biodegrade. A range of tests show, however, that PFBS will not be toxic to birds, algae, aquatic invertebrates, fish or sewage micro-organisms.

PFBS is not bioaccumulative or toxic to aquatic organisms. As PFBS is persistent, levels may build up and be distributed widely in the environment over time due to the potential for substitution in products that currently contain perfluorooctane sulfonate (PFOS). However, PFBS will stay mostly in the water column due to the much higher water solubility compared with higher PFAS homologues. PFBS is not classifiable for the aquatic environment under the Globally Harmonized System for Hazard Classification and Communication (GHS) (United Nations, 2003).

PFBS is rapidly excreted in animals (monkeys) by the kidneys with up to 87% excreted in the urine within 24 hours post dosing. No difference is observed between male and female animals. 3M is currently developing urinary data for PFBS in potentially exposed workers. This study has not been finalized and was not provided during this assessment. Studies show PFBS is highly bound to human

albumin with indications of a saturated binding to albumin in serum and negligible binding to the other liver-manufactured proteins gamma globulin, alpha globulin, fibrinogen, alpha-2-macroglobulin, transferrin and beta lipoproteins.

The mammalian (animal) toxicology of potassium PFBS shows low acute oral and dermal toxicity. No information on acute inhalation toxicity is available. PFBS is not irritating to skin. Potassium PFBS is found to be an eye irritant with the potential to cause severe eye damage. There is no evidence of skin sensitisation.

A 90-day study in rats (0, 60, 200 and 600 mg/kg bw/day) indicates no serious damage to health by prolonged exposure to potassium PFBS at doses up to 200 mg/kg bw/day. The target organs in rats following repeated exposure are the kidney and stomach with treatment-related microscopic changes observed in the kidneys and stomach of the male and female rats. However, histopathological examination of the kidney by an independent expert reported that no consistent changes were seen in the kidneys and the renal effects were not secondary to treatment.

The results of two in vitro studies and a chromosomal aberration test show no evidence of mutagenicity due to potassium PFBS. Animal data to date does not indicate potassium PFBS is a developmental toxin nor a substance toxic to reproduction, fertility or lactation.

A pre-natal developmental toxicity in rats (0, 100, 300 and 1000 mg/kg bw), shows a No Observed Adverse Effect Level (NOAEL) of 300 mg/kg bw/day for maternal toxicity based on reduced body-weight gains and feed consumption at 1000 mg/kg bw/day and a NOAEL of 1000 mg/kg bw/day for developmental toxicity.

Potassium PFBS is not currently listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999). As a result of the hazard assessment, in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), it is recommended that potassium PFBS be classified "Hazardous" with the following risk phrases: R36 – Irritating to Eyes. The corresponding safety phrases are S25 – Avoid contact with eyes, and S26 – In case of contact with eyes, rinse immediately with plenty of water and contact a doctor or Poisons Information Centre.

With respect to the current use, the use of PFBS-based chemicals in the performance chemical category is supported, however, uses that would result in wide dispersion in the aquatic environment is not supported by NICNAS. NICNAS will annotate the Australian Inventory of Chemical Substances (AICS) to restrict use accordingly.

The consequences of NICNAS annotating the AICS of the restricted use of PFBS is that any planned dispersive use of PFBS and PFBS-based chemicals will need to be notified to NICNAS for assessment for prior approval.

Contents

PREFACE iii

OVERVIEW v

ACRONYMS AND ABBREVIATIONS ix

1. INTRODUCTION 1

2. CHEMICAL IDENTITY AND COMPOSITION 3
   - 2.1 Chemical identity 3

3. PHYSICAL AND CHEMICAL PROPERTIES 4
   - 3.1 Physical-chemical properties 4
   - 3.2 Comments on physical-chemical properties 4
   - 3.3 Conclusion 8

4. EFFECTS ON LABORATORY MAMMALS 9
   - 4.1 Toxicokinetics 9
   - 4.2 Protein binding 11
   - 4.3 Acute toxicity 16
     - 4.3.1 Acute toxicity – oral 16
     - 4.3.2 Acute toxicity – dermal 17
     - 4.3.3 Acute toxicity – inhalation 18
   - 4.4 Irritation and corrosivity 18
     - 4.4.1 Irritation-skin 18
     - 4.4.2 Irritation - eye 19
   - 4.5 Skin sensitisation – guinea pig maximisation test 21
   - 4.6 Repeat dose toxicity 22
     - 4.6.1 Repeat dose range-finding toxicity 22
     - 4.6.2 Repeat dose toxicity 26
     - 4.6.3 Repeated dose toxicity 90 days 29
   - 4.7 Genotoxicity 37
     - 4.7.1 In vitro-mutagenicity in bacteria 37
     - 4.7.2 Chromosome aberration test in cultured Chinese Hamster Ovary cells 40
   - 4.8 Prenatal developmental toxicity 43
   - 4.9 Two generation reproduction study 46

5. HUMAN HEALTH HAZARD CLASSIFICATION 66
5.1 Acute toxicity 66
5.2 Irritation 66
5.2.1 Skin irritation 66
5.2.2 Eye irritation 66
5.3 Skin sensitisation 67
5.4 Repeated dose toxicity 68
5.5 Genotoxicity 69
5.6 Reproductive, developmental and lactation toxicity 69

6. ENVIRONMENTAL FATE 71
6.1 Summary of environmental fate 71
6.1.1 Photolysis 71
6.1.2 Laboratory incineration study 71
6.1.3 Biodegradability 73
6.1.4 Bioaccumulation 73
6.1.5 Environmental Levels 75
6.1.6 Sources of perfluoralkyl substances in the environment 76
6.1.7 Conclusion 77

7. EFFECTS ON ORGANISMS IN THE ENVIRONMENT 78
7.1 Avian 78
7.2 Toxicity to aquatic organisms 81
7.2.1 Aquatic plants 81
7.2.2 Aquatic invertebrates 82
7.2.3 Fish 86
7.2.4 Sewage micro-organisms inhibition of microbial activity 88
7.3 Conclusion 89

8. DISCUSSION AND CONCLUSIONS 90

9. RECOMMENDATIONS 93
9.1 Recommendations for regulatory bodies 93
9.1.1 NOHSC 93
9.2 Recommendations for potassium PFBS importers and formulators of PFBS-containing products and State and Territory Authorities 93
9.2.1 Hazard communication – Material Safety Data Sheet 93
9.2.2 Hazard communication – labels 94
9.2.3 Recommendation to importers and users 94
APPENDIX 95
REFERENCES 97

Acronyms and Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ADG Code	Australian Dangerous Goods Code
AFFF	Aqueous Film Forming Foam
AICS	Australian Inventory of Chemical Substances (NICNAS)
AMSA	Australian Maritime Safety Authority
ANOVA	Analysis of Variance
AS	Australian Standard
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BP	boiling point
bw	bodyweight
°C	Celsius
C^0_p	standard heat capacity at constant pressure
CAS	Chemical Abstracts Service
Cl	clearance
cm	centimetre
CMC	carboxymethylcellulose
DEH	Australian Government Department of the Environment and Heritage
DMSO	Dimethyl sulfoxide
DO	dissolved oxygen
EC50	median effective concentration
EINECS	European Inventory of Existing Commercial Chemical Substances
EU	European Union
FOB	Functional Observational Battery
g	gram
DG	day of gestation

dL	decalitres
DL	day of lactation
DP	day of post weaning
FtS	fluorotelomer sulfonate
g/m^3	gram per cubic metre
GHS	Globally Harmonised System (of Classification and Labelling of Chemicals)
GLP	good laboratory practice
HPLC/MS/MS	High Performance Liquid Chromatography Mass Spectrometry/Mass Spectrometry
hr	hour
IC(NA) Act	Industrial Chemicals (Notification and Assessment) Act 1989 (Commonwealth)
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous
K	Kelvin
kg	kilogram
kJ	kiloJoule
Koc	organic carbon partition coefficient
Kow	octanol/water partition coefficient
km	kilometre
L	litre
LC50	median lethal concentration
LD50	median lethal dose
LC/MS/MS	liquid chromotagraphy tandem mass spectometry
LEL	lower explosive limit
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
LOQ	level of quantification
∝g	microgram
m	metre

m^3	cubic metre
mg	milligram
mg/kg	milligram per kilogram
bw/d	body weight per day
mg/L	milligram per litre
mg/m^3	milligram per cubic metre
min	minute
mL	millilitre
mm	millimetre
MSDS	Material Safety Data Sheet
n	number
NA	not applicable
ng	nanogram
ng/m^3	nanogram per cubic metre
ng/mL	nanogram per millilitre
ng/L	nanogram per litre
No.	number
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NMeFOSE	N-methyl perfluorooctane sulfonamidoethanol
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOHSC	National Occupational Health and Safety Commission
NOS	not otherwise specified
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances (US EPA)
PFAS	perfluoroalkyl sulfonate
PFBS	perfluorobutane sulfonate
PFHxS	perfluorohexane sulfonate
PFOS	perfluorooctane sulfonate

PFCAs	perfluorocarboxylic acids
PFPeS	perfluoropentane sulfonate
pg	picogram
pKa	dissociation constant
RCG	Relative Cell Growth
RFT	Range Finding Test
RMI	Relative Mitotic Index
STP	sewage treatment plant
T	temperature
$t_{1/2}$	half life
TG	Test Guideline
TM	Trademark
TSCA	Toxic Substances Control Act (US)
∝g/mL	microgram per millilitre
UEL	upper explosive limit
UN Number	United Nations (Identifications) Number
US EPA	United States Environmental Protection Agency
$V_{d\ ss}$	volume of distribution at steady state
WHO	World Health Organization
<	approximately

1. Introduction

NICNAS called for information on perfluoroalkyl sulfonate (PFAS) chemicals in September 2002. This was because of concerns that the PFAS group of industrial chemicals may be hazardous to human health and the environment.

A PFAS chemical is a generic term used to describe any fully fluorinated carbon chain length sulfonate chemical. It may be simple salts or polymers that contain the PFAS chemical as only a portion of the entire polymer.

As a result of the September 2002 call for information, the 3M Corporation provided copies of studies concerning the effects of potassium perfluorobutane sulfonate (potassium PFBS), CAS No 29420-49-3, in the environment and on laboratory mammals. The studies provided by the 3M Corporation addressed the following issues: physical and chemical properties; fate; hydrolysis; photolysis; incineration; biodegradability; bioaccumulation; toxicokinetics; acute oral and dermal toxicity; irritation/corrosivity; sensitisation; repeat-dose oral toxicity; genotoxicity, reproductive toxicity, avian toxicity and aquatic toxicity of potassium PFBS.

The studies allowed for an assessment of the environmental and health hazards of potassium PFBS and classification of the chemical in accordance with the National Occupational Health and Safety Commission *Approved Criteria for Classifying Hazardous Substances* (the Approved Criteria) (NOHSC, 2004). The Appendix of this report includes a classification of potassium PFBS in accordance with the Globally Harmonized System for Hazard Classification and Communication (GHS) (United Nations, 2003).

Potassium PFBS, also known as 1-butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-, potassium salt; 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonic acid, potassium salt; and potassium 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonate, is a simple salt of a PFAS chemical and is listed in the Australian Inventory of Chemical Substances (AICS).

As a man-made substance, potassium PFBS does not occur naturally in the environment. Potential applications for potassium PFBS and PFBS-based chemicals, which have unique physical and surfactant properties and hence potentially a range of speciality applications, may include heat, chemical and abrasion resistance and as dispersion, wetting and surface treatments.

Potential exposure routes for PFBS include production and use of PFBS and PFBS-based chemicals and degradation of certain fluoropolymers to PFBS. NICNAS notes it is likely that some importers and users may not know if products contain PFBS and its derivatives, including fluoropolymers which may degrade to PFBS, because such chemical ingredients may not be mentioned on Material Safety Data Sheets and labels.

Information collected by NICNAS to date shows no manufacture in Australia of potassium PFBS, PFBS derivatives or fluoropolymers that may degrade to PFBS. Additionally, to date no use in Australia of potassium PFBS as such is reported. However, certain fluoropolymers that include monomers based on PFBS are reported. These fluoropolymers, which have certain consumer and industrial applications, have

been assessed under the NICNAS New Chemicals program and are currently in use in Australia under certificate.

These PFBS-based chemicals have applications in industrial and consumer carpet protection treatments, industrially applied corrosion resistant paints and coatings and high performance industrial chemical applications in the metal processing industry. Under section 64(2)(e) of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), there is a requirement that introducers of these chemicals notify the Director, NICNAS of any additional information that has become available (within 28 days of the occurrence) as to the adverse health effects or adverse environmental effects of these chemicals.

In Australia, the major use identified to date of PFBS-based chemicals is in the high performance industrial chemical category. Based on the current use pattern, potential public exposure to PFBS is expected to be predominantly by secondary exposure via the environment by means of degradation of certain fluoropolymers to PFBS.

There are a number of uses overseas of PFBS-based chemicals in the performance chemical category. These applications include electronic grade fluorochemical surfactants in etch solutions, photoresists, photoresist strippers and edge bead removers, anti-reflective coatings, spin-on glass films and as flame retardant additives. PFBS-based chemicals are regarded as potential replacements for perfluorooctane sulfonate (PFOS)-based surfactants.

A range of PFBS-based surfactants have been reviewed by the United States Environmental Protection Agency (US EPA) and placed on the Toxic Substances Control Act (TSCA) inventory. PFBS-based surfactants have also been commercialised in Europe. PFBS and PFBS-based surfactants are not included in the U.S. EPA's PFAS Final Significant New Use Rule (SNUR 67 FR 11008) or Supplemental Proposed Significant New Use Rule (SNUR 67 FR 11014) (US EPA, 2002). A 3M Technical Data Bulletin states to minimise the exposure to the environment of PFBS, that the company is limiting sales of PFBS-based surfactants to non-dispersive applications or applications with low emissions to the environment (3M Specialty Material, 2002).

During the course of this assessment, an automated literature search was established relating to potassium PFBS using the Current Contents Search® (CC Search®) database which provides access to the contents and bibliographic data of more than 8000 international journals and 2000 books covering the scientific disciplines from 1993 to the present. In addition, an automated literature search was established using the OVID database, which covers scientific, technical, and medical journals from 1988 to the present. Relevant publications were also evaluated in this report.

There is ongoing national and international activity in relation to certain perfluorinated chemicals. More information about these activities can be obtained from NICNAS and the web site at: www.nicnas.gov.au

2. Chemical Identity and Composition

2.1 Chemical identity

Chemical Name:	potassium perfluorobutane sulfonate
CAS No.:	29420-49-3
EINECS No.:	249-616-3
Synonyms:	1-butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-, potassium salt 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulfonate de potassium Potassium 1,1,2,2,3,3,4,4,4-nonafluorobutane-1-sulphonate perfluorobutanesulfonic acid potassium salt potassium nonaflate potassium nonafluorobutanesulfonate potassium perfluoro-1-butanesulfonate potassium perfluorobutanesulfonate potassium PFBS
Molecular Formula:	$C_4HF_9O_3S^-.K^+$
Structural Formula:	CF_3□$(CF_2)_3$□SO_3^-□K^+
Molecular Weight:	339.21

3. Physical and Chemical Properties

3.1 Physical-chemical properties

Property	Value	Reference
Appearance	White powder (potassium salt)	Wildlife International, 2002
Boiling point	76-84^{o}C/0.13 kPa; 200^{o}C/101 kPa (acid)	Kirk Othmer, 1978-1984
Melting point	270.4^{o}C (potassium salt)	Ammons, 2000
Density	Not available	
Water solubility	46.2 g/L at 20^{o}C for potassium salt 52.6-56.6 g/L at 22.5-24^{o}C	Wildlife International, 2000; 3M Environmental Laboratory, 2001
Vapour pressure	<1.22 X 10^{-5} Pa at 20 ± 1^{o}C for potassium salt	Wildlife International, 2002
Henry's law constant	H <8.91 X 10^{-8} Pa.m^{3}/mol Kaw <3.65562 X 10^{-11} (unitless), for potassium salt	3M Environmental Laboratory, 2002
Partition coefficient (Log Kow)	Not determined	
Adsorption/Desorption (Koc)	No adsorption of PFBS potassium salt to 3 soils or a sediment, low adsorption to sludge	Centre Analytical Laboratories, 2001
Dissociation Constant	Fully dissociated in water over the environmental pH range of 4-9	

Details of the studies are given in the following section.

3.2 Comments on physical-chemical properties

The data have been generated on a mix of the PFBS free acid (boiling point, water solubility and adsorption/desorption), or the potassium salt of PFBS. However, this is not a concern in environmental terms, as due to its strong acidity, it is estimated that PFBS will always be ionised in the environmental pH range of 4-9, and the potentially greater volatility of the free acid should not be a consideration.

Appearance at 20°C and 101.3 kPa:	White powder (potassium salt)
Boiling Point:	76-84°C/0.13 kPa; 200°C/101 kPa (estimated) (acid)
Method:	Not stated
Remarks:	The above results are listed in a table in an article on perfluoroalkane sulfonic acids in the standard reference Kirk-Othmer's Encyclopedia of Chemical Technology. The article is written by a 3M employee.
Test Facility:	Not stated
Melting Point:	270.4°C
Method:	Not stated
Remarks:	No details provided except that this was an average of 3 determinations performed on 3 lots using a BUCHI Melting Point B-545 instrument. However, considering the boiling point above this would appear to be for the potassium salt.
Test Facility:	Not stated
Density:	Not available
Vapour Pressure:	$<1.22 \times 10^{-5}$ Pa at 20 ± 1°C for potassium salt
Method:	OECD TG 104 and US EPA Product Property Test Guidelines Series OPPTS 830.7950 – spinning rotor gauge method
Remarks:	The method is based on the measurement of the rotational frequency of a stainless steel ball, which is magnetically suspended in a vacuum chamber, and where the deceleration rate of the ball rotation is proportional to the vapour pressure of the sample. The measurement cycle was repeated twice for a total of three vapour pressure determinations. While the vapour pressures for two reference samples (hexachlorobenzene and DDT) were consistent with published values, those for the PFBS samples were less than the background vapour pressure. It may be concluded that the potassium salt of PFBS is very slightly volatile (Mensink et al., 1995).
Test Facility:	Wildlife International Ltd (2002)
Henry's Law Constant:	H $<8.91 \times 10^{-8}$ Pa.m^3/mol; Kaw $<3.65562 \times 10^{-11}$ (unitless)
Method:	Standard relationships were used.
Remarks:	The above values were calculated from the vapour pressure and water solubility values for PFBS potassium salt.

	The report calculates that at equilibrium with air greater than 99.999999996% will be in the water phase. It may be concluded that the potassium salt of PFBS is very slightly volatile from water (Mensink et al., 1995).
Test Facility:	3M Environmental Laboratory (2002)
Water Solubility:	46.2 g/L at 20 ± 0.5°C
Method:	OECD TG 105 Water Solubility (Shake Flask Method)
	US EPA Product Property Test Guidelines Series: 830.7840
Remarks:	The preliminary test consisted of adding increasingly larger amounts of NANOpure^{R} water to a known amount of test substance (PFBS potassium salt) at room temperature. Based on this the definitive test consisted of the addition of NANOpure^{R} water to an excess amount of the test substance, shaking at 30°C for 1, 2 or 3 days, followed by leaving at 20°C for a further 24 hours with occasional shaking. Subsamples were removed and centrifuged vigorously to remove undissolved, suspended material and the supernatant analysed by HPLC. The result quoted is the mean of the results from 2 and 3 days shaking at 30°C.
	It may be concluded that the potassium salt of PFBS is readily soluble in water (Mensink et al., 1995).
Test Facility:	Wildlife International, Ltd (2000)
Water, Methanol And Acetone Solubility:	52.6-56.6 g/L at 22.5-24°C (water), >10% in methanol and acetone at 22.5°C
Method:	OECD TG 105 Water Solubility (Shake Flask Method)
	US EPA Product Property Test Guidelines Series: 830.7840
Remarks:	The Solubility Screen Test Method was used to determine the solubility range of two batches of potassium PFBS in water, methanol and acetone. As the solubility of the latter two was greater than 10%, no further testing was performed.
	As a solubility of >22 g/L was indicated in water (purity unclear) the definitive tests were performed in a very similar fashion to the above study.
Test Facility:	3M Environmental Laboratory (2001)
Hydrolysis As A Function Of pH:	Not determined, but expected to be stable
Remarks:	The submission indicates that a hydrolysis study on perfluorooctane sulfonate (PFOS) conducted over 49 days at 50°C showed no detectable loss of the initial concentration over time at pH 1.5, 3, 5, 7, 9 and 11. An environmental half-life of greater than 41 years was estimated. As PFOS and PFBS share the same functionality and the same type of chemical bonds, a

similar stability may be expected.

Partition Coefficient (N-Octanol/Water): Log Pow at 20°C has not been determined

Remarks: The submission indicates that as PFBS is a surface active agent the partition co-efficient between octanol and water cannot be measured using standard methodologies, and notes the demonstrated lack of bioaccumulation in fish (see below). A result for octanol solubility to allow a simple comparison with the water solubility is also not available, but such comparisons should be treated with caution due to the surfactant nature of PFBS.

Adsorption/ Desorption – Main Test: Freundlich Kf = 0.3 for an activated sludge. Kf or Koc not able to be calculated for 3 soils and 1 sediment as no detectable adsorption occurred.

Method: OECD TG 106 Adsorption-Desorption Using a Batch Equilibrium Method.

US EPA Fate, Transport and Transformation Test Guidelines, OPPTS835.1110 Activated Sludge Sorption Isotherm.

Soil Type	Organic Carbon Content (%)	pH	Koc (mL/g)
Don Uglem loam	4.9	7.4	Not determinable
Broeren Clay loam	2.6	6.0	As above
Kittson County clay	2.6	7.2	As above
Goose River sediment	1.3	7.7	As above
NIST sludge	Not performed	Not performed	Kf = 0.3

Remarks: In the preliminary test the Don Uglem loam and clay soils were mixed with 0.01M $CaCl_2$ solution in 3 different ratios (1:1, 1:5 and 1:25). These were shaken at 17.0 °C to 19.1°C for up to 48 hours to achieve equilibrium. During this the concentration of PFBS in both the water and soil was determined by LC/MS for both soils and the 3 soil/solution ratios.

For the measurement of sorption kinetics the remaining soil (Broeren clay loam), river sediment and sludge, at solution ratios of 1:1 for the first two and 1:5 for the sludge, were shaken with 0.01M $CaCl_2$ for 24 hours, with analyses of the solution determined by LC/MS over 24 hours.

Adsorption isotherms were then attempted to be measured over the 50-1000 ∝g/L range using the same solution ratios for the soils/sediment and sludge as for the sorption kinetics, and the aqueous phases analysed after 24 hours. For determination of the desorption isotherms following removal of the adsorption solutions from the solids, these were remixed with fresh 0.01M $CaCl_2$ with analyses of the solution by LC/MS after 24 hours.

While the mass balances were acceptable, PFBS was only detected on the sludge, for which Freundlich isotherm

	calculations could only be performed. This was independent of solution:soil ratios. The desorption phase of the experiment indicated PFBS was readily lost from the sludge (desorption Kf = 0.001) The lack of adsorption to the three soils and the sediment places PFBS in the very high mobility class (Koc <50). This was also the case for the activated sludge as the initial PFBS concentration in solution was independent of the determined sorption value.
Test Facility:	Centre Analytical Laboratories (2001)
Dissociation Constant:	pKa not available but PFBS is a very strong acid.
Remarks:	Expected to be a very strong acid, in line with trifluoromethanesulfonic acid, which is noted to be one of the strongest simple protic acids known (Kirk-Othmer (1980). Therefore PFBS will always occur in the ionised state in the environmental pH range of 4-9.

3.3 Conclusion

PFBS is a strongly acidic, highly water soluble substance which has a low vapour pressure and is poorly adsorbed to soils and sediments, and is therefore expected to remain in the water compartment on release into the environment.

4. Effects on Laboratory Mammals

4.1 Toxicokinetics

Test Substance:	potassium perfluorobutane sulfonate (unspecified purity)
Test Facility:	Southern Research Institute (2001)
Method/guideline Followed:	Not stated. Toxicocokinetic parameters were estimated using the WinNonlin program. Monkeys were housed in individual metal cages during the quarantine time (which was a minimum of 35 days prior to the study start) and during the study period (31-days post dosing). All animals were checked twice daily for signs of mortality/morbidity and were examined shortly after dose administration for clinical signs of toxicity. Cage side clinical observations were performed daily. Body weight was measured on study days 0, 4, 7 and 14. Monkeys were retained in the stock colony at the end of the study.
GLP:	No
Year:	2001
Species/Strain:	Cynomologus monkey/Macaca fascicularis
Sex:	Male and female
Age and body weight range of animals used:	unspecified age, body weight range: male 4.4 to 5.8 kg; female 3.3 to 3.9 kg
Number of animals:	3 male and 3 female
Route of administration:	Intravenous
Vehicle:	0.9% sodium chloride solution containing 5 mg/mL of test material.
Doses:	10 mg/kg, single dose. The dose was administered by injection into a superficial arm or leg vein at a volume of 2 mL/kg.
Parameters, body fluids and tissues monitored and/or sampled:	serum pre-dose baseline, 2-, 4-, 24-, and 48-hours and on study days 4, 7, 11, 14 and 31 post dose; urine 24-hour collection pre-dose base line and on study days 1, 7 and 14 post dose; pharmacokinetic parameters of clearance (Cl), half life ($t_{1/2}$), and Area Under the Curve (AUC).

Statistical methods used: Mean values and standard deviations for each calculated pharmacokinetic parameter were reported.

Mean values and standard deviations for each serum and urine time point were not specified in the study.

Results: All monkeys survived to the end of the study. No adverse clinical signs were observed during the study period. Body weights remained constant during the study period.

No sex-related differences in serum levels of the test material were observed. The serum test material concentrations ranged from 19628 to 61740 ng/mL (equivalent to approximately 19.6 ppm to 61.7 ppm) at 2-hrs post dosing (earliest time point) in individual animals. Serum test material concentrations decreased 48-hours post dosing to a range of 463 ng/mL to 8172 ng/mL (equivalent to approximately 0.46 ppm to 8.17 ppm) in individual animals. The test material was not detected in serum in any individual monkey at the study end (Study Day 31).

For five of the six animals, approximately 34% to 87% of the test material was recovered in the urine within 24 hours post dosing. For the remaining monkey, less than 1% of the dose was recovered at the 24-hour time interval. This was attributed to loss or spillage of the excreted urine by the animal during the collection interval.

On day 14 post-dosing, individual monkeys excreted less than 0.01% of the test material within a 24-hour urine sample collection period.

A summary of the calculated pharmacokinetic parameters is given in Table 4.1:

Table 4.1 - Calculated kinetic parameters in Cynomologus monkeys

Pharmacokinetic Parameter	Male		Female	
	Mean	Standard Deviation	Mean	Standard Deviation
$t_{1/2\,\langle}$ (day)[a]	0.04	0.02	0.06	0.01
$t_{1/2\,®}$ (day)[b]	0.55	0.21	0.47	0.18
$t_{1/2\,®}$ (day)[c]	4.0	1.9	3.5	3.1
$AUC_{0\text{-infinity}}$ [d]	24 258	14 918	35 401	23 037
Cl (mL/day/kg)[e]	511	245	368	207
Vd_{ss} (mL/kg)[f]	254	31.5	255	29.5

[a] half-life of the ⟨-phase
[b] half-life of the ®-phase
[c] half-life of the ®-phase
[d] Area under the serum concentration versus time curve calculated from time zero to infinity
[e] Total body clearance
[f] Volume of distribution at steady state

A three compartment pharmacokinetic model was calculated from the serum concentration of the test material. A terminal-phase half-life of four days was observed.

Conclusion: No gender specific differences in the pharmacokinetics of the test material in the cynomologus monkey were observed in this study.

Urinary excretion of the unchanged test material in the cynomologus monkey was a major route of excretion observed in this study with up to 87% of the test material excreted in 24 hours and a (terminal) elimination half life of four days. The reason for the small steady state volume of distribution of the test material is uncertain. Further investigations on the small steady volume of distribution will assist in better understanding the persistence or otherwise of the test material in mammals.

4.2 Protein binding

Test Substance: potassium perfluorobutane sulfonate (unspecified purity)

Test Facility: Southern Research Institute (2003)

Method/guideline followed: Not stated. Undertaken in accordance with the test facility's Standard Operating Procedures for handling human and primate plasma.

Protein binding studies of perfluorobutane sulfonate, perfluorohexane sulfonate, perfluorooctane sulfonate and perfluoroctanoate (the test materials) in human, rat and monkey plasma at concentrations of 1, 10, 100, 250 and 500 ppm of the test material.

Additionally, protein binding studies of perfluorobutane sulfonate, perfluorohexane sulfonate, perfluorooctane sulfonate and perfluoroctanoate (at a final concentration of 10 ∝g/mL of the relevant test material) to human-derived plasma protein fractions (albumin, gamma globulin, alpha-globulin, fibrinogen, alpha-2-macroglobulin, transferrin, and beta lipoproteins).

All samples (unspecified number) analysed by High Performance Liquid Chromatography Mass Spectrometry/Mass Spectrometry (HPLC/MS/MS are reportedly within the concentration range of the standard curve (unspecified linearity slope variance).

The range of reliability for the HPLC/MS/MS analyses of monkey serum are as follows:

- Perfluorobutane sulfonate: 0.5 - 500 ng/mL
- Perfluoroctanoate: 20 – 1000,000 ng/mL
- Perfluorohexane sulfonate: 5 – 20,000 ng/mL
- Perflurooctane sulfonate: 20 – 10,000 ng/mL

Monkey serum samples were diluted with control blank serum to ensure the concentration of the test material was within the range of reliability prior to analysis.

Remarks: The study methodology reports the use of human, rat and monkey serum rather than plasma as is cited in the study title.

The range of reliability for the HPLC/MS/MS analyses of rat and human serum and human plasma protein fractions and purity of the test materials are unspecified.

Any potential for interference between the test materials and/or internal standard and/or HPLC/MS/MS matrix/mobile phase is not reported.

The reported protein binding methodology does not appear to be a classical equilibrium dialysis system, i.e., using two chambers separated by an artificial semi-permeable membrane (AED), or ultrafiltration method (Martin, 1993; Hinderling, 1997). It is unclear if the partitioning of the compounds was performed under controlled physiological conditions, e.g., pH = 7.4; temperature = 37°C. The time to equilibration is not reported. The study does not investigate red blood cell partitioning. The study does not represent an investigation of the dissociation potential of the test materials from human proteins.

GLP: not stated

Year: 2003

Species *Serum/plasma samples*: Samples (0.5 mL) were obtained from cynomologus monkeys (unspecified sample size) treated with the potassium perfluorobutane sulfonate (unspecified dose). It would appear the PFBS containing serum/plasma was the result of spiked samples – this is because the attached analytical methodology probably relates to that used in mammalian toxicity studies and is not specific to the protein binding study. Details of the species and treatment of the rats is not included in the study report. The source of the human plasma is unspecified.

Plasma protein fractions: derived (unspecified) from humans.

Sample preparation: *Human derived plasma protein*: Protein binding of the test materials was evaluated in separately spiked samples (final concentration of 10 ∝g/mL of the relevant test material) in the seven human derived protein fractions (albumin, gamma globulin, alpha-globulin, fibrinogen, alpha-2-macroglobulin, transferrin, beta lipoproteins) at concentrations of 10% and 100% of physiological plasma protein concentrations (Table4.2):

Table 4.2 - Plasma protein fraction concentrations

Plasma Protein Fraction	Concentration (~10% physiological concentration) mg/mL	Concentration (~ 100% physiological concentration) mg/mL
albumin	443	4600
gamma globulin	196	1768
alpha-globulin	136	1212
fibrinogen	38	408
alpha-2-macroglobulin	67	200
transferrin	42	297
beta lipoproteins	20	200

Samples were fortified with the internal standard, mixed with an ion-pairing reagent (tetrabutylammonium hydrogen sulfate). A resulting ultra-filtrate was analysed by HPLC/MS/MS to determine the amount of unbound test material.

Rat, human and monkey plasma/serum: Monkey plasma/serum samples apparently derived from perfluorobutane sulfonate treated animals were fortified with an internal standard, mixed with an ion-pairing reagent (tetrabutylammonium hydrogen sulfate); followed by extraction with and evaporation of ethyl acetate prior to reconstitution in a mobile phase mixture suitable for analysis by HPLC/MS/MS.

Details of the source of and sample preparation for human and rat serum are unspecified in the study report, but appears to be serum samples separately spiked with the test materials at concentrations of 1, 10, 100, 250 and 500 ppm.

Parameter measured: amount of unbound test material

Parameter reported: percent bound of test material.

Statistical methods: None specified. Mean values and standard deviations for measured parameter were not reported.

Results: The results of the analyses of the protein binding of the four test materials in the seven above-mentioned protein fractions at 10% and 100% protein fraction concentrations are given in Table 4.3 and 4.4, respectively. Additionally, the results of the evaluations of the protein binding of the four test materials in rat, human and monkey serum at 1, 10, 100, 250 and 500 ppm is given in Table 4.5.

At test material concentrations of 10∝g/mL (equivalent to 10 ppm), the data in Table 4.3 and 4.4 shows human albumin binding for

perfluorohexane sulfonate, perfluorooctane sulfonate and perfluoroctanoate of 96% – 99% at 10% and 100% physiological albumin concentrations compared to 71% and 93% albumin binding of perfluorobutane sulfonate at 10% and 100% physiological albumin concentrations, respectively. There is insufficient data in the study report to ascertain the significance or otherwise of the difference in the values of the percent binding of the four test materials to human albumin.

The percent protein binding of perfluorobutane sulfonate (10 ∝g/mL equivalent to 10 ppm) to human-derived gamma globulin, alpha-globulin, fibrinogen, alpha-2-macroglobulin, transferrin and beta lipoproteins (10% or 100% physiological concentration of the protein) is no more than 11% - with the percent binding ranges from less than < 0.1% for binding to transferin to 11.3% for binding to alpha-2-macroglobulin.

At concentrations of up to 100 ppm (equivalent to 100 ∝g/mL) of the test material, the data in Table 4.5 shows the percent binding of perfluorobutane sulfonate in rat, human and monkey serum is typically greater than 98%. At perfluorobutane sulfonate concentration of 250 ppm and 500 ppm (equivalent to 250 ∝g/mL and 500 ∝g/mL) the percent binding of the test material in rat, human and monkey serum ranged from 85% - 94%. The slightly lower value of the percent of perfluorobutane sulfonate bound to rat, human and monkey serum at concentrations of the test material above 100 ppm compared to below 100 ppm is suggestive of a saturation of the binding above 100 ppm.

Conclusion: High levels of binding of perfluorobutane sulfonate to human albumin (94% binding at 100% physiological concentrations of albumin) and an apparent saturated binding of the test material to albumin at concentrations of perflurobutane sulfonate above 100 ppm – 250 ppm in rat, human and monkey serum was observed in this study.

Table 4.3 - Percent (%) binding to human plasma protein fractions (approximately 10% physiological concentrations)

Test material (10∝g/mL)	Albumin	Gamma globulin	Alpha-globulin	Fibrinogen	Alpha-2-macroglobulin	Transferrin	Beta lipoproteins
Perfluorobutane sulfonate	70.8	< 0.1	3.1	< 0.1	11.3	< 0.1	< 0.1
Perfluorohexane sulfonate	> 99.5	15.0	33.6	15.6	9.9	8.0	25.8
Perfluorooctane sulfonate	99.0	6.3	49.9	< 0.1	12.5	7.2	90.1
Perfluoroctanoate	96.4	3.5	28.5	5.4	7.9	1.0	19.6

Table 4.4 - Percent (%) binding to human plasma protein fractions (approximately 100% physiological concentrations)

Test material (10∝g/mL)	Albumin	Gamma globulin	Alpha-globulin	Fibrinogen	Alpha-2-macroglobulin	Transferrin	Beta lipoproteins
Perfluorobutane sulfonate	93.5	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Perfluorohexane sulfonate,	> 99.9	26.1	13.7	< 0.1	< 0.1	6.4	64.1
Perfluorooctane sulfonate	99.8	24.1	59.4	< 0.1	< 0.1	< 0.1	95.6
Perfluoroctanoate	99.7	3.0	11.0	< 0.1	< 0.1	2.1	39.6

Table 4.5 - Percent (%) protein binding to rat, human and monkey plasma

Test Material	Perfluorobutane sulfonate			Perfluorohexane sulfonate			Perfluorooctane sulfonate			Perfluoroctanoate		
Concentration (ppm/∝g/mL))	Rat	Monkey	Human	Rat	Monkey	Human	Rat	Monkey	Human	Rat	Monkey	Human
1	<100	<100	<100	<100	<100	<100	<100	<100		<100	<100	<100
10	98.4	99.7	99.0	<100	<100	<100	99.8	99.9	99.9	99.5	99.8	99.9
100	95.9	96.6	98.0	99.9	99.9	100	99.7	99.9	99.9	98.6	99.8	99.9
250	85.3	95.7	94.3	99.1	99.8	99.9	99.5	99.9	99.9	97.6	99.8	99.6
500	84.1	91.8	88.4	98.2	99.5	99.4	99.0	99.9	99.9	97.3	99.5	99.4

4.3 Acute toxicity

4.4 Acute toxicity – oral

Test substance: potassium perfluorobutane sulfonate (approximately 98% - 99% purity)

Test Facility: Primedica Redfield (2000a)

Method: The method was analogous with OECD 423 Acute Oral Toxicity - Acute Toxic Class Method. No protocol deviations from the OECD test method were reported.

A single oral dose was administered by gavage to fasted animals on Study Day 1 (SD 1). The animals were held post dosing for a fourteen-day recovery period.

As no deaths occurred in the high-dose (2000 mg/kg bw) group, the mid- (1000 mg/kg bw) and low-dose (500 mg/kg bw) groups were not tested.

Results:

Mortality:

Group designation	Number and sex of animals	Dose mg/kg bw	Mortality
High dose	5 males + 5 females	2000	none
Mid dose	5 males + 5 females	1000	□
Low-dose	5 males + 5 females	500	□

LD50: >2000 mg/kg bw

Clinical signs: The adverse clinical observations recorded were localised limb alopecia in a male rat on Study Day 4 and Study Day 6 to 15; urine stained abdominal fur in a male rat approximately 4-hours post dose; and liquid faeces in a female rat at approximately 3- and 4-hours post dose. These observations are not regarded as being related to the test material because they occurred in three different animals.

Body weight: During the study period (Study Day 1 to 15), mean body weight increased in males (+114 ±15.9 gram) and females (+49.4 ± 17.4 gram). No body weight loss for either males or females was observed. This suggested no adverse effects on body weights or body weight changes of a single oral dose of 2000 mg/kg bw of the test material.

Effect on Organs: No effects on organs were observed other than dilation of the right kidney in one male and one female rat. The dilation was attributed to hydronephrosis and not the test material. Unilateral urethral obstruction (UUO)-induced hydronephritis may occur as a congenital condition in rats (Cruz et al., 2002).

Conclusion: The test material is of low acute toxicity via the oral route with an oral LD50 > 2000 mg/kg bw.

4.5 Acute toxicity – dermal

Test substance: potassium perfluorobutane sulfonate (approximately 98%-99% purity)

Test Facility : Primedica Redfield (2000b)

Method: The method was analogous with OECD Guideline 402 Acute Dermal Toxicity – Limit Test. No protocol deviations from the OECD test method were reported.

The submitted study proposed three study groups each consisting of 5 male and 5 female rats dosed at a single dermal application of 500 mg/kg bw (low-dose), 1000 mg/kg bw (mid-dose) and 2000 mg/kg bw (high-dose) with an exposure period of approximately 24 hours with a fourteen-day recovery. As no deaths occurred at the high dose level (2000 mg/kg bw), the mid-dose (1000 mg/kg bw) and low-dose (500 mg/kg bw) groups were not dosed and the study was terminated. A description of the study design is as follows:

Group designation	Number and sex of animals	Dose mg/kg bw
High-dose	5 males + 5 females	2000
Mid-dose	5 males + 5 females	1000
Low-dose	5 males + 5 females	500

The test material was applied as a powder uniformly to approximately 10% of the body surface area for a 24-hour exposure period and was held in contact with the skin with a porous gauze dressing and non-irritating tape. After 24 hours, the test material was removed with warm water and gauze. Clinical observations were made for 14 days.

The method included provision for all animals that died prior to scheduled termination to be weighed and necropsied. On Study Day 15, all animals (non-fasted) were humanely euthanised via carbon dioxide asphyxiation and submitted for a complete necropsy examination. A complete necropsy examination was defined in the study details as examination of all external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. No histopathological examinations were performed.

Mean and standard deviations were calculated for all quantitative data.

GLP: Yes

Year: 2000

Species/Strain:	Rat/Sprague-Dawley
Vehicle:	1% carboxymethyl cellulose (medium viscosity) in deionised water.
Type of dressing:	semi-occlusive
Results:	
LD50:	> 2000 mg/kg bw
Clinical signs (local):	Clinical observations included red material around the eyes, nose and mouth on Day 1 only.
Clinical signs (systemic):	No treatment-related adverse clinical observations, mortality, changes in body weight or gross pathology were found. One procedural (unspecified) death resulted in the replacement of a single male rat.
Conclusion:	The test material is of low acute toxicity via the dermal route with a dermal LD > 2000 mg/kg bw.

4.6 Acute toxicity – inhalation

No information on acute inhalation toxicity was provided.

4.7 Irritation and corrosivity

4.8 Irritation-skin

Test substance:	potassium perfluorobutane sulfonate (approximately 98%-99% purity)
Test Facility:	Primedica Redfield (2000c)
Method:	The method was analogous with OECD 404 Acute Dermal Irritation/Corrosion. No protocol deviations from the OECD test method were reported. A single dermal application of 500 mg/kg bw of test material was applied to three 14-week old female New Zealand rabbits to approximately 6 cm^2 of skin and covered with a gauze patch, which was held in place with non-irritating tape, for a four-hour exposure period, followed by a fourteen day recovery period. The application to approximately 6 cm^2 of skin is consistent with OECD 404 Acute Dermal Irritation/Corrosion. Untreated skin served as a control. The animals were examined for signs of erythema and oedema and the responses scored at 60 minutes and then at 24, 48 and 72 hours after patch removal. The duration of the observation period was sufficient to evaluate the reversibility of the effects observed. No animals required termination due to severe distress and/or pain at any stage of the test.
GLP:	Yes

Species/Strain: Rabbit/New Zealand White

Number and sex) of animals: 3 female nulliparous and non-pregnant animals.

Vehicle: 1% carboxymethyl cellulose (medium viscosity) in deionised water.

Observation Period: 72 hours

Type of Dressing: semi-occlusive

Results:

Lesion	Mean Score* Animal No.			Maximum Value
	1	**2**	**3**	
erythema	0	0	0	0
oedema	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for each animal using the Draize scoring method (Draize et al., 1944).

During the study no adverse findings were reported with the exception of one rabbit exhibiting decreased appetite on Study Day 2 to 4.

Conclusion: A single exposure to 500 mg of the test material failed to induce erythema, oedema, or other dermal findings during the scoring periods.

4.9 Irritation - eye

Test substance: potassium perfluorobutane sulfonate (approximately 98%-99% purity)

Test Facility: Primedica Redfield (2000d)

Method: The method was analogous with OECD 405 Acute Eye Irritation/Corrosion

A single ocular application in the left eyes of three 16-week old female New Zealand rabbits with approximately 80 mg of powdered test material on Study Day 1. Both eyes were flushed with approximately 0.5 mL of 0.9% saline after the 24-hour score was recorded. Ocular irritation was scored at approximately 1, 24, 48 and 72 hours post dose, and then daily to the study end at 21 days to determine the progress, reversibility and/or irreversibility of any lesions. Fluoroscein staining was used to score the irritation.

The dose level of 80 mg is less than the maximum dose level of 100 mg described in OECD Guideline No. 405.

GLP: Yes

Year: 2000

Species/Strain: Rabbit/New Zealand White

Number of Animals: 3

Observation Period: 21 days

Results:

Ocular Irritation Score

1-hour post-dose						
Rabbit	Cornea Opacity	Cornea Area [1]	Iris	Conjunctivae Redness	Chemosis	Conjunctivae Discharge
1	1	1	2	2	4	3
2	1	1	1	2	1	3
3	2	3	2	2	2	3
24-hour post-dose						
Rabbit	Cornea Opacity	Cornea Area	Iris	Conjunctivae Redness	Chemosis	Conjunctivae Discharge
1	1	1	1	2	3	3
2	2	2	1	2	2	3
3	1	1	1	2	2	3
48-hours post-dose						
Rabbit	Cornea Opacity	Cornea Area	Iris	Conjunctivae Redness	Chemosis	Conjunctivae Discharge
1	0	0	1	2	2	3
2	2	2	1	2	2	3
3	3	3	1	2	3	3
72-hours post-dose						
Rabbit	Cornea Opacity	Cornea Area	Iris	Conjunctivae Redness	Chemosis	Conjunctivae Discharge
1	0	0	1	1	1	3
2	2	2	1	2	2	3
3	2	3	1	2	3	3
Study Day 21						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	4	4	2	2	3	1

[1] The study used the ocular irritation scoring system described in Hackett RB, McDonald TO (1991) Eye Irritation. In: Dermatotoxicology, 4th Edition, Marzulli FN and Maibach HI (eds), pp 749-815, 1991. This is consistent with the OECD Guideline 405, which specifies that the area of cornea opacity should be noted. The corneal area scoring is as follows:

Corneal Area Score	*Area of ulceration or opacity*
0 (zero)	no ulceration or opacity
1	1 – 1/4 (but not zero) of cornea involved
2	> 1/4 but < 1/2 of cornea involved
3	> 1/2 but < 3/4 of cornea involved
4	> 3/4 of cornea involved

Under the Approved Criteria, ocular lesions are considered significant when the results in the case where three animals are used in the test, the mean values, on two or more animals, are equivalent to any of the following:

- cornea opacity equal to or greater than 2 but less than 3;
- iris lesion equal to or greater than 1 but greater than 2;
- redness of the conjunctivae equal to or greater than 2.5;
- oedema of the conjunctivae (chemosis) equal to or greater than 2.

All scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

Conclusions	The test material is considered irritating to the eye. There is insufficient numerical data to describe the degree of eye irritation potential of the test material according to the modified version of the system described by Kay and Calandra (Kay & Calandra, 1962).

4.10 Skin sensitisation – guinea pig maximisation test

Test substance:	potassium perfluorobutane sulfonate (approximately 97% purity)
Test Facility:	Redfield Laboratories (2002)
Method:	The method was analogous with OECD 406 Skin Sensitisation. On Study Day 22 during the challenge and re-challenge phase, the type of tape used to secure the animal chambers was changed. This deviation is not expected to affect the integrity of the OECD test method.
GLP:	Yes
Year:	2002
Species/Strain:	Guinea Pig/Hartley-derived
Number of animals:	Test group: 10 males, 20 females; 15 in control group: 5 males, 10 females. Twice the number of female than male animals was used because the female portion of the study was repeated due to equivocal results. However, only data for 15 females used in the repeat portion of the study is included in the report.
Vehicle:	sterile water for injection
Induction phase:	Induction Concentration: intra-dermal: 125 mg/mL (equivalent to a 12.5 mg intra-dermal dose) topical: 10% (equivalent to a 50 mg topical dose)
Signs of irritation:	For intra-dermal induction, the clinical observations were consistent with tissue damage and healing caused by the administration of Freund's Complete Adjuvant. The progression of events consisted

primarily of raised area, multiple scabbing, erythema, and skin thickening.

Challenge phase

1st challenge : topical application: 33.3% (equivalent to a 166 mg topical dose)

2nd challenge: topical application (males): 33.3%. Due to the lack of positive results (a score greater than 0), the females were not re-challenged.

All animals of the test and control groups were pre-treated with 10% SLS in paraffin liquid 1 day prior to topical induction.

Results: No control or test group animal exhibited dermal scores in excess of 1 (discrete or patchy erythema) in males for either the challenge or re-challenge phase. Additionally, scores of 0 (zero) were observed in females for the challenge. As a result of the lack of a positive result for the challenge, the females were not re-challenged.

Conclusion: There was no evidence of reactions indicative of skin sensitisation to the test material under the conditions of the test.

4.11 Repeat dose toxicity

4.12 Repeat dose range-finding toxicity

Test substance: potassium perfluorobutane sulfonate (> 97% purity)

Test Facility: Primedica Redfield (2000e)

Method: Analogous to a repeated dose range finding study described in the OECD Guideline No. 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

The purpose of the study was to identify dose levels for a 28-day oral repeat dose study.

The highest dose level chosen should aim to induce toxic effects but not death or severe suffering.

GLP: No. Clinical observations were conducted under the test facility's quality assurance protocol and standard operating procedures.

Year: 2000

Species/Strain: Rat/Sprague-Dawley

Route of administration: Oral – gavage

Duration: Total exposure days: 10 days

Dose: The dose levels are as follows:

Group	Number and sex of animals	Dose mg/kg bw/day
I (control)	5 males + 5 females	0
II	5 males + 5 females	100
III	5 males + 5 females	300
IV	5 males + 5 females	1000

Frequency of dose: Dose regimen: 7 days per week.

Vehicle: 1% carboxymethylcellulose (medium viscosity) in deionised water

Statistical methods: Statistical differences between the control and treated groups were determined along with an analysis of variance of the treated groups of the same sex compared to the control group at common time points. Statistically significant probabilities were reported as either pδ0.05 or pδ0.01.

Method: The test material was administered daily by oral gavage in graduated doses to four groups of healthy young adult animals aged six to eight weeks, one dose level per group for a period of 10 consecutive days. The dose levels comprised of 0, 100, 300 and 1000 mg/kg bw/day of the test material. Individual doses were calculated using the most recent collected body weights. The dose volume was 25 mL/kg.

Animals in the control group (0 mg/kg bw/day) were handled in an identical manner to the test group subjects. The control group animals received the vehicle in the highest volume used in administering the test material to the 100, 300 and 1000 mg/kg bw/day dosage groups.

During the period of administration the animals were observed closely, each day for signs of toxicity. Animals that died or were euthanased, via carbon dioxide asphyxiation, during the test were necropsied and at the conclusion of the test on Study Day 11 surviving animals were euthanased and necropsied. Details of the clinical observations undertaken during the course of the study are listed below.

Observations of clinical mortality and morbidity were recorded twice daily (morning and evening). Body weights and feed consumptions were recorded on Study Days 1, 7, and 10.

Blood samples were processed and evaluated for the following parameters: total protein, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, triglycerides, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, creatinine kinase, lactate dehydrogenase, triglyceride, lipase, amylase, sorbitol dehydrogenase, calcium, phosphorus, sodium, potassium and chloride.

Haematological parameters monitored were leukocytes (white blood cells; WBC), erythrocytes (red blood cells; RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), nucleated red blood cell count (NRBC), lymphocytes, segmented neutrophils, monocytes, eosinophils, basophils, abnormal lymphocytes, activated partial thromboplastin time (APTT) and prothrombin time.

All animals were submitted to a complete necropsy. A complete necropsy was defined as examination of the external surfaces of all the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

Organ weights were recorded for the following organs prior to fixation: adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes and thymus. Paired organs were weighed together.

The following organs and tissues were examined in situ, dissected free and fixed in 10% neutral buffered formalin: adrenal glands, aorta, bone marrow (sternum), brain (brain stem, cerebellum, cerebum), cervix, epididymies, oesophagus, eyes (with optic nerve), femur (with articular surface), Harderian gland, heart, large intestine, small intestine, kidneys, lacrimal gland, liver, lungs (with bronchi), lymph nodes (mandibular, mesenteric), mammary gland, ovaries, oviducts, pancreas, pituitary gland, prostate gland, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin (abdominal), spinal cord (cervical, thoracic, lumbar), spleen, stomach (forestomach, glandular), testes, thymus, thyroid/parathyroid, tongue, uterus, urinary bladder, Zymbal's gland and vagina.

Tissues, identified in the above paragraph, from the control group and the 1000 mg/kg bw group and all gross lesions found were processed for microscopic evaluation. Tissues were evaluated for the presence of non-neoplastic lesions. The test allowed for non-neoplastic lesions to be graded as to severity as trace (1), mild (2), moderate (3) and severe (4) and for the microscopic evaluation of all tissues identified in the above paragraph for all animals found dead or sacrificed in extremis.

Results:

Clinical signs: There were no adverse clinical signs observed at any treatment level for either male or female rats.

Body weight: There were no significant effects of the test material on male or female body weights and body weight changes.

Feed consumption: There were no significant effects of the test material on absolute feed consumption.

Haematology and Biochemistry findings: No dose dependent effects on clinical chemistry findings were observed in this study.

A significant increase ($p < 0.01$) in glucose levels and segmented neutrophils was observed in female rats in the 1000 mg/kg bw/day treatment group compared to the controls. The mean glucose levels increased from 107 ± 17.1 mg/dL in the control group to 163 ± 23.5 mg/dL in the treatment group and the mean segmented neutrophils increased from $0.5 \pm 0.25 \times 10^3/mm^3$ to $1.2 \pm 0.34 \times 10^3/mm^3$ in the treatment group. There was no clear association of these observations with other reported findings including histopathology.

All other heamotological measurements in the treatment groups were comparable to the control group.

In the 100 mg/kg bw/day treatment group, mean albumin levels in males significantly ($p < 0.01$) decreased from 4.4 ± 0.10 g/dL in the control group to 3.9 ± 0.39 g/dL. In the 300 mg/kg bw/day treatment group males, gamma-glutamyltransferase (GGT) significantly ($p < 0.051$) increased from 0 ± 0 U/L in the control group to 4 ± 2.4 U/L. In the 100 and 300 mg/kg bw/day treatment group, mean chloride levels significantly increased to 89 ± 2.2 mmol/L ($p < 0.05$) and 90 ± 1.5 mmol/L ($p < 0.01$), respectively, in males compared to control values of 87 ± mmol/L. In the 300 mg/kg bw/day treatment group, mean alkaline phosphatase significantly decreased ($p < 0.01$) from 22126.3 U/L in the control group to 14041.2 U/L in the females.

It is unlikely that the above-mentioned clinical chemistry changes are related to the test material, as the changes were not observed at 1000 mg/kg bw/day in either gender.

Mortality: There was no mortality observed at any treatment level for either male or female rats.

The lack of mortality in the range finding study is consistent with the results of the acute oral toxicity study, which reported an LD50 for the test material greater than 2000 mg/kg.

Necropsy findings:

Gross pathology and Histopathology:

Three singular incidences of enlarged mandibular lymph nodes were noted in one male control rat and one female rat in each of the 300 or 1000 mg/kg bw/day treatment groups. Enlarged mandibular lymph nodes correlated with either mild lymphoid hyperplasia (vehicle control male and high dose female, respectively) or mild plasmacytosis (female that received 300 mg/kg).

In the 100 mg/kg bw/day treatment group, one female rat had diverticulum in the small intestine, one male rat had small seminal vesicles and one male rat had kidney dilation. The kidney dilation and diverticulum of the small intestine correlated microscopically with unilateral moderate hydronephrosis and diverticulum, respectively.

In the 1000 mg/kg bw/day treatment group, one male rat had focal liver changes. Other changes observed were focus changes in the lungs (one male and one female); inflammation of the trachea (one female); inflammation of the oesphagus (two females) with one of these animals also presenting with oesophageal haemorrhage; a non-neoplastic ultimobranchial cyst of the thyroid (one female) and oedema and inflammation of the glandular stomach (one female each).

In male animals that received 1000 mg/kg bw/day of the test material, lung foci correlated microscopically with moderate focal haemorrhage. In female animals that received 1000 mg/kg bw/day of test material, lung foci correlated microscopically with mild chronic focal inflammation accompanied by mild crystalline material.

The study facility reported lesions representative of early cardiomyopathy in the vehicle control rats and the rats that received 1000 mg/kg bw/day of the test material, but this is not regarded as treatment related. The study reports this finding is a common spontaneous lesion in Sprague-Dawley rats. No reference was provided.

None of the findings were related to the test material.

Organ weights: Absolute and relative organ weights were similar to the corresponding control groups for male and female rats across treatment groups with the exception of absolute and relative liver weights and the percent brain weight and liver weights.

Mean percent increase in absolute liver weight, liver percent body weight and percent brain weight to liver weight in male (female percentages are in parenthesis) that received 1000 mg/kg bw/day of the test material were 36% at $p < 0.01$ (female: 22% at $p < 0.05$), 34% at $p < 0.01$ (female: 18% at $p < 0.01$) and 40% at $p < 0.01$ (female: 22% at $p < 0.05$), respectively. These changes were not associated with clinical chemistry and histopathology changes. The biological and toxicological significance of these observations is unclear due to the lack of supportive histopathological findings.

Conclusion: As a guide for dose levels in the 28-day oral repeat dose study, a maximum dose of 1000 mg/kg bw/day is indicated in this study.

4.13 Repeat dose toxicity

Test substance: potassium perfluorobutane sulfonate (> 97%)

Test Facility: Primedica Redfield (2001)

Method: The method is analogous to the OECD Guideline No. 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.

GLP: Yes (with the exception of the analysis of the formulated test material).

Year: 2001

Species/Strain: Rat/Sprague-Dawley

Route of Administration:

Oral – gavage

Duration: Total exposure days: 28 days

Dose:

Group	Number and sex of animals	Dose mg/kg bw/day
Control	10/sex (5)	0
Low-dose	10/sex	100
Mid-dose	10/sex	300
High-dose	10/sex (5)	900

Number in parenthesis indicates the number of recovery animals (14 to 15-day recovery period)

Frequency of Dose: Dose regimen: 7 days per week.

Vehicle: 1% carboxymethylcellulose (medium viscosity) in deionised water

Statistical methods used:

Statistical differences between the control and treated groups were determined along with an analysis of variance of the treated groups of the same sex compared to the control group at common time points. Statistically significant probabilities were reported as either $p<0.05$ or $p<0.01$.

Description of method: The purpose of the study was to evaluate the toxicity potential associated with the oral administration (gavage) of the test material in Sprague-Dawley rats for a 28-day repeated dose with a 14 to 15-day recovery period.

The highest dose level chosen was aimed to induce toxic effects but not death or severe suffering, with the descending sequence of dose intended to demonstrate any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). The dosage levels were chosen based on the results of the above-mentioned oral dose-finding study.

Results:

Clinical signs: Clinical observations included localised alopecia on the back, limbs, neck and underside; scabbed area on back and neck; soft or liquid faeces; and urine stained abdominal fur. All these observations are considered to be incidental because they were not dosage related.

Body weight: No treatment-related effects on body weight and body weight gain were noted. During the recovery period, a significant decrease ($p < 0.05$) of approximately 7% of control values in mean body weight in males treated at 900 mg/kg bw/day was recorded on Study Day 36. The mean body weight in the control and 900 mg/kg bw/day treatment groups was 421.0±24.4 and 393±24.4

gram, respectively. This corresponded to 6 out of the 10 animals in the 900 mg/kg bw/day treatment groups on Study Day 36 with a mean body weight less than the control value. This observation was not regarded as treatment related because it resolved at the conclusion of the recovery period.

Food consumption: No treatment-related effects on food consumption were noted.

Haematology:

The reported significant haematological findings were considered coincidental and not associated with the administered test material in this study as the incidences were not dosage dependent. The changes are described below.

A significant increase ($p < 0.05$) in Activated Partial Thromboplastin Time (male: $p < 0.05$, approximately 3% above the control values; females: $p < 0.05$, approximately 13.5% above the control values) and Prothrombin Time (male: $p < 0.01$, approximately 6% above the control values; female $p < 0.05$, approximately 5% above the control values), and a significant decrease in platelet levels (male: $p < 0.05$, approximately 11% below the control values; female: $p < 0.05$, approximately 20% below control values) was observed during the recovery period in animals treated at 900 mg/kg bw/day. The biological and toxicological significance of these observations is unclear due to the lack of supportive histopathological findings.

Biochemistry findings:

A significant decrease in serum phosphorus and potassium in male rats treated at 300 mg/kg bw/day and 900 mg/kg bw/day were observed. The decrease for potassium was approximately 20% of the control values at a level of significance of $p < 0.05$ in both treatment groups. The decrease for phosphorus was approximately 16% of control values at a level of $p < 0.05$ in both treatment groups.

A significant increase ($p < 0.05$) in chloride levels in males treated at 900 mg/kg bw/day (approximately 4% above the control values) was observed. The biological and toxicological significance of these observations is unclear.

Other observations such as a significant increase in serum potassium (approximately 16% of the control values; $p < 0.05$) in females treated at 100 mg/kg bw/day and a significant decrease in alkaline phosphatase (approximately 30% of the control values; $p < 0.05$) during the recovery period in females treated at 900 mg/kg bw/day are not dose related in nature and did not appear to be related to the administration of the test material.

Mortality: No mortality was recorded during the main study or the recovery period.

Necropsy findings:

Gross pathology:

One gross lesion (small right testes in a animal treated at 100 mg/kg bw/day) was recorded at necropsy, but was not considered to be related to the administration of the test material.

Organ weight changes:

Significant increases in absolute and relative male liver weights (approximately 25% and 30%, respectively) and female kidney weights (approximately 11% and 9%, respectively) were observed in animals that received 900 mg/kg bw/day compared to controls. These findings were not observed in the recovery animals. These changes appear to be associated with the test material. The biological and toxicological significance of these observations is unclear due to the lack of supportive or histopathological findings. However, the liver and kidney may be seen as the potential target organs for toxicity and suggests liver weight changes are relevant

Histopathology: The incidental microscopic lesions observed in the test animals are not considered to be related to the administration of the test material.

Motor activity: There were no test-material related effects on motor activity and audio/visual evaluations. An examination of peripheral neuropathy function showed a gender specific statistically significant increase in tail flick latency (suggestive of hyperactivity) for males treated at 100 mg/kg bw/day ($p<0.01$), 300 mg/kg bw/day ($p<0.05$), and 900 mg/kg bw/day ($p<0.01$).

Conclusion: A No Observed Effect Level (NOEL) was established as 100 mg/kg bw/day in males in this study based on significant decreases in serum phosphorus and potassium at doses of 300 and 900 mg/kg bw/day when compared to controls. A No Observed Adverse Effect Level (NOAEL) was established as 300 mg/kg bw/day in females and males in this study based on significant increase in kidney and liver weights, respectively, in animals that received 900 mg/kg bw/day compared to controls. The interpretation of gender specific observation of tail flick in male rats in this study is uncertain.

4.14 Repeated dose toxicity 90 days

Test Substance: potassium perfluorobutane sulfonate (> 98%)

Test Facility: Argus Research (2003a)

Method: The test facility reported the methodology as the U.S. Environment Protection Agency Health Effects Test Guidelines; 90-Day Oral Toxicity in Rodents. Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.3100, August 1998. A copy of the protocol was provided but has not been confirmed for consistency with the above-mentioned methodology. The method is analogous with the OECD 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

The test facility reported no protocol deviations to the above-mentioned methodology that adversely affected the integrity of study.

GLP: Yes

Year: 2003

Species/Strain: Rat/Crl:CD∨(SD)IGS BR VAF/Plus∨

Route of Administration:

Oral – gavage

Exposure Information: Total exposure days: 90 to 93 day.

Dose regimen: 7 days per week.

Doses: The test material was administered once daily for 90-93 days with the dosage 10 mL/kg adjusted for the most recently recorded body weight to the following treatment groups:

Group	Number and sex of animals	Dose mg/kg bw/day
Control	10/sex	0
Low-dose	10/sex	60
Mid-dose	10/sex	200
High-dose	10/sex	600

Vehicle: 0.1% carboxymethylcellulose (medium viscosity) in deionised water.

Statistical methods used:

Statistical differences between the control and treated groups were determined along with an analysis of variance of the treated groups of the same sex compared to the control group at common time points. Statistically significant probabilities were reported as either pδ0.05 or pδ0.01.

Description of method: The method involved eighty rats approximately 37 days old randomly assigned to four dosage groups.

Observations for clinical signs and deaths were made daily before dosage and approximately 60 minutes after dosage administration, and on the day of sacrifice. Detailed clinical observations were conducted for all male and female rats once before the first dosage and at least one weekly thereafter. Signs noted included, but were not limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity, e.g., lacrimation, pilo-erection, pupil size, and unusual respiratory pattern. Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes, e.g.,

excessive grooming, repetitive circling, or bizarre behaviours, e.g., self-mutilation, walking backwards, were recorded.

Ophthalmological examinations were performed for all rats prior to dosage and at termination of the study. Body weights for male and female rats were recorded weekly during the dosage period and at sacrifice. Feed consumption values were recorded weekly during the dosage period.

A functional observational battery and motor activity assessment were conducted on five male and five female rats per group. The following parameters were assessed:

- lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, pilo-erection, respiration, and urination and defecation (autonomic responses);
- sensor-motor response to visual, auditory, tactile and painful stimuli (reactivity and sensitivity);
- reaction to handling and behaviour in the open field (excitability);
- gait pattern in the open field, severity of gait abnormalities, air righting reaction, and landing foot splay (gait and sensorimotor coordination);
- forelimb and hindlimb grip strength; and
- abnormal clinical signs including but not limited to convulsions, tremors and other unusual behaviour, hypotonia, emaciation, dehydration, unkempt appearance and deposits around the eyes, nose or mouth.

All surviving rats were sacrificed on the day following the last administration of the test material. Rats were exsanguinated following sacrifice and blood was collected for haemotological and clinical biochemical evaluations.

The haemotological parameters investigated were erythrocyte count (RBC), haematocrit (HCT), haemoglobin (HCG), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total leukocyte count (WBC), differential leukocyte count, platelet count, mean platelet volume (MPV) and cell morphology. The sera was analysed for total protein, triglycerides, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, total bilirubin, urea nitrogen (BUN), creatinine, alanine aminotransferase, asparatate aminotransferase, alkaline phosphatase, calcium, phosphorus, sodium, potassium and chloride.

Upon termination of exposure, histopathological examination on all prepared tissues from the control and 600 mg/kg bw/day treatment group was performed.

A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross necropsy included an initial physical examination of the external surfaces and all orifices as well as an internal examination of tissues and/organs in situ. In addition, the cranial, thoracic and abdominal cavities were examined. Selected organs were weighed and retained. All gross lesions were examined histologically. Histological examination was performed on all tissues from the control (0 mg/kg bw/day) and 600 mg/kg bw/day dosage groups and also the nasal cavities and turbinates, stomachs and kidneys of the male and female rats in the 60 and 200 mg/kg bw/day dosage groups.

The following organs were excised, trimmed and weighed prior to fixation with paired organs weighed as pairs: adrenals, brain, kidneys, heart, thymus, spleen, liver, testes, epididymies, ovaries and uterus. The following tissues or representative samples were retained in neutral buffered 10% formalin: adrenals, aorta, bone marrow (sternum), brain, (cerebrum, cerebellum, medulla), epididymies, oesophagus, eyes (with optic nerve), femur, heart, large intestines (colon, cecum, rectum), small intestine (duodenum, jejunum, ileum), kidneys, larynx, pharynx and nose, liver, lungs, lymph nodes (mandibular, mesenteric), mammary glands (with skin, female rats only), ovaries, pancreas, Peyer's patches, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, uterus, urinary bladder and vagina.

Results:

Clinical signs: One male rat died on Study Day 85 in the 600 mg/kg bw/day treatment group. One to two days preceding the death in the 600 mg/kg bw/day treatment group, red perioral substance and urine-stained fur, decreased motor activity, coldness to the touch, ptosis, dehydration, brown substance around the mouth and un-groomed appearance was observed in the animal. Necropsy revealed thin walls of the stomach in the cardiac region, gaseous distension of the stomach and intestines, red thymus and a small spleen. The death is not considered related to the administration of the test material because it was a single event with a sudden onset of adverse clinical symptoms. All other male rats survived until scheduled sacrifice. All female rats survived to scheduled sacrifice.

The incidence of a red perioral substance (slight to extreme in 6 rats) and urine-stained abdominal fur (3 rats) were significantly increased ($p \delta 0.01$) in male rats in the 600 mg/kg bw/day treatment group compared to the controls.

Chromorhinorrhoea occurred in two and three rats in the 200 and 600 mg/kg bw/day dosage groups, respectively.

All other clinical observations in the male and female rats were considered unrelated to the test material because the incidences were not dosage dependent and/or observations occurred in only

one (male) or one or two (female) animals per group. The effects were observed randomly across all doses. These clinical observations in the male rats included localised alopecia of the limbs and neck, discoloured fur, swollen ears or snout, excess salivation, red substance in cage pan, decreased motor activity, dehydration, ptosis, brown substance in cage pan, un-groomed coat, cold to touch, soft or liquid faeces, scab on the neck, head or forelimb, ulceration on neck, chromdacryorrhea, missing/broken incisors and abrasion on neck or head. These clinical observations in the female rats included missing/broken or misaligned incisors, localised alopecia of the limbs and back, chromdacryorrhoea, urine-stained abdominal fur, red, slight perioral substance, excess salivation, swollen ears, rales, red substance in cage pan, soft or liquid faeces, chromorhinorrhea, bent tail and abrasion on forepaw.

Body Weights: Terminal body weights for the male and female rats were comparable among the four dosage groups and did not differ significantly.

In the male rats, body weight gains were significantly reduced ($p \delta 0.01$) on Study Days 15 to 22 in the 60 mg/kg bw/day and 200 mg/kg bw/day treatment groups by approximately 20% and 21%, respectively, compared to controls.

In female rats, body weight gains were significantly increased ($p \delta 0.01$) on Study Days 43 to 50 in the 60 mg/kg bw/day treatment group by approximately 103% compared to controls.

These changes in body weights are not considered treatment related. This is because the incidence was not dosage related and occurred only once.

Feed Consumption: Absolute and relative feed consumption values for male and female rats were comparable among the four dosage groups and did not differ significantly.

Opthalmologic findings: Opthalmological examination of the male rats at study termination revealed one male rat in the 200 mg/kg bw/day dosage group with chromodacryorrhea of both eyes; all opthalmological examinations were normal prior to dosage administration.

Ophthalmological examination of the female rats prior to dosage treatment revealed one female rat in the 60 mg/kg bw/day dosage group with iritis of the left eye, all opthalmological examinations were normal at study termination.

No significant dose related opthalmological changes as a result of the administration of the test material were observed in either gender.

Haematology: Average values for haemoglobin concentration and haematocrit were significantly decreased ($p \delta 0.05$ or $p \delta 0.01$) in male rats in the 200 and 600 mg/kg bw/day treatment groups by approximately 5% to 7% compared to controls. Average values for red blood cells

were significantly decreased ($p \delta 0.05$) in male rats in the 600 mg/kg bw/day treatment group by approximately 7% compared to controls. At necropsy, no concurrent dose-related focal necrosis of the inter-sternal cartilage was observed, with only two of ten female rats (minimal) in the 600 mg/kg bw/day treatment group and four of ten male rats (2 minimal, one mild and one moderate) in the control group displaying focal necrosis.

The mean corpuscular haemoglobin concentration was significantly increased ($p \delta 0.05$) in female rats in the 60 mg/kg bw/day treatment group by approximately 2% compared to controls. This change is not considered treatment related because it was not dose related and was not observed in the other groups. No treatment-related changes in Prothrombin Time and Activated Partial Thromboplastin were observed in either gender.

Average values for leukocytes, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, mean platelet volume, prothrombin time, activated partial thromboplastin, nucleated red blood cell count, segmented neutrophils and bands, monocytes, oesinophils, basophils, lymphocytes and other cells were comparable among the four dosage groups for male and female rats.

Biochemistry findings: A significant increase ($p \delta 0.01$) in serum chloride in male rats in the 600 mg/kg bw/day day treatment group of approximately 3% compared to controls was observed. Average total protein and albumin values were significantly decreased ($p \delta 0.05$) in female rats in the 600 mg/kg bw/day day treatment group by approximately 7% and 10%, respectively, compared to controls. Average values for glucose, cholesterol, total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphate, calcium, inorganic phosphorus, triglycerides, sodium, potassium, globulin and the albumin/globulin ratio were unaffected in either gender by dosages of the test material as high as 600 mg/kg bw/day.

Organ weights changes: A significant decrease ($p \delta 0.05$ or $\delta 0.01$) in absolute weight of the spleen in male rats in the 60, 200 and 600 mg/kg bw/day treatment groups of approximately 16%, 11% and 13%, respectively, compared to controls, was observed. In addition, in male rats a significant decrease ($p \delta 0.05$ or $\delta 0.01$) compared to controls in the weight of the spleen (approximately 10%-13%) and the spleen weight to brain weight (approximately 15%-18%) in the 60 and 600 mg/kg bw/day treatment groups, respectively, was observed. The toxicological significance of these organ weight changes in male rats, however, is unclear.

The weights of the epididymis, testes, brain, liver, kidneys, adrenals, thymus and heart and the ratios of these organ weights to the terminal body weights and the brain weights for the male rats were unaffected by dosages of the test material as high as 600 mg/kg bw/day.

In the female rats, the weights of the brain, liver, kidney, adrenals, spleen, ovaries, uterus and heart, and the ratios of these organ weights to the terminal body weights and the brain weights, were unaffected by dosages of the test material as high as 600 mg/kg bw/day.

Necropsy findings: The results of the necropsy examination in the male rat found dead in the 600 mg/kg bw/day dosage group is described above. All the surviving male and female rats appeared normal at necropsy.

Histopathology: No treatment-related microscopic changes were observed in any of the tissues specified for examination in the 60 and 200 mg/kg bw/day treatment groups in either gender. Treatment-related microscopic changes, however, were observed in the kidneys and stomach of the male and female rats in the 600 mg/kg bw/day treatment group.

The treatment-related changes in the kidney were increased incidence of hyperplasia of the epithelial cells of the medullary and papillary tubules and ducts in the inner medullary region. This occurred in 60% and 80% of female and male rats, respectively, in the 600 mg/kg bw/day treatment group compared to no more than 10% of the control animals. Six (one minimal and five mild) of ten female and eight (four minimal, three mild and one moderate) of ten male rats in the 600 mg/kg bw/day treatment group presented with the increased incidence of hyperplasia. The tubules had a dark appearance with increased amounts of small interstitial cells with prominent dark nuclei. Other treatment-associated changes that occurred at lower or single incidences in the kidneys of rats of this group were minimal foci of papilliary oedema in three of ten male and three of ten female rats, and moderate papilliary necrosis in one of ten male rats.

In addition to the results from the study laboratory, kidney slides of both the control and treated animals from the 90-day study of potassium PFBS were also examined by an independent pathologist. The overall conclusion from the independent pathologist indicates no renal effect secondary to treatment (3M, 2005).

The treatment-related changes in the stomach were increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the fore-stomach in the male and female rats in the 600 mg/kg bw/day treatment group, i.e., in 80% and 90% of male and female rats, respectively, compared to more than 10% of the control animals. This represented eight (5 minimal and 3 mild) of 10 male and nine (3 minimal and six mild) of 10 female rats in the 600 mg/kg bw/day dosage group with the necrosis. In this group, five (4 minimal and 1 mild) of 10 male and seven (6 minimal and 1 mild) of 10 female rats presented with hyperplasia/hyperkeratosis of the limiting ridge of the stomach compared to no incidences in the control, 60 and 200 mg/kg bw/day dosage groups.

The changes in the stomach in the male and female rats was considered to be treatment-related in the 600 mg/kg bw/day treatment group.

Microscopic examination of the nasal cavity and nasal turbinates revealed a few equivocal microscopic changes that occurred at low and sporadic incidences in rats in the 200 mg/kg bw/day and 600 mg/kg bw/day treatment groups. These changes occurred primarily in the posterior nasal cavity/turbinates and involved the olfactory mucosa. These histomorphological changes included single or low incidences of multifocal necrosis or atrophy of the olfactory mucosa, focal acute/subacute or chronic inflammation, adhesions of the turbinate to either an adjacent turbinate or to the lateral nasal wall, focal hyperostosis of turbinate bone and/or foci of olfactory epithelial hyperplasia.

The test facility noted the lesions in the nasal cavity/turbinates were of uncertain significance and origin mainly because they occurred in the 200 m/kg bw/day and 600 m/kg bw/day treatment groups at very low and sporadic incidences and were multifocal in distribution. The test facility also noted that the mechanism of the lesions in the nasal cavity/turbinates were not typical or consistent with a systemic toxic effect, and many of the lesions were more suggestive of local irritating effect on the nasal mucosal membranes. The cause in the study of any irritating effect on the nasal mucosa in the rats, therefore, is uncertain. Insufflation of the test material into the nasal cavities was not reported in the study but cannot be unequivocally discounted.

Motor Activity and Functional Observational Battery:

There were no statistically significant or biological differences among the four dosage groups in the measures of the functional observational battery (FOB). There were no alterations in home cage behaviour, autonomic functions (lacrimation, salivation, palpebral closure, prominence of the eye, pupillary reaction to light, pilo-erection, respiration, defecation and urination), sensorimotor functions (responses to visual, auditory, tactile and painful stimuli, excitability, gait and sensorimotor coordination (gait pattern in the open field, severity of gait abnormalities, air righting reaction and landing foot splay) and forelimb and hindlimb grip strength and abnormal clinical observations.

The 200 mg/kg bw/day dosage was associated with increased reductions in red blood cells, haemoglobin concentration and haematocrit in male rats. The 600 mg/kg bw/day dosage was associated with a decrease in red blood cells only. The toxicological significance of these changes are not clear.

Increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach, along with minimal or mild thickening of the mucosa of the limiting ridge due to hyperplasia and hyperkeratosis in the male and female rats was considered to be treatment-related in the 600 mg/kg bw/day

treatment group. These effects may be due to a direct irritation effect of PFBS. Treatment-related microscopic changes were observed in the kidneys and stomach of the male and female rats in the 600 mg/kg bw/day treatment group.

Conclusion: The NOAEL in rats was established as 200 mg/kg bw/day in this study based on necrosis in the limiting ridge of the forestomach.

A significant ($p \delta 0.05$) decrease in average total protein and albumin values of 7% and 10%, respectively, was seen in males, and a significant ($p \delta 0.01$) increase in serum chloride was seen in the males. Kidney hyperplasia was also reported at 600 mg/kg bw/day. However, histopathological of the kidney by an independent expert reported that no consistent changes were seen in the kidneys and the renal effects were not secondary to treatment.

4.15 Genotoxicity

4.16 In vitro-mutagenicity in bacteria

Test Substance: potassium perfluorobutane sulfonate (100% purity)

Test Facility: Sitek Research Laboratories (2001a)

Method: The study method was the Plate Incorporation/Preincubation Mutation Assay. The experimental procedures for the study method were essentially those of Ames, B. N., McCann, J., Yamasaki, E., *Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test* (Ames et al., 1975); Maron, D. R. and Ames, B. N. *Revised methods for the Salmonella mutagenicity test*. (Maron, 1980); Green M. H. L., and Muriel, W. J., *Mutagen testing using trp + reversion in Escherichia coli*. (Green & Muriel., 1977); and Venitt, S., and Parry, J. M., (eds) *Mutagenicity Testing: A Practical Approach* (Venitt & Parry, 1984).

The method was analogous with OECD Guideline 471 Bacterial Reverse Mutation Test.

A dose finding test was performed using strains TA100 and WP2uvrA using seven doses ranging from 5.0 to 5000 µg/plate with and without induced rat liver S-9 (one plate per dose). A definitive mutagen assay and a second confirmatory mutagen assay were performed.

Fresh cultures of bacteria were grown up to the late exponential/early stationary phase of growth and checked for high titre viability. Five strains of bacteria are used. The selected *S. typhimurium* strains have GC base pairs at the primary reversion site to detect mutagens and cross-linking agents. WP2uvrA was included as some mutagens and cross-linking agents may not be detected by the *S. typhimurium* strains.

The amino acid requirement for growth was demonstrated for the culture preparation. All of the *Salmonella. typhimurium* strains were confirmed positive for histidine dependence. E coli strain WP2uvrA was confirmed positive for trypthophan dependence. All *Salmonella typhimurium* strains were confirmed positive for the rfa mutation. The R-factor strains, TA98 and TA100 were confirmed positive for the pKM101 plasmid. The titre of the stock solutions for each strain indicated the stock cultures contained approximately between 5.0×10^8 and 1.0×10^9 bacteria per mL.

A response was considered a positive response if either strain TA98 or TA100 exhibited a mean reversion frequency that was at least double the mean reversion frequency of the corresponding solvent control in at least one dose, or if either strain TA1535, TA1537 or WP2uvrA exhibited a three-fold increase in the mean reversion frequency compared to the solvent control in at least one dose. In addition, the response must have been dose dependent, or increasing concentrations of the test article must have showed increasing mean reversion frequencies. In evaluation of the results, consideration was given to the degree of toxicity exhibited by the dose causing the two-to three-fold or greater increase in the reversion frequency and the magnitude of the increase in reversion frequency.

The negative control was a solvent DMSO control with the plated *S. typhimurium* strains TA1535, TA1537 and the E. coli strain WP2uvrA without the test substance, and otherwise treated in the same ways as the treatment groups, both with and without metabolic activation.

Strain specific positive controls were used. The positive control chemicals used in the presence and absence of exogenous metabolic activation were as follows:

Strain	S-9	Chemical	Concentration (∝g/plate)
TA98	□	2-nitrofluorene	5.0
TA98	+	2-aminoanthracene	1.25
TA100	□	sodium azide	1.0
TA100	+	2-aminoanthracene	1.25
TA1535	□	sodium azide	1.0
TA1535	+	2-aminoanthracene	1.25
TA1537	□	9-aminoacridine	50
TA1537	+	2-aminoanthracene	1.25
WP2uvrA	□	methyl methanesulfonate	4000
WP2uvrA	+	2-aminoanthracene	10

GLP: Yes

Year: 2001

Species/Strain: *Salmonella typhimurium*:

TA98, TA100, TA1535 and TA1537

Escherichia coli: WP2 uvrA.

Metabolic Activation System:

Rat liver S9 fraction (induced with Aroclor 1254 or phenobarbital)

Concentration Range in Main Test:

a) With metabolic activation: 0 - 5000 µg/plate.

b) Without metabolic activation: 0 - 5000 µg/plate.

Vehicle: DMSO

Results: Summaries of the results of the range finding, definitive mutagen assay and confirmatory mutagen assay were provided. Original data tables were not provided.

No dose-dependent increases in mutation frequency were detected with the test material in any strain in the presence and absence of induced rat liver S-9. Negative and positive controls gave appropriate responses.

When using the positive control reference chemicals, an appropriate positive control response was a mean reversion frequency for the strains TA98 and TA100 that was at least double the mean reversion frequency of the corresponding solvent control in at least one dose, or a three-fold increase in the mean reversion frequency for the strains TA1535, TA1537 or WP2uvrA compared to the solvent control in at least one dose.

Conclusion The test material was not mutagenic to bacteria under the conditions of the *Salmonella typhimurium*/*Escherichia coli* Plate Incorportion/Preincubation Mutagen Assay.

4.17 Chromosome aberration test in cultured Chinese Hamster Ovary cells

Test Substance: potassium perfluorobutane sulfonate (100% purity)

Test Facility: Sitek Research Laboratories (2001b)

Method: Test for chemical induction of chromosome aberration in cultured chinese hamster ovary cells (CHO) with and without metabolic activation was analogous with the OECD Guideline for the Testing of Chemicals No. 473: In Vitro Mammalian Chromosome Aberration Test (OECD 1997a).

The test facility reported the method used as: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline S2A Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. Recommended for Adoption at Step 4 of the ICH Process on 19 July 1995 by the ICH Steering Committee. OECD Proposal for Updating Guideline 473 (In vitro Mammalian Chromosomal Aberration Test) Draft February 1997.

Based on a Range Finding Test (RFT) the toxicity of the test material was assessed in a definitive chromosome aberration assay with concurrent untreated (water), solvent dimethyl sulfoxide (DMSO) and positive controls performed with and without metabolic activation. As the results for the definitive assay were negative in both systems, a confirmatory assay was performed without activation only. The cytotoxicity was evaluated on the basis of the reduction in the Relative Cell Growth (RCG) and the Relative Mitotic Index (RMI).

The in vitro CHO chromosome aberration test used cultures of established CHO cell lines. The doubling time of the cell line used was approximately 12 hours and its model chromosome number was 21.

The CHO cell cultures were exposed to three analysed concentrations of the test material both with and without metabolic activation. Duplicate assays were performed at each concentration and for negative/solvent control cultures. At 3- and 18-hours after exposure of cell cultures to the test material, the assay cultures were treated with the metaphase-arresting substance colchicine, harvested, stained, and metaphase cells were analysed microscopically for the presence of chromosome aberrations. Treatment of the cell cultures commenced at approximately 48 hours after mitogenic stimulation.

Concurrent positive and negative controls both with and without metabolic activation were included in each experiment. The negative controls consisted of untreated cells. Historical data for the untreated negative controls was provided to re-confirm no deleterious or mutagenic effects within the untreated negative control cell cultures of the CHO cell line.

Negative controls were used for every harvest time. To demonstrate the sensitivity of the CHO test system, positive controls used known clastogens appropriate to the metabolic activation condition. These were the clastogens cyclophosphamide at 7.5 ∝g/mL and mitomycin C at 0.2 ∝g/mL in conditions with and without metabolic activation, respectively.

GLP: Yes

Year: 2001

Cell Type/Cell Line: Chinese Hamster Ovary-W-B1 cell line

Metabolic Activation System:

Phenobarbital and ®-naphthoflavone induced rat liver S9 fraction
and the cofactor pool.

Vehicle: McCoy's 5A culture medium.

Test substance Solvent:

Dimethyl Sulfoxide (DMSO)

Metabolic Activation	Definitive Test Substance Concentration (µg/mL)	Exposure Period	Harvest Time
Absent			
Test 1	0*, 1000*, 2500*, 5000*	3 hours	18 hours
Test 2	0*, 1000*, 2500*, 5000*	3 hours	18 hours
Present			
Test 1	0*, 1000*, 2500*, 5000*	3 hours	18 hours
Test 2	0*, 1000*, 2500*, 5000*	3 hours	18 hours

*Cultures selected for chromosome aberration analysis and the determination of Relative Cell Growth (RCG) and Relative Mitotic Index (RMI). One hundred metaphases were scored from each of the two replicate cultures at each concenetration and the controls. The determination of RMI included a test substance concentration of 500 µg/mL. The confirmatory assay (without activation) included test substances concentration of 50, 100, 500, 1000, 2500, 5000 µg/mL.

Results: The results of the Range Finding Study showed there was no obvious reduction (more than 50% versus that of the solvent control) in the RCG even at the highest concentration of 5000 µg/mL in both the non-activated and activated systems. RCGs ranged from 84%-109% and 128%-304% for the non-activated and activated systems, respectively, for the test material concentrations of 5.0- 5000 µg/mL.

The definitive chromosome aberration assay showed no obvious reduction (more than 50%) at any concentration in either system. The RCGs ranged from 104%-133% in the non-activated system and from 84%-110% in the activated system. The RMI data showed that the RMIs ranged from 100%-144% in the non-activated system and from 61%-94% in the activated system. Therefore,

chromosome aberrations were scored from the three highest concentrations of 1000, 2500 and 5000 µg/mL in both the non-activated and activated systems. In addition, the corresponding untreated (water), solvent (DMSO) and positive controls (mitomycin C and cyclophosphamide) were also scored.

The averages of the percentage of the cells with aberrations scored from the assay are summarised below:

	Average Percentage of Cells with Aberrations	
Treatment	**Without activation**	**With activation**
Untreated (water)	0.0%	0.0%
Solvent (DMSO) Control	0.0%	0.0%
Test material (1000, 2500 and 5000 µg/mL)	0.0%	0.0%
Positive Control	25.5%	26.0%

Using the Chi-square test, statistical analysis showed in the definitive assay that none of the test material concentrations in the non-activating and activating system were found to have induced a statistically significant increase in the percentage of cells with aberrations over the solvent controls.

The results of the confirmatory assay without activation showed that the RCGs ranged from 46%-165%. The RMIs ranged from 17%-88%. No metaphases were found at the concentration of 2500 µg/mL. Therefore, chromosome aberrations were scored from three concentrations of 500, 1000 and 5000 µg/mL. In addition, the corresponding untreated, solvent and positive (mitomycin C at 0.2 µg/mL) controls were also scored. The results of the confirmatory chromosome aberration assay are summarised below:

	Average Percentage of Cells with Aberrations
Treatment	**Without activation**
Untreated (water)	0.0%
Solvent (DMSO) Control	0.0%
Test material (1000, 2500 and 5000 µg/mL)	0.0-0.5%%
Positive Control (mitomycin C at 0.2 µg/mL)	29.0%

Using the Chi-square test, statistical analysis showed in the confirmatory assay that none of the test material concentrations in the non-activating system were found to have induced a statistically significant increase in the percentage of cells with aberrations over the solvent controls.

No cytotoxicity was observed at doses up to 5000 µg/mL in either the presence or absence of S9 fraction. No increase in the frequency of cells containing chromosomal aberrations was observed over

control levels either with or without S9. Positive controls gave the expected responses.

Conclusion — The test material was not clastogenic to Chinese Hamster Ovary-W-B1 cells treated in vitro under the conditions of the test.

4.18 Prenatal developmental toxicity

Test Substance: potassium perfluorobutane sulfonate (> 98%)

Test Facility: Argus Research (2002)

Method: Organization for Economic Cooperation and Development (1981). *OECD Guidelines for Testing Chemicals*. Section 4, No. 414: Teratogenicity, adopted 12 May 1981. U.S. Environmental Protection Agency (1988). Health Effects Test Guidelines; Prenatal Developmental Toxicity Study. Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.3700, August 1998. U.S. Environmental Protection Agency (1977). Toxic Substances Control Act (TSCA) Test Guidelines; Final Rule. Prenatal Developmental Toxicity, 799.9370 (cross referenced to OPPTS 870.3700), *Federal Register*, August 15, 1997.

The method is analogous to OECD Guideline 414 Prenatal Developmental Toxicity Study.

The test facility reported no protocol deviations from the above-mentioned methodology that adversely affected the integrity of the study.

This study was designed to provide general information concerning the effects of prenatal exposure on the pregnant test animal and on the developing animal and included assessment of maternal effects as well as death, structural abnormalities, or altered growth in the foetus.

One hundred presumed pregnant female rats were randomly assigned to four dosage groups (described below), 25 rats per group. The animals were dosed according to a constant dose volume of 10 ml/kg of body weight and observed daily from day 0 through day 21 of gestation for body weight and abnormal clinical signs.

The female rats were observed for viability twice each day of the study. Rats were also examined for clinical observations of effects of the test material, abortions, premature deliveries and deaths before and approximately 60±10 minutes after dosage administration and on the day of scheduled sacrifice.

Body weights were recorded daily during the dosage period and prior to sacrifice. Feed consumptions were recorded on days 0, 6, 9, 12, 15, 18 and 20 of gestation. All animals were sacrificed on day 21 by carbon dioxide asphyxiation and examined for the number of corpora lutea, implantation sites, number of viable and non-viable foetuses, and number of early and late resorptions. Gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The

gravid uterus was excised and weighed. Each foetus was weighed and sexed and subjected to external gross necropsy.

Approximately one-half of the foetuses were examined for soft tissue alterations. The remaining foetuses were subjected to a skeletal examination.

GLP: Yes

Year: 002

Species/Strain: Rat/Crl:CD∨(SD)IGS BR

VAF/Plus∨ Number of animals per dose:

5

Route of Administration:Oral – gavage

Vehicle: 0.1% carboxymethylcellulose (CMC), medium viscosity, in deionised water

Dosing regimen: Four groups of single-mated female rats were administered the test material in 0.1% CMC by oral gavage on gestation days 6-20. Doses were adjusted daily on the basis of the individual body weights recorded before intubation.

Doses: 0, 100, 300 and 1000 mg/kg bw.

Statistical methods used:

Animals were assigned to cages according to computer-generated random units. The statistical methods used for analysis of the data were: Bartlett's Test of Homogenicity of Variance and the Analysis of Variance (ANOVA) for e.g, maternal body weight, body weight changes, feed consumption values and litter averages for percent male foetuses, percent resorbed conceptuses, foetal body weights, foetal anomaly data and foetal ossification data; and Dunnett's Test to identify statistical significances of the individual groups for significant (p δ 0.05) ANOVAs. Count data obtained at Caesarean-sectioning were evaluated using the Kruskal-Wallis test. Statistically significant probabilities were reported as either $p<0.05$ or $p<0.01$.

Results: *Mortality*: Two rat deaths occurred in the 1000 mg/kg bw/day treatment group. One death was attributed to an intubation accident. No cause of death was determined for the other rat but was considered unrelated to the test material because it was a single event.

One rat in each of the control and 300 mg/kg bw/day dosage groups began delivery before Caesarean-sectioning on day 21 of gestation (DG 21) and were sacrificed. All other rats survived until scheduled sacrifice.

Clinical Observations and Necropsy: All clinical observations were considered unrelated to the test material because the incidences

were not dosage dependent or the observations occurred in only one or three rats. These clinical observations included localised alopecia on the limbs in rats in the control (vehicle) group, 100 mg/kg bw/day and 300 mg/kg bw/day dosage groups, and cold to touch and dehydration in the 1000 mg/kg bw/day dosage group rat that died as the result of an intubation error. The only necropsy observations were perforation of the eosophagus and red fluid in the thoracic cavity in the 1000 mg/kg bw/day dosage group rat that died as the result of an intubation error. These necropsy observations are considered unrelated to the test material.

Maternal Body Weights, Body Weight Changes and Gravid Uterine Weights: Significant reductions in maternal body weight gains were observed during DGs 6 to 9 ($p \delta 0.05$; approximately 44% of control values) and 18 to 21 ($p \delta 0.01$; approximately 13% of control values) in the 1000 mg/kg bw/day dosage group. There was a significant reduction in maternal body weights gains for the entire gestation period ($p \delta 0.01$; approximately 32% of control values) in the 1000 mg/kg bw/day dosage group. (Correlation of the significant decreases in maternal body weight and maternal body weight gains with significant reductions in absolute and relative feed consumptions is shown below).

Gravid uterine weights were slightly reduced (91% of control values) in the 1000 mg/kg bw/day treatment group but was not statistically significant.

Maternal Absolute and Relative Feed Consumption Values: Absolute and relative feed consumption values were significantly reduced ($p \delta 0.05$ $p \delta 0.01$) on DGs 9 to 12 (relative) and 18 to 21 in the 1000 mg/kg bw/day treatment group compared to controls. These reductions ranged from 5% to 12% of control values. Absolute and relative feed consumption values were significantly ($p \delta 0.01$) reduced by approximately 7% and 5%, respectively, in the 1000 mg/kg bw/day treatment group for the treatment period (DGs 6 to 21) compared to controls.

Litter Observations and Caesarean-sectioning: Foetal body weights (total, male and female) were significantly ($p \delta 0.01$) reduced in the 1000 mg/kg bw/day treatment group compared to controls. These reductions were all approximately 8% to 9% of controls. No other effects were observed. The litter averages for implantations and percent live male foetuses were comparable among the four dosage groups and did not significantly differ. No dams had a litter consisting of only resorbed conceptuses, there were no dead foetuses and all placentae appeared normal. The litter average for corpora lutae was significantly ($p \delta 0.01$) reduced in the 300 mg/kg bw/day dosage group by approximately 14% compared to controls, but the reduction is not considered treatment related because it was not dosage dependent and the corpora lutae formation occurred prior to dosage administration.

No gross external soft tissue or skeletal foetal malformations or variations were considered related to the test material. There were no dosage-dependent or significant differences in the litter or foetal incidences of any gross external, soft tissue or skeletal alterations. A significant (p δ 0.01) increase in the percentage of foetuses with any alteration from 1.1% (4 foetuses) in the controls compared to 4.1% (13 foetuses) in the 100 mg/kg bw/day dosage group is not considered treatment related as it occurred at maternally toxic doses.

Conclusions: A NOAEL of 300 mg/kg bw/day for maternal toxicity was identified in this study, based on reduced body-weight gains and feed consumption at 1000 mg/kg bw/day.

A NOAEL of 1000 mg/kg bw/day for developmental toxicity was indicated in this study as the reduction in foetal body weights in the 1000 mg/kg bw/day treatment group was less than 10% of the control value and most likely associated with maternal toxicity.

4.19 Two generation reproduction study

Test Substance: potassium perfluorobutane sulfonate (> 97% purity)

Test Facility: Argus Research (2003b)

Test method: The method is reported to conform with the U.S. Environmental Protection Agency (1988). Health Effects Test Guidelines; Reproduction and Fertility Effects. Office of Prevention, Pesticide and Toxic Substances (OPPTS) 870.3800, August, 1998. U.S. Environmental Protection Agency (1997) Toxic Substances Test Guidelines; Final Rule Reproduction and Fertility Effects, 799.9380 (cross referenced to OPPTS 870.3800) *Federal Register*, August 15 1997.

The method is analogous to OECD Guideline 416 Two-Generation Reproduction Toxicity Study.

The test facility reported no protocol deviations from the above-mentioned methodology, which adversely affected the outcome or interpretation of the study.

Test animals: The test material was administered to groups of 30 female and male parental (P) animals via oral gavage suspensions beginning at approximately six weeks of age until the day before sacrifice. Dosing continued prior to and during mating, the resultant pregnancy, and through weaning of the resultant F1 offspring. Dosing details are given further below.

After weaning, 30 F1 offspring pups per sex per dosage group were selected to become P2 adults. The substance was administered to the selected F1 offspring during growth into adulthood, mating, and production of the F2 generation, until weaning of the F2 generation offspring. The F1 generation rats were given the same dosage of test substance as their respective sires and dams.

Following 10 weeks of exposure, the F1 generation adults were mated to produce the F2 litters with continual dosing of the test material during their growth into adulthood, mating and production of an F2 generation, and through the weaning of the F2 generation until the day before sacrifice. All F1 pups not selected for the production of the F2 generation were sacrificed.

F1 and F2 generation pups may have been exposed to the test material during maternal gestation or via maternal milk during the postpartum period.

Test design: The observation periods comprised of approximately 10 weeks pre-mating, 2 weeks cohabitation, 3 weeks presumed gestation, 3 weeks lactation and 3 weeks postpartum. The individual housing for the P generation rats and F1 generation litter mating consisted of a male:female ratio per cage of 1:1.

Day 0 of pregnancy was defined as the day a vaginal copulatory plug or spermatozo in a smear of vaginal contents was found. Animals were allowed to litter normally and rear their offspring to weaning. Litter standardization was not performed.

P and F1 generation rat clinical observations and/or general appearance, body weight and feed consumption values were recorded weekly. During postpartum, P and F1 female rat body weight and feed consumption values were recorded on Days 1, 5, 8, 11, 15 and 22 (terminal body weight). Oestrus cycle was evaluated by examination of vaginal cytology beginning 21 days before scheduled cohabitation period and continued until evidence of a vaginal copulatory plug or spermatozoa. The duration of gestation was calculated from Day 0 of presumed gestation to the day the first pup was observed.

Litter observations included the number and sex of pups, stillbirths, live births and gross alterations. Litter size and viability on Days 5, 8, 15 and 22 postpartum, viability indices (percentage of pups born that survive to Days 1 and 5 postpartum), lactation index, percentage of pups that survive from Day 8 to 22 postpartum, and percent survival and sex ratio tabulated on Days 1, 5, 8, 15 and 22 post partum were recorded. The number of implantation sites and general condition of the dam and litter during the postpartum period was recorded.

Pre- and post-weaning litters were observed for dead pups at least twice daily. Pre- and post-weaning clinical observations and/or general appearance were recorded daily. Maternal behaviour was observed on Day 1, 5, 8, 15 and 22 postpartum. Litter pre- and post-weaning body weights and feed consumption values were recorded. Sexual maturation of F1 and F2 offspring were evaluated for female and male rats via the age of vaginal patency (beginning on Day 24 postpartum) and the age of preputial separation (beginning on Day 35 postpartum), respectively.

All study animals found dead or moribund were examined for gross lesions and for the cause of the moribund condition or death. Adult P and F1 generation rats were necropsied and examined for gross lesions. Tissues examined included the external and internal portions of all hollow organs, the external surfaces of the brain and spinal column, the nasal cavity and neck and associated organs and tissues, the thoracic, abdominal and pelvic cavities with associated organs and tissues, the lungs and the musculo/skeletal carcass

Three pups per sex per F1/F2 generation pups not selected for continued examination were randomly selected and examined for gross lesions. All F1 pups not selected for production of the F2 generation were sacrificed on day 22. F2 pups were sacrificed on day 22 and a gross necropsy was performed.

The following organs were individually weighed and organ-to-body weight and organ-to-brain weight ratios calculated in all P and F1 parental rats: brain, kidneys, spleen, ovaries, testes, thymus, seminal fluids (with coagulating glands and fluid), liver, adrenal glands, pituitary, uterus with oviducts and cervix, epididymis and prostate.

The following organs were weighed from the first randomly selected pup per sex per litter for F1 and F2 generation pups (Day 22 post partum): brain, spleen, liver, thymus, kidneys.

The method allowed any gross lesions found, the pituitary, liver, adrenal glands, kidneys, vagina, testis (right), epididymis (right), prostate and seminal vesicles (with coagulating glands) in all P and F1 generation parental male and female rats at necropsy to be retained for histological evaluation. Any gross lesions found, the kidneys, liver and gross lesions from the selected F1 and F2 generation (three pups per sex per litter) on Day 22 postpartum were also retained for histological evaluation.

Examination of the left caudia epididymis was used to evaluate sperm concentration, motility and morphology in P and F1 generation male rats. The left testis was used for evaluation of testicular spermatid concentration. The weight of the right and left testis and epididymis, seminal vesicles (with and without fluid) and prostate were recorded. Histopathology on the right and left epididymis, prostate and seminal vesicles was undertaken.

GLP: Yes

Year: 2003

Species/strain: Rat/Crl:CD∨(SD)IGS BR

VAF/Plus∨ Route of administration: oral – gavage

Doses: 0, 30, 100, 300, and 1000 mg/kg bw

Doses administered prior to mating and during gestation and lactation were based on the individual animal body weight and

adjusted weekly. Certified Rodent Diet∨ #5002 and (reverse osmosis) water were available ad libitum.

Vehicle: 0.1% carboxymethylcellulose (medium vicosity) in reverse osmosis membrane processed deionised water

Sex:
P Males 30 virgin rats per dosage group
P Females: 30 virgin rats per dosage group

F1 Males: 30 virgin rats per dosage group
F1 Females: 30 virgin rats per dosage group

Control group and treatment:
Yes. Concurrent no treatment. Concurrent vehicle.

Frequency of treatment: 7 days per week for two generations (one litter per generation).

Duration of test: Through weaning of F2 animals:

P Males:	approximately 84 days
P Females:	approximately 126 days
Litter of the P-generation/'F1 litter':	approximately 21 days
F1 Male:	approximately 84 days
F1 Females:	approximately 126 days

Pre-mating exposure period for males (P and F1):

P Males: 70 days
F1 Males: 70 days

Pre-mating exposure period for females (P and F1):

P Females: 70 days
F1 Females: 70 days

Statistical methods used:

The statistical methods used for analysis of the data were: Bartlett's Test of Homogenicity of Variance and the Analysis of Variance (ANOVA), i.e., body weight, body weight changes, feed consumption values, organ weight data and litter averages for percent male pups, pup body weights, percent pup mortality data; and Dunnett's Test to identify statistical significances of the individual groups for significant (p δ 0.05) ANOVAs. Count data obtained at natural delivery of dams were evaluated using the Kruskal-Wallis test. Statistically significant probabilities were reported as either $p<0.05$ or $p<0.01$.

Results:

Mortality: P1 Adults – One male rat at 1000 mg/kg bw/day on Study Day 55, one female rat in the control group on Study Day 23 and one female rat moribund sacrificed at 300 mg/kg bw/day on lactation day (LD) 15.

F1 Generation Males: Deaths were seen in the control and all the treated groups. Deaths recorded were: one in the control and 100 mg/kg bw/day groups each, and three in the 30, 300 and 1000 mg/kg bw/day dosage groups, respectively.

F1 Generation Females: One female rat in the 1000 mg/kg bw/day dosage group on post weaning day 35.

No P1 or F1 generation deaths are attributed to the test material.

P Generation Male Rat Findings:

Clinical Signs: Significant increases ($p \delta 0.01$) in the incidences of excess salivation, perioral substance and urine-stained abdominal fur occurred in the 1000 mg/kg bw/day dosage group. The incidence of chromorhinorrea was also increased in this dosage group. No more than two males in the 300 mg/kg bw/day dosage group had observations of excess salivation and perioral substances. No other treatment-related clinical observations were observed because the incidences were not dose dependent and/or occurred in only one or two male rats in any dosage group. These clinical observations included soft or liquid faeces, localised alopecia, missing/broken and/or misaligned incisors, chromodacryorrhea, scabs, red perinasal substance, ulceration on the neck or back, swollen and purple ears, abrasions on the neck, head or axilla, red substance on the penis, swollen snout and dehydration.

Feed Consumption: Absolute and relative feed consumptions were unaffected by dosages of the test material as high as 1000 m/kg.

Body Weight, Organ Weights, and Ratios of Organ Weights: Terminal body weights were unaffected by dosages of the test material as high as 1000 mg/kg.

Body weight gains were significantly ($p \delta 0.01$) reduced compared to controls in the 1000 mg/kg bw/day dosage group on Study Days 43 to 50 and 50 to 57 by approximately 25% and 29%, respectively. A significant ($p \delta 0.01$) reduction in body weight gain from +6.5±5.5gram in the control group to –0.9 ±8.6 gram occurred in this group on Study Days 105 to 112 compared to controls. However, no differences in body weights and terminal body weight were observed in the 1000 mg/kg bw/day group over the study period.

Body weight gains were significantly greater in the 100 mg/kg bw/day dosage group on Study Days 36 to 43 (+24.7±6.9 gm $p \delta 0.05$ compared to controls of +20.3±6.7 gm), in the 300 mg/kg bw/day dosage groups on Study Days 64 to 70 (+14.2±9.0 gm $p \delta 0.01$) compared to controls (+8.9±4.2 gm) and in the 1000 mg/kg bw/day day dosage group on Study Days 36 to 57. These increases were considered unrelated to the test substance because they were not dosage dependent and/or did not persist.

The absolute weight of the liver was significantly ($p \delta 0.01$) increased in the 300 and 1000 mg/kg bw/day dosage group by approximately 11% and 18%, respectively, compared to controls, and was considered treatment related. The ratios of the liver weight to the terminal body weight and liver weight to the brain weight

were significantly ($p \delta 0.01$) increased in the 300 and 1000 mg/kg bw/day dosage groups and were considered treatment related. The percentage increases in the relative liver weights in the
300 and 1000 mg/kg bw/day dosage groups were approximately 10% and 21%, respectively, compared to controls. The percentage increases in the ratio of the liver weight to the brain weight in the 300 and 1000 mg/kg bw/day dosage groups were approximately 13% and 18%, respectively.

The absolute weight of the brain was significantly reduced ($p \delta 0.05$) by approximately 4% in the 30 mg/kg bw/day dosage group compared to control values, but was not considered treatment-related because it was only observed in this group. The ratios of the liver weight and left kidney weight to the brain weight in the 30 mg/kg bw/day dosage group were significantly increased ($p \delta 0.05$ or $p \delta 0.01$) by approximately 9% and 8%, respectively, reflecting the significantly reduced brain weight in this group and are, therefore, not regarded as treatment related.

No other treatment-related changes in terminal body weights and/organ weights and ratios (%) of organ weights to terminal body weight and brain weight in the P generation male dosage groups were observed. The changes observed were incidental and not dosage dependent. The absolute weights of the left epididymis, left cauda epididymis, left testes, right testis minus tunica albuginea, seminal vesicles with and without fluid, right epididymis, right testis, prostate, pituitary, brain, left and right kidneys, left and right adrenals, spleen and thymus and the ratios of these organ weights to the terminal body weight and the brain weight were unaffected by dosages of the test material as high as 1000 mg/kg.

Necropsy and Histopathology: Of the 30 male rats examined, the number of P-generation male rats reported normal on necropsy in the 0 (control), 30, 100, 300 and 1000 mg/kg bw/day dosage groups was 27, 29, 28, 23, and 24, respectively. All necropsy observations were considered unrelated to the test material because the incidences were not dosage dependent and/or the observations occurred in only one or two rats in any dosage group. The necropsy observations were as follows:

Controls: small testes and epididymis, red pin point areas in the thymus, and brown area in the pituitary; 30 mg/kg bw/day group: red pin point areas in the thymus; 100 mg/kg bw/day group: small testes and red pin point areas in the thymus; 300 mg/kg bw/day group: small testes and epididymis, red pin point area in the thymus, slight or moderate dilation of the kidneys, dark red substance in the right pelvis, and absent right adrenal. In the 1000 mg/kg bw/day group, the necropsy observations were small testes, large testes, small epididymis, red pin point areas in the thymus, slight or moderate dilation of the kidney, and dark red substance in right pelvis.

Necropsy of the one male P-generation rat found dead revealed red perinasal and perioral substance and thin clear fluid in the thoracic cavity.

One male rat in each of the control and 1000 mg/kg bw/day dosage groups with small and/or flacid testes and/or epididymis reportedly mated but did not impregnate the cohort female rat.

Treatment-related microscopic changes were observed in the kidney and liver of male rats in the 300 and 1000 mg/kg bw/day dosage groups. The changes in the liver consisted of increased size (hypertrophy) of hepatocytes. The liver cells were enlarged due to an increased amount of finely granular eosinophilic cytoplasm. The hypertrophy generally was present in all areas of the lobules but tended to be more prevalent and more prominent in the centrilobular region.

In the kidney, the primary treatment-related microscopic changes consisted of an increased incidence and severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papillary region. There was also an increased incidence of focal papillary oedema in the 1000 mg/kg bw/day dosage group (minimal in 9 of 30 rats) compared to controls (minimal in 1 of 30 rats). Mild (2 of 30 rats) and moderate (1 of 30 rats) papillary haemorrhage occurred only in the 1000 mg/kg bw/day dosage group.

No treatment-related microscopic changes were observed in the reproductive organs of any male P generation rat selected for histopathologic evaluation that had been given 1000 mg/kg bw/day of the test material. All microscopic changes observed in other tissues specified for examination were considered incidental and unrelated to the administration of the test material.

Reproduction and Fertility: In the P-generation male rats, all mating and fertility parameters, i.e., number of days to inseminate, rats that mated, fertility index, rats with confirmed mating dates during the first and second week of cohabitation, and rats pregnant per rat in cohabitation were unaffected by dosages of the test material as high as 1000 mg/kg bw/day. The slight, non-significant reduction in the fertility index and the number of pregnant rats per number of rats in cohabitation in the 1000 mg/kg bw/day dosage group was considered unrelated to the test material because it was within the historical range of the testing facility.

Sperm motility for the P-generation male rats was unaffected by dosages of the test material as high as 1000 mg/kg bw/day. Group mean values were comparable, with no statistical differences among the five dosage groups, and ranged from 94% to 96%.

A statistically significant ($p \delta 0.05$) reduction of approximately 18% in the number of spermatids per gram of testis was observed in the 1000 mg/kg bw/day P-generation dosage group (147.9±38.9 million sperm/gm) compared to the controls (121.8±35.7 million sperm/gm). This is considered coincidental and not biologically

meaningful. This is because there was no statistically significant treatment-related effects on P-generation male mating and fertility parameters. (It is also noteworthy there was no similar statistical reduction in the number of spermatids per gram of testis observed in the F1 generation rats treated at 1000 mg/kg).

No statistically significant difference was observed in sperm morphology across the treatment groups in the P-generation males. While a statistically significant (pδ0.05) increase in the percent of abnormal sperm in the 1000 mg/kg bw/day day dosage group of the F1 generation was observed, this is not considered biologically significant because the mean percent of abnormal sperm in the P and F1 generation male rats was comparable, i.e. 1.5% to 2.0% and 1.3% to 1.9%, respectively, across the dosage groups. Consequently, the comparable low incidence of head and/or tail abnormalities observed for the P and F1 generation male rats in all treatment groups is not considered treatment related.

P Generation Female Rat Findings:

Clinical Signs: Significant (p δ 0.01) increases in the incidence of dried or wet red perioral substances (7 rats) and salivation (5 rats) occurred in the P generation females in the 1000 mg/kg bw/day dosage group during the cohabitation period compared to the control values. The incidence of dried or wet red perioral substances (3 rats) continued to be significantly (p δ 0.01) increased in this dosage group during the gestation period. All other clinical observations during the pre-cohabitation, gestation and lactation periods were considered unrelated to the test material because the incidences were not dose-dependent and/or the observations occurred in only one or two female rats in any dosage groups. These observations included missing/broken teeth and/or misaligned incisors, chromorhinorrhea, localised alopecia, swollen digits or paws, soft or liquid faeces, swollen snout, swollen and purple ears, clear perinasal substances, exophthalmos, rales, limited use of hindlimb, dehydration, scab on head or mouth, clear or red perivaginal substance, urine-stained abdominal fur, sheath or tip of tail missing and mass on the mouth.

Feed Consumption: No treatment-related effects on absolute and relative feed consumption values were observed during the cohabitation, gestation and lactation period. The changes are not considered toxicologically significant as they were transient and did not persist.

Terminal Body Weights, Body Weight and Body Weight Changes, Organ Weights, and Ratios of Organ Weights: Terminal body weights of the P-generation female rats were comparable among the five dosage groups and did not differ significantly.

In the 1000 mg/kg bw/day dosage group, body weight gains were significantly reduced (p δ 0.01) during the cohabitation period on Study Days 64 to 70 by 14% (+0.5±4.4 gm) compared to control values (+4.3±5.2 gm) and during the gestation period for the days of

gestation (DGs) 0 to 7 by 30% (+20.7±15.3 gm) compared to control values (+29.8±5.4 gm). Body weights changes was significantly reduced ($p \delta 0.05$) in the 30 mg/kg bw/day dosage group during the DGs 0 to 7 by 12% (+26.3±7.1 gm) compared to control values (+29.8±5.4 gm). Additionally body weights were significantly ($p \delta 0.01$ or $p \delta 0.01$) reduced by approximately 5% during the lactation period on the days of lactation (DLs) 8 and 11 in the 1000 mg/kg bw/day dosage group.

Body weight and body weight gains during the pre-cohabitation and lactation periods were unaffected by dosages of the test material as high as 1000 mg/kg bw/day day. The significant reduction ($p \delta 0.05$) in body weight gain on the days of gestation(GDs) 0 to 7 in the 30 mg/kg bw/day dosage group was not considered treatment related because it was not dosage dependent.

All organ weights (pituitary, liver, left and right adrenals, left and right kidneys, spleen, thymus, left and right ovary and uterus with oviducts) and the ratios of these organ weights to terminal body weight and brain weight, were unaffected by dosages of the test material as high as 1000 mg/kg. The weight of the brain was significantly decreased ($p \delta 0.01$) in the 1000 mg/kg bw/day dosage group by approximately 4% compared to the control value.

Necropsy and Histopathology: All necropsy observations were considered unrelated to the test material because the incidences were not dosage dependent and the observations occurred in only one female rat in any dosage group. These observations included small or large spleen, firm tan area on the spleen and tissue (pup) in the stomach.

As previously described, one death occurred in the P-generation female cohort in the control group during gestation (DG 23) and in the 300 mg/kg bw/day dosage group during lactation (DL 15). All tissues examined at necropsy in the P-generation rat found dead on gestation day 23 appeared normal. The test facility attributed the death of the dam to slow or difficult labour. On necropsy, the P-generation female rat which was moribund and sacrificed on LD 15 revealed a small spleen (0.20 gm). All other tissues appeared normal. The only adverse clinical observations reported in this rat prior to sacrifice by the test facility were red peri-vaginal substance on DL 2 and dehydration on DL 15. This rat began to lose weight after DL 8 and feed consumption values were unremarkable.

No treatment-related microscopic changes were observed in the liver of P1 female rats in doses up to 1000 mg/kg.

Treatment-related microscopic changes were observed in the kidney of female rats in the 300 and 1000 mg/kg bw/day dosage group. The change in the kidney consisted of an increased incidence and severity of hyperplasia of the tubular and ductular epitheium in the inner medulla/papillary region. The affected tubules were more densely cellular with variable-sized nuclei. There was also increased incidence of minimal or mild focal papillary oedema in

the 300 and 1000 mg/kg bw/day dosage group and medullary tubular dilation was seen in one 300 mg/kg bw/day dosage group rat and two 1000 mg/kg bw/day dosage group rats. In addition, three incidents of minimal to moderate focal papillary necrosis were observed in the 300 mg/kg bw/day treatment group although none were observed in the 1000 mg/kg bw/day treatment group.

No treatment-related microscopic changes were observed in the reproductive organs of any female P generation rat selected for histopathologic evaluation as high as 1000 mg/kg bw/day of the test material. All microscopic changes observed in other tissues specified for examination were considered incidental and unrelated to the administration of the test material.

Reproduction and Fertility: Natural deliveries were unaffected by dosages of the test material as high as 1000 mg/kg bw/day. These observations included the number of pregnant animals, percent delivered litters, duration of gestation, number of implantation sites per delivered litter, dams with still born pups, dams with live-born pups, gestation index, i.e., the number of rats with live offspring per number of pregnant rats, dams with all pups dying between lactation days 1 to 4, and dams with all pups dying between postpartum days 5 to 21.

Pregnancy occurred in 25 of 29 rats in each dose group. All pregnant rats delivered a litter. The age of vaginal patency and average number of estrous stages per 21 days were comparable among the treatment groups and did not differ significantly. Evaluation of estrous stages at sacrifice did not reveal any differences among the five dosage groups.

All mating and fertility parameters (number of days of cohabitation, rats that mated, fertility index, rats with confirmed mating dates during the first and second week of cohabitation and rats pregnant per rats in cohabitation) were unaffected by dosages of the test material as high as 1000 mg/kg bw/day. The slight non-significant reduction in the fertility index and the number of pregnant rats per number of rats in cohabitation in the 1000 mg/kg bw/day dosage group was considered unrelated to the test substance because it was within the historical control range of the Test Facility.

F1 Generation Litters Finding:

No significant differences were found in the number of pups found dead or presumed cannibalised across all treatment groups compared to control. No significant decrease in the viability index was observed across the treatment groups compared to controls. While a significant decrease ($p < 0.01$) in lactation index was observed in the 100 and 300 mg/kg bw/day treatment groups, no difference was found in the 1000 mg/kg bw/day treatment group compared to controls. The lactation index was 97.4% and 97.7% in the 100 and 300 mg/kg bw/day treatment groups compared to 99.4% in the control group.

The number of pups surviving per litter, the percentage of male pups, litter size and average pup body weight per litter were comparable and did not significantly differ on LDs, 1, 5, 8, 15 or 22 for any of the dosage groups when compared to the control group.

No clinical or necropsy observations in the F1 generation pups were attributable to dosages of the test material as high as 1000 mg/kg bw/day. The incidences were not dosage dependent and/or the observations occurred in no more than one or two litters. The clinical observations included cold to touch, not nursing, umbilical hernia, not nesting, alopecia on back or head, abrasion on back, scab on the back or head, dehydration, missing or black tip of tail, decreased motor activity, corneal opacity, bruised eye or black or absent portion of tail.

Necropsy observations included no milk in the stomach, liver adhered to diaphragm, pedunculated areas of the liver, dilation of the ureter, opaque tan material in the anterior chamber of the eye, large spleen, diaphragmatic hernia, moderate dilation of the renal pelvis and extreme dilation of the lateral ventricles of the brain.

Terminal body weights of the F1 generation pups selected for organ weight evaluation were comparable among the five treatment dosage groups and did not significantly differ. The weights of the brain, spleen, liver, kidney and thymus and ratio of these organ weights to the terminal body weight and brain weight were unaffected by dosages of the test material as high as 1000 mg/kg.

F1 Generation Male Rats Findings:

Clinical Signs: A significant ($p \delta 0.01$) increase in the incidence of excess salivation occurred in the 1000 mg/kg bw/day dosage group.

All other clinical observations were considered unrelated to the test material because the incidences were not dosage dependent and/or the observations occurred in only one or two male rats in the dosage group. These observations are chromodacryorrhea, chromorhinorrhea, missing/broken and/or misaligned incisors, urine-stained abdominal fur, tip or sheath of tail missing, soft or liquid faeces, localised alopecia on limbs, neck or back, scabs on various body regions, swollen ear(s), un-groomed snout, rales, purple or discoloured ear(s), un-groomed coat, axillary mass, red or brown perioral substance, abrasions on head, dehydration, limited use of forelimb, red urine, microphthalmia, abdominal distension, red substance in the cage pan, emaciation, decreased motor activity, cold to touch, gasping, respiration distress and hypernea.

Feed Consumption: Absolute (g/day) and relative (g/kg/day) feed consumption (days of post weaning (DPs) 1 to 71) were unaffected by dosages of the test material as high as 1000 mg/kg bw/day. Relative feed consumption values were significantly increased ($p \delta 0.05$ or $p \delta 0.01$) on DPs 22 to 29 (approximately 5%) and 36 to 43 (approximately 4%) in the 300 mg/kg bw/day dosage group and on DPs 36 to 43 (approximately 7.5%), 43 to 50

(approximately 5%) and 64 to 71 (approximately 9%) in the 1000 mg/kg bw/day dosage group. None of these significant changes were considered treatment related because they did not persist.

Terminal Body Weights, Body Weight and Body Weight Changes, Organ Weights, and Ratios of Organ Weights: Body weight and body weight gains were unaffected by dosages of the test material as high as 300 mg/kg. Details of the significant changes observed are described below.

Terminal body weight of the F1 generation male rats were significantly (p δ 0.01) reduced in the 1000 mg/kg bw/day dosage group by approximately 8% compared to the control group for the entire pre-cohabitation period and post weaning period.

Body weight gains for the F1 generation male rats were reduced in the 1000 mg/kg bw/day dosage group during most of the period prior to mating, reaching the level of significance (p δ 0.01) on days 8 to 15 of post weaning (DPs), a decrease of approximately 10% compared to the control group.

Body weights were significantly reduced on days of post weaning (DP) 36 (p δ 0.05), DP 43 (p δ 0.01), DP 57 (p δ 0.01) and DP 64 (p δ 0.01) in the 1000 mg/kg bw/day dosage group compared to control values. In addition to these significant reductions in body weights (p δ 0.05 or p δ 0.01) on DPs 36, 43, 57, 64, there were also significant (p δ 0.05 or p δ 0.01) reductions in body weight on DPs 106, 113, 120, 127, 134 and on the day of sacrifice in the 1000 mg/kg bw/day dosage group. All these body weight reductions were less than 10% of control values.

As a result of these reductions, body weight gains were significantly (p δ 0.01) reduced by approximately 8% compared to controls in the 1000 mg/kg bw/day dosage group for the entire pre-cohabitation (DP1 to pre-cohabitation) and the entire post weaning period (DP1 to termination).

The absolute weights of the seminal vesicles with and without fluid were significantly (p δ 0.05 or p δ 0.01) decreased in the 1000 mg/kg bw/day dosage group by approximately 14% and 16%, respectively. The absolute liver weight was not affected. The ratio of liver weight to the terminal body weight was significantly increased (p δ 0.01) in the 1000 mg/kg bw/day dosage group by approximately 11%. The ratio of organ weights collected at necropsy to the brain weights were comparable to control values and did not significantly differ. The absolute weight of the pituitary was significantly decreased (p δ 0.05) in the 300 mg/kg bw/day dosage group by approximately 15% but was not considered treatment related because it was not observed in the treatment groups.

The absolute weight of all other organs (left epididymis, left cauda epididymis, left testis, right epididymis, right testis, prostate,

pituitary, brain, left and right kidneys, left and right adrenals, spleen and thymus) and the ratios of these organ weights to the terminal body weight and brain weight were unaffected by dosages of the test material as high as 1000 mg/kg bw/day.

Necropsy and Histopathology: All necropsy observations were considered unrelated to the test material because the incidences were not dosage dependent and the observations occurred in only one male rat in any dosage group. These observations included a large adrenal gland, slight or marked dilation of the renal pelvis, a pitted area on the capsule of the kidney, swollen ankle of the left hindlimb, small, purple and flaccid testes, a small epididymis, a hole in the palate and firm, tan areas in the liver.

Microscopic examination of the liver and kidneys of the male rats revealed treatment-related effects similar to those in the P generation male rats. The changes in the liver consisted of hypertrophy of hepatocytes in the 300 and 1000 mg/kg bw/day dosage groups. The liver cells were enlarged due to increased amount of finely granular eosinophilic cytoplasm. The hypertrophy generally was present in all areas of the lobules, but tended to be more prevalent and more prominent in the centrilobular region.

In the kidney, the primary treatment-related microscopic change consisted of an increased incidence and severity of hyperplasia of the tubular and ductular epithelium of the inner medulla/papillary area in the 1000 mg/kg bw/day dosage group. The affected tubules had increased amounts of small cells with prominent dark nuclei. There was also an increased incidence of minimal papillary oedema in the 1000 mg/kg bw/day dosage group. Papillary haemorrhage also occurred in low incidence in the F1 generation male rats, the incidences are described as follows. Sporadic incidences of focal papillary necrosis were observed in two out of the twenty-eight F1 generation male rats examined in the 300 mg/kg bw/day dosage group and was attributed to the test material. Relatively low incidences of pelvic haemorrhage, hyperplasia of the pelvic/papillary urothelium, pelvic mineralisation and cortical tubular basophilia were observed in the treated P and F1 generation animals. These changes are reported as occurring spontaneously in this strain of rat. The possibility, however, that their incidence or occurrence could have been exacerbated in association with the other treatment-related renal effects is a consideration.

No treatment-related microscopic changes were observed in the reproductive organs of any male F1 generation rat selected for histopathologic evaluation that had been given 1000 mg/kg bw/day of the test material. All microscopic changes observed in other tissues specified for examination were considered to be incidental and unrelated to the administration of the test material.

Reproduction and Fertility: Dosages of the test material as high as 1000 mg/kg bw/day did not significantly affect any mating and fertility parameters evaluated in the F1 generation male rats. All

mating and fertility parameters (number of days to inseminate, rats that mated, Fertility Index, rats with confirmed mating dates during the first and second week of cohabitation and rats pregnant in cohabitation) were comparable among the five dosage groups and did not significantly differ.

Sperm motility evaluated for the F1 generation male rats were unaffected by dosages of the test material as high as 1000 mg/kg. Group means were comparable among the five treatment groups and ranged from 92 to 95% motile sperm. No treatment-related differences were observed in total sperm count (sperm per gm of epididymis and sperm per gram of testis).

A statistically significant (pδ0.05) increase in the percent of abnormal sperm in the 1000 mg/kg bw/day dosage group of the F1 generation male rats to 1.9% compared to control value of 1.5% was observed. This increase was not considered biologically meaningful since the group mean percent of abnormal sperm in the P (1.6% to 2.0%) and F1 (1.3% to 1.9%) generation male rats were comparable among the five dosage groups. This low incidence of head and/or tail abnormalities observed for F1 generation male rats in all dosage groups was not, therefore, considered treatment-related.

A significant (pδ0.01) increase in the day of preputial separation by approximately two days compared to the control group was observed in the F1 generation males treated at 1000 mg/kg. The average day when preputial separation was evident in the 1000 mg/kg bw/day dosage group was 49.3 days of age compared to the control group value of 47.7 days of age. This observation was considered related to the significantly (p δ 0.01) reduced body weights in the 1000 mg/kg bw/day dosage group. Additionally, the day of preputial separation was significantly delayed (p δ 0.05) in the 30 mg/kg bw/day dosage group.

F1 Generation Female Rat Finding:

Clinical Signs: A significant increase (p δ 0.01) in the incidence of excess salivation (5/10) and red perioral substance (7/10) during the pre-cohabitation period occurred in the F1 generation female rats in the 1000 mg/kg bw/day dosage group. This is considered to be treatment related. Red perioral substance continued to be significantly increased in this dosage group during the gestation period.

All other clinical observations during the pre-cohabitation, presumed gestation and lactation periods were considered unrelated to the test material because the incidences were not dosage dependent and/or the observations occurred on only one or two female rats in any dosage group.

During pre-cohabitation, clinical observations in the Controls included: localised alopecia (2). In the 30 mg/kg bw/day treatment group: missing/broken teeth (1), swollen and/or purple ear(s) (1), and localised alopecia (2). In 100 mg/kg bw/day treatment group:

swollen paw (1), misaligned incisors (1), missing/broken teeth (1), swollen and/or purple ear(s) (1) and localised alopecia (5). In the 300 mg/kg bw/day treatment group: limited use of the left hindlimb (1), swollen paw (1), missing/broken teeth (1), swollen and/or purple ear(s) (1), swollen digit(s) (1), chromorhinorrhea (2), and localised alopecia (5). In the 1000 mg/kg bw/day treatment group: exophthalmos (1), clear perinasal substance (1), swollen digit(s) (1), chromorhinorrhea (1), rales (1) and localised alopecia (2).

During presumed gestation, clinical observations in the Controls included: localised alopecia (2). In the 30 mg/kg bw/day treatment group: localised alopecia (two), swollen and purple ears (1), swollen paw (1) and clear perivaginal substance (1). In the 100 mg/kg bw/day group: localised alopecia (5), missing/broken incisors (2), misaligned incisors (2), swollen and purple ears (1), and swollen paw (1). In the 300 mg/kg bw/day group: localised alopecia (3), chromorhinorrhea (1), rales (1), missing/broken incisors (1), misaligned incisors (1), swollen and purple ears (1), swollen paw (1), limited use of left hindlimb (1) and swollen digit (1). In the 1000 mg/kg bw/day group: chromorhinorrhea (1), rales (1) and dehydration (1).

During the lactation period, clinical observations in the Controls included: urine-stained abdominal fur (1), swollen ears (1), purple ears (1), misaligned incisors (1), chromorhinorrhea (1), chromodacryorrhea (1), corneal opacity (1) and right axilla ulceration (1). In the 30 mg/kg bw/day group: urine-stained fur (1), swollen ears (1), purple ears (1), missing/broken incisors (1), soft or liquid faeces (1) and chromorhinorrhea (2). In the 100 mg/kg bw/day: urine-stained fur (2), misaligned incisors (3), missing/broken incisors (1), soft or liquid faeces (2), exophthalmos (1), traumatised cornea (1) and localised limb alopecia (1). In the 300 mg/kg bw/day group: urine-stained abdominal fur (2), swollen and purple ears (3), misaligned incisors (3), missing incisors (2), missing forepaw digit (1), soft or liquid faeces (1) and chromorhinorrhea (1). In the 1000 mg/kg bw/day group: urine-stained fur (4), swollen and purple ears (2), misaligned incisors (1), missing/broken incisors (1), missing forepaw digit (1), soft or liquid faeces (1) and dehydration (1).

Feed Consumption: No treatment-related effects on absolute and relative feed consumption values were observed during the cohabitation, gestation and lactation period. Absolute feed consumption values were significantly decreased ($p \delta 0.05$) on days of post weaning (DPs) 8 to 15 in the 300 and 1000 mg/kg bw/day dosage groups compared to the control group. These significant changes were not considered toxicologically important because they did not persist.

Terminal Body Weights, Body Weight and Body Weight Changes, Organ Weights, and Ratios of Organ Weights: Body weight and body weight gains, for the F1 generation female rats during the cohabitation, gestation and lactation periods were unaffected by

dosages of the test material as high as 1000 mg/kg. A significant reduction (p δ 0.05) in body weight gain on Study Days 8 to 15 (pre-cohabitation) in the 300 mg/kg bw/day dosage group was not considered treatment related because it was not dosage dependent. The significant increases (p δ 0.05 or p δ 0.01) in body weight in the 30, 100, 300 and/or 1000 mg/kg bw/day dosage groups on days of gestation (DG) 14, 18 and/or 21 and/or days of lactation (DLs) 5, 8, and/or 22 were not considered treatment related because they were not dosage dependent.

Terminal body weights for the F1 generation female rats were significantly increased (p δ 0.05 or p δ 0.01) in the 30, 100, 300 and 1000 mg/kg bw/day dosage groups but were not considered treatment related because they were not dosage dependent. The terminal body weights were 106%, 107%, 105% and 105% of the control value in the 30, 100, 30 and 1000 mg/kg bw/day dosage groups, respectively.

All organ weights (pituitary, brain, liver, left and right kidneys, left and right adrenals, thymus, left and right ovary and uterus) and ratios of these organ weights to the terminal body weights and to the brain weights were unaffected by dosages of the test material as high as 1000 mg/kg.

The absolute weights of the spleen were significantly increased (p δ 0.05) by approximately 113% in the 30, 100 and 300 mg/kg bw/day dosage groups compared to the control group value, but were not considered treatment-related because they were not dosage dependent. No significant change in absolute weights of the spleen were found in the 1000 mg/k/day dosage group compared to control group values.

Necropsy and Histopathology: All necropsy observations were considered unrelated to the test material because the incidences were not dosage dependent and the observations occurred in only one female rat in any dosage group. These observations included slight dilation of the renal pelvis, small thymus, dark-red raised area on the left lateral lobe of the liver, large adrenals and yellow abdominal adipose tissue connected proximately to the spleen and pancreas. Pup tissue was found in the stomach of one 1000 mg/kg bw/day dosage group dam that was sacrificed due to no surviving pups. One rat in each of the 0 (control), 30 and 300 mg/kg bw/day dosage groups is reported to have slight hydrometra and all three rats were not pregnant.

Treatment-related microscopic changes were observed in the kidney of female rats in the 300 and 1000 mg/kg bw/day dosage groups. The primary treatment-related microscopic change consisted of increased incidence and severity of hyperplasia of the tubular and ductular epitheleum of the inner medulla/papilla region in the 300 and 1000 mg/kg bw/day dosage group. The affected tubules had increased amounts of small cells with prominent dark nuclei. There was also an increased incidence of minimal or mild foal papilliary

oedmea in the 300 and 1000 mg/kg bw/day dosage groups. Unlike the P generation females, there was no incidence of necrosis.

No treatment-related microscopic changes were observed in the liver of F1 female rats.

No treatment-related microscopic changes were observed in the reproductive organs of the female F1 generation rats selected for histopathologic evaluation as high as 1000 mg/kg bw/day of test substance. All microscopic changes observed in other tissues specified for examination were considered to be incidental and unrelated to the administration of the test material.

Reproduction and Fertility: Pregnancy occurred in 24 to 28 rats in each dosage group of 30 animals. Natural delivery observations were unaffected by dosages of the test material as high as 1000 mg/kg. Values for the number of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more live-born pups/number of pregnant rats), number of dams per stillborn pups, dams with all pups dying, live-born pups and viability index (number of live pups on day 5 postpartum per number of live-born pups on day 1 postpartum) were comparable among the five treatment dosage group and did not differ significantly. The number of pups surviving per litter, the percentage male pups, litter size and average pup weights were comparable and did not differ significantly on days of lactation (DLs) 1, 5, 8, 15 or 22 for any of the dosage groups when compared to the control group.

The average number of oestrus stages per 21 days were comparable among the five dosage groups and did not differ significantly. The number of rats with six or more consecutive days of diestrus was significantly increased ($p \delta 0.05$) from 7 in the control group to 15 in the 100 mg/kg bw/day dosage group and significantly decreased ($p \delta 0.05$) from 7 in the control group to nil in the 1000 mg/kg bw/day dosage group. These changes are not considered treatment related because they are not dosage dependent and did not affect fertility or mating.

All mating and fertility parameters (number of days cohabitation, rats that mated, fertility index, rats with confirmed mating dates during the first and second week of cohabitation and rats pregnant per rats in cohabitation) were unaffected by dosages of the test material as high as 1000 mg/kg bw/day.

F2 Generation Findings:

The percentage of still-born pups was significantly increased ($p \delta 0.01$) in the 300 mg/kg bw/day dosage group and significantly reduced ($p \delta 0.01$) in the 1000 mg/kg bw/day dosage group. The changes detailed below were considered unrelated to the test material because they were not dosage dependent.

The number of pups found dead or presumed cannibalised were significantly increased (p δ 0.05 or p δ 0.01) on LD 1 in the 30, 100 and 300 mg/kg bw/day dosage groups to 0.8%, 1.5% and 1.5%, respectively, compared to the control value of 0%; significantly reduced (p δ 0.05 or p δ 0.01) on LDs 2 to 5 in the 30, 100 and 300 mg/kg bw/day dosage groups to 2.3%, 1.3% and 1.8%, respectively, compared to control value of 4.5%; and significantly reduced (p δ 0.05) to 0.3% in the 30 and 100 mg/kg bw/day dosage groups and significantly increased (p δ 0.05) in the 1000 mg/kg bw/day dosage group to 5.1% compared to control value of 2.7%. The lactation index (number of live pups on day 22 postpartum per number of live pups on day 5 postpartum) was significantly reduced (p δ 0.01) to 93.1% in the 1000 mg/kg bw/day dosage group compared to the control value of 97.0%.

Necropsy observations in the F2 generation pups occurred in only one or two litters and included coldness to the touch, dehydration, not nesting, purple lower midline/chest/head/neck/hind-paw/entire body, exophthalmos, corneal opacity, yellow skin, no milk in the stomach, scab or abrasion on hind-limb, emaciation and missing of black tip of the tail.

F2 generation weanling rats had comparable terminal body weights on LD 22 (the values did not significantly differ). None of the significant differences in organ weights or organ weight ratios for the F2 generation pups were considered treatment related because such effects were not dosage dependent.

The changes in the number of pups found dead or cannibalised were not considered related to the test material because they were not dosage dependent and/or did not affect any other measure of pup viability (surviving pups per litter, live litter size at weaning). No clinical necropsy observations for the F2 generation pups were attributable to the dosage of the test material as high as 1000 mg/kg bw/day because the incidences were not dosage dependent and the observations occurred in only one or two litters.

Observations of no milk in stomach occurred in 8 (100%), 5 (55.6%), 3 (42.8%), 6 (75.0%) and 14 (77.8%) pups that were found dead and necropsied in the 0, 30, 100, 300 and 1000 mg/kg bw/day dosage groups, respectively. The dermal layer was missing and the amniotic sac was intact in two dead pups from the same litter in the 100 mg/kg bw/day dosage group, and one dead pup in a 300 mg/kg bw/day dosage group litter had yellow fluid present in the abdominal cavity. Necropsy observations for F2 generation pups on LD 22 included slight dilation of the renal pelvis, small misshapen xiphoid, numerous clear fluid filled cysts in the renal cortex and slight dilation of the lateral ventricles of the brain.

Terminal body weights of the F2 generation pups selected for organ weight evaluation were comparable among the five dosage groups and did not differ significantly. The weights of the brain, spleen, liver, kidneys and thymus and the ratios of these organ weights to

the terminal body weight and brain weight were unaffected by dosages of the test material as high as 1000 mg/kg.

Absolute weights of the spleen, and ratios of the spleen weight to the terminal body weight and to the brain weight for the female pups, and for both sexes combined, were significantly increased (p δ 0.05 or p δ 0.01) by 64%, 35% and 49%, respectively, in the 30 mg/kg bw/day dosage group compared to the control group. The absolute weights of the kidneys of the female pups and the ratios of the kidney weight to the brain weight for the female pups and both sexes combined (ratio only) were also significantly increased (p δ 0.05 or p δ 0.01) by approximately 12%, 12% and 10%, respectively, in the 30 mg/kg bw/day dosage group compared to the control group. The ratio of the thymus weight to the brain weight for the female pups was significantly (p δ 0.05) increased in the 30 mg/kg bw/day dosage group by approximately 25% compared to the control group. The ratio of the thymus weight to the terminal body weight for the male pups was significantly increased (p δ 0.01) in the 100 mg/kg bw/day dosage group by 25% compared to the control group. The ratio of the spleen weight to the brain weight for the male pups was significantly (p δ 0.05) increased by approximately 26% in the 30 mg/kg bw/day dosage group compared to controls. These significant changes are not considered related to the administration of the test material as the changes were not dosage dependent.

Conclusion:

P generation male NOAEL:
100 mg/kg bw/day. Based on treatment-related microscopic changes in the liver and kidney at 300 mg/kg bw/day.

P generation female NOAEL:
100 mg/kg bw/day Based on treatment-related microscopic changes in the kidney at 300 mg/kg bw/day.

P generation male and female reproductive NOAEL:
1000 mg/kg bw/day
No effect in reproductive parameters at 1000 mg/kg bw/day.

*F1 litter (development) NOAEL*1000 mg/kg bw/dayNo treatment-related effects were observed in the F1 litter at 1000 mg/kg bw/day.

F1 generation male NOAEL:
100 mg/kg bw/day Based on treatment-related microscopic changes in the liver and kidney at 300 mg/kg bw/day.

F1 generation female NOAEL:
100 mg/kg bw/day
Based on treatment-related microscopic changes in the kidney at 300 mg/kg bw/day.

F1 generation male and female reproductive NOAEL
1000 mg/kg bw/day
No effect in mating, fertility or maternal delivery parameters at 1000 mg/kg bw/day.

F2 litter NOAEL
1000 mg/kg bw/day
No treatment-related effects were observed in the F2 generation pups at 1000 mg/kg bw/day.

5. Human Health Hazard Classification

Health hazards

This section discusses the classification of the health effects of potassium perfluorobutane sulfonate (PFBS) according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (the Approved Criteria) (NOHSC, 2004). The Approved Criteria are cited in the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994a) and provide mandatory criteria for determining whether a workplace chemical is hazardous.

No human data is available. The classification for health effects is based on the experimental studies (animal and in vitro tests) described in this report. In extrapolating results from experimental studies to humans, consideration has been given to relevant issues such as quality of data, weight of evidence, metabolic and mechanistic profiles, inter- and intra-species variability and relevance of exposure levels.

5.1 Acute toxicity

The test material has a low acute oral and dermal toxicity to rats with an oral and dermal LD50 of greater than 2000 mg/kg. There is no evidence of non-lethal irreversible effects from single exposures to potassium PFBS in animals.

Classification status

Potassium PFBS is of low toxicity via the oral and dermal route. Potassium PFBS does not meet the Approved Criteria for classification as a hazardous substance for acute oral and dermal toxicity.

5.2 Irritation

5.3 Skin irritation

In a study for skin corrosion/irritation potential in three female New Zealand rabbits conducted by a method analogous to OECD Guideline No. 404, a single application of 500 mg of powdered potassium PFBS failed to induce erythema, oedema, or other possible dermal findings during the 72-hour scoring period. No adverse findings were reported with the exception of one rabbit exhibiting decreased appetite on Study Day 2 to 4.

Classification status

Potassium PFBS is non-irritating to skin and does not meet the Approved Criteria for classification as irritating or corrosive to skin.

5.4 Eye irritation

In a study for acute eye irritation/corrosion conducted by a method analogous to OECD 405 Acute Eye Irritation/Corrosion, a single ocular application in the left eyes of three 16-week old female New Zealand rabbits with approximately 80 mg of

powdered potassium PFBS resulted in conjunctival redness, chemosis, and discharge; iris inflammation and corneal opacity which was evident 21-days post administration.

Ocular irritation was scored at approximately 1, 24, 48 and 72 hours post dose, and then daily to the study end at 21 days to determine the progress, reversibility and/or irreversibility of any lesions. The test material was an eye irritant in rabbits.

The test material meets the Approved Criteria for eye irritation due to a mean value for oedema of the conjunctivae (chemosis) being equal to or greater than 2 in two or more animals and occurring within 72 hours after exposure and persisting for at least 24 hours.

Post 72-hours exposure, symptoms involving corneal opacity, the iris, chemosis and conjunctival redness resolved in two of the three animals by Study Day 10. Discharge in these animals did not resolve until Study Day 21.

The remaining animal displayed resolving symptoms involving corneal opacity, the iris, chemosis and conjunctival redness post 72 hours exposure. By Study Day 13, this animal displayed worsening symptoms regarding these parameters. The reasons for this worsening is unknown.

By the end of the study period on Study Day 21, this animal displayed an opaque cornea (Grade 4) covering greater than three quarters of the cornea and the iris was not discernible through the opacity. The iris score of Grade 2 was consistent with symptoms of no reaction to light, haemorrhage, and gross destruction with any or all of these symptoms reportedly present. The redness score of Grade 2 was consistent with diffuse crimson colour with individual vessels not being easily discernible. The chemosis score of Grade 3 was consistent with swelling of the eye lids with the lids half closed. A small amount of discharge (Grade 1) was also present in this animal but was insufficient to moisten the lids and hairs adjacent to the lids.

In accordance with the Approved Criteria, the above-mentioned observations in only one animal on Study Day 21 is not sufficient to consider the test substance a risk of causing serious damage to eyes.

Classification status

Potassium PFBS is irritating to the eye. Potassium PFBS meets the Approved Criteria for classification as irritating to eyes (R36).

5.5 Skin sensitisation

In one skin sensitisation study in guinea pigs conducted according to OECD Guideline No. 406 there was no evidence of positive responses at challenge concentrations of 33% of the test material.

Classification status

There was no evidence of reactions indicative of skin sensitisation to potassium PFBS under the conditions of the test. Potassium PFBS does not meet the Approved Criteria for classification as a skin sensitiser.

Repeated dose toxicity

The results of three repeat dose toxicity studies in rats were submitted, with a duration of 14 days (range-finding study), 28 and 90 days and daily oral gavage dosing at rates up to 1000, 900 and 600 mg/kg bw/day, respectively.

The above mentioned 14-day range finding study was part of the 28-day repeated dose study. The range finding study was undertaken to provide information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. As such, the range-finding study has not been used for the purpose of health classification in this report.

A NOEL was established as 100 mg/kg bw/day in the male rats and NOAEL of 300 mg/kg bw/day in the female rats in the 28 day repeated dose study. A significant decrease in serum phosphorus and potassium in male rats treated at 300 mg/kg bw/day and 900 mg/kg bw/day was observed. The decrease for potassium was approximately 20% of the control values at a level of significance of $p < 0.05$ in both treatment groups. The decrease for phosphorus was approximately 16% of control values at a level of $p < 0.05$ in both treatment groups. Increases in absolute and relative male liver weights (approximately 25% and approximately 30%, respectively) and female kidney weights (approximately 11% and approximately 9%, respectively) were significantly increased in animals that received 900 mg/kg bw/day compared to controls.

A NOAEL in male and female rats was established at 200 mg/kg bw/day in the 90-day repeated dose study. Treatment-related microscopic changes were observed in the stomach of the male and female rats in the 600 mg/kg bw/day treatment group. The treatment-related changes in the stomach were increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach in the male and female rats in the 600 mg/kg bw/day treatment group. This occurred in 80% and 90% of male and female rats, respectively, compared to more than 10% of the control animals. Kidney hyperplasia was also reported at 600 mg/kg bw/day. However, histopathological of the kidney by an independent expert reported that no consistent changes were seen in the kidneys and the renal effects were not secondary to treatment.

Increased incidence of necrosis of individual squamous epithelial cells in the limiting ridge of the forestomach, along with minimal or mild thickening of the mucosa of the limiting ridge due to hyperplasia and hyperkeratosis in the male and female rats was considered to be treatment-related in the 600 mg/kg bw/day treatment group. Treatment-related microscopic changes were observed in the stomach of the male and female rats in the 600 mg/kg bw/day treatment group. The NOAEL in female rats was established as 200 mg/kg bw/day in the 90-day study.

Classification status

A NOAEL in male and female rats was established as 200 mg/kg bw/day in the 90-day study on the basis of necrosis in the limiting ridge of the forestomach.

Guidance in the NOHSC Approved Criteria for classification for severe effects after repeated exposure states that clinical observations do not, themselves, indicate ‘serious damage’ and would not normally justify classification with the R48 risk phrase ‘Danger of serious damage to health by prolonged exposure’. For such a classification the Approved Criteria require the demonstration of a clear functional

disturbance or morphological change(s) of toxicological significance at oral doses less than or equal to 50 mg/kg bw/day in repeat or prolonged exposure scenarios. Such a guidance value applies directly when severe lesions have been observed in a sub-chronic (90 day) toxicity test. No such effects were observed in the reported 90-day study.

Based on the results of the 90-day study reported, potassium PFBS does not meet the Approved Criteria for classification as a danger of serious damage to health by prolonged exposure.

Genotoxicity

Two in vitro studies were provided for potassium PFBS. The studies were a *Salmonella typhimurium* reverse mutation assay and a chromosomal aberration test. The chromosomal aberration test was conducted with negative results in this test (Chinese Hamster Ovary-W-B1 cells in vitro, with and without S9 activation at doses up to 5000 ∝g/plate). The *Salmonella typhimurium* reverse mutation assay was conducted with and without S9 activation at doses up to 5000 ∝g/plate. Potassium PFBS tested negative in this assay.

Classification status

Potassium PFBS is not mutagenic to bacteria or clastogenic to Chinese Hampster Ovary-W-B1 cells under the conditions of the tests. Based on the above results, potassium PFBS does not meet the Approved Criteria for classification as a mutagenic substance.

5.6 Reproductive, developmental and lactation toxicity

In the study of female rats administered 0, 100, 300 and 1000 mg/kg bw/day of the test material on gestation days 6 to 20 significant reductions in body weight gain and reduced absolute and relative feed consumption during gestation was observed at 1000 mg/kg bw/day in female rats. No signs of maternal toxicity were reported at doses of 300 mg/kg bw/day and below. NOAEL of 300 mg/kg bw/day for maternal toxicity was indicated in this study.

Significant reduction in foetal body weight (9%) was reported at 1000 mg/kg bw/day. A NOAEL of 1000 mg/kg bw/day for developmental toxicity was also indicated in this study because the reduction in foetal body weights in the 1000mg/kg bw/day treatment group was less than 10% of control value.

In a two generation reproductive toxicity study in rats administered 0, 30, 100, 300 and 1000 mg/kg bw/day of the test material, there was no evidence of adverse effects on reproduction, fertility and lactation at the highest dose of 1000 mg/kg bw/day. The NOAEL for reproductive toxicity in males and female P generation is 1000 mg/kg bw/day. The P generation male and female rats NOAEL toxicity was 100 mg/kg bw/day based on treatment-related microscopic changes in the kidney and liver for P-generation males and kidneys for P-generation females, respectively. F1 litter developmental NOAEL was 1000 mg/kg bw/day, the highest dose in the study.The F1 generation male and female rats NOAEL was 100 mg/kg bw/day based on treatment-related microscopic changes in the kidney and liver for males and kidneys for females, respectively, at 300 mg/kg bw/day.

The reproductive NOAEL in the male and female F1 generation rats was 1000 mg/kg bw/day. This dosage had no effect on mating, fertility or maternal delivery parameters. No adverse effects were observed in F2-generation pups in doses as high as 1000 mg/kg bw/day.

Classification status

Based on the above results, potassium PFBS does not meet the Approved Criteria for classification as a substance toxic to reproduction, development or lactation.

6. Environmental Fate

6.1 Summary of environmental fate

Table 6.1 – Summary of environmental fate studies

Property	Result	Reference:
Hydrolysis	Unlikely to occur	Nil
Photolysis	Unlikely to occur based on an available study for PFOS	Nil
Incineration	Perfluoroalkylsulfonyl compounds do not form combustion products that are either perfluoroalkyl-sulfonates, perfluorinated acids or precursors to these	(UDRI, 2003)
Biodegradability	Expected to be persistent	Nil
Bioaccumulation	BCF <1	(Wildlife International, 2001a)
Environmental contamination	Levels are low at this time, but may be expected to rise with increased use	Nil

6.2 Photolysis

Photolysis could occur by two types of mechanisms: direct and indirect photolysis. The first, direct absorption of a photon by the target species leading to a chemical change, could not occur as PFBS has no absorbance over the 290-900 nm wavelength range. The second, the chemical or electronic excitation transfer from a light absorbing species to the test substance, which then undergoes some type of chemical change, involves production of a radical species which then reacts with the target substance. With the perfluorinated sulfonates, the ultimate result would be the rupture of the weakest bond in the molecule, i.e., the C-S bond.

In a study on photolytic decomposition of PFOS (details not provided), it is stated neither direct or indirect photolytic decomposition was observed, and none of the predicted degradation products were detected. A minimum half-life for PFOS was calculated to be >3.6 years. Because PFBS has the same functionalities, the reaction rate with radical species should be very similar, and photolytic decomposition is not likely to be a significant pathway for environmental breakdown.

6.3 Laboratory incineration study

In order to determine whether incineration is a potential source of perfluoroalkyl sulfonates, a laboratory-scale study roughly simulating a full-scale-hazardous waste

incinerator was performed at the University of Dayton Research Institute (UDRI, 2003).

Combustion tests were completed for seven alkyl sulfonyl fluorocarbons, including PFBS and PFOS potassium salts as well as 4 compounds/polymers in which these moeities were contained in the sulfonamide form. The aim of the study was to examine whether perfluoroalkylsulfonyl compounds/polymers containing the PFAS moiety (in the sulfonamide form) can form combustion products that are then either perfluoroalkyl sulfonates or are precursors to perfluoroalkyl sulfonates through transformation reactions likely to occur in the environment.

The batch-charged continuous flow reactors developed at UDRI to study the thermal stability of organic materials were employed. These volatilise a small quantity of material (typically less than 1 mg), which is then mixed with flowing dry air and transported through a high temperature quartz tubular reactor where the sample vapours are thermally stressed under controlled conditions of time, temperature and excess air. The materials surviving this exposure are then analysed by in-line gas chromatography/mass spectrometry (GC/MS).

Preliminary testing indicated that larger amounts of material (mg quantities), a higher temperature (ca 1250^{o}C) and longer duration exposures (40 sec) in a specially designed pyroprobe were needed to fully gasify the materials. The sponsors (3M) also required that the experiments were sensitive enough to detect low-level (0.1%) transformation to perfluoroalkyl sulfonates in the exhaust gases from the fluorinated portions of the test compounds. These conditions are said to be representative of the range of conditions that occur in a full-scale incinerator, though they are quite severe compared with the conditions in the primary zone of an incinerator, eg a rotary kiln.

The results indicate that, based on LC/MS measurements, the 4 compounds/polymers which contained the PFBS/PFOS moeities in the sulfonamide form do not release perfluorinated alkyl sulfonates above the detection limit (ca 10 ppb). This was substantiated by extracted ion analysis that indicated negligible amounts of volatile sulfonate-containing degradation products. Perfluorinated alkyl sulfonate precursors also could not be detected by GC/MS, strongly suggesting that the C-S bond was completely destroyed (and did not reform) in the combustion tests.

In PFBS and PFOS combustion studies small amounts of these (maximum of 1.1%) were detected in the reactor/transfer line system as well as in the polyurethane foam (PUF) sample cartridges. GC/MS analysis was also conducted to assess the formation of other combustion products, specifically products of incomplete combustion. The most abundant combustion by-product was benzene, which was observed from all samples except PFOS. Other aromatic hydrocarbons commonly observed included styrene, benzaldehyde, benzonitrile, phenol and naphthalene.

Several fluorinated organic intermediates were also observed in the reactor effluent. These were limited to simple C1-C2 fluorinated alkanes and alkenes as well as simple fluorinated aromatics, consistent with the molecular structure of the starting materials, but no higher molecular weight polyfluorinated aromatic hydrocarbons were detected. There was also no evidence that fluorinated acids were significant combustion products.

The results of this laboratory-scale incineration study indicate that properly operating full-scale municipal or hazardous waste incineration facilities can adequately dispose of PFBS or PFOS containing materials, and that incineration of these fluorinated

compounds is not likely to be a significant source of release of perfluorinated alkyl sulfonates into the environment.

6.4 Biodegradability

No biodegradability test data have been provided. Submitted data indicates that PFBS appears to be stable under environmental conditions. Note that Ellis et al., (2001) found that the related trifluoroacetic acid is extremely persistent and showed no degradation in laboratory sediment-water systems or over two years in an outdoors microcosm study, though in one year it appeared to undergo transient partitioning to an unknown pond phase during the colder months. Moody et al., (2002) also discuss the available literature and conclude that the perfluorocarbon chain is not biodegradable, with any alterations limited to the nonfluorinated portions of the molecule. It is concluded that PFBS will be persistent in the environment.

6.5 Bioaccumulation

The following test using the potassium salt has been provided examining this important aspect (Wildlife International, 2001a).

Test substance:	PFBS potassium salt
Test facility:	Wildlife International, 2001a
Method:	OECD TG 305 Bioconcentration: Flow-through Fish Test. US EPA Series 850 – Ecological Effects Test Guidelines (OPPTS No 850.1730)
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Exposure period:	Exposure: 28 days Depuration: 16 days
Auxiliary solvent:	Nil
Concentration range:	
- Nominal	0.5 and 5.0 mg/L
- Actual	0.53 and 5.2 mg/L
Analytical monitoring:	Water samples were collected on pre-test days –2 and –1, and on uptake days 0 (0 and 4 hours), 1, 3, 7, 14, 21 and 28, and on depuration days 1, 3, 7, 10 and 14. Tissue samples were also collected on selected water sampling days, and both were analysed for PFBS by LC/MS to allow calculation of the BCF values, as well as uptake rates and depuration rates in edible tissue, non-edible tissue and whole fish.
Method:	During the uptake phase 85 bluegills (acclimated for 48 hours previously) were exposed in one of three groups: 1) a dilution water control, 2) a nominal concentration of 0.5 mg/L (measured 0.53 mg/L) and 3) a nominal concentration of 5.0 mg/L (measured 5.2 mg/L). The test apparatus was a continuous-flow diluter, which delivered approximately 6.3 volume additions of test water to the test chambers (106 L

stainless steel filled aquaria with approximately 80 L of test solution). Due to the substance's high solubility in water, no auxiliary solvent needed to be used. The temperature stayed within 21.9 to 22.1°C, dissolved oxygen from 7.4 to 8.8 mg/L, pH between 8.0 to 8.5 and alkalinity between 164 and 184 mg/L (as $CaCO_3$).

Heads, fins and viscera were removed from the fish bodies and considered to be the non-edible tissue, with the remainder the edible portion. Both tissues were blended in an homogeniser set at approximately 9500 rpm. This method ensures that any PFBS in the blood or liver, where PFOS is know to accumulate rather than in the lipid fraction (Jones et al., 2003), will be measured.

Results: Concentrations of PFBS in the control and the depuration water were all below the limit of quantitation (LOQ, 0.125 mg/L), while measured concentrations of PFBS were between 96% to 119% and 101% to 109% of nominal for the low and high levels, respectively. Fish in the control and lower concentration appeared normal and healthy throughout the test, with no treatment-related signs of toxicity. At the higher concentration one fish died on day 23 of the uptake phase.

Concentrations of PFBS in edible and non-edible fish tissues appeared to reach steady-state between day 3-7, and were not significantly different thereafter.

Bioconcentration factor: Steady-state BCF values ranged from 0.113 in edible tissue to 0.272 in non-edible tissue at the lower concentration, and 0.16 and 0.43 respectively at the higher concentration, indicating that tissue concentrations never reached exposure concentrations. PFBS was rapidly eliminated during the depuration phase of the test, with estimates to reach 50% clearance between 1.3-2.9 days, depending on the fish tissue and level of exposure.

Results: The low bioconcentration potential has been confirmed by a recent literature paper (Martin et al., 2003a), which reported from a study of the bioconcentration and tissue distribution of perfluorinated acids in rainbow trout that only sulfonates with more than four perfluoroalkyl carbons could be detected in blood, liver and carcass at all sampling times. Martin et al. (2003a) concluded that shorter polyfluorinated acids (PFAs) are expected to have insignificant bioconcentration potential, in contrast to higher homologues. In a concurrent paper (Martin et al., 2003b) it was reported that the half-life of PFBS in the liver of rainbow trout was 3.3 days in a dietary accumulation study.

Conclusion: It may be concluded from the above study that the bioaccumulation potential for PFBS is low.

6.6 Environmental Levels

Compared to the higher homologues (C6 and greater), there has been very limited detection of PFBS in the environment, partly due to previous insensitive detection limits, and also probably due to low levels of use to date. The published data are summarised in Table 6.2.

Table 6.2 - Levels of PFBS detected in the environment

Location	Result	Reference:
Japanese lakes and bays etc	Not detected in water, fish, birds and humans (insensitive detection limits, <7.6 to <151 ng/g in liver or ng/mL in blood)	Taniyasu et al. (2003)
Tokyo Bay, South China Sea and Sulu Sea	Apparently not detected (much more sensitive detection limits, = 3.8 ng/g)	Yamashita et al. (2004)
Mid Atlantic Ocean, Western, Central and Eastern Pacific Ocean	As above	Yamashita et al. (2004)
Canada, Toronto International Airport	0.0090 and 0.0077 ∝g/g (both close to the detection limit of 0.0038 ∝g/g = 3.8 ng/g,) in fish livers taken from 2 sites of a creek contaminated following an AFFF spill. Also detected in water samples collected separately, but not quantified	Moody et al. (2002)
United States Naval Air Station Fallon, Nevada Tyndall Air Force Base, Florida	 Groundwater, ≤LOQ – 210 ∝g/L Groundwater, 10 – 144 ∝g/L	Schultz et al. (2004)

In a recent publication (Taniyasu et al., 2003) PFBS was tested for in water, fish, birds and humans obtained from a number of locations in Japan, but it was not detected. However, the HPLC/MS/MS detection limits, ranging from <7.5 to <151 (ng/g in liver, wet wt or ng/mL in blood) seem rather insensitive compared with those for perfluorohexane sulfonate (PFHxS), which was either detected in about 33% of fish blood samples (maximum concentrations in blood and liver were 121 ng/mL and 19 ng/g wet wt) or had much lower detection limits (<7.6 ng/g in liver, wet wt or ng/mL in blood). It is unclear whether this reflects a much lower use level in the past.

The same authors (Yamashita et al., 2004) developed a much more sensitive assay using liquid chromatography-tandem mass spectrometry, which is able to analyse perfluorinated acids, including PFBS, PFOS and PFHxS down to the parts per quadrillion level. Marine water samples collected from a range of ocean environments (see Table 6.2) were analysed for PFBS, but as opposed to the higher homologues, no detections were reported.

Moody et al. (2002) detected PFBS by LC/MS/MS only in two samples at concentrations of 0.0090 and 0.0077 ∝g/g (both close to the detection limit of 0.0038 ∝g/g = 3.8 ng/g, and therefore more sensitive than above) of fish livers taken from 2

sites (of the 6 tested) in a creek running beside an airport. By contrast PFHxS was detected in all samples, ranging from 0.011 to 0.29 ∝g/g. PFBS was not detectable either upstream or downstream of the site where an aqueous film forming foam (AFFF) had been spilt at this airport, in contrast to PFHxS where the levels were 0.046 and 0.29 ∝g/g respectively. Perfluoropentanesulfonate (PFPeS) was also detected at 4 of the 6 sites, though again levels were relatively low (range 0.0046 to 0.013 ∝g/g).

Perfluoroalkanesulfonates and perfluorocarboxylates are known to be present in AFFFs, as typified by PFOS levels being much higher (range 2.00 to 72.9 ∝g/g) at the eight sampling sites at this airport. Higher homologs and perfluorooctanoic acid were also detected.

Water samples were also analysed for perfluoroalkanesulfonates and perfluorocarboxylates by LC/MS/MS, but only levels of PFOS, PFOA and PFHxS were reported, with the latter at a maximum concentration of 134 ∝g/L. The paper notes other homologs were observed in water samples, including PFBS, but were not quantified.

Schultz et al. (2004) also used a direct injection, liquid chromatography tandem mass spectrometry (LC MS/MS) assay to quantify a suite of perfluoralkyl sulfonates (PFAS) and fluorotelomer sulfonate (FtS) surfactants in groundwater collected from two US military bases where fire-training activities using AFFF had been conducted. Levels of PFBS in ground water at the Naval Air Station Fallon, Nevada were ≤LOQ–210 ∝g/L, while they were 10–144 ∝g/L at Tyndall Air Force Base, Florida. These were in the same order or somewhat lower than the higher homologues. Total PFAS ranged from 0-1680 ∝g/L at the former and 273-3500 ∝g/L at the latter, which also had a total FtS content of 1100-14600 ∝g/L compared with none detected at Fallon (probably not used at this site). Total perfluorocarboxylic acids (PFCAs) were 0-7090 ∝g/L at Fallon and 0-298 ∝g/L at Tyndall.

6.7 Sources of perfluoralkyl substances in the environment

As noted in Section 6.1.5, levels of PFBS in the global environment currently appear to be very low, reported as at or below the limits of detection, except through contamination by fire-training activities using Aqueous Film Forming Foam (AFFF). Whether this situation will remain so if PFBS use increases, for example by its substitution in some products that currently include perfluorooctane sulfonate (PFOS) or related higher homologues, is unclear.

The current information/hypotheses on how the higher homologues have become widely disseminated through the environment is briefly summarised below, followed by comments on possible implications for PFBS. Information is currently only available for these homologues since such chemicals have been the most widely used to date.

The vapour pressure and Henry's law constant of PFOS indicates it has very low volatility and therefore unlikely to enter directly into the atmosphere (Stock et al., 2004). It has been hypothesized that PFOS and the perfluoroalkyl acid chemical perfluorooctanoic acid (PFOA) must therefore be globally distributed via more volatile, neutral airborne contaminants that undergo long-range transport and then degrade to yield the free acids.

Polyfluorinated sulfonamides are widely distributed throughout the North American troposphere with mean concentrations ranging from 22 to 403 pg/m^3. The dominant polyfluorinated contaminant is dependent on the sampling location (Stock et al., 2004).

For example, high mean concentrations of the polyfluorinated sulfonamide chemical N-methyl perfluorooctane sulfonamidoethanol (NMeFOSE) of 359 pg/m^3 were identified in the air of Griffin, Georgia which is located in the main carpet manufacturing and treatment area of the US. The authors speculate that the polyfluorinated sulfonamide probably enters the environment from carpet treatment products, many of which consist of fluorinated molecules linked to polymeric materials. It is possible that free chemical may be left in the carpet fibres. An alternative hypothesis is that the chemically-bound chemical NMeFOSE–is released from carpets due to chemical, physical, and/or biological degradation processes.

Support for the hypothesis of release from carpets comes from Shoeib et al., (2004) who measured NMeFOSE and the related polyfluorinated sulfonamide chemical N-ethyl perfluorooctane sulfonamidoethanol (NEtFOSE) in indoor and outdoor air. Mean indoor air concentrations for these were 2590 and 770 pg/m^3, respectively, and the ratios between indoor and outdoor air were 110 and 85, respectively. Paper products were also suggested by Stock et al., (2004) as a possible source of polyfluorinated sulfonamides in air.

These findings have major implications for the substitution of some PFOS containing compounds/polymers with PFBS containing chemicals/polymers in Australia. The lower molecular weight PFBS precursors would be expected to be more volatile and therefore more readily subject to long-range transport, before they break down to PFBS. While not bioaccumulative, PFBS is persistent, and levels would build up in environment, staying mostly in the water column due to the much higher water solubility compared with higher homologues.

6.8 Conclusion

PFBS is not bioaccumulative and will stay mostly in the water column due to much higher water solubility compared with higher homologues. In water it is expected to be very persistent as it will not hydrolyse, photolyse or biodegrade. As use increases, for example, in its substitution for PFOS, levels of PFBS may build up and be distributed widely in the environment, given that its precursors are likely to be more volatile, yet structurally similar to PFOS.

7. Effects on Organisms in the Environment

Data covering testing on birds and a range of aquatic organisms have been provided, and are summarised below. All results point to a very low ecotoxicity for PFBS.

7.1 Avian

Three studies have been provided, two are 5-day dietary tests on two different species, and the third is a reproduction test on bobwhite quail.

Table 7.1 - Toxicity to Birds

Study Type	Study Guideline	Species	Age	Results (as PFBS K salt)	Reference
Acute dietary	OECD TG 205	Bobwhite quail	10 days	LC50 >10 000 ppm in diet NOEC 3160 ppm	Wildlife International (2003a)
	OECD TG 205	Mallard duck	8 days	LC50 >10 000 ppm in diet NOEC 5620 ppm	Wildlife International (2003b)
Reproduct -ion	OECD 206	Bobwhite quail	Adult	NOEC 900 ppm	Wildlife International (2005)

Comments on acute dietary studies

Both species of birds (12 per treatment group) were exposed to potassium perfluorobutane sulfonate blended in a feed/corn oil mix (comprised of 98% basal ration and 2% corn oil) at a dietary concentration of 0 (control), 1000, 1780, 3160, 5620 and 10 000 ppm. This was administered to quail for five days at 39^{o}C, and to mallards for five days at 31^{o}C, both with a 16 hours light:8 hours dark photoperiod. After a further three days on untreated basal diet, half of the control and treated birds were euthanised and subjected to gross necropsy. The remaining birds were maintained on basal rations until day 22, when they were euthanised, liver weights recorded and samples of liver tissue and blood recorded.

.

Samples of the test diets were collected to verify (by HPLC/MS/MS) the test concentrations administered and to confirm the stability and homogeneity of the test substance in the diets. These aspects were satisfactory. Symptoms of toxicity/abnormal behaviour were recorded daily throughout the study, and bodyweights were measured at the initiation of the test, and on days 5, 8, 15 and 22. Average feed consumption was determined for each group during the exposure and post-exposure periods.

There were no mortalities in the control groups. One control bobwhite quail was noted limping on days 5 and 6, apparently due to an injury. In addition there were no

treatment-related mortalities, with either species, at all test concentrations. All the mallards at all concentrations were normal in appearance and behaviour through the test. However, with the bobwhite quail toe picking was observed in between 2-5 birds in all the treatment groups, with toe, foot and ankle lesions as the result of pen-mate aggression. These lesions were associated with lameness in several birds, and lethargy in one case. One bobwhite at the 1780 ppm treatment was found dead on day 3 with such lesions. Also one bird each at 1780 and 5620 ppm was noted with slight lateral head curl during the last week of the test. None of these injuries/deaths was considered treatment related.

For the bobwhite quail there were no apparent treatment-related effects on bodyweight compared with the controls among birds in the 1000, 1780 and 3620 ppm groups throughout the test. There was a statistically significant ($p<0.01$) reduction in body weight gain in quail in the 1780 ppm group during days 1-5, but as this was not concentration responsive it was not considered treatment related. However, there was a statistically significant ($p<0.01$) reduction in body weight gain in quail in the 5620 and 10000 ppm groups during days 1-8 that was considered treatment related. A statistically significant ($p<0.01$) reduction in body weight gain among mallards that was considered treatment-related was only observed the 10 000 ppm group. Here there were occasional apparent differences in lower groups but these were not considered treatment-related as they were not dose responsive.

With both species there were no apparent treatment-related effects on feed consumption at any concentration. Only with the bobwhite was a slight reduction in feed consumption noted for all test concentrations during exposure, but as these were not concentration responsive, or associated with a reduction in body weight gain, these were not considered treatment-related.

With the bobwhite quail gross necropsy noted only the toe, foot and ankle lesions plus one bird at 1000 ppm had an encapsulated feather mass in the abdominal cavity and a small spleen. Gross necropsy of mallards revealed one bird in the control group with a haematoma, and another with a pale and mottled spleen. All of these findings were considered incidental to treatment.

While there were reductions in the mean absolute liver weight at 1000, 5620 and 10 000 ppm at day 8 with bobwhite quail, when examined as a percentage of body weight (relative liver weight) these differences were no longer apparent or statistically significant. No differences were apparent by day 22. For mallards there were no statistically significant differences in liver weight between the control and treatment groups.

For both species the dietary LC50 value was determined to be >10000 ppm, the highest concentration tested. For the bobwhite the NOEC was considered to be 3160 ppm due to the reduction in body weight gain at 5620 and 10000 ppm, while for the mallard the NOEC was 5620 ppm due to the reduction in body weight gain only at 10 000 ppm.

Comments on the reproduction study

The study was conducted according to OECD TG 206 and FIFRA guidelines Subsection 71-4. Eighteen week old northern bobwhite quail were exposed to T-7485 (PFBS potassium salt) at nominal dietary concentrations of 100, 300 and 900 ppm for a period of approximately 21 weeks, along with a control group fed with non-treated

diet. Each group consisted of sixteen pairs of birds, with one male and one female per pen.

Effects on adult health (daily for mortality, abnormal behavior and signs of toxicity), body weight gain (at test initiation, 2, 4, 6 and 8 weeks, as well as at test termination) and feed consumption (each week) were evaluated. At the beginning of week 8, the photoperiod was increased to induce egg production. Each week some eggs were selected for egg shell thickness measurement, with all other eggs candled for detection of egg shell cracks or abnormality prior to incubation. During the 21 day incubation eggs were candled twice more to detect infertile eggs or embryo mortality. They were then placed in a hatcher and when complete the hatchlings were removed and their group weight (by pen) determined. When 14 days old, the average body weights (again by parental pen) of all surviving offspring was determined. On completion of the test analyses were performed to determine statistically significant differences between groups.

Egg contents, sera and liver samples from all surviving adults, and from selected offspring, were collected for possible analysis. In addition samples of liver, kidney and gonads were collected from adults and selected offspring for histopathological examination. All birds that died during the course of the study were subjected to a gross necropsy.

Analysis of diet samples revealed no abnormalities, with all concentrations within 12% of nominal at all times during the test.

There were no treatment-related mortalities in any of the treatment groups. However, there were two adult bird mortalities considered to be incidental, with one each in the control and 100 ppm treatment groups. Another bird in the 300 ppm treatment group was euthanised. Necropsy showed a number of symptoms unrelated to treatment. In addition, no overt signs of toxicity were observed at any of the concentrations tested. Clinical observations noted in all groups included those normally associated with injuries and penwear, including foot, leg and head lesions, feather loss, lameness, loss of co-ordination, ruffled appearance, wing drop and lethargy. Most were transient in nature. Gross necropsy of all surviving adults revealed no treatment related observations.

There were no apparent treatment-related effects on or statistically significant differences between adult body weights at any of the concentrations tested. There were also no apparent treatment-related effects upon feed consumption in all groups. While there were no statistically significant differences between the control and 100 and 900 ppm treatment groups, there was a slight but statistically significant ($p < 0.01$) increase in consumption at 300 ppm during week 16 of the test, which was not considered to be treatment related.

Again compared with controls, there were no apparent treatment-related effects on reproductive performance or statistically significant differences in any of the reproductive parameters measured (eggs laid, percent eggs cracked, egg shell thickness, live 3 week embryos, number of hatchlings including as a percentage of eggs set, 14 day survivors of these 2 parameters and offspring body/liver weights) of all treatments. There was a slight though not significantly different reduction in the total number of eggs laid at 900 ppm, but this was primarily attributable to a single hen with foot lesions that did not lay any eggs for the last 5 weeks of the study. Finally, there were no abnormal serum, liver or egg analytical results, and the results of the histopathology of the liver, kidney and gonad samples showed only minor

lesions for some adults and offspring, which were considered incidental and unrelated to treatment.

Based on the results of the above study it was concluded the no-observed-effect concentration for northern bobwhite exposed to PFBS in the diet was 900 ppm, the highest concentration tested.

7.2 Toxicity to aquatic organisms

Seven studies on toxicity to aquatic organisms have been assessed and are summarised in Table 7.2.

Table 7.2 - Toxicity to aquatic organisms

Species	Test Duration	Result (mg/L)	Reference:
Algae (*Selanastrum capricornutum)*	96 h	EC50 = 2347-5733	Wildlife International (2001b)
Daphnia magna	48 h	EC50 = 2180	Wildlife International (2001c)
Mysid shrimp *(Mysidopsis bahia)*	96 h	EC50 = 372	Wildlife International (2001d)
Daphnia magna	21 days	NOEC = 502	Wildlife International (2001e)
Fathead minnow (*Pimephales promelas*)	96 h	EC50 = 1938	Wildlife International (2001f)
Bluegill sunfish (*Lepomis macrochirus*)	96 h	EC50 = 6452	Wildlife International (2001g)
Sewage micro-organisms	3 h	EC50 = >1000	Wildlife International (2001h)

7.3 Aquatic plants

Algal growth inhibition test

Test substance:	PFBS potassium salt
Test facility:	Wildlife International, Ltd (2001b)
Method:	OECD TG 201 Alga, Growth Inhibition Test.
	US EPA Series 850–Ecological Effects Test Guidelines, OPPTS Number 850.5400: Algal Toxicity Tiers I and II.
Species:	*Selanastrum capricornutum*
Exposure period:	96 hours
Concentration range:	
- Nominal	313, 625, 1250, 2500, 5000 and 10000 mg/L
- Actual	285, 563, 1077, 2216, 4561 and 9478 mg/L (mean measured)
Auxiliary solvent:	Nil
Water hardness:	Not stated
Analytical monitoring:	By LC/MS at test initiation, after approximately 72 hours and at test termination

Method: Tests were conducted in sterile, 250 mL polycarbonate Erlenmeyer flasks containing 100 mL of test or algal control medium. Cell density at test initiation was approximately 1.0 X 10^4 cells/mL. Temperature was held at 24 ± 2°C and the algae were held under continuous cool-white fluorescent lighting (target intensity 4300 ± 430 lux) throughout. Measurements of pH ranged from 6.9 to 7.1 on day 0, to 7.5-8.7 at 96 hours. Cell counts were conducted using a hemacytometer and microscope for examination of atypical cell morphology such as changes in shape, size or colour. Cell densities, area under the growth curves, growth rates and percent inhibition values were calculated using "The SAS System for Windows, Release 6.12." At the end of the 96 hours exposure, algistatic effects were differentiated from algicidal effects by performing a recovery phase.

Results:

Biomass		**Growth**	
EbC50 (mg/L at 96 h)	**96 h NOEC (mg/L)**	**ErC50 (mg/L at 96 h)**	**96 h NOEC (mg/L)**
2347 (95% CL 2018-2707)	1077	5733 (95% CL 5659-5817)	1077

Results: All test solutions remained clear and colourless throughout. Samples collected at the beginning of the test had measured concentrations that ranged from 88% to 99% of nominal. These were 80%-92% after 72 hours and 87%-94% at test termination. After 96 hours there were no signs of aggregation, flocculation or adherence of the algae to the test flasks at any concentration. However, algal cells in the 2216, 4561 and 9478 mg/L treatment groups appeared large compared with controls. The 9478 mg/L group was maximally inhibited at the end of the 96 hour exposure period. When aliquots were diluted with algal medium and cultured for six days, the effect on algal growth was found to be algistatic based on growth in the recovery phase.

Conclusion: PFBS potassium salt is practically non-toxic to algae. Any effect will be algistatic rather than algicidal.

7.3.2 Aquatic invertebrates

Acute toxicity to daphnids

Test substance: PFBS potassium salt

Test facility: Wildlife International, Ltd (2001c)

Method: OECD TG 202 Daphnia sp. Acute Immobilisation Test - static.

US EPA Series 850–Ecological Effects Test Guidelines, OPPTS Number 850.1010.

Species: *Daphnia magna*

Exposure period: 48 hours

Auxiliary solvent: Nil

Water hardness: 181 mg/L as $CaCO_3$ (at test initiation)

Analytical monitoring: By LC/MS at test initiation, after approximately 24 hours and at test termination.

Method: Tests were conducted in 300 mL plastic beakers containing 225 mL of test or control medium. A 1 L primary stock solution was prepared at a concentration of 4000 mg/L through inverting and stirring to aid solubilisation of the test substance. Aliquots were proportionally diluted to prepare the other concentrations. The temperature was held at 20 ± 1°C and the daphnids were subjected to a photoperiod of 16 hours light: 8 hours darkness with a light intensity of about 144 lux throughout. Measurements of pH ranged from 7.9 to 8.5 and dissolved oxygen from 8.4 to 8.6 mg/L.

Daphnid neonates used in the test were less than 24 hours old and were not fed during the test. Observations were made at approximately 4, 24 and 48 hours after test initiation to determine the numbers of mortalities and immobile organisms. EC50 values were calculated by the Probit method and the NOEC was determined by visual inspection of the mortality, immobility and clinical observations.

Results:

Concentration mg/L		Number of *D. magna*	Number Immobilised	
Nominal	**Actual**		**24 h**	**48 h**
control		20	0	1
250	234	20	0	1
500	470	20	0	0
1000	886	20	0	1
2000	1707	20	0	4
4000	3767	20	20	20

EC50: 2183 (95% CL 1707-3767) mg/L (mean measured) at 48 hours

NOEC: 886 mg/L (mean measured) at 48 hours

Results: All test solutions remained clear and colourless throughout. Samples collected at the beginning of the test had measured concentrations that ranged from 83% to 96% of nominal. These were 89%-99% after 24 hours and 79%-92% at test termination. The single mortality/immobility observed at the 234 and 886 mg/L test concentrations was not considered treatment related.

Conclusion: The notified chemical is practically non-toxic to daphnia.

Acute toxicity to mysid shrimp

Test substance: PFBS potassium salt

Test facility: Wildlife International, Ltd (2001d)

Method: US EPA Series 850–Ecological Effects Test Guidelines, OPPTS Number 850.1035 (1) Acute Toxicity Test for Estuarine and Marine Organisms.

Species: Mysid shrimp *(Mysidopsis bahia)*

Exposure period: 96 hours

Auxiliary solvent: Nil

Salinity: 20-21 parts per thousand

Analytical monitoring: By LC/MS at test initiation, after approximately 48 hours and at test termination.

Method: Tests were conducted in 2000 mL polyethylene buckets containing 1500 mL of test or control medium. A primary stock solution was prepared at a concentration of 1000 mg/L using an electric mixer to aid in the solubilisation of the test substance, and then proportionally diluted to make up the test concentrations. The temperature was held at 25 ± 2°C and the mysids were subjected to a photoperiod of 16 hours light: 8 hours darkness using fluorescent tubes that emitted wavelengths similar to natural sunlight. Measurements of pH ranged from 8.0 to 8.3 and dissolved oxygen remained >6.5 (88% of saturation) mg/L throughout the test.

Juvenile mysids were used and fed live brine shrimp nauplii daily during the test to prevent cannibalism. Observations were made at approximately 4, 24, 48, 72 and 96 hours after test initiation to determine the numbers of mortalities and individuals exhibiting clinical signs of toxicity or abnormal behaviour. LC50 values were calculated by the Probit method except at 24 hours, where the binomial method was used. The NOEC was determined by visual inspection of the mortality and clinical observations.

Results:

Concentration mg/L		Number of *M bahia*	Number Dead	
Nominal	**Actual**		**48 h**	**96 h**
control		20	0	0
31	32	20	0	0
63	64	20	0	0
125	127	20	0	0
250	269	20	1	3
500	554	20	9	18
1000	1071	20	20	20

EC50: 372 (95% CL 314-440) mg/L (mean measured) at 96 hours

NOEC: 127 mg/L (mean measured) at 96 hours

Remarks: All test solutions remained clear and colourless throughout. Samples collected at the beginning of the test had measured concentrations that ranged from 99% to 110% of nominal. These were 99%-112% after 48 hours and 102%-112% at test termination. At the 554 mg/L level, some of the surviving mysid were either lethargic or swimming erratically through 24 to 96 hours.

Conclusion: The notified chemical is practically non-toxic to the mysid shrimp.

Chronic toxicity to Daphnids

Test substance: PFBS potassium salt

Test facility: Wildlife International, Ltd (2001e)

Method: OECD TG 211 *Daphnia magna* Reproduction Test – semi-static.

US EPA Series 850–Ecological Effects Test Guidelines, OPPTS

	Number 850.1300 *Daphnia magna* Life-Cycle (21-day Renewal) Chronic Toxicity Test.
Species:	*Daphnia magna*
Exposure period:	21 days
Auxiliary solvent:	Nil
Water hardness:	128-140 mg/L as $CaCO_3$
Analytical monitoring:	By LC/MS at test initiation, after approximately 24 hours and at test termination.
Method:	Tests were conducted in 250 mL plastic beakers containing 200 mL of test or control medium. A 4 L primary stock solution was prepared at a concentration of 2000 mg/L by employing stirring with a mixer until all the test substance had dissolved. Aliquots were proportionally diluted to prepare the other concentrations. The temperature was held at 20 ± 1°C except for two occasions when it was 18 °C. This occurred during renewal of the test solutions and was probably the result of the temperature probe being exposed to air. The daphnids were subjected to a photoperiod of 16 hours light: 8 hours darkness with a light intensity of ranging from 357 to 512 lux. Measurements of pH ranged from 7.9 to 8.7 and dissolved oxygen concentrations remained >7.9 mg/L (87% of saturation) throughout. Daphnid neonates used in the test were less than 24 hours old and were fed during the test. Solutions were renewed every 2-3 days. The first generation daphnids were observed daily for survival, the onset of reproduction and clinical signs of toxicity. The criteria for death included white opaque colouration, lack of movement of appendages, absence of heartbeat and lack of response to gentle prodding. Immobilisation was defined as lack of movement except for minor activity of the appendages. The presence of eggs in the brood pouch, aborted eggs, males or ephippia was also recorded. With the onset of reproduction, the number of second generation neonates was counted (including number of live, dead or immobile and aborted eggs) on each Monday, Wednesday and Friday during the test. At each observation period the second generation daphnids were discarded, except on day 14 where they were collected and used for the second generation acute exposure. At test termination the length and dry weight of each surviving first generation daphnid was determined. Statistical analyses (using TOXSTAT Version 3.5 statistical software) were performed on survival of first-generation daphnids, the number of live young and the length and dry weight of the surviving first generation daphnids.

Results:

Concentration mg/L		Number of *D. magna*	Percent mortality		
Nominal	**Actual**		**7 days**	**14 days**	**21 days**
control		20	0	0	10
63	60	20	0	0	0
125	121	20	0	0	0
250	247	20	0	0	0
500	502	20	0	0	0
1000	995	20	0	30	30
2000	1876	20	20	80	80

LOEC: (reproduction/length)	995 mg/L (mean measured) at 21 days
NOEC:	502 mg/L (mean measured) at 21 days
MATC:	707 mg/L (mean measured) at 21 days
Results:	All test solutions remained clear and colourless throughout. Freshly prepared samples had measured concentrations that ranged from 85% to 103% of nominal, whereas measured values from old solutions were 86% to 106% of nominal. Surviving daphnids were only affected at the two highest concentrations, and were either discoloured or smaller. Daphnids in all groups started producing neonates on day 8. Reproduction and mean length were significantly lower from the 995 mg/L treatment. The second generation acute exposure test indicated a 48 h NOEC of 995 mg/L, with all daphnids surviving at this concentration.
Conclusion:	The notified chemical is practically non-toxic to daphnids after chronic exposure.

7.4 Fish

Acute toxicity to fathead minnow

Test substance:	PFBS, potassium salt
Test facility:	Wildlife International, Ltd (2001f)
Method:	OECD TG 203 Fish, Acute Toxicity Test static. US EPA Series 850–Ecological Effects Test Guidelines, OPPTS Number 850.1075
Species:	Fathead minnow (*Pimephales promelas*)
Exposure period:	96 hours
Auxiliary solvent:	Nil
Water hardness:	128 mg $CaCO_3$/L
Analytical monitoring:	Nil
Method:	Juvenile fish of average length 35 ± 5 mm, and mean wet weight 320 ± 100 mg were tested in 25 L polyethylene aquaria containing 15 L of test solution (loading rate 210 mg of fish per L of test solution). There were 10 fish per replicate, with 2 replicates per test concentration, and

a 16 h light: 8 h dark daily photoperiod at approximately 222 lux.

Test solutions were prepared by addition of the test substance to the dilution water, followed by stirring to aid in the solubilisation of the test material. The temperature remained within 20.86°C-22.6°C, the pH within 8.3 to 8.5 and the dissolved oxygen content remained >7.5 mg/L (86% of saturation). Daily observations of mortality and other clinical signs of toxicity were made. The LC50s were determined by the binomial method, and the NOEC by visual interpretation of the mortality and clinical observation data.

Results:

Concentration mg/L		Number of Fish	Mortality				
Nominal	Actual		2 h	24 h	48 h	72 h	96 h
0	-	20	0	0	0	0	0
204	220	20	0	0	0	0	0
408	437	20	0	0	0	0	0
816	888	20	0	0	0	0	0
1632	1655	20	0	3	5	6	6
3263	3341	20	0	20	20	20	20

LC50: 1938 (95% CL 888-3341) mg/L (mean measured) at 96 hours.

NOEC: 888 mg/L (mean measured) at 96 hours.

Results: All test solutions appeared clear and colourless throughout. Samples collected at the beginning of the test had measured concentrations that ranged from 100% to 105% of nominal. These were 99%-109% after 48 hours and 99%-123% at test termination. There were no other sub-lethal observations on remaining fish except that one fish was lying on the bottom after 2 hours at the 437 mg/L concentration.

Conclusion: The test substance is practically non-toxic to the fathead minnow.

Acute toxicity to bluegill sunfish

Test substance: PFBS, potassium salt

Test facility: Wildlife International, Ltd (2001g)

Method: OECD TG 203 Fish, Acute Toxicity Test static.

US EPA Series 850–Ecological Effects Test Guidelines, OPPTS Number 850.1075

Species: Bluegill sunfish (*Lepomis macrochirus*)

Exposure period: 96 hours

Auxiliary solvent: Nil

Water hardness: 148 mg $CaCO_3$/L

Analytical monitoring: Nil

Method: Juvenile fish of average length 44 ± 10 mm, and mean wet weight 1000 ± 600 mg were tested in 25 L polyethylene aquaria containing 15 L of test solution (loading rate 700 mg of fish per L of test solution). There were 10 fish per replicate, with 2 replicates per test concentration, and a 16 h light: 8 h dark daily photoperiod at

approximately 220 lux.

Test solutions were prepared by addition of the test substance to the dilution water, followed by stirring to aid in the solubilisation of the test material. The temperature remained within 22.1-23.9°C, the pH within 8.0 to 8.4 and the dissolved oxygen content remained >5.4 mg/L (62% of saturation). Daily observations of mortality and other clinical signs of toxicity were made. The LC50s were determined by the Probit method (24 h) and the binomial method at other times, and the NOEC by visual interpretation of the mortality and clinical observation data.

Results:

Concentration mg/L		Number of Fish	Mortality				
Nominal	Actual		2 h	24 h	48 h	72 h	96 h
0	-	20	0	0	0	0	0
612	629	20	0	0	0	0	0
1224	1311	20	0	0	0	0	0
2448	2715	20	0	0	0	0	0
4895	5252	20	0	2	2	3	3
9790	9433	20	0	15	20	20	20

LC50: 6452 (95% CL 5252-9433) mg/L (mean measured) at 96 hours.

NOEC: 2715 mg/L (mean measured) at 96 hours.

Results: All test solutions appeared clear and colourless throughout. Samples collected at the beginning of the test had measured concentrations that ranged from 93% to 114% of nominal. These were 95%-116% after 48 hours and 99%-124% at test termination. There were no other sub-lethal observations on remaining fish except after 3 hours at the highest 9433 mg/L concentration where fish were either lethargic or lying on the bottom.

Conclusion: The test substance is practically non-toxic to the bluegill sunfish.

7.5 Sewage micro-organisms inhibition of microbial activity

Test substance: PFBS potassium salt

Test facility: Wildlife International, Ltd (2001h)

Method: OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum: Activated sludge from the Denton Wastewater Treatment Plant, Maryland, which receives predominantly wastes from domestic sources.

Exposure period: 3 hours

Concentration range: (Nominal) 1, 3, 10, 30, 100, 300 and 1000 mg/L

Method: The inoculum was sieved using a 2 mm screen and the total suspended solids (TSS) concentration adjusted to 4000 mg/L by settling for 30 minutes and removing the supernatant. Subsequently 9.6 mL of synthetic sewage, 120 mL of inoculum, the appropriate amount of test or reference substance with enough water to bring the total volume to

	300 mL, were mixed. There were two inoculum controls. Incubation was between 21%-22°C. A reference test using between 3 and 50 mg/L 3,5-dichlorophenol was performed concurrently. After 3 hours aeration the contents of the vessels were transferred to a BOD vessel and the respiration rate measured over a period of 10 minutes and calculated in mg O_2/L/hour. The EC50 was determined by the probit method.
Results:	
IC50:	>1000 mg/L after 3 hours
Results:	The inhibitory effects on respiration ranged from –6.4 (100 mg/L) to 17.5% (300 mg/L) over the test concentration range, but there was no clear dose response. As respiration rates of the two inoculum controls (5%) did not vary by more than 15% and the 3-h EC50 of the reference substance (15.6 mg/L) was within the 5 to 30 mg/L limits considered acceptable, the test was considered as valid.
Conclusion:	The test substance is not inhibitory to sewage micro-organisms.

7.6 Conclusion

A range of tests show that PFBS will not be toxic to birds, algae, aquatic invertebrates (daphnids and mysid shrimp), fish or sewage micro-organisms. However, a recent literature paper (MacDonald et al., 2004) has concluded that PFOS toxicity thresholds for the chironomid *Chironomus tentans* are 2-3 orders of magnitude lower than those reported for other aquatic organisms. The authors note that this is possibly through some kind of interaction with haemoglobin, which is present at all levels of dissolved oxygen (DO) in chironomids as opposed to daphnids, where haemoglobin is produced only in response to declining DO levels. Whether this would also be the case with PFBS is unclear, but it does for the first time identify a species which is much more sensitive to some polyalkylfluorinated sulfonates.

8. Discussion and Conclusions

Uses

No manufacture of potassium PFBS, PFBS derivatives or fluoropolymers that may degrade to PFBS is reported to date in Australia.

Additionally, no use in Australia of potassium PFBS as such is reported, though certain fluoropolymers that include monomers based on PFBS are reported. These PFBS-based chemicals have applications in industrial and consumer carpet protection treatments, industrially applied corrosion resistant paints and coatings and high performance industrial chemical applications in the metal processing industry. The main use of PFBS-based chemicals in Australia is in the high performance industrial chemical category.

Based on the current use pattern in Australia, potential public exposure to PFBS is expected to be predominantly by means of secondary exposure via the environment as a result of the degradation of certain fluoropolymers to PFBS.

Health Effects

PFBS is a strongly acidic, highly water soluble substance with a low vapour pressure. The surfactant and strong acidic nature of potassium PFBS cannot be discounted from playing a role in the toxicology of potassium PFBS.

PFBS is rapidly excreted by the renal route in cynomologus monkeys. A single-dose (10 mg/kg bw) intravenous toxicokinetics study of PFBS in male and female cynomologus monkey (n = 6) showed urinary excretion to be a major excretion route for PFBS – with PFBS serum levels below the quantification limit of 0.5 ∝g/mL in all monkeys at the study end (Study Day 31), and approximately 34% - 87% of the administered dose recovered in the urine within 24 hours post-dosing. No-gender specific differences in the elimination of the PFBS were observed in this study.

The results of a non-classical protein binding study indicated PFBS is highly bound to human albumin with indications of a saturated binding of the test material to albumin in serum. Binding of the PFBS to the other liver-manufactured proteins gamma globulin, alpha globulin, fibrinogen, alpha-2-macroglobulin, transferrin and beta lipoproteins was found to be negligible (< 0.1%).

The protein binding study did not support a proposition for the lack of protein binding of PFBS (3M, 2004). This is because among the plasma proteins, albumin is the most important because of its high concentration relative to other proteins and its ability to bind acidic (and basic) compounds (Martin et al., 1993). Investigations of the dissociation rates of PFBS from plasma proteins, and equilibrium partitioning characteristics of the test material with red blood cells, may assist in better understanding the toxicokinetics of PFBS. It is anticipated that ongoing investigations into the binding characteristics of perfluorinated chemicals to human proteins may assist in better understanding the mechanism of excretion of these chemical in general (Jones et al., 2003). 3M is currently developing urinary data for PFBS in potentially exposed workers. This study has not been finalized and was not provided during this assessment.

The mammalian (animal) toxicology of potassium PFBS shows low acute oral and dermal toxicity as well as evidence of being non-irritating to skin. No information on acute inhalation toxicity is available. Potassium PFBS is found to be an eye irritant and meets the Approved Criteria for classification as irritating to eyes (NOHSC, 2004). Potassium PFBS has the potential to cause severe eye damage and corrosion (as defined by the Globally Harmonized System for Hazard Classification and Communication (GHS) (United Nations, 2003)). Data to date shows no evidence of skin sensitisation due to potassium PFBS at concentrations of 33% of the test material.

A 90-day study in rats (0, 60, 200 and 600 mg/kg bw/day) does not indicate a danger of serious damage to health by prolonged exposure to potassium PFBS, as defined in the NOHSC Approved Criteria, at doses up to 200 mg/kg bw/day. In the 90-day study, treatment-related microscopic changes were observed in the stomach of the male and female rats in the 600 mg/kg bw/day treatment group with a NOAEL in male and female rats established as 200 mg/kg bw/day.

The results of two in vitro studies (*Salmonella typhimurium* reverse mutation assay and a Chinese Hamster Ovary-W-B1 cells chromosomal aberration test with and without S9 activation) show no evidence of mutagenicity due to potassium PFBS.

Animal (rat) data to date does not indicate potassium PFBS is a developmental toxin nor a substance toxic to reproduction, fertility or lactation.

Based on the results of a pre-natal developmental toxicity in rats (0, 100, 300 and 1000 mg/kg bw), a NOAEL of 300 mg/kg bw/day for maternal toxicity was identified based on reduced body-weight gains and feed consumption at 1000 mg/kg bw/day. In addition, a NOAEL of 1000 mg/kg bw/day for developmental toxicity was indicated in this study, as the observed reduction in foetal body weights in the 1000 mg/kg bw/day treatment group was less than 10% of the control value.

The results of a 2-generation reproductive toxicity study in rats showed a P generation male and female NOAEL of 100 mg/kg bw/day based on treatment-related microscopic changes in the liver and kidney, and treatment-related microscopic changes in the kidney in male and female rats, respectively, at 300 mg/kg bw/day. A P generation male and female reproductive NOAEL of 1000 mg/kg bw/day was indicated based on no adverse effects observed in reproductive parameters at does up to 1000 mg/kg bw/day. The study also showed an F1 litter (developmental) NOAEL of 1000 mg/kg bw/day.

In the F1 generation, the study showed an F1 generation male and female NOAEL:of 100 mg/kg bw/day based on treatment-related microscopic changes in the liver and kidney, and treatment-related microscopic changes in the kidney in male and female rats, respectively, at 300 mg/kg bw/day. An F1 generation male and female reproductive NOAEL was observed as no effect in mating, fertility or maternal delivery parameters was observed at 1000 mg/kg bw/day. An F2 litter NOAEL of 1000 mg/kg bw/day was observed based on no treatment-related effects observed in the F2 generation pups at doses of the test material up to 1000 mg/kg bw/day.

Environmental Effects

PFBS is a strongly acidic, highly water soluble substance which has a low vapour pressure and is poorly adsorbed to soils and sediments and is therefore expected to remain in the water compartment on release into the environment.

In water it is expected to be very persistent as it will not hydrolyse, photolyse or biodegrade. However, a range of tests show that it will not be toxic to birds, algae, aquatic invertebrates, fish or sewage micro-organisms.

PFBS is not bioaccumulative or toxic to aquatic organisms, however it is persistent, and levels may build up and be distributed widely in the environment over time, staying mostly in the water column due to the much higher water solubility compared with the higher homologues.

Potassium PFBS was assessed against the screening criteria detailed in Annex D of the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP). Potassium PFBS does not meet all the criteria for POPs.

9. Recommendations

This section provides the recommendations arising from the hazard assessment of potassium PFBS. Recommendations are directed principally at regulatory bodies and importers and formulators of PFBS-containing products. Implicit in these recommendations is that best practice is implemented to minimise occupational and public exposure and environmental impact.

With respect to the current use, the use of PFBS-based chemicals in the performance chemical category is supported, however, uses that would result in wide dispersion in the aquatic environment is not supported by NICNAS. NICNAS will annotate the Australian Inventory of Chemical Substances (AICS) to restrict use accordingly.

The consequences of NICNAS annotating the AICS of the restricted use of PFBS is that any planned dispersive use of PFBS and PFBS-based chemicals will need to be notified to NICNAS for assessment for prior approval.

9.1 Recommendations for regulatory bodies

9.2 NOHSC

Potassium PFBS is not currently listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999).

In accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), potassium PFBS is classified "Hazardous" with the following risk phrases:

- R36 – Irritating to Eyes;

The following safety phrases are also recommended for potassium PFBS:

- S25 – Avoid contact with eyes
- S26 – In case of contact with eyes, rinse immediately with plenty of water and contact a doctor or Poisons Information Centre.

This classification for potassium PFBS should be adopted by NOHSC as part of their process for updating the *List of Designated Hazardous Substances* (NOHSC, 1999). Industry should label potassium PFBS for this hazard classification (See Recommendation 9.2.2).

9.3 Recommendations for potassium PFBS importers and formulators of PFBS-containing products and State and Territory Authorities

9.4 Hazard communication – Material Safety Data Sheet

Under the *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994a) and the Commonwealth, State and Territory regulations introduced in accordance with these national model regulations, employees shall have

ready access to Material Safety Data Sheets (MSDS) for hazardous substances at their workplace. MSDS provide information to those who use the hazardous substance.

In order to ensure conformity with this code, it is recommended that importers of potassium PFBS review their MSDS for compliance and pay particular attention to the following points:

- risk phrases and hazard information should be updated to reflect the hazard classification in Recommendation 9.1.1 above.

9.5 Hazard communication – labels

In accordance with the *National Code of Practice for the Labelling of Workplace Substances* (NOHSC, 1994b) it is recommended that importers of potassium perfluorobutane sulfonate review their labels for compliance and pay particular attention to the following points:

- risk phrases and hazard information should be updated to reflect the hazard classification in Recommendation 9.1.1; and
- safety phrases should be included as noted in Recommendation 9.1.1.

9.6 Recommendation to importers and users

PFBS is very persistent in water and does not biodegrade or hydrolyse. It is therefore recommended that uses of PFBS-based chemicals and products be limited to non-dispersive applications and those with low emissions to the environment.

NICNAS annotation of the AICS to restrict use of PFBS will have the consequence that industry will be obliged to notify NICNAS for assessment for prior approval for any planned dispersive use of PFBS and PFBS-based chemicals.

Appendix

In this report, potassium perfluorobutane sulfonate has been classified against the NOHSC *Approved Criteria for Classifying Hazardous Substances* (Approved Criteria) (NOHSC, 2004) and, in the case of physicochemical hazards, the *Australian Code for the Transport of Dangerous Goods by Road and Rail* (ADG Code) (FORS, 1998). However, classifications under the Globally Harmonized System for Hazard Classification and Communication (GHS) (United Nations, 2003) will come into force when the GHS is adopted by the Australian Government and promulgated into Commonwealth legislation. GHS documentation is available at

http://www.unece.org/trans/danger/publi/ghs/officialtext.html

The classification of potassium perfluorobutane sulfonate against the GHS can be found below.

Health Hazards	Classification	Hazard Communication
Eye irritant	Category 1	*Symbol*: Corrosive *Signal word*: Danger *Hazard Statement*: Causes severe eye damage

Under the GHS, a single harmonized hazard category is adopted for substances that have the potential to seriously damage the eyes. Category 1 - irreversible effects on the eye – includes observations in animals of grade 4 cornea lesions and other severe reactions such as destruction of the cornea observed at any time during the test and persistent corneal opacity, discolouration of the cornea by a dye substance, adhesions, pannus and interference with the function of the iris or other effects that impair sight. In this context, persistent lesions are considered those which are not fully reversible within an observation period of normally 21 days.

Category 1 also contains substances fulfilling the criteria of corneal opacity ε 3 or iritis detected in a Draize eye test with rabbits, because severe lesions like these usually do not reverse within a 21 day observation period. (United Nations, 2003)

Irreversible effects categories

An eye irritant Category 1 (irreversible effects on the eye) is a test material that produces:

- at least in one animal, effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
- at least in 2 of 3 tested animals, a positive response of:

  - corneal opacity = 3, and/or
  - iritis > 1.5,

  calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.

In a single eye irritation study, Grade 4 corneal opacity was observed in one rabbit at 21 days. The iris was not discernible through the opacity covering greater than one-quarter of the cornea. This finding is consistent with the criteria described above. Potassium PFBS therefore meets the classification of an eye irritant – Catergory 1.

Environmental Hazards

PFBS is not classifiable for the aquatic environment under the GHS (United Nations, 2003).

References

3M (2004) Ecotoxicology, environmental fate and health testing of perfluorobutane sulfonate (unpublished report submitted by the 3M Corporation).

3M (2005) Internal communication.

3M Environmental Laboratory (2001) Characterisation Study PFBS: a.) Primary Standard – Test Control Reference #TCR-99030-023 (lot 101), b.) Test Control Reference #TCR-00017-071 (Lot 102) Phase: Solubility Determination. 3M Environmental Laboratory Study #FACT-TCR009 (LIMS #E00-1877), 28 March 2001. 3M Environmental Laboratory Minnesota (unpublished report submitted by the 3M Corporation)

3M Environmental Laboratory (2002), Determination of the air-water partition co-efficient of perfluorobutane sulfonate (PFBS) from its vapour pressure and water solubility (18 June 2002) (un-numbered unpublished report submitted by the 3M Corporation)

3M Specialty Material (2002) Technical data bulletin: environmental, health, safety and regulatory profile of perfluorobutane sulfonate (PFBS) <http://multimedia.mmm.com/mws/mediawebserver.dyn?TTTTTTB_LdgTmwUTfwUTTTj7zDsssssr- >, accessed 2004.

Ames, BN, McCann J, Yamasaki, E (975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutagen Research, **31:** 347-367.

Ammons BC (2000) Internal memo to Shannon, T., dated 07/06/2000.

Argus Research (2002) Oral (Gavage) Developmental toxicity study of potassium perfluorobutane sulfonate (PFBS) in rats. United States of America (unpublished report submitted by the 3M Corporation).

Argus Research (2003a) Oral (Gavage) Repeated dose 90-day toxicity study of potassium perfluorobutane sulfonate (PFBS) in rats. United States of America (unpublished report submitted by the 3M Corporation).

Argus Research (2003b) Oral (gavage) two-generation (one litter per generation) reproduction study of potassium perfluorobutane sulfonate (PFBS) in rats. United States of America (unpublished report submitted by the 3M Corporation).

Centre Analytical Laboratories (2001), Adsorption and desorption study to determine the mobility and distribution of perfluorobutanesulfonic acid in soil (Centre study number 023-048, 8 March 2001) Centre Analytical Laboratories Pennsylvania (unpublished report submitted by the 3M Corporation)

Cruz RC, Steinhardt GF, Liapis H., et al. (2002) The role of matrix metalloproteinases (MMPs) in unilateral ureteral obstruction (UUO) in newborn rats. The FASEB Journal (Journal of the Federation of American Societies for Experimental Biology, 16 (5): A1097 Part 2.

Draize JH. et al. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol. Exptl. Thera. **82**:377-390.

Ellis DA, Hanson,ML, Sibley PK, Shahid T, Fineberg NA, Solomon KR, Muir DCG & Mabury SA (2001) The fate and persistence of trifluoroacetic and chloracetic acids in pond waters. Chemosphere, **42**: 309-318.

European Commission (2003) Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No.1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market - Part II. Institute for Health and Consumer Protection, European Chemicals Bureau, European Communities. EUR 20418 EN/3.

FORS (1998) Australian code for the transport of dangerous goods by road or rail (ADG Code), 6th ed. Canberra, ACT, Federal Office of Road Safety.

Green MHL & Muriel WJ (1977) Mutagen testing using trp + reversion in Escherichia coli. In: BJ Kilbey et al. (eds) Handbook of mutagenicity test procedures. Amsterdam, Elsevier North Holland Biomedical Press, pp 65-94.

Hackett RB, McDonald TO (1991) Eye irritation. In: Marzulli, FN and Maibach, HI (eds) Dermatotoxicology, 4th edition, pp749-815.

Hinderling PH (1997) Red blood cells: A neglected compartment in pharmacokinetics and pharmacodynamics. Pharmacological Reviews, **49**(3): 279-295.

Jones PD, Hu W, De Coen W, Newsted JL & Geisy JP (2003) Binding of perfluorinated fatty acids to serum proteins. Environmental Toxicology and Chemistry, **22**(11):2639-2649.

Kay JH & Calandra JC (1962) Interpretation of eye irritation tests. Journal of the Society of Cosmetic Chemists, **13:**281-289.

Kirk-Othmer Encyclopedia of Chemical Technology, 3rd edition (1978-1984) M Grayson (ed). Vol 10, p.952 (R A Geunther contributor). New York, John Wiley and Sons.

MacDonald MM, Warne AL, Stock NL, Mabury SA, Solomon KR & Silbey, PK (2004), Toxicity of perfluorosulfonic acid and perfluorooctanoic acid to *Chironomus tentans*, Environmental Toxicology and Chemistry, **23**(9):2116-2123.

Maron DR & Ames BN (1980) Revised methods for the Salmonella mutagenicity test Mutation Research, **113**: 173-215.

Martin A, Bustamonte P, & Chun AHC (1993) Physical pharmacy (4th edition). Philadelphia, Lippincott Williams and Wilkins.

Martin JW, Mabury SA, Solomon KR & Muir DCG (2003a), Bioconcentration and tissue distribution of perfluorinated acids in Rainbow Trout (Oncorhynchus mykiss), Environmental Toxicology and Chemistry, **22**(1): 196-204.

Martin JW, Mabury SA, Solomon KR & Muir DCG (2003b), Dietary accumulation of perfluorinated acids in Juvenile Rainbow Trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry, **22**(1): 189-195.

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H & Linders JBHJ (1995) Manual for summarising and evaluating the environmental aspects of pesticides. Bilthoven, The Netherlands, National Institute of Public Health and Environmental Protection.

Moody CA, Martin JW, Kwan WC, Muir DCG and Mabury SA (2002), Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. Environmental Science and Technology, **36(**14): 545-551.

NOHSC (National Occupational Health and Safety Commission) (1994a) National model regulations for the control of workplace hazardous substances [NOHSC:1005(1994)]. Canberra, Australian Government Publishing Service.

NOHSC (National Occupational Health and Safety Commission) (1994b) National Code of Practice for the Labelling of Workplace Hazardous Substances [NOHSC:2012(2004)]. Canberra, Australian Government Publishing Service.

NOHSC (National Occupational Health and Safety Commission) (2004) Approved criteria for classifying hazardous substances (3rd edition) [NOHSC:1008(2004)]. Canberra, National Occupational Health and Safety Commission.

NOHSC (1999) List of designated hazardous substances [NOHSC:10005(1999)]. Sydney, National Occupational Health and Safety Commission.

Organization for Economic Cooperation and Development (1981). OECD Guidelines for Testing Chemicals. Section 4, No. 414: Teratogenicity, adopted 12 May 1981

Organization for Economic Co-operation and Development (OECD 1987) Guideline 402 Acute dermal toxicity – limit test

Organization for Economic Co-operation and Development (OECD 1992) Guideline 406 Skin sensitisation

Organization for Economic Co-operation and Development (OECD 1995) Guideline 407 Repeated dose 28-day oral toxicity study in rodents

Organization for Economic Co-operation and Development (OECD 1997a) Guideline for the Testing of Chemicals Number 473 In vitro mammalian chromosome aberration test

Organization for Economic Co-operation and Development (OECD 1997b) Guideline 471 Bacterial reverse mutation test

Organization for Economic Co-operation and Development (OECD 1998) Guideline 408 Repeated dose 90-day oral toxicity study in rodents

Organization for Economic Co-operation and Development (OECD 2001a) Guideline 423 Acute oral toxicity - acute toxic class method

Organization for Economic Co-operation and Development (OECD 2001b) Guideline 414 Prenatal developmental toxicity study.

Organization for Economic Co-operation and Development (OECD 2001c) Guideline 416 Two-generation reproduction toxicity study.

Organization for Economic Co-operation and Development (OECD 2002a) Guideline 405 Acute eye irritation/corrosion.

Organisation for Economic Cooperation Development (OECD 2002b) Hazard assessment of perfluorooctane sulfonate (PFOS) and its salts. Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Geneva, 2002

Primedica Redfield (2000a) Acute oral toxicity study of potassium perfluorobutane sulfonate administered to Sprague Dawley rats (Study number: 132-002). United States of America (unpublished report submitted by the 3M Corporation).

Primedica Redfield (2000b) Acute dermal toxicity study of potassium perfluorobutane sulfonate applied Sprague Dawley rats (Study number: 132-010). United States of America (unpublished report submitted by the 3M Corporation).

Primedica Redfield (2000c) Acute dermal irritation study of potassium perfluorobutane sulfonate applied to New Zealand White rabbits (Study Number: 132-004). United States of America (unpublished report submitted by the 3M Corporation).

Primedica Redfield (2000d) Acute ocular irritation study of potassium perfluorobutane sulfonate applied to New Zealand White rabbits (Study number: 132-005). United States of America (unpublished report submitted by the 3M Corporation).

Primedica Redfield (2000e) A repeated dose range finding toxicity study of potassium perfluorobutane sulfonate in Sprague-Dawley rats (Study number: 132-006). United States of America (unpublished report submitted by the 3M Corporation).

Primedica Redfield (2001) A 28-day oral (gavage) toxicity study of potassium perfluorobutane sulfonate in Sprague-Dawley rats (Study number: 132-007). United States of America (unpublished report submitted by the 3M Corporation).

Redfield Laboratories (2002) Delayed contact hypersensitivity study of potassium perfluorobutane sulfonate in Hartley Guinea Pigs (Maximisation Test) (Study number: 132-015). United States of America (unpublished report submitted by the 3M Corporation).

Shoeib M, Harner T, Ikonomonu M & Kannan K (2004) Indoor and outdoor concentrations and phase partitioning of perfluoroalkyl sulfonamides and ploybrominated diphenyl ethers. Environmental Science and Technology, **38(**5): 1313-1320.

Sitek Research Laboratories (2001a) Evaluation of potassium perfluorobutane sulfonate in the Salmonella typhimurium/Escherichia coli Plate Incorporation/Preincubation mutation assay in the presence and absence of induced rat liver S-9 (Study Number: 132-008). United States of America (unpublished report submitted by the 3M Corporation).

Sitek Research Laboratories (2001b) Test for chemical induction of chromosome aberration in cultured Chinese Hamster Ovary (CHO) cells with and without metabolic activation (Study Number: 132-009). United States of America (unpublished report submitted by the 3M Corporation).

Southern Research Institute (2001) A pharmacokinetic study of perfluorobutanesulfonate in the cynomologos monkey. United States of America (unpublished report submitted by the 3M Corporation).

Southern Research Institute (2003) Protein binding of perfluorobutane sulfonate (Study number 9921.7) (unpublished report submitted by the 3M Corporation).

Stock NL, Lau FK, Ellis DA, Martin JW, Muir DCG & Mabury SA (2004) Perfluorinated telomer alcohols and sulfonamides in the North American troposphere. Environmental Science and Technology, **38**(4): 991-996.

Schultz MM, Barofsky DF & Field JA (2004) Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. Environmental Science & Technology, **38**(6): 1828-1835

Taniyasu S, Kannan K, Hanari N & Yamashita N (2003) A survey of perfluorooctane sulfonate and related perfluorinated organic compounds in water, fish, birds and humans from Japan. Environmental Science and Technology, **37(**12):2634-2639.

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UNECE), New York and Geneva.

United Nations Environment Programme (UNEP) Stockholm Convention on Persistent Organic Pollutants (POPS), convention text accessed 5 April 2005: http://www.pops.int/documents/convtext/convtext_en.pdf

United States Environment Protection Agency. Toxic Substances Control Act (TSCA) inventory <http://www.epa.gov/opptintr/newchems/invntory.htm> .

US EPA (2004) PBT Profiler Definitions Office of Pollution Prevention and Toxic, www.pbtprofiler.net (last updated August 27 2004)

U.S. Environmental Protection Agency (1977). Toxic Substances Control Act (TSCA) Test Guidelines; Final Rule. Prenatal Developmental Toxicity, 799.9370 (cross referenced to OPPTS 870.3700), *Federal Register*, August 15, 1997.

U.S. Environmental Protection Agency (1988). Health Effects Test Guidelines; Prenatal Developmental Toxicity Study. Office of Prevention, Pesticides and Toxic Substances (OPPTS) 870.3700, August 1998.

United States Environmental Protection Agency (2002) Perfluoroalkyl Sulfonates; Significant New Use Rule (SNUR 67 FR 11008) Supplemental Proposed Significant New Use Rule (SNUR 67 FR 11014). Federal Register, Vol 67, No. 47, March 11 2002, pp 11008 - 11014. <http://www.epa.gov/fedrgstr/EPA-TOX/2002/March/Day-11/t5746.pdf >

University of Dayton Research Institute (2003) Laboratory-scale thermal degradation of perfluoroalkyl sulfonates and perfluoroalkyl sulfonamides. University of Dayton Research Institute Project No UDR-TR-2002-000153, 1 July 2003, Dayton Ohio (unpublished report submitted by the 3M Corporation).

Venitt S & Parry JM (eds) (1984) Mutagenicity testing: a practical approach. Oxford, IRL Press.

Wildlife International, Ltd (2000) Determination of the water solubility of perfluorobutanesulfonate, potassium salt (PFBS) by the shake flask method. Wildlife International, Ltd, Project Number: 454C-118 (3M Environmental Lab Project Number: E00-1429, 30 August 2000). Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001a) Perfluorobutanesulfonate, potassium salt (PFBS): a flow-through bioconcentration test with the Bluegill. Wildlife International, Ltd Project Number: 454A-117 (3M Environmental Lab Project Number: E00-1429, 9 May 2001). Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001b) PFBS: A 96-hour toxicity test with the freshwater alga (Selenastrum capricornutum), Wildlife International, Ltd Project Number: 454A-129 (3M Environmental Lab Project Number: E00-1429), 20 March 2001. Wildlife International, Ltd, Maryland (unpublished report submitted by the 3M Corporation)

Wildlife International, Ltd (2001c), Perfluorobutane sulfonate, Potassium salt (PFBS): A 48-hour static acute toxicity test with the Cladoceran (Daphnia magna), Wildlife International, Ltd Project Number: 454A-118A (3M Environmental Lab Project Number: E00-1429), 20 March 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001d), PFBS: A 96-hour static acute toxicity test with the saltwater mysid (Mysidopsis bahia), Wildlife International, Ltd Project Number: 454A-128 (3M Environmental Lab Project Number: E00-1429), 21 March 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001e) PFBS: A semi-static life-cycle toxicity test with the Cladoceran (Daphnia magna), Wildlife International, Ltd Project Number: 454A-130 (3M Environmental Lab Project Number: E00-1429), 11 April 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001f), Perfluorobutane sulfonate, Potassium Salt (PFBS). A 96-hour Static Acute Toxicity Test with the Fathead minnow (Pimephales promelas), Wildlife International, Ltd Project Number: 454A-115 (3M Environmental Lab Project Number: E00-1429), 20 March 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001g) Perfluorobutane sulfonate, potassium salt (PFBS). A 96-hour static acute toxicity test with the Bluegill (Lepomis macrochirus), Wildlife International, Ltd Project Number: 454A-114 (3M Environmental Lab Project Number: E00-1429), 20 March 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2001h) PFBS: An activated sludge, respiration inhibition test, Wildlife International, Ltd Project Number: 454E-102A (3M Environmental Lab Project Number: E00-1429), 20 March 2001. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2002) Determination of the vapor pressure of perfluorobutanesulfonate, potassium salt (PFBS) using the spinning rotor gauge method, Wildlife International, Ltd Project Number: 454C-127 (3M Environmental Lab Project Number: E00-1429), 29 April 2002. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2003a), A dietary study with the Northern Bobwhite, Wildlife International, Ltd Project Number: 454-112 (3M Environmental Lab Project Number: E00-1429), 10 January 2003. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2003b) A dietary study with the Mallard, Wildlife International, Ltd Project Number: 454-113 (3M Environmental Lab Project Number: E00-1429), 9 January 2003. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Wildlife International, Ltd (2005) T-7485: A reproduction study with the Northern Bobwhite, Wildlife International, Ltd Project Number: 454-116 (3M Environmental Lab Project Number: E03-0583), 27 May 2005. Maryland, Wildlife International, Ltd (unpublished report submitted by the 3M Corporation).

Yamashita N, Kannan K, Taniyasu S, Horii Y, Okazawa T, Petrick G & Gamo T (2004) Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography-tandem mass spectrometry. Environmental Science & Technology, **38(**21): 5522-5528.