



**Rotterdam Convention on the Prior
Informed Consent Procedure for
Certain Hazardous Chemicals and
Pesticides in International Trade**

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Chemical Review Committee

Seventh meeting

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Item 4 (c) (iv) of the provisional agenda*

Technical work: review of notifications

**of final regulatory action: perfluorooctane sulfonate,
its salts and its precursors**

Perfluorooctane sulfonate, its salts and its precursors

Note by the Secretariat

Addendum

Supporting documentation provided by the European Union

The Secretariat has the honour to provide, in the annex to the present note, documentation received from the European Union to support its notification of final regulatory action for perfluorooctane sulfonate, its salts and its precursors as industrial chemicals. The documentation has not been formally edited.

* UNEP/FAO/RC/CRC.7/1.

Annex

COMMISSION REGULATION (EU) No 757/2010**of 24 August 2010****amending Regulation (EC) No 850/2004 of the European Parliament and of the Council on persistent organic pollutants as regards Annexes I and III****(Text with EEA relevance)**

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC ⁽¹⁾, and in particular Article 14(1) thereof,

Whereas:

(1) Regulation (EC) No 850/2004 implements in the law of the Union the commitments set out in the Stockholm Convention on Persistent Organic Pollutants (hereinafter 'the Convention') approved by Council Decision 2006/507/EC of 14 October 2004 concerning the conclusion, on behalf of the European Community, of the Stockholm Convention on Persistent Organic Pollutants ⁽²⁾ and in the Protocol to the 1979 Convention on Long-Range Transboundary Air Pollution on Persistent Organic Pollutants (hereinafter 'the Protocol') approved by Council Decision 2004/259/EC of 19 February 2004 concerning the conclusion, on behalf of the European Community, of the Protocol to the 1979 Convention on Long Range Transboundary Air Pollution on Persistent Organic Pollutants ⁽³⁾.

(2) Following nominations of substances received from the European Union and its Member States, Norway and Mexico, the Persistent Organic Pollutants Review Committee established under the Convention has concluded its work on the nine proposed substances, which have been found to meet the criteria of the Convention. At the fourth meeting of the Conference of the Parties to the Convention on 4–8 May 2009 (hereinafter 'COP4') it was agreed to add all nine substances to the Annexes to the Convention.

(3) In view of the decisions taken at COP4 it is necessary to update Annexes I and III to Regulation (EC) No 850/2004. Annex I to Regulation (EC) No 850/2004 should be amended to take into account that substances can be listed only in the Convention.

(4) The COP4 decided to list eight of the substances in Annex A (elimination) to the Convention. The ninth substance, Perfluorooctane sulfonic acid and its derivatives (hereinafter PFOS) is still widely used worldwide and COP4 decided to list it in Annex B (restriction) with a range of exemptions. Regulation (EC) No 850/2004 has a similar structure with Annex I (prohibition) and Annex II (restriction). The Convention contains obligations to prohibit or restrict production, use, import and export of the substances listed in its Annexes A and B. By listing the substance covered by the COP4 decisions in Regulation (EC) No 850/2004, the scope of the restriction is brought in conformity with the COP4 decision as Regulation (EC) No 850/2004 includes conditions for production, use and waste management in addition to restricting placing on the market.

(5) Placing on the market and use of PFOS has been restricted in the Union by virtue of Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) ⁽⁴⁾. The existing restriction on PFOS in the Union contains only few exemptions compared to those included in the COP4 decision. PFOS was also listed in Annex I to the revised Protocol adopted on 18 December 2009. Therefore PFOS should be listed together with the other eight substances in Annex I to Regulation (EC) No 850/2004. Derogations that were applicable for PFOS when listed in Annex XVII are carried over and listed in Annex I to Regulation (EC) No 850/2004 with only few amendments. The derogations should be subject to the use of best available technique where applicable. The specific derogation to use PFOS as wetting agents for use in controlled electroplating systems is time limited in accordance with the COP4 decision. If technically justified the deadline can be prolonged, subject to approval by the Conference of the Parties to the Convention. Member States must report every four years on the use of the allowed derogations. The European Union as a Party to the Convention should report to it based on the Member State reports. The Commission should continue to review the remaining derogations and the availability of safer alternative substances or technologies.

(6) The provisions in Article 4(1)(b) of Regulation (EC) No 850/2004 regarding substances occurring as an unintentional trace contaminant should be defined for PFOS to ensure a harmonised enforcement and control of that Regulation, while at the same time guaranteeing conformity with the Convention. By virtue of Annex XVII to Regulation (EC) No 1907/2006 PFOS was

⁽¹⁾ OJ L 158, 30.4.2004, p. 7.

⁽²⁾ OJ L 209, 31.7.2006, p. 1.

⁽³⁾ OJ L 81, 19.3.2004, p. 35.

⁽⁴⁾ OJ L 396, 30.12.2006, p. 1.

allowed to be used in quantities below certain thresholds. Until further information becomes available, the thresholds in Annex XVII to Regulation (EC) No 1907/2006 for PFOS in articles correspond to a level below which PFOS can not be meaningfully used while enabling control and enforcement through existing methods. These thresholds should therefore limit the use of PFOS to a level corresponding to unintentional trace contaminants. For PFOS as substances or in preparations, this Regulation should establish a threshold corresponding to a similar level. To rule out intentional use, this level should be lower than the level applied in Regulation (EC) No 1907/2006.

- (7) Placing on the market and use of pentabromodiphenyl ether and octabromodiphenyl ether have been restricted in the Union by virtue of Annex XVII to Regulation (EC) No 1907/2006 with a maximum concentration limit of 0,1 % by weight below which it is not considered restricted. The COP4 decided to list congeners present in the commercial forms of pentabromodiphenyl ethers and octabromodiphenyl ethers having POPs characteristics. For reasons of coherence the listing in Regulation (EC) No 850/2004 should follow the approach of Annex XVII to Regulation (EC) No 1907/2006 for those derivatives identified by COP4 as having POP characteristics; therefore hexabromodiphenyl ether, heptabromodiphenyl ether, tetrabromodiphenyl ether and pentabromodiphenyl ether derivatives should be listed in Annex I to Regulation (EC) No 850/2004.
- (8) The provisions in Article 4(1)(b) of Regulation (EC) No 850/2004 regarding substances occurring as an unintentional trace contaminant should be defined for polybrominated diphenyl ethers (PBDEs) to ensure a harmonised enforcement and control of that Regulation, while at the same time guaranteeing conformity with the Convention. This Regulation should establish a fixed threshold for considering unintentional trace contaminants regarding PBDEs in substances, preparations and articles. Subject to further information that becomes available and a review by the Commission, in line with the objectives of this Regulation, the thresholds in Annex XVII to Regulation (EC) No 1907/2006 for PBDEs in articles produced from recycled materials should limit the use of PBDEs to unintentional trace contaminants in that they are considered to correspond to a level below which PBDEs can not be meaningfully used while enabling control and enforcement through existing methods. For PBDEs as substances, in preparations or in articles, this Regulation should establish a threshold corresponding to a similar level.

- (9) It is necessary to clarify that the prohibition in Article 3 of Regulation (EC) No 850/2004 does not apply to

articles containing PBDEs and PFOS already in use on the date of entry into force of this Regulation.

- (10) DDT and Hexachlorocyclohexanes (HCH), including lindane, should be listed without derogations. Part A of Annex I to Regulation (EC) No 850/2004 allows Member States to maintain existing production and use of DDT for the production of dicofol. No Member State is currently using the derogation. In addition, dicofol was denied for inclusion in Annex I to Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market⁽¹⁾ as well as in Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market⁽²⁾. That derogation should therefore be deleted. HCH, including lindane, is listed in Part B of Annex I Regulation (EC) No 850/2004, with two specific derogations for certain specific uses. The derogations expired on 1 September 2006 and on 31 December 2007 and should therefore be deleted.
- (11) In conformity with the COP4 decisions pentachlorobenzene should be added to Annexes I and III to Regulation (EC) No 850/2004 so that it becomes subject to a general prohibition as well as the release reduction provisions in that Regulation. Chlordecone and hexabromobiphenyl should be moved to Annex I, Part A as they are now listed to both international instruments.
- (12) In accordance with Article 22 of the Convention, amendments to Annexes A, B and C thereto enter into force one year from the date of communication by the depositary of an amendment, which will fall on 26 August 2010. Consequently and for reasons of coherence this Regulation should apply from the same date. This Regulation should therefore enter into force as a matter of urgency.
- (13) The measures provided for in this Regulation are in accordance with the opinion of the Committee established by Directive 67/548/EEC,

HAS ADOPTED THIS REGULATION:

Article 1

Annexes I and III to Regulation (EC) No 850/2004 are amended in accordance with the Annex to this Regulation.

⁽¹⁾ OJ L 123, 24.4.1998, p. 1.

⁽²⁾ OJ L 230, 19.8.1991, p. 1.

Article 2

This Regulation shall enter into force on the day of its publication in the *Official Journal of the European Union*.

It shall apply from 26 August 2010.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 24 August 2010.

For the Commission
The President
José Manuel BARROSO

ANNEX

(1) Annex I to Regulation (EC) No 850/2004 is replaced by the following:

‘ANNEX I

Part A — Substances listed in the Convention and in the Protocol as well as substances listed only in the Convention

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
Tetrabromodiphenyl ether $C_{12}H_6Br_4O$			<ol style="list-style-type: none"> For the purposes of this entry, Article 4(1)(b) shall apply to concentrations of Tetrabromodiphenyl ether equal to or below 10 mg/kg (0,001 % by weight) when it occurs in substances, preparations, articles or as constituents of the flame-retarded parts of articles. By way of derogation, the production, placing on the market and use of the following shall be allowed: <ol style="list-style-type: none"> without prejudice to subparagraph (b), articles and preparations containing concentrations below 0,1 % of tetrabromodiphenyl ether by weight when produced partially or fully from recycled materials or materials from waste prepared for re-use; electrical and electronic equipment within the scope of Directive 2002/95/EC of the European Parliament and Council (*). Use of articles already in use in the Union before 25 August 2010 containing Tetrabromodiphenyl ether as a constituent of such articles shall be allowed. Article 4(2), third and fourth subparagraphs shall apply in relation to such articles.
Pentabromodiphenyl ether $C_{12}H_5Br_5O$			<ol style="list-style-type: none"> For the purposes of this entry, Article 4(1)(b) shall apply to concentrations of pentabromodiphenyl ether equal to or below 10 mg/kg (0,001 % by weight) when it occurs in substances, preparations, articles or as constituents of the flame-retarded parts of articles. By way of derogation, the production, placing on the market and use of the following shall be allowed: <ol style="list-style-type: none"> without prejudice to subparagraph (b), articles and preparations containing concentrations below 0,1 % of pentabromodiphenyl ether by weight when produced partially or fully from recycled materials or materials from waste prepared for re-use;

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
			<p>(b) electrical and electronic equipment within the scope of Directive 2002/95/EC.</p> <p>3. Use of articles already in use in the Union before 25 August 2010 containing Pentabromodiphenyl ether as a constituent of such articles shall be allowed. Article 4(2), third and fourth subparagraphs shall apply in relation to such articles.</p>
<p>Hexabromodiphenyl ether</p> <p>C₁₂H₄Br₆O</p>			<p>1. For the purposes of this entry, Article 4(1)(b) shall apply to concentrations of hexabromodiphenyl ether equal to or below 10 mg/kg (0,001 % by weight) when it occurs in substances, preparations, articles or as constituents of the flame-retarded parts of articles.</p> <p>2. By way of derogation, the production, placing on the market and use of the following shall be allowed:</p> <p>(a) without prejudice to subparagraph (b), articles and preparations containing concentrations below 0,1 % of hexabromobiphenyl ether by weight when produced partially or fully from recycled materials or materials from waste prepared for re-use;</p> <p>(b) electrical and electronic equipment within the scope of Directive 2002/95/EC.</p> <p>3. Use of articles already in use in the Union before 25 August 2010 containing Hexabromodiphenyl ether as a constituent of such articles shall be allowed. Article 4(2), third and fourth subparagraphs shall apply in relation to such articles.</p>
<p>Heptabromodiphenyl ether</p> <p>C₁₂H₃Br₇O</p>			<p>1. For the purposes of this entry, Article 4(1)(b) shall apply to concentrations of heptabromodiphenyl ether equal to or below 10 mg/kg (0,001 % by weight) when it occurs in substances, preparations, articles or as constituents of the flame-retarded parts of articles.</p> <p>2. By way of derogation, the production, placing on the market and use of the following shall be allowed:</p> <p>(a) without prejudice to subparagraph (b), articles and preparations containing concentrations below 0,1 % of heptabromodiphenyl ether by weight when produced partially or fully from recycled materials or materials from waste prepared for re-use;</p>

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
			<p>(b) electrical and electronic equipment within the scope of Directive 2002/95/EC.</p> <p>3. Use of articles already in use in the Union before 25 August 2010 containing Heptabromodiphenyl ether as a constituent of such articles shall be allowed. Article 4(2), third and fourth subparagraphs shall apply in relation to such articles.</p>
<p>Perfluorooctane sulfonic acid and its derivatives (PFOS)</p> <p>$C_8F_{17}SO_2X$</p> <p>(X = OH, Metal salt ($O-M^+$), halide, amide, and other derivatives including polymers)</p>			<p>1. For the purposes of this entry, Article 4(1)(b) shall apply to concentrations of PFOS equal to or below 10 mg/kg (0,001 % by weight) when it occurs in substances or in preparations.</p> <p>2. For the purposes of this entry, Article 4(1) (b) shall apply to concentrations of PFOS in semi-finished products or articles, or parts thereof, if the concentration of PFOS is lower than 0,1 % by weight calculated with reference to the mass of structurally or micro-structurally distinct parts that contain PFOS or, for textiles or other coated materials, if the amount of PFOS is lower than 1 $\mu\text{g}/\text{m}^2$ of the coated material.</p> <p>3. Use of articles already in use in the Union before 25 August 2010 containing PFOS as a constituent of such articles shall be allowed. Article 4(2), third and fourth subparagraphs shall apply in relation to such articles.</p> <p>4. Fire-fighting foams that were placed on the market before 27 December 2006 may be used until 27 June 2011.</p> <p>5. If the quantity released into the environment is minimised, production and placing on the market is allowed for the following specific uses provided that Member States report to the Commission every four years on progress made to eliminate PFOS:</p> <p>(a) until 26 August 2015, wetting agents for use in controlled electroplating systems;</p> <p>(b) photoresists or anti reflective coatings for photolithography processes;</p> <p>(c) photographic coatings applied to films, papers, or printing plates;</p>

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
			<p>(d) mist suppressants for non-decorative hard chromium (VI) plating in closed loop systems;</p> <p>(e) hydraulic fluids for aviation.</p> <p>Where derogations in points (a) to (e) above concern the production or use in an installation within the scope of Directive 2008/1/EC of the European Parliament and of the Council (**), the relevant best available techniques for the prevention and minimisation of emissions of PFOS described in the information published by the Commission pursuant to Article 17(2), second subparagraph, of Directive 2008/1/EC shall apply.</p> <p>As soon as new information on details of uses and safer alternative substances or technologies for the uses in points (b) to (e) becomes available, the Commission shall review the derogations in the second subparagraph so that:</p> <p>(i) the uses of PFOS will be phased out as soon as the use of safer alternatives is technically and economically feasible,</p> <p>(ii) a derogation can only be continued for essential uses for which safer alternatives do not exist and where the efforts undertaken to find safer alternatives have been reported on,</p> <p>(iii) releases of PFOS into the environment have been minimised by applying best available techniques.</p> <p>6. Once standards are adopted by the European Committee for Standardisation (CEN) they shall be used as the analytical test methods for demonstrating the conformity of substances, preparations and articles to paragraphs 1 and 2.</p>
DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane)	50-29-3	200-024-3	—
Chlordane	57-74-9	200-349-0	—
Hexachlorocyclohexanes, including lindane	58-89-9	200-401-2	—
	319-84-6	206-270-8	
	319-85-7	206-271-3	
	608-73-1	210-168-9	

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
Dieldrin	60-57-1	200-484-5	—
Endrin	72-20-8	200-775-7	—
Heptachlor	76-44-8	200-962-3	—
Hexachlorobenzene	118-74-1	200-273-9	—
Chlordecone	143-50-0	205-601-3	—
Aldrin	309-00-2	206-215-8	—
Pentachlorobenzene	608-93-5	210-172-5	—
Polychlorinated Biphenyls (PCB)	1336-36-3 and others	215-648-1 and others	Without prejudice to Directive 96/59/EC, articles already in use at the time of the entry into force of this Regulation are allowed to be used
Mirex	2385-85-5	219-196-6	—
Toxaphene	8001-35-2	232-283-3	—
Hexabromobiphenyl	36355-01-8	252-994-2	—

(*) OJ L 37, 13.2.2003, p. 19.

(**) OJ L 24, 29.1.2008, p. 8.

Part B — Substances listed only in the Protocol

Substance	CAS No	EC No	Specific exemption on intermediate use or other specification
—			

(2) In Annex III the following substance is added:

‘Pentachlorobenzene (CAS No 608-93-5)’

Unclassified

ENV/JM/RD(2002)17/FINAL



Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

21-Nov-2002

English - Or. English

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

CO-OPERATION ON EXISTING CHEMICALS

HAZARD ASSESSMENT OF PERFLUOROOCTANE SULFONATE (PFOS) AND ITS SALTS

JT00135607

Document complet disponible sur OLIS dans son format d'origine
Complete document available on OLIS in its original format

**ENV/JM/RD(2002)17/FINAL
Unclassified**

English - Or. English

Preface

In the margins of the ninth meeting of the Task Force on Existing Chemicals (29-30 May 2000) several Member countries agreed to informally work together to collect information on the environmental and human health hazards of perfluorooctane sulfonate (PFOS) to produce a hazard assessment. The decision followed the announcement by a major US manufacturer – 3M – to globally phase out the manufacture and use of these chemicals beginning in 2001. The US and the UK agreed to lead the activity with the Secretariat assisting by requesting readily available exposure information from Member countries as well as from non-Member countries through IFCS.

An informal meeting was hosted by the US on 26-27 October 2000 (Crystal City, Virginia, US) to:

- review the current status of assessment activities;
- learn about actions being taken in other countries; and
- identify planned or ongoing work on this issue.

In preparation for the meeting 3M circulated a draft initial assessment report including robust study summaries of key studies, together with exposure information.

At the 31st Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (7-10 November 2000), it was agreed that, since this was a matter of sufficient interest to all Member countries, this activity should be undertaken under the existing Chemicals Programme, overseen by the Task Force. As PFOS is not an HPV Chemical, it was not dealt with under the HPV Chemicals Programme.

A draft hazard assessment was posted on the OECD web site for comment in December 2000. The OECD established an electronic discussion group to exchange comments and information. A special session on PFOS and its salts was held on 25 January 2001 in Orlando, USA, as part of the 11th SIAM meeting. At this session an overview of the draft hazard assessment was presented.

The draft hazard assessment was revised twice since December 2000 to incorporate comments that were received, as well as to incorporate newly completed studies. Comments were received from 3M, World Wildlife Fund, Health Canada, Environment Canada, and Australia.

At the 11th meeting of the Task Force on Existing Chemicals (27-28 May 2002), the revised hazard assessment was discussed. The Task Force agreed with the conclusions and recommendations of the hazard assessment. The Task Force also agreed that the Secretariat should gather information from governments and BIAC on risk management activities currently undertaken or planned in Member countries on PFOS.

At the 34th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (5-8 November 2002), the final draft of the assessment was endorsed. The Joint Meeting recommended that this document be derestricted under the authority of the Secretary General.

This hazard assessment of perfluorooctane sulfonate (PFOS) and its salts includes all information that was available by July 2002. A quantitative risk assessment was not conducted as this should entail regional exposure information. The hazard information on PFOS should be used with caution in evaluating the potential hazards of other perfluorinated compounds. The perfluorinated compounds represent a very unique chemistry whose toxicological properties are presently not well understood and clearly the presence of different length (perfluorinated) carbon chains and functional groups are likely to influence toxicity. It is not clear at this time whether the hazard concerns of PFOS can be extrapolated to other perfluorinated compounds except under circumstances where the compound may degrade to PFOS.

Assessment activities on PFOS and its salts are also on-going in other international fora, e.g. OSPAR.

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RECOMMENDATIONS OF THE HAZARD ASSESSMENT

Perfluorooctane sulfonate (PFOS) is a candidate for further work.

Sufficient information exists to address hazard classification for all SIDS human health endpoints. PFOS is persistent, bioaccumulative and toxic to mammalian species. There are species differences in the elimination half-life of PFOS; the half-life is 100 days in rats, 200 days in monkeys, and years in humans. The toxicity profile of PFOS is similar among rats and monkeys. Repeated exposure results in hepatotoxicity and mortality; the dose-response curve is very steep for mortality. This occurs in animals of all ages, although the neonate may be more sensitive. In addition, a 2-year bioassay in rats has shown that exposure to PFOS results in hepatocellular adenomas and thyroid follicular cell adenomas; the hepatocellular adenomas do not appear to be related to peroxisome proliferation. Further work to elucidate the species differences in toxicokinetics and in the mode of action of PFOS will increase our ability to predict risk to humans. Epidemiologic studies have shown an association of PFOS exposure and the incidence of bladder cancer; further work is needed to understand this association.

Sufficient information exists to address hazard classification for all SIDS environmental endpoints. PFOS is persistent in the environment and has been shown to bioconcentrate in fish. It has been detected in a number of species of wildlife, including marine mammals. Its persistence, presence in the environment and bioaccumulation potential indicate cause for concern. It appears to be of low to moderate toxicity to aquatic organisms but there is evidence of high acute toxicity to honey bees. No information is available on effects on soil- and sediment-dwelling organisms and the equilibrium partitioning method may not be suitable for predicting PNECs for these compartments. PFOS has been detected in sediment downstream of a production site and in effluents and sludge from sewage treatment plants.

Given the apparent widespread occurrence of PFOS, national or regional exposure information gathering and risk assessment may need to be considered. In addition, data on its toxicity to soil and sediment-dwelling organisms could be generated as a post-SIDS activity. There is currently no information on effects on soil- or sediment-dwelling organisms and PFOS has been detected in sediment and its presence in sewage sludge could lead to soil exposure if spread on agricultural land.

SUMMARY AND CONCLUSIONS OF THE HAZARD ASSESSMENT

Perfluorooctane sulfonate (PFOS) and its salts are fully fluorinated organic compounds. The number of production sites is not clear, but there is production in the US, Europe and Japan. In recent years (to 2000), approximately 4,500 metric tons of PFOS-related chemicals have been produced annually. The major global producer of PFOS intends to cease production by the end of 2002.

The majority of PFOS-related chemicals are high molecular weight polymers in which PFOS represents a fraction of the total molecular weight. PFOS-related chemicals are used in a variety of products, including as surface-treatments of fabric for soil/stain resistance, coating of paper as part of a sizing agent formulation and in specialised applications such as fire fighting foams.

PFOS has a solubility of approximately 550 mg/l in pure water at 24-25°C. The solubility decreases significantly with increased salt content, for example the potassium salt of PFOS has a solubility in fresh water of 370 mg/L and of 25 mg/l in filtered sea water. Due to the surface-active properties of PFOS, the Log Kow cannot be measured. The potassium salt of PFOS has a low vapour pressure, 3.31×10^{-4} Pa at 20 °C.

Human Health

In human blood samples, PFOS has been detected in the serum of occupational and general populations. In the U.S., the highest reported blood serum level of PFOS was observed in 1995 in a manufacturing employee in Decatur, Alabama (12.83 ppm). Mean PFOS levels have been dropping in that plant and a plant in Belgium since 1995, the most recent being 1.32 ppm and 0.80 ppm, respectively, in 2000. In the general population, serum collected from blood banks and commercial sources have indicated mean PFOS levels of 30-53 ppb. In individual serum samples obtained from adults and children in various regions of the U.S., mean PFOS levels were approximately 43 ppb.

Several occupational studies have been conducted on volunteers at the 3M plants in Decatur, Alabama and Antwerp, Belgium. Cross-sectional studies, based on the results of a voluntary medical surveillance program for employees at each plant, did not report consistent associations between workers' PFOS levels less than 6 ppm and certain hematology, hormonal, and other clinical chemistry parameters in 1995 and 1997. In 2000 when the analysis included male employees from both plants, mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly ($p < .05$) higher for workers with the highest PFOS serum levels (1.69 – 10.06 ppm). Serum triiodothyronine was significantly higher and thyroid hormone binding ratio was significantly lower in workers with the highest PFOS serum levels ($p < .05$ for both). The association with T3 also remained significant and positive in multivariable regression analyses adjusted for potential confounders. A longitudinal analysis of these data did not reveal statistically significant associations over time between PFOS and cholesterol, triglycerides, and other lipid and hepatic parameters. Hormones were not included in these analyses. There are several limitations to both the cross-sectional and longitudinal studies, such as the voluntary nature of the medical surveillance, the small number of employees participating across sampling periods, the different labs and analytical techniques used to measure serum PFOS, and the differences in PFOS levels, demographics, and clinical chemistries between employees in the Decatur and Antwerp plants.

In a mortality study, which followed workers for 37 years, mortality risks for most of the cancer types and non-malignant causes were not elevated. However, a statistically significant risk of death from bladder cancer was reported. Three male employees in the cohort died of bladder cancer (0.12 expected), and all of them had been employed at the plant for more than 20 years. All of them had also worked in high exposure jobs for at least 5 years. In order to screen for morbidity outcomes, an "episode of care" analysis was undertaken for employees who had worked at the plant between 1993 and 1998. Many different types of cancer and other non-malignant conditions were examined. Increased risks were not reported for most of the conditions or did not reach statistical significance. However, an increased risk of episodes was reported for neoplasms of the male reproductive system, the overall category of cancers and benign growths, and neoplasms of the gastrointestinal tract. These risk ratios were highest in employees with the highest and longest exposures to fluorochemicals.

Animal studies show that PFOS is well absorbed orally and distributes mainly in the serum and the liver. No further metabolism is expected. Elimination from the body is slow and occurs via the urine and feces. There are species differences in the elimination half-life of PFOS. The half-life in serum is 7.5 days in adult rats and 200 days in Cynomolgus monkeys. In humans, it appears to be quite longer. A recent half-life analysis was conducted on 9 retired 3M chemical workers. PFOS samples were collected over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. The mean half-life for PFOS was 8.67 years (range 2.29 – 21.3 years, SD = 6.12).

PFOS has shown moderate acute toxicity by the oral route with a rat LD50 of 251 mg/kg. A one-hour LC50 of 5.2 mg/l in rats has been reported. PFOS was found to be mildly irritating to the eyes and non-irritating to the skin of rabbits. PFOS has not been shown to be genotoxic in a variety of assay systems.

Numerous repeat-dose oral toxicity studies on PFOS have been conducted in rats and primates. In general, exposure to PFOS results in hepatotoxicity and mortality; the dose-response curve for mortality is very steep for rats and primates. Adverse signs of toxicity observed in 90-day rat studies included increases in liver enzymes, hepatic vacuolization and hepatocellular hypertrophy, gastrointestinal effects, hematological abnormalities, weight loss, convulsions, and death. These effects were reported at doses of 2 mg/kg/day and above. In a dietary 2-year bioassay in Sprague-Dawley rats, hepatotoxicity, characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm. Hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration were found in all the male treated groups (0.5, 2, 5, or 20 ppm). Based on the pathological findings in the liver, the LOAEL was 5 ppm and the NOAEL was 2 ppm in female rats. In males, the LOAEL was 0.5 ppm, and a NOAEL was not established.

Adverse signs of toxicity observed in Rhesus monkey studies included anorexia, emesis, diarrhea, hypoactivity, prostration, convulsions, atrophy of the salivary glands and the pancreas, marked decreases in serum cholesterol, and lipid depletion in the adrenals. The dose range for these effects was reported between 1.5-300 mg/kg/day. No monkeys survived beyond 3 weeks into treatment at 10 mg/kg/day or beyond 7 weeks into treatment at doses as low as 4.5 mg/kg/day. In a 6-month study of Cynomolgus monkeys, low food consumption, excessive salivation, labored breathing, hypoactivity, ataxia, hepatic vacuolization and hepatocellular hypertrophy, significant reductions in serum cholesterol levels, and death were observed at 0.75 mg/kg/day. No effects were observed at doses of 0.15 or 0.03 mg/kg/day. No effects were noted in animals at any dose level following a 52-week recovery period. The average concentration of PFOS in the serum following 26 weeks of treatment was 11.1 ± 1.52 , 58.5 ± 4.67 and 160 ± 23.9 $\mu\text{g/ml}$ for the females in the 0.03, 0.15 and 0.75 mg/kg/day groups, respectively; for males, the average concentrations were 15.9 ± 5.54 , 68.1 ± 5.75 and 194 ± 8.93 $\mu\text{g/ml}$ in the 0.03, 0.15 and 0.75 mg/kg/day groups, respectively. After the 52-week recovery period, the serum levels were 21.4 ± 2.01 and 41.4 ± 1.15 $\mu\text{g/ml}$ for the females in the 0.15 and 0.75 mg/kg/day groups, respectively; for males, the average concentrations were 19.1 ± 0.805 and 41.1 ± 25.9 $\mu\text{g/ml}$ in the 0.15 and 0.75 mg/kg/day groups, respectively.

The potential carcinogenicity of PFOS has been examined in a dietary 2-year bioassay in Sprague-Dawley rats. There was a significant increase in the incidence of hepatocellular adenomas in males and females at the highest dose of 20 ppm; the females at 20 ppm also had a significant increase in combined hepatocellular adenomas and carcinomas. In addition, there was a significant increase in thyroid follicular cell adenomas and combined thyroid follicular cell adenomas and carcinomas in the male recovery group at 20 ppm. There was no evidence of peroxisome proliferation in the livers of the treated animals.

Postnatal deaths and other developmental effects were reported at low doses in offspring in a 2-generation reproductive toxicity study in rats. At the two highest doses of 1.6 and 3.2 mg/kg/day, pup survival in the first generation was significantly decreased. All first generation offspring (F1 pups) at the highest dose died within a day after birth while close to 30% of the F1 pups in the 1.6 mg/kg/day dose group died within 4 days after birth. As a result of the pup mortality in the two top dose groups, only the two lowest dose groups, 0.1 and 0.4 mg/kg/day, were continued into the second generation. The NOAEL and LOAEL for the second generation offspring (F2 pups) were 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in pup body weight.

The liver and serum from the F0 and F1 animals was analyzed for PFOS. Qualitatively, the results for the F0 animals indicate that all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of

the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group.

Based on the results of the two-generation reproductive toxicity study, a cross-fostering study was conducted as a means of determining whether the reductions in pup viability were a result of *in utero* exposure to PFOS or as a result of exposure during lactation; thus the potential for a distinction to be made between prenatal and postnatal effects following continuous maternal treatment. Under the limited conditions of the study, the data appear to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse effect on pup viability.

Several mechanistic studies are being conducted to understand the neonatal death (3M Company, 2001c). Preliminary results indicate that reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the death of the offspring.

Developmental effects were also reported in prenatal developmental toxicity studies in the rat and rabbit, although at slightly higher dose levels. Signs of developmental toxicity in the offspring were evident at doses of 5 mg/kg/day and above in rats administered PFOS during gestation. Significant decreases in fetal body weight and significant increases in external and visceral anomalies, delayed ossification, and skeletal variations were observed. A NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for developmental toxicity were indicated. In the same study, evidence of treatment-related signs of maternal toxicity were also observed at doses of 5 mg/kg/day and above and mainly consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough hair coat, and bloody crust, as well as decreases in body weight gains and food consumption. Reductions in the mean terminal body weights minus the gravid uterine weights were also observed at doses \geq 5 mg/kg/day. A NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for maternal toxicity were indicated. In rabbits, significant reductions in fetal body weight and significant increases in delayed ossification were observed in the offspring of pregnant females administered PFOS during gestation at doses of 2.5 mg/kg/day and above. A NOAEL of 1.0 mg/kg/day and a LOAEL of 2.5 mg/kg/day for developmental toxicity were indicated. Maternal toxicity in the does was evident at doses of 1.0 mg/kg/day and above, and consisted of an increase incidence of abortions and scant feces, as well as significant reductions in mean maternal body weight gains and food consumption. A NOAEL of 0.1 mg/kg/day and a LOAEL of 1.0 mg/kg/day for maternal toxicity were indicated.

Environment

There is currently little information on the life-cycle steps that may lead to release of PFOS to the environment. However, PFOS has been detected in surface water and sediment downstream of a production facility and in wastewater treatment plant effluent, sewage sludge and landfill leachate at a number of cities in the US. Sampling of several wildlife species from a variety of sites across the United States has shown widespread distribution of PFOS and it was detected in the ppb range in the plasma of several species of eagles, wild birds, and fish. PFOS has been detected in marine mammals at a number of locations across the world.

PFOS is persistent in the environment. It does not hydrolyse, photolyse or biodegrade under environmental conditions and is not expected to volatilize, based on an air/water partition coefficient of $< 2 \times 10^{-6}$ Pa.m³/mol. PFOS has been shown to bioconcentrate in the tissues of bluegill sunfish and carp. In bluegill sunfish, BCF (BCFK) values between 1124 and 4013 were determined and PFOS depurated slowly with estimated 50% clearance times of up to 116 days. In carp, BCF values were determined to be between 200 and 1500.

The substance shows moderate acute toxicity to aquatic organisms, the lowest LC₅₀ for fish is a 96-hour LC₅₀ of 4.7 mg/l to the fathead minnow *Pimephales promelas* for the lithium salt. For aquatic invertebrates, the lowest EC₅₀ for freshwater species is a 48-hour EC₅₀ of 27 mg/l for *Daphnia magna* and for saltwater species, a 96-hour LC₅₀ value of 3.6 mg/l for the Mysid shrimp *Mysidopsis bahia*. Both tests were conducted on the potassium salt. For algae, the potassium salt gave a 96h NOEC of >3.2 mg/l with *Skeletonema costatum*.

Long-term toxicity data is available for fish and aquatic invertebrates. The lowest NOEC for fish is a 42 day NOEC (survival) of 0.3 mg/l in an early life stage test with *Pimephales promelas* using the potassium salt. The lowest NOEC for aquatic invertebrates is a 35-day NOEC_{reproduction} of 0.25 mg/l for *Mysidopsis bahia* using the potassium salt. For freshwater species, there is a 28-day NOEC_{reproduction} of 7 mg/l for *Daphnia magna*, also using the potassium salt.

A growth inhibition test has been carried out on PFOS potassium salt with *Lemna gibba* (Duckweed). The test gave a 7-day IC₅₀ of 108 mg/l for inhibition of frond production and a 7-day NOEC of 15.1 mg/l based on the inhibition of frond production and evidence of sub-lethal effects.

PFOS does not appear to be toxic to sewage sludge microorganisms. In an activated sludge respiration inhibition test, the 3-hour IC₅₀ value for PFOS (potassium salt) was >905 mg/l (nominal concentration).

No data are available for effects on soil-dwelling or sediment-dwelling species. The use of equilibrium partitioning models to derive a PNEC for these compartments may not be applicable to this anionic surfactant.

PFOS has been tested on two species of bird, the Mallard duck, *Anas platyrhynchos*, and the Northern Bobwhite quail, *Colinus virginianus*. The lowest acute dietary LC₅₀ value of 220 mg/kg of food was determined in the test with the quail. The lowest NOEC of 37 mg/kg of food for effects on body weight was, in contrast, obtained in the test with the duck.

There are data available from acute oral and contact toxicity tests on the Honey bee (*Apis mellifera*) using PFOS potassium salt. These studies indicate moderate and high orders of toxicity of PFOS to bees when administered via these routes. The acute oral test yielded a 72-hour LD₅₀ for ingestion of PFOS of 0.40 µg/bee and a 72-hour NOEL of 0.21 µg/bee. The contact test yielded a 96-hour LD₅₀ of 4.78 µg/bee and a 96-hour NOEL of 1.93 µg/bee.

The results of an amphibian teratogenesis study carried out with *Xenopus laevis* (African clawed frog) show PFOS potassium salt to be acutely toxic to (96-hour LC₅₀ = 13.8 mg/l), and cause malformations in (96-hour EC₅₀ = 12.1 mg/l), frog embryos. The minimum concentration that inhibited growth of the embryos was determined to be 7.97 mg/l. A teratogenic index of 1.1 was determined from the ratio of the 96-hour LC₅₀ to the 96-hour EC₅₀, indicating a low potential for PFOS to be a developmental hazard in this species.

1.0 Identity

Chemical Name: Perfluorooctane Sulfonate

The perfluorooctane sulfonate anion (PFOS) does not have a specific CAS number. The acid and salts have the following CAS numbers:

acid (1763-23-1)
ammonium (NH_4^+) salt (29081-56-9)
diethanolamine (DEA) salt (70225-14-8)
potassium (K^+) salt (2795-39-3)
lithium (Li^+) salt (29457-72-5)

Molecular formula: $\text{C}_8\text{F}_{17}\text{SO}_3$

Structural formula: $\text{CF}_3\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-CF}_2\text{-S(=O)(=O)O}^-$

Synonyms: 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro;
1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid;
1-Octanesulfonic acid, heptadecafluoro-;
1-Perfluorooctanesulfonic acid;
Hepatadecafluoro-1-octanesulfonic acid;
Perfluoro-n-octanesulfonic acid;
Perfluorooctanesulfonic acid;
Perfluorooctylsulfonic acid

1.1 Physicochemical Properties

Due to the surface-active properties of PFOS and the test protocol itself, PFOS forms three layers in octanol/water and hence, an n-octanol/water (K_{ow}) partition coefficient cannot be determined. Consequently, the various physicochemical properties (e.g., bioconcentration factor, soil adsorption coefficient), which can usually be estimated for conventional organic compounds utilizing K_{ow} equations, cannot be estimated, and a calculated (estimated) log K_{ow} cannot be trusted. Even if the log K_{ow} were known, it may not be appropriate for predictive purposes, e.g., bioconcentration. Studies on laboratory rats indicate that PFOS does not bioconcentrate in the lipid fraction. Instead, it tends to bind to certain proteins. In two studies, PFOS was reported to have a mean solubility of 519 mg/L and 570 mg/L in pure water at 24-25°C. Solubility decreases significantly with increased salt content (12.4 mg/L in natural seawater at 22-23°C, and 20.0 mg/L in a 3.5% NaCl solution at 22-24°C (3M Company, 2001a). In a related study, PFOS was reported to have a mean solubility of 56.0 mg/L in pure octanol (3M Company, 2001b). These data suggest that any PFOS discharged to a water source would tend to remain in that medium, unless it is adsorbed onto particulate matter or assimilated by organisms. If PFOS does bind to particulate matter the material would ultimately end up in the sediment. Further study is underway to determine the presence of PFOS in sediments from various locations and the binding potential of PFOS to sediments.

The available physicochemical properties for the potassium salt of PFOS are as follows (3M Report, 1999):

Melting point: $\geq 400^\circ\text{C}$
Boiling point: not calculable
Vapor pressure: 3.31×10^{-4} Pa at 20°C (3.27×10^{-9} atm)

Air/water partition coefficient in pure water: 0 ($<2 \times 10^{-6}$)

Solubility: pure water: 570 mg/L

Solubility: fresh water: 370 mg/L

Solubility: unfiltered seawater: 12.4 mg/L

Solubility: filtered seawater: 25 mg/L

The specific gravities (water = 1) and pH values (in parentheses) of the PFOS salts are as follows (3M Report, 1999):

DEA, ~ 1.1 (~ 7)

NH_4^+ , ~ 1.1 (~ 7)

Li^+ , ~ 1.1 (6-8)

K^+ , ~ 0.6 (7-8)

The above water solubility values correspond to the following Henry's Law constants ($\text{atm}\cdot\text{m}^3/\text{mol}$), calculated herein, utilizing the vapor pressure of 3.27×10^{-9} atm given in the 3M Report (1999):

Pure water: 3.05×10^{-9}

Fresh water: 4.7×10^{-9}

Unfiltered seawater: 1.4×10^{-7}

Filtered seawater: 2.4×10^{-8}

As a point of reference, the Henry's Law constant for pure water at 20°C is 4.34×10^{-7} .

2.0 General Information on Exposure

The starting material for PFOS-related chemicals is perfluorooctanesulfonyl fluoride (POSF). POSF is manufactured through a process known as Simons Electro-Chemical Fluorination (ECF) in which an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedstock of 1-octanesulfonyl fluoride. The ECF process replaces the carbon-hydrogen bonds on molecules of the organic feedstock with carbon-fluorine bonds. Perfluorination occurs when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields between 30-45 percent straight chain (normal) POSF, along with a variable mixture of byproducts and impurities. The output of the ECF process is not a pure chemical, but instead a mixture of isomers and homologues including higher and lower straight-chain homologues; branched-chain perfluoroalkyl fluorides of various chain lengths; straight-chain, branched, and cyclic perfluoroalkanes and ethers; and other byproducts (3M Company, 2000b).

According to information available to the U.S. Environmental Protection Agency (USEPA), 3M Company is the dominant producer of POSF. In 1997, 3M reported the manufacture or importation into the United States of approximately 1,848 metric tons of POSF. For 2000, 3M forecasts a volume of 1,820 metric tons manufactured or imported into the United States. After accounting for 3M operations in Antwerp, Belgium, 3M estimates a total global POSF production of 3,665 metric tons for 2000 (3M Company, 2000d). Production of POSF by 3M is expected to decline to zero by the end of 2002 as 3M scales back the production POSF-derived chemicals (3M Company, 2000d).

Since most POSF is incorporated into higher molecular weight polymers, it comprises only a portion of the mole fraction of the entire polymer in the final product. However, it should be noted that the secondary reactions used to produce POSF derivatives do not necessarily produce pure products. Typically, 1-2% of the final product is comprised of unreacted or partially reacted fluorinated starting materials or intermediates that are carried forward into the final product as impurities (3M Company, 2000b).

To date, 3M has not provided information on the total cumulative production volumes of POSF or PFOS-related chemicals since initial commercialization over 40 years ago. Precise production volume information for manufacturers other than 3M outside the United States has also been difficult to obtain. 3M, however, has asserted that it is the dominant global producer of PFOS chemicals, responsible for the large majority of total global production volumes (3M Company, 2000e). The limited production volume information provided by OECD member countries for companies located outside the United States supports this conclusion.

Aside from the United States and Belgium, other OECD Member countries that reportedly have production capacity include Italy and Japan. There may also be some production in non-OECD countries. Following are companies that have been identified in various chemical buyer's guides as offering PFOS-related chemicals for sale (Directory of World Chemical Producers, 2000; ChemSources USA, 2000; OPD Chemical Buyers Directory, 2000). This information has not been corroborated independently, except for Miteni S.p.A. of Italy and Dianippon Ink & Chemicals, Inc. of Japan.

OECD member countries

- Miteni S.p.A. (Italy)
- EniChem Synthesis S.p.A. (Italy)
- Dianippon Ink & Chemicals, Inc. (Japan)
- Midori Kaguka Co., Ltd. (Japan)
- Tohkem Products Corporation (Japan)
- Tokyo Kasei Kogyo Company, Ltd. (Japan)
- Fluka Chemical Co, Ltd. (Switzerland)
- BNFL Fluorochemicals Ltd. (United Kingdom)
- Fluorochem Ltd. (United Kingdom)

Non-OECD countries

- Milenia Agro Ciencias S.A. (Brazil)
- Changjiang Chemical Plant (China)
- Indofine Chemical Company, Inc. (India)
- Scientific Industrial Association P & M Ltd. (Russian Federation)

2.1 Production and Use of PFOS

The majority of PFOS-related chemicals are high molecular weight polymers in which PFOS represents a fraction of the total molecular weight. PFOS-related chemicals are used in a variety of products, as shown in the figure following this section. These products can be divided into three main categories of use:

- Surface treatments,
- Paper protection, and
- Performance chemicals.

Each of these categories and the associated 3M production volume is described in more detail below. Note that in many cases the production volumes represent total solid metric tons of fluorochemical-containing compound, not PFOS itself. Less than 91 metric tons of PFOS and its salts are commercialized as finished products (3M, 2000b).

In addition, varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials

or intermediates) are carried forward into final products at concentrations of 1-2% or less as a result of the ECF manufacturing process. These residuals also have the potential to degrade or metabolize to PFOS (3M, 2000b).

2.1.1 PFOS-Based Surface Treatment Applications

PFOS-related chemicals produced for surface treatment applications provide soil, oil, and water resistance to personal apparel and home furnishings. Specific applications in this use category include protection of apparel and leather, fabric/upholstery, and carpet. These applications are undertaken in industrial settings by customers such as textile mills, leather tanneries, finishers, fiber producers, and carpet manufacturers. PFOS-related chemicals are also used in aftermarket treatment of apparel and leather, upholstery, carpet, and automobile interiors by the general public or professional applicators (3M Company, 2000b). In 2000, the global production volume of PFOS-related chemicals for this use category is estimated at approximately 2,160 metric tons (3M Company, 2000b).

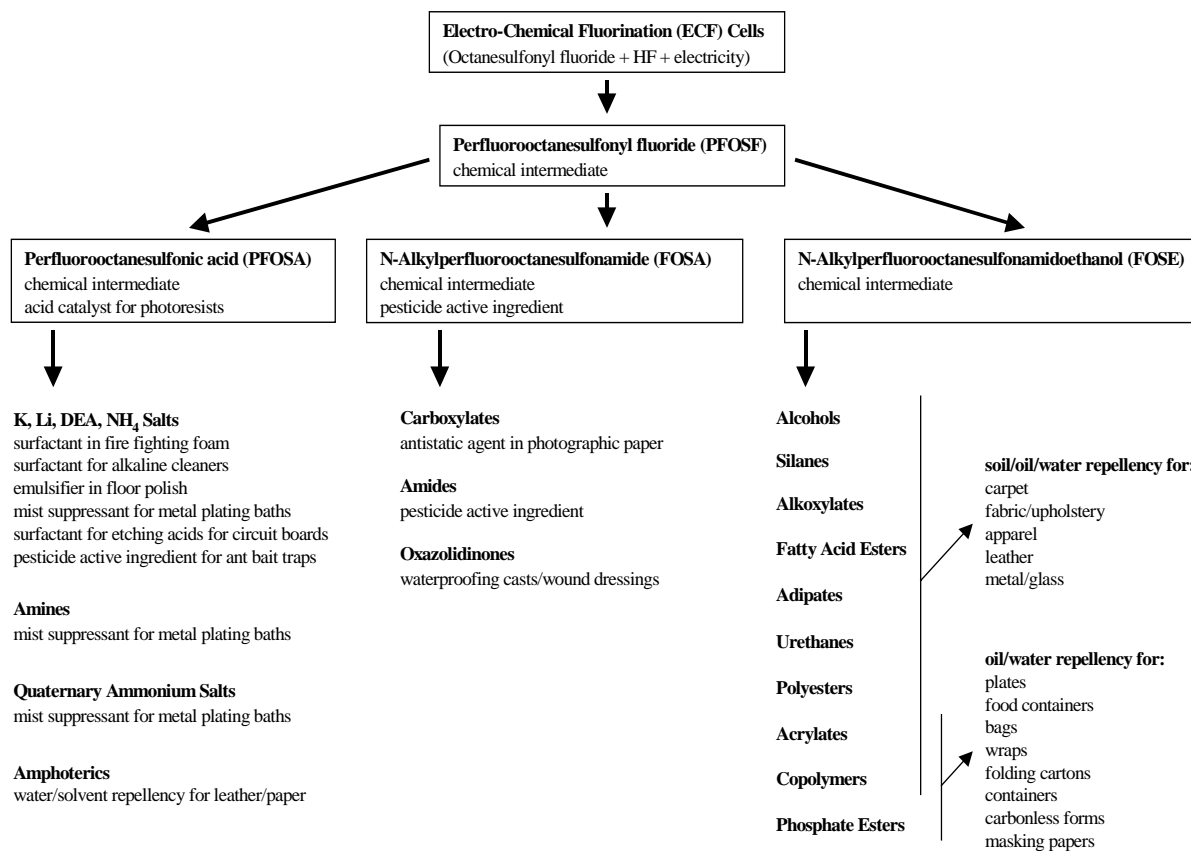
2.1.2 PFOS-Based Paper Protection Applications

PFOS-related chemicals produced for paper protection applications provide grease, oil, and water resistance to paper and paperboard as part of a sizing agent formulation. Specific applications in this use category include food contact applications (plates, food containers, bags, and wraps), as well as non-food contact applications (folding cartons, containers, carbonless forms, and masking papers). The application of sizing agents is undertaken mainly by paper mills and, to some extent, converters who manufacture bags, wraps, and other products from paper and paperboard (3M Company, 2000d). In 2000, the global production volume of PFOS-related chemicals for this use category is estimated at approximately 1,490 metric tons (3M Company, 2000b).

2.1.3 PFOS-Based Performance Chemical Applications

PFOS-related chemicals in the performance chemical category are used in a variety of specialized industrial, commercial, and consumer applications. This category includes various salts of PFOS that are commercialized as finished products. Specific applications in this category include fire fighting foams, mining and oil well surfactants, acid mist suppressants for metal plating and electronic etching baths, photolithography, electronic chemicals, hydraulic fluid additives, alkaline cleaners, floor polishes, photographic film, denture cleaners, shampoos, chemical intermediates, coating additives, carpet spot cleaners, and as an insecticide in bait stations (3M Company, 2000b; Boeing, 2000). In 2000, the global production volume of PFOS chemicals for this use category is estimated at approximately 831 metric tons (3M Company, 2000d). Of this volume, approximately 151 metric tons will be used in fire fighting foams.

Perfluorooctyl Sulfonates: Major Product Categories



2.2 Environmental Exposure and Fate

PFOS does not hydrolyze, photolyze or biodegrade under environmental conditions and it is persistent in the environment.

2.2.1. Volatility

PFOS is not expected to volatilize, based on an assigned air/water partition coefficient of $< 2 \times 10^{-6}$ Pa.m³/mol. This assignment was made by Prof. Donald Mackay based on experimental data generated by 3M. In that study, the Hc was too small to measure experimentally using pure water. The interpretation by Mackay states “[PFOS] is thus essentially non-volatile from aqueous solution. This is probably because of its ionic nature. The simple expedient is to assign it a Kaw of zero, i.e. is a type 2 involatile chemical in our nomenclature.” This determination is in agreement with the Henry’s Law constant of 3.05×10^{-9} calculated herein for pure water. Overall, the conclusion is that PFOS is a substance with very low and possibly negligible volatility.

2.2.2 Combustion

To begin to understand the potential degradation pathways for perfluorinated chemicals, the incineration process was thermodynamically modeled. The most pertinent finding of the reported analysis is that the carbon-sulfur bond in the perfluorooctane sulfonate molecule is a fairly weak bond. To validate the models prediction, laboratory combustion studies on a series of perfluoroalkyl compounds, including PFOS and two polymeric product formulations are being conducted. An assessment will be made of combustion by-products through a range of temperatures. These data will be important to determine whether PFOS may enter the atmosphere as a result of incomplete combustion of waste.

2.2.3 Photolysis/Oxidation

PFOS does not appear to photolyze (Hatfield, T. 2001). Screening studies on the aqueous photolytic degradation of PFOS, EtFOSE alcohol, MeFOSE alcohol, EtFOSA and MeFOSA as well as a surfactant and foamer product, all appeared to undergo indirect photolysis to FOSA, PFOA, a hydride and olefins; PFOS was not detected (Hatfield, T. 2001, 3M Report No. W2775). One product, an aromatic perfluorooctane sulfonate, did photodegrade to form PFOS (3M Report, 1999).

2.2.4 Biodegradation

The biodegradability of PFOS was examined in a MITI-I test (Kurume Laboratory (2002). No significant degradation of PFOS was observed in 28 days, either as net oxygen demand from degradation of test substance (i.e., ultimate degradation), loss of total organic carbon (TOC; another way to measure ultimate degradation), or loss of parent compound identity (primary degradation). Average percentage biodegradation after 28 days was observed to be 0% by oxygen demand; 6% by removal of total organic carbon; and 3% by liquid chromatography-mass spectrometry (LC-MS; measures primary degradation). These values are indicative of no significant degradation, within the accuracy limits for this test. Validity of the test was confirmed by degradation of the control substance aniline, for which % of theoretical oxygen demand exceeded the pass criterion of 45% after 7 days and 60% after 14 days respectively (74% and 85% of theoretical at days 7 and 14, respectively).

2.3 Environmental Monitoring

3M’s Multi-City Study reported on PFOS concentrations from water, sludge, sediment, POTW effluent and landfill leachate samples taken in six cities (3M, 2001a). Four of the cities (Decatur AL, Mobile AL,

Columbus GA, Pensacola FL) were supply cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland TN, Port St. Lucie FL) were control cities that do not have significant fluorochemical activities. Across all cities, POTW effluent concentrations ranged from 0.041 to 5.29 ppb. The POTW sludge (dry wt.) range was less than 0.2 ug/kg to 3,120 ppb; the drinking water range was non-detect to 0.063 ppb; the landfill leachate range was non-detect to 53.1 ppb; the surface water range was non-detect to 0.138 ppb; the sediment range was non-detect to 1.13 ppb (dry wt.); and the quiet water range was non-detect to 2.93 ppb. The control cities samples generally inhabited the lower end of the above ranges, except for the POTW effluent and sludge findings for Cleveland, which were intermediate in their ranges, and the quiet water samples at Port St. Lucie, which were the highest. 3M states that all the PFOS surface water concentrations are below laboratory-derived NOEC (No Observed Effect Concentrations) for aquatic organisms.

The Multi-City Study also included a market basket sampling of PFOS residue in a total of over 200 samples taken from green beans, apples, pork muscle, cow milk, chicken muscle, chicken eggs, bread, hot dogs, catfish, and ground beef (3M, 2001a). Measurable quantities of PFOS, to 0.852 ng/g, were found in four milk samples and one ground beef sample; one of the four milk samples was from a control city, the balance of the samples with measurable PFOS were from cities with fluorochemical manufacture or use.

Giesy reported the results of a global monitoring survey of PFOS in marine mammals; they were located in Florida, California and Alaskan U.S. coastal waters, the northern Baltic Sea, the Mediterranean Sea, the Arctic (Spitsbergen), and Sable Island in Canada. PFOS was detected in the liver and blood of marine mammals from most locations. The largest liver concentration was 1520 ng/g, wet wt., in bottlenose dolphin from Florida; the largest blood concentration was 475 ng/mL in ringed seal from the northern Baltic Sea.

The plasma of piscivorous (fish-eating) birds was sampled in the late 1980's and early 1990's or obtained from the U.S. Fish and Wildlife Service (date of liver samples was not listed), and these samples were later sent to 3M for analysis of PFOS levels (3M Company, 2000a). Species sampled for PFOS plasma levels included the bald eagle, albatross, and sea eagle. Plasma levels ranged from below the limit of detection (1 ppb) up to a maximum of 1047 ppb, present in a bald eagle. Levels in the livers sampled from six bird species ranged from below the limit of detection up to a maximum of 2055 ppb.

Giesy reported on PFOS in fish and fish-eating water birds. Fish were sampled from the U.S., certain European countries, the North Pacific Ocean and Antarctic locations (Giesy, 2001a). The highest concentration was 923 ng/g, wet wt., in the muscle of fish from a Belgium estuary. The highest concentration of PFOS in U.S. Great Lakes fish was 297 ng/g, wet wt., in the muscle of carp. Muscle tissue of fish from several inland lakes in Michigan U.S. did not contain detectable PFOS, nor did fish from the North Pacific and Antarctic oceans. Fish-eating bird samples were collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, Japanese and Korean coasts (Giesy, 2001b). PFOS concentrations collected from the plasma of bald eagles in the midwestern USA ranged from not quantifiable to 2220 ng/mL, with a mean of 330 ng/mL. The largest PFOS concentration in liver samples from USA birds was 1780 ng/g, wet wt. from a Brandts cormorant. PFOS concentrations in the sera of North Pacific Ocean albatrosses ranged from 3 to 34 ng/mL. PFOS concentrations in blood or livers of birds from the Mediterranean and Baltic Seas were relatively less than those found in U.S. birds.

Giesy reported on PFOS in mink and river otter livers from the U.S. (Giesy, 2001c). PFOS was found in all samples, with the largest concentration of 4800 ng/g, wet wt., found in mink liver. PFOS concentrations in river otter livers from Washington and Oregon states ranged from 34 to 994 ng/g, wet wt.

Giesy reported that PFOS was detected in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast at 51 of 77 sampling locations at a concentration range of <42 to 1225 ng/g, dry wt. (Giesy,

2001d).

Giesy reported on the concentrations of PFOS in surface water, sediments, clams, and fish collected from locations upstream and downstream of the 3M facility at Decatur AL (Giesy, 2001e). Of the three downstream sampling locations, the two closest to the 3M facility had PFOS surface water concentrations significantly greater than the two upstream sites (means of 150 ug/L and 82 ug/L, vs. 0.009 (est.) and 0.053 ug/L); the nearest two locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 5930 ug/kg, 1299 ug/kg vs. 0.18(est.) and 0.98). Clam and fish samples were collected at two locations, one upstream and one downstream of the 3M facility. The average fish whole body PFOS concentration for the upstream location was 59.1 ug/kg (wet wt.), while that for the downstream location was 1332 ug/kg. The average PFOS concentration in clams at the upstream location was 15.6(est.) ug/kg; that for the downstream location was 14.1(est.) ug/kg.

Hansen (2002) reported concentrations of PFOS measured from surface water samples taken from the Tennessee River up- and downstream of the outfall from the fluorochemical manufacturing facility at Decatur AL (the 3M facility mentioned above). There were 20 sampling sites above and 20 sites below the outfall location, spaced at approximately 2 mile intervals. Upstream of the facility the average concentration of PFOS was 32 ± 11 ng/L; the downstream concentrations were observed to increase at a point approximately six miles below the outfall; the average PFOS concentration from that point downstream was 114 ± 19 ng/L. The report states that the consistency of the PFOS concentrations within these two regions suggests the absence of either major environmental sinks or additional sources of PFOS in the areas sampled.

The 3M Environmental Lab reported on analyses of PFOS in naive rat livers, rat chow, and fish meal (a component of rat chow). Rat livers from two of three suppliers had endogenous levels of PFOS above the limit of detection (15 ng/g). PFOS levels in the male rats with measurable levels increased with age, while the levels in female rats showed no correlation with age. In the rat chow study, two of the four chows examined had PFOS above the 2-10 ng/g level of quantitation, 18 and 12 ng/g, respectively. The fish meal study analyzed PFOS levels in six types of fishmeal formulated from at least three types of fish. Three of the six types had PFOS levels above the limit of quantitation of 3.5 ng/g; the maximum concentration was 15.7 ng/g.

The 3M Environmental Laboratory also summarized PFOS analyses of avian and aquatic feed matrices. PFOS levels were not detected in the two daphnid feeds, and were below the limits of quantitation in the avian feed. Dry artemia (brine shrimp) cysts used for fish feed had PFOS levels of 9-10 ug/kg.

2.4 Human Biomonitoring

For many years, PFOS has been measured in the serum of workers occupationally exposed to PFOS. It was also recently detected in the serum of the general population, but at much lower levels. The results of the most recent analyses of blood samples are described below and summarized in Table 1.

2.4.1 Occupational Exposures

PFOS serum levels have been measured in workers involved in both the manufacturing of perfluorochemicals and the processing of these compounds into products, such as fire protection and surface protection products. Biomonitoring data are available from manufacturing plants in Alabama and Belgium, a processing facility in Japan, and corporate offices in Minnesota.

Fluorochemical Manufacturing

Two 3M plants, located in Decatur, Alabama and Antwerp, Belgium, have produced POSF from electrochemical cell fluorination and then subsequently used POSF to produce various products through polymerization processes. These POSF-based products degrade or metabolize to PFOS. A third manufacturing plant, located in Cottage Grove, Minnesota, has not produced POSF, but has manufactured some POSF-based products. Serum PFOS levels were measured in manufacturing plant employees in 1995 (n = 178), 1997 (n = 149), and 2000 (n = 521) using high performance liquid chromatography/mass spectrometry (Olsen et al, 1999; Olsen et al, 2001d; Olsen et al, 2001e; Olsen et al., 2001f). Employees volunteered to participate for medical surveillance. Arithmetic means and ranges were reported for all surveillance periods. Geometric means have only been reported in 2000. These data are provided in Table 1.

Mean serum PFOS levels have been declining at both plants since 1995. Decatur serum levels have been higher than Antwerp levels during every surveillance period. The PFOS levels in workers across both plants in 1995 ranged from 0.10 ppm to 12.83 ppm. The range of PFOS levels of workers in 1997 was 0.10 - 9.93 ppm. The largest number of employees at each plant participated in the 2000 medical surveillance program (521 out of 840 employees). The mean serum PFOS level at the Decatur plant was 1.32 ppm (range = 0.06 – 10.06 ppm). Most of the employees at both facilities had PFOS serum levels that were <6 ppm across all of the sampling periods.

Except for the year 2000, the participation rate of the total number of potentially exposed employees in the medical monitoring program from which these data were derived was not provided. It was reported that the employees were offered a medical monitoring program, but it is not clear how many of them actually volunteered to participate. In addition, age of the employees and duration of employment were not reported for most of the surveillance years, and it is unclear how many of the total number of employees worked at each plant. Therefore, it should be noted that these data only provide a snapshot of the serum levels of participating workers during each sampling period.

In order to address the voluntary nature of the monitoring program at the 3M plants, a random sample of employees from the Decatur plant was chosen to measure seven different fluorochemicals in employees' blood (Olsen et al., 1999). Limited job information, years worked, and building location were collected to provide a better understanding of the distribution of fluorochemical serum levels in employees. The random sample consisted of 187 chemical plant workers (126 randomly chosen workers and 61 volunteers) and 76 film plant workers (60 randomly chosen and 16 volunteers). The levels in these employees, who were sampled in late 1998, were similar to those reported above. Mean PFOS levels were 1.505 ppm (range, 0.091-10.600) for the random sample, 1.259 ppm for the volunteers, and 1.424 ppm for all participants. Geometric means were 0.944 ppm (95% CI 0.787-1.126) for chemical plant employees and 0.136 ppm (95% CI 0.114-0.161) for film plant employees. When compared by job description, workers in the chemical plant had higher PFOS levels than workers in the film plant. PFOS levels in males were positively, although modestly, associated with number of years worked in the chemical plant.

Fluorochemical Processing

Biomonitoring data were submitted on workers in Japan processing perfluorinated chemicals into fire protection products and surface products (Burris et al., 1999). In 1999, PFOS, PFOA and PFHS (perfluorohexanesulfonate) were measured in employees' serum at the Sagamihara plant. Serum was drawn from both production employees (n = 32) who regularly handle fluorochemicals and management employees (n = 32) who are not regularly exposed. Serum was also drawn from management employees (n = 30) at the Head Office in Tokyo. The highest PFOS level in 32 production employees was 0.628 ppm, with an arithmetic mean of 0.135 ppm. The highest PFOS level in either of the management groups was

0.0967 ppm, while the average level was approximately 0.04 ppm. The results of biomonitoring performed at the Sagami-hara plant indicate lower levels of PFOS than at either of the manufacturing facilities cited above.

Although the exposures would not necessarily be considered “occupational” for either group, PFOS levels in corporate staff/managers at a 3M plant in St. Paul, Minnesota were similar to those reported in the corporate managers in Japan (3M Report, 1999). Thirty-one employees, none of whom had worked in fluorochemical production or research and development, were sampled in 1998. All of the participating employees had measurable levels of PFOS in their serum. The mean PFOS level was 0.047 ppm, with a range of 0.028 to 0.096 ppm. In this group of 3M employees, age was significantly associated with increased serum PFOS. No other data on these workers were provided.

2.4.2 Non-occupational Exposures

PFOS has been measured in the serum of the general U.S. population and in small numbers of historical samples (dating as far back as 1957) from sources throughout the world. In 1998, PFOS levels in pooled serum from 2 commercial sources in the U.S. ranged from 43- 45 ppb and 26 - 45 ppb (3M Report, 1999). There were approximately 500 donors in the first source and an estimated 200 donors in the second. No other data, such as geographic location or age of the donors, were provided. 3M also analyzed thirty-five lots of individual or pooled human sera samples from U.S. chemical or biological supply companies in 1999 (3M Letter, 1999). These samples yielded an average of 35 ppb PFOS, ranging from 5 to 85 ppb.

Sera pooled from 18 regional blood banks in various geographic regions of the U.S. were analyzed for PFOS in 1998 (3M Report, 1999). There were 68 pools and an estimated 340-680 donors. The overall mean PFOS serum level across the pools was 29.7 ppb. The PFOS levels varied quite a bit depending on the geographic location of the blood bank. The range of the levels across geographic regions was 9 to 56 ppb, while the range of the averages was 14 to 52 ppb.

Pooled sera from blood banks in Belgium (6 pools), the Netherlands (5 pools), and Germany (6 pools) also have been analyzed for PFOS. Each pool had 15-20 donors. The Netherlands had the highest PFOS levels (mean = 53 ppb) and Belgium had the lowest (mean = 17 ppb).

Individual blood samples from 3 different age populations were recently analyzed for PFOS and other fluorochemicals using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS) (Olsen et al., 2002a, 2002b, 2002c). The studies’ participants included adult blood donors, an elderly population participating in a prospective study in Seattle, WA, and children from 23 states participating in a clinical trial. Overall, the PFOS geometric means were similar across all 3 populations (34.9 ppb, 31.0 ppb, and 37.5 ppb, respectively). The geometric means and 95% tolerance limits and their upper bounds were comparable across all 3 studies. However, the upper ranges for the children and adults were much higher than for the elderly population. It is not clear whether this is the result of geographic differences in PFOS levels or some other factor. It should be noted that PFOS and PFOA were highly correlated in all three studies ($r = .63$, $r = .70$, and $r = .75$). The details of each study are provided below.

Serum PFOS levels in 645 blood donors (332 males, 313 females), aged 20-69 years, were obtained from 6 American Red Cross blood banks (Olsen et al., 2002a). The blood banks were located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD. Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex. The only demographic factors known for each donor were age, gender, and location.

The geometric mean serum PFOS level for all locations and gender was 34.9 ppb (95%CI, 33.3-36.5 ppb).

The range was < LLOQ (4.3 ppb) to 1656 ppb. Males had significantly higher ($p < .05$) geometric mean PFOS levels than females. The geometric mean for all males was 37.8 ppb (95% CI, 35.5-40.3) and was 31.3 ppb for all females (95% CI, 30.0 – 34.3). Age was not an important predictor of adult serum fluorochemical concentrations. When stratified by geographic location, the highest geometric mean for PFOS was in the samples from Charlotte, NC (51.5 ppb, range: 19.3 – 166.0) and the lowest from Boston (28.0 ppb, range: 4.3 – 87.2).

The highest serum PFOS measurement in this sample was 1656 ppb from a male blood donor, 67 years old from Portland. The next highest donor level was 329 ppb from a male donor, 62 years old also from Portland. The next 8 highest serum PFOS values (range 139 – 226 ppb) were measured in 4 females and 4 males representing Charlotte (n=4), Hagerstown (n=2), Los Angeles (n=1) and Minneapolis/St. Paul (n=1). In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 100.0 ppb.

Serum PFOS levels were reported for 238 (118 males and 120 females) elderly volunteers in Seattle participating in a study designed to examine cognitive function in adults aged 65-96 (Olsen et al., 2002b). Age, gender and number of years' residence in Seattle were the only data available on the participants. Most of the participants were under the age of 85 and had lived in the Seattle area for over 50 years.

The geometric mean of PFOS for all samples was 31.0 ppb (95% CI, 28.8-33.4). The range was 3.4 – 175 ppb. There was no significant ($p < .05$) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ($p < .05$) associated with PFOS in men but not in women. The mean of the 95% tolerance limit for PFOS was 84.1 ppb with an upper 95% confidence limit of 104.0 ppb.

A sample of 599 children, ages 2-12 years old, participating in a study of group A streptococcal infections, was analyzed for PFOS levels (Olsen et al., 2002c). The samples were collected in 1994-1995 from children residing in 23 states and the District of Columbia. The geometric mean of PFOS for all of the participants was 37.5 ppb (95% CI, 33.3-36.5). The range was 6.7 to 515.0 ppb. Male children had significantly ($p < .01$) higher geometric mean PFOS levels than females: 40.1 ppb and 35.2 ppb, respectively. In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. When stratified by age, the geometric mean tended to rise for each age group from age 2 (28.6 ppb) through age 9 (42.8 ppb) where it was highest, and then started to decrease gradually to 32.8 ppb at 12 years. In simple linear regression analyses, age was not significantly ($p < .05$) associated with PFOS. Although the data were not provided, a graphical presentation of log PFOS levels for each state by gender were similar across the states, however, it is difficult to interpret these data given the limited sample size for each gender/location subgroup.

In another study, Olsen et al. (2001g) obtained samples from 31 cadavers (16 males and 15 females) over an 18-month period from the International Institute for the Advancement of Medicine (IIAM). The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were harvested from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Serum samples were obtained from 5 ml of blood; liver samples consisted of 10 g of tissue. Samples were frozen at IIAM and shipped frozen to 3M for analysis. The samples were extracted using an ion-pairing extraction procedure and were quantitatively assayed using HPLC-ES/MS and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the extraction procedure. The average fortified sample recovery of PFOS from human sera was 89% (SD 21%) and the average fortified sample recovery of PFOS from human liver was 78% (SD 24%).

Serum PFOS levels that were determined to be less than the limit of quantitation (LOQ) were assigned a value midpoint between zero and the LOQ. The mean serum PFOS level for the 24 serum donors analyzed was 17.7 ng/mL; the geometric mean for serum PFOS was 14.7 ng/mL. Of the 30 PFOS liver samples analyzed, 15 (50%) were determined to be <LOQ. These samples were assigned a value midpoint between zero and the LOQ. The mean liver PFOS level was 18.8 ng/g; the geometric mean for liver PFOS was 15.2 ng/g. Mean PFOS levels for male and female donors were similar for both serum (male = 18.2 ng/mL; female = 17.2 ng/mL) and liver (male = 19.2 ng/g; female = 28.4 ng/g). Although the data were not shown, the report stated that no associations were observed between measured PFOS levels and age. The average PFOS serum and liver data for each of the 23 paired samples (serum and liver from the same individual) showed a good correlation. The mean liver to serum ratio was 1.3:1. Of the 13 male donors with paired samples, the mean liver to serum ratio was again 1.3:1; the mean serum level was 18.2 ng/mL and the mean liver level was 20.8 ng/g. For the 10 female donors with paired samples, the mean liver to serum ratio was 1.3:1 with mean serum levels of 16.9 ng/mL and mean liver levels of 16.3 ng/g, respectively.

Table 1. Mean PFOS serum levels in human populations

Occupational Exposures		
Plant Location	Mean (ppm)	Range (ppm)
Decatur, Alabama		
1995 (n = 90)	2.44	0.25 - 12.83
1997 (n = 84)	1.96	0.10 - 9.93
1998 (n = 126)	1.51	0.09 - 10.6
2000 (n = 263)	1.32	0.06 - 10.06
	(GM = 0.91)*	(33.3 - 36.5)**
Antwerp, Belgium		
1995 (n = 93)	1.93	0.10 - 9.93
1997 (n = 65)	1.48	0.1 - 4.8
2000 (n = 258)	0.80	0.04 - 6.24
	(GM = 0.44)*	(0.38 - 0.51)**
Building 236 (n = 45)	0.182	<0.037 - 1.036
Sagamihara, Japan (1999) (processing PFOS) n = 32	0.135	0.0475 - 0.628
General Population Exposures		
Source	Mean (ppb)	Range (ppb)
Non-occupational (n = 31) (corporate staff or managers) St. Paul, Minnesota (1998)	47	28 - 96
Non-occupational (1999) (plant management, Japan) Sagamihara n = 32	40.3	31.9 - 56.6
Tokyo n=30	52.3	33 - 96.7
Commercial Sources, U.S. (1998)		
Intergen n = ~500 donors	44	43 - 44
Sigma n = ~ 200 donors	33	26 - 45
Other Commercial Sources, U.S. (1999) n = 35 lots	35	5 - 85
U.S. Blood Banks (1998) n = ~340-680 donors	29.7	9 - 56
European Blood Banks (1999)		
Belgium (6 pooled samples)	17	4.9 - 22.2
Netherlands (5 pooled samples)	53	39 - 61
Germany (6 pooled samples)	37	32 - 45.6
U.S. Blood Banks (2000) American Red Cross (ages 20-69) n = 645	34.9 (geometric mean)	4.3 - 1656
Samples in U.S.children (ages 2-12) (1995) (n = 599)	37.5 (geometric mean)	6.7 - 515
Samples in elderly in Seattle, WA (ages 65-96) (1999) (n = 238)	31.0 (geometric mean)	3.4 - 175

*GM is geometric mean; ** 95% Confidence Interval of the GM

3.0 Human Health Hazards

3.1 Metabolism and Pharmacokinetics

3.1.1 Absorption

PFOS is well absorbed following ingestion. After a single oral dose of PFOS-14C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours (Johnson, Gibson and Ober, 1979a). The digestive tract and contents contained on average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the PFOS-14C dose was absorbed from solution after administration to non-fasted rats.

3.1.2 Distribution

At 24 to 48 hours after a single oral dose of PFOS-14C (4.2 mg/kg) in rats, approximately 86% of the radioactivity recovered was found in the carcass (Johnson, Gibson and Ober, 1979a). The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ~0.2%. The concentrations of total carbon-14 in red blood cells and plasma were compared. The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-14C, there is no selective retention of carbon-14 in red blood cells.

At 89 days after a single intravenous dose of PFOS-14C (4.2 mg/kg) in male rats, mean tissue C-14 concentrations above one ug equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1 (Johnson, Gibson and Ober, 1979b). Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 ug/g. There was a difference in C-14 content of subcutaneous fat (0.2 ug/g) and abdominal fat (≤ 0.08 ug/g). Very little C-14 was found in whole eye (0.16 ug/g) and no detectable C-14 was found in brain. Only liver and plasma contained a substantial percentage of dose at 89 days post dose, 25.21% and 2.81%, respectively. The low levels of radioactivity found for kidney, lung, testes, and spleen are due in part to blood still contained in these organs when homogenized.

There have been several studies conducted to examine the levels of PFOS in the dam, fetus and neonate. These studies are described in detail in sections 3.4 and 3.5. In general, the levels of PFOS are higher in the liver than in the serum of dams, fetuses and neonates, and placental transfer of PFOS has been demonstrated

3.1.3 Elimination

Urinary excretion is the primary route of elimination for PFOS in the rat. By 89 days after a single intravenous dose of PFOS-14C (4.2 mg/kg) in male rats, mean urinary excretion was 30.2 \pm 1.5% of total C-14 administered (Johnson, Gibson, and Ober, 1979b). Mean cumulative fecal excretion was 12.6 \pm 1.2%. Radioactive content in feces was too low to measure after 64 days.

The half-life for elimination of total PFOS carbon-14 from plasma after a single oral dose (4.2 mg/kg) in

male rats is 7.5 days (Johnson, Gibson and Ober, 1979a). This determination was based upon analysis of plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after the single oral dose.

There is evidence of enterohepatic circulation of PFOS. After 21 days of cholestyramine treatment, the mean percentage of PFOS-14C dose eliminated via feces (75.8 ± 5.0) was 9.5-fold the mean percentage of dose eliminated via feces by control rats (8.0 ± 0.8 , Johnson, Gibson and Ober, 1984). After adjustment for the amount of carbon-14 excreted in urine (18% for controls and 5% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 19% for cholestyramine-treated rats and 74% for control rats. After PFOS-14C administration, the mean liver carbon-14 content at 21 days represents 11% and 40% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 21 days of cholestyramine treatment. The authors conclude that the high concentration of PFOS-14C in liver at 2 to 3 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 by nearly 10-fold suggest that there is a considerable enterohepatic circulation of PFOS-14C.

3.1.4 Half-life in Humans

In order to determine the half-life of PFOS, 3 retirees who worked in 3Ms Chemical Division were followed for five and a half years (3M Company, 2000c). Serum PFOS levels suggested a mean elimination half-life ($t_{1/2}$) of 1,428 days (approximately 4 years). A half-life study on a larger group of retirees ($n = 27$) is currently underway, in which serum samples are drawn every 6 months over a 5-year period. Two interim reports describing the results thus far have been submitted (Burris et al, 2000; Burris et al., 2002). The first interim report suggested a serum half-life of 139-640 days. However, there were several limitations to these analyses including: 1) the limited data available and the range of serum PFOS levels measured; 2) serum was analyzed after each collection period with only one measurement per time period on different days using slightly different analytical techniques; and 3) the reference material purity was not determined until after the first 3 samples had been analyzed. An effort was made to minimize experimental error, including systematic and random error in the analytical method, involving 9 of the original 27 subjects. Serum samples were collected from each of the subjects over 4 time periods spanning 180 days, measured in triplicate with all time points from each subject analyzed in the same analytical run. This would allow for statistical evaluation of the precision of the measurement and assure that all systematic error inherent in the assay equally affected each sample used for half-life determination.

Of the 9 retirees included in this analysis, there were 7 males and 2 females, all from the Decatur plant. The average age of the retirees was 61 years, the mean number of years worked at Decatur was 27.7 years, and the average number of months retired was 18.9. Average BMI of this group was 27.9. The mean PFOS value at study initiation was 0.89 ppm (range 0.11 – 3.53 ppm, SD = 1.07).

The mean serum half-life for PFOS was 8.67 years (range 2.29 – 21.3 years, SD = 6.12). Age, BMI, number of years worked or years since retirement were not significant predictors of serum half-lives in multivariable regression analyses. This analysis has attempted to reduce experimental error in the determination of a half-life for PFOS. However, several issues should be noted. First, the effect of continued non-occupational, low-level exposure on the half-life is unknown. Second, PFOS is a metabolic product of other compounds found in the retirees' blood; therefore, PFOS is likely being produced in the body while the study was ongoing. Third, it is not known if there are interactions between PFOS and other fluorochemicals in the body. Fourth, this estimate is much higher than that reported in lab animals. However, it may not be appropriate to directly compare the results of the animal half-life data with these data due to potentially different protein binding sites and affinities. Fifth, systematic error of the analytical method could be as high as $\pm 20\%$ and still satisfy the data quality criteria.

3.2 Acute Toxicity

Four reports of acute studies of PFOS have been submitted, one inhalation toxicity of rats, two oral studies of rats, and one dermal and eye irritation study of rabbits.

In a study to determine the median lethal concentration (LC_{50}), Rusch et al. (1979) administered PFOS dust in air to Sprague-Dawley rats, 5/sex/group, levels of 1.89 to 45.97 mg/l PFOS to eight test groups. A Wright dust-feed mechanism with dry air at a flow rate of 12 to 16 liters per minute was used to administer the PFOS dust. Rats were exposed for 1 hour. The test group rats weighed 201-299 g at study initiation. The control group rats weighed 203-263 g at study initiation. The test group rats were exposed to 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09 or 45.97 g/l. The control rats were exposed to dry air at a flow rate of 12 liters per minute. All other protocols were the same as the test group rats. The rats were observed for abnormal signs prior to exposure, at 15-minute intervals during the 1-hour exposure, at removal from the exposure chamber, hourly for four hours after exposure, and daily thereafter for 14 days. Individual body weights were recorded on Day 0 (prior to exposure), Day 1, Day 2, Day 4, Day 7, and Day 14. It is reported that all animals dying spontaneously were necropsied as soon as possible after death. Blood samples were collected on Day 14 from all surviving animals, but analyses were not provided. The LC_{50} of 5.2 mg/l and 95% confidence limits of 4.4 and 6.4 mg/l were calculated using the method of Litchfield and Wilcoxon.

The highest dose group, 45.97 mg/l, was not used in the LC_{50} calculations and terminated on Day 2. At that point, only 5 animals survived and blood samples were taken at termination. The 13.9 mg/l group was also terminated early (Day 1) because of a mechanical problem during exposure. These animals were also not used in the LC_{50} determination.

In the 24.09 mg/l exposure group, all animals died by Day 6. At 7.05 and 6.49 mg/l there was 80% mortality. At 4.88, 2.86, and 1.89 mg/l there was 20%, 10%, and 0% mortality, respectively. The rats in all these groups showed signs of toxicity including emaciation, red material around the nose or other nasal discharge, yellow material around the anogenital region, dry rales or other breathing disturbances, and general poor condition. Abnormal in-life observations were reported to be less frequent in the lower exposure groups.

At necropsy, the most common abnormality was discoloration of the liver and lung. Discoloration of the lung was also observed in control rats and therefore may not be treatment related. Therefore, the most significant treatment related abnormality was varying degrees of discoloration of the liver. Among animals that died prematurely, decreased body weight, discoloration of the lung, and discoloration and distention of the small intestine were also observed.

In a study to determine the acute oral LD_{50} , Dean et al. (1978) administered CD rats, 5/sex/group, doses of 0, 100, 215, 464, and 1000 mg/kg PFOS by gavage. The powdered test material was suspended in a 20% acetone/80% corn oil mixture. All dose levels were administered as volumes of 10 ml/kg body weight. The rats weighed 172-212 g at the beginning of the study immediately prior to dosing and weights were recorded at Day 7 and Day 14. The rats were observed for abnormal signs during the four hours after exposure, and daily thereafter for 14 days. It is reported that all animals dying spontaneously were grossly necropsied, as well as all rats that survived to the end of the 14 day study.

Acute oral LD_{50} values and 95% confidence limits were calculated for males (233 [160-339] mg/kg), females (271 [200-369] mg/kg) and combined male and female rats (251 [199-318] mg/kg).

All rats in the 464 and 1000 mg/kg dose groups died before the end of the study. Three animals in the 215 mg/kg group died prematurely. It appears signs of toxicity most frequently observed included:

hypoactivity, decreased limb tone, and ataxia. At necropsy, observations included: yellow-stained urogenital region, stomach distention and signs of irritation of the glandular mucosa, and lung congestion.

In a second oral study, Gabriel (1978; cited in Dean et al., 1978) administered two groups of Sherman-Wistar albino rats (5 /sex/group) two doses of PFOS by gavage in water. The rats weighed between 200 - 300 grams. It was determined the acute oral LD₅₀ was greater than 50 mg/kg and less than 1500 mg/kg.

All of the rats administered 1500 mg/kg died before the 14-day observation period ended, with the last rat dead on Day 9. None of the rats administered 50 mg/kg died before the end of the study. It is stated that rats were observed for signs of toxicity and mortality but it is not clear how frequently they were observed and no individual animal data was provided. It is stated that the high dose group rats were “depressed” at 2- 3 hours after dosing and “severely depressed or semi-comatose” by 24 hours; staying in this state until death occurred. No individual pathology data were provided. The author states: “Gross pathologic examination revealed nothing remarkable.”

Biesemeier and Harris (1974) examined the potential for PFOS to cause skin and eye irritation. Six albino rabbits had their hair clipped from their backs and flanks, and it appears five tenths of one gram (0.5 g; the procedure states either 0.5 g or 0.5 ml and the test material as supplied by 3M was a solid) of the test material was placed on abraded or intact prepared test sites, then covered with gauze patches. After 24 hours and 72 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale. No reference is provided for method or scoring of results. Concentration or total dose of test material was not provided. In all six rabbits, it is reported the primary skin irritation scores were 0; which indicates no reddening or swelling detected.

In the eye irritation study, six albino New Zealand White rabbits were placed in collars so they could not rub their eyes. It appears one tenth of a gram (0.1 g; the procedure states either 0.1 g or 0.1 ml was used and T117 was a solid as supplied by 3M) of the test substance was instilled in one eye, the other eye was left untreated as a control. The concentration or total dose of the test substance were not provided. It is reported that the reaction to the test material was read against a scale of damage to the cornea, iris, and the bulbar and palpebral conjunctivae at 1, 24, 48, and 72 hours after treatment. The scale criteria were not presented or referenced. Each time the eyes were scored, any accumulated discharge or residue of test material was flushed from the eye. There is no reference provided for the method. It appears that scores were maximal at 1 hour and 24 hours after treatment then decreased over the rest of the study (Biesemeier and Harris, 1974).

3.3 Mutagenicity

PFOS was tested for its ability to induce mutation in the Ames Salmonella/Microsome Plate Test and in the D4 strain of *Saccharomyces cerevisiae* (Litton Bionetics, Inc., 1979). It was also tested in: (1) a *Salmonella – Escherichia coli*/Mammalian-microsome reverse mutation assay (Mecchi, 1999); (2) an *in vitro* assay for chromosomal aberrations in human whole blood lymphocytes (Murli, 1999); and (3) an assay for unscheduled DNA synthesis (UDS) in rat liver primary cell cultures (Cifone, 1999). It was negative in all assays in which it was tested. PFOS does not induce reverse mutation at the histidine locus of *S. typhimurium* or at the tryptophan locus of *E. coli* when tested with or without metabolic activation. It does not induce chromosomal aberrations in human lymphocytes when tested with or without metabolic activation and does not induce UDS in primary cultures of rat hepatocytes. PFOS was also tested in an *in vivo* mouse micronucleus assay (Murli, 1996). PFOS did not induce micronuclei in the bone marrow of Crl:CD-1 BR mice and is negative in the mouse bone marrow micronucleus assay.

T-2247 CoC which is a 50% by weight solution of the diethanolammonium salt of perfluorooctanesulfonate in water was tested for its ability to induce gene mutation in 5 strains of *S.*

typhimurium and also for its ability to induce recombination in *S. cerevisiae* strain D3. (Simmon, 1978). The chemical was negative in all 5 strains of *Salmonella* and in *S. Cerevisiae* D3 when tested with and without metabolic activation. T-2247 CoC was tested with *S. typhimurium* strains TA98 and TA100 in a desiccator assay for the detection of volatile compounds. It was nonmutagenic when tested under these conditions.

3.4 Repeated Dose Toxicity

Three 90-day subchronic studies of PFOS have been conducted, two gavage studies in rhesus monkeys and one dietary study in rats. In addition, a four week and a 26 week capsule study in cynomolgus monkeys have been conducted. Both reports were unaudited drafts at the time of this review.

In the rat subchronic study, Goldenthal et al. (1978b) administered CD rats, 5/sex/group, dietary levels of 0, 30, 100, 300, 1000 or 3000 ppm PFOS (FC-95) for 90 days. The males weighed 196-232 g and the females weighed 165-206 g at study initiation. The dietary levels were equivalent to doses of 0, 2, 6, 18, 60 and 200 mg/kg/day. The rats were observed daily for general clinical signs; body weights and food consumption were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. The rats were sacrificed after 90 days of treatment and a gross necropsy was conducted. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All of the rats in the 300, 1000 and 3000 ppm groups died. Death occurred between days 13-25 and days 18-28 for the males and females, respectively, in the 300 ppm group. At 1000 ppm, death occurred between days 8-14, and at 3000 ppm, the rats died between days 7-8 of treatment. The rats in all groups showed signs of toxicity including emaciation, convulsions following handling, hunched back, red material around the eyes, yellow material around the anogenital region, increased sensitivity to external stimuli, reduced activity and moist red material around the mouth or nose.

Three males and two females in the 100 ppm group died prior to scheduled sacrifice. Two of the males and the two females died during week 5 and the third male died during week 11 of the study. At study termination, mean body weights were reduced by 16.7% and 16.3% in the male and female groups, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for males and females at 100 ppm. After 30 days of treatment, hematologic values were comparable among the control and 100 ppm groups. Clinical chemistry analyses at one month showed a significant increase in mean glucose in males, blood urea nitrogen values in males and females, and creatinine phosphokinase and alkaline phosphatase values for females. After 90 days of treatment at 100 ppm, the two surviving males had significantly reduced erythrocyte, hemoglobin, hematocrit and leukocyte counts; the three surviving females had significantly reduced hemoglobin and reticulocyte counts, as well as slightly lower erythrocyte, hematocrit and leukocyte counts. Two of the surviving females showed slight to moderate increases in plasma glutamic oxalacetic and pyruvic transaminase activities. Urinalysis results were comparable among treated and control groups at 30 and 90 days. Relative liver weight was significantly increased in the males and absolute and relative liver weights were significantly increased in the females. Relative kidney weights were significantly increased in both sexes.

All rats in the 30 ppm group survived until the end of the study. At study termination, mean body weights were reduced by 8.7 and 8% in the males and females, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for the males at 30 ppm. Hematologic values were comparable among the control and 30 ppm group at 30 and 90 days. One female showed a slightly elevated glucose level and one male showed a slightly increased alkaline phosphatase level at 30 days. At 90 days, one male showed moderate increases in glucose, blood urea nitrogen and γ -glutamyl transpeptidase activity. The females had significant increases in absolute and relative liver weights. The males had significant decreases in absolute and relative adrenal weights, absolute thyroid/parathyroid weight and absolute pituitary weight. The biological significance of the changes in male organ weights is unclear since similar changes were not noted in higher dose groups.

At necropsy, treatment related gross lesions were present in all treated groups and included varying degrees of discoloration and/or enlargement of the liver and discoloration of the glandular mucosa of the stomach. Histologic examination also showed lesions in all treated groups. Centrilobular to midzonal cytoplasmic hypertrophy of hepatocytes and focal necrosis was observed in the liver; the incidence and relative severity were greater in the males. In addition, especially among rats in the 300, 1000 and 3000 ppm groups, treatment related histologic lesions were noted in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. In the thymus, this consisted of depletion in the number and size of the lymphoid follicles and in the bone marrow hypocellularity was noted. The spleen was slightly atrophied with a corresponding decrease in the size and number of lymphoid follicles and cells and a similar depletion was noted in the mesenteric lymph nodes. Mucosal hyperkeratosis and/or acanthosis was observed in the forestomach and mucosal hemorrhages were noted in the glandular portion of the stomach. Decreases in the height and thickness of the villi were noted in the small intestine. Atrophy of the skeletal muscle was noted, as well as epidermal hyperkeratosis and/or acanthosis was noted in the skin.

In the first rhesus monkey study, Goldenthal et al. (1979) administered rhesus monkeys, 2/sex/group, doses of 0, 10, 30, 100 or 300 mg/kg/day PFOS (FC-95) in distilled water by gavage. The males weighed 3.05-3.80 kg at study initiation and the females weighed 2.75-4.10 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study. The study was terminated after 20 days due to the death of the monkeys. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All of the treated monkeys died. The monkeys in the 300 mg/kg/day group died between days 2-4, the monkeys in the 100 mg/kg/day group died between days 3-5, the monkeys in the 30 mg/kg/day group died between days 7-10, and the monkeys in the 10 mg/kg/day group died between days 11-20 of treatment. The monkeys from all the groups showed similar signs of toxicity including decreased activity, emesis with some diarrhea, body stiffening, general body trembling, twitching, weakness, convulsions and prostration. At necropsy, several of the monkeys in the 100 and 300 mg/kg/day groups had a yellowish-brown discoloration of the liver; histologic examination showed no microscopic lesions. Congestion, hemorrhage and lipid depletion of the adrenal cortex was noted in all treated groups. No other lesions were noted.

In the second study, Goldenthal et al. (1978a) administered rhesus monkeys, 2/sex/group, doses of 0, 0.5, 1.5 or 4.5 mg/kg/day PFOS (FC-95) in distilled water by gavage for 90 days. The males weighed 2.55-3.55 kg at study initiation and the females weighed 2.7-3.75 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. The monkeys were sacrificed after 90 days of treatment and a gross necropsy was conducted. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

All monkeys in the 4.5 mg/kg/day group died or were sacrificed *in extremis* between week 5 and 7 of the study. Beginning on the first or second day of the study, these monkeys exhibited signs of gastrointestinal tract toxicity including anorexia, emesis, black stool and dehydration. All of the monkeys had decreased activity and just prior to death showed marked to severe rigidity, convulsions, generalized body trembling and prostration. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.7 kg at week 5. After 30 days of treatment, there was a significant reduction in serum cholesterol and a 50% reduction in serum alkaline phosphatase activity. At necropsy, mean organ weights were comparable among the control and treated monkeys. Histologic examination showed several treatment related lesions. All the male and females had marked diffuse lipid depletion in the adrenals. One male and two females had moderate diffuse atrophy of the pancreatic exocrine cells with decreased cell size and loss of zymogen granules. Two males and one female had moderate diffuse atrophy of the serous alveolar cells characterized by decreased cell size and loss of cytoplasmic granules.

All monkeys in the 1.5 mg/kg/day group survived until the end of the study. During the first week of the study, the monkeys had decreased activity. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included black stool, diarrhea, mucous in the stool and bloody stool; at the end of the study, anorexia, dehydration or general body trembling were noted. Although statistical significance was not achieved, the mean body weight of the males dropped from 3.15 kg at the beginning of the study to 2.93 kg at the end of the study, and the mean body weight of the females dropped from 3.22 kg to 2.75 kg. One of the females had very low serum cholesterol and another had a reduction in inorganic phosphate. Necropsy revealed no treatment related lesions.

All monkeys in the 0.5 mg/kg/day group survived until the end of the study. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included diarrhea, soft stools, anorexia and emesis. Occasionally, decreased activity was noted in three of the monkeys. Necropsy revealed no treatment related lesions.

Serum samples from monkeys in the 0, 0.5 and 1.5 mg/kg/day dose group were individually analyzed for PFOS levels; liver samples from the 0, 0.5, 1.5 and 4.5 mg/kg/day dose groups were also analyzed for PFOS levels. Except for the control animals where animal 7355M was used in both the serum and liver control groups, different animals were used for serum and liver determinations. PFOS levels in serum were developed by pyrolysis; precision is estimated to be $\pm 10 - 25\%$.

Levels of PFOS in serum

Monkey #	Dosage Group	PFOS Levels in Serum (ppm)
7355M	0	40
7358M	0	20
7368F	0	15
7460M	0.5 mg/kg/day	150
7466F	0.5 mg/kg/day	150
7462M	1.5 mg/kg/day	250
7500F	1.5 mg/kg/day	275

No explanation is offered for the relatively high serum levels in the controls. Sample 7355M was run on two separate days and gave the same results.

Levels of PFOS in liver

Monkey #	Dosage Level	PFOS in Liver (mg)	PFOS in Liver (ppm)
7355M	0	3000	50
7368F	0	1500	20
7463M	0.5 mg/kg/day	7000	100
7466F	0.5 mg/kg/day	8000	100
7462M	1.5 mg/kg/day	45000	650
7500F	1.5 mg/kg/day	40000	600
7484M	4.5 mg/kg/day	40000	650
7502F	4.5 mg/kg/day	80000	1000

Livers were stored refrigerated for several months prior to analysis. This resulted in the separation of some liquid from the samples. At the time of analysis, only partial samples were taken in which the ratio of solid to liquid was estimated visually. Recovery of PFOS from the liver samples was estimated from experiments where a known amount of PFOS was added to a control liver. Here as in the serum, control values were higher than expected. Because of this, a 0.133 g liver sample was analyzed for total fluorine. The fluorine value calculated as PFOS was equivalent to 11 ppm in the liver.

PFOS values in serum and liver are approximately the same at the 0.5 mg/kg/day level but are substantially higher in the liver at the 1.5 mg/kg/day level. Serum levels were not determined at the 4.5 mg/kg level. That may be because only a single female survived until scheduled sacrifice in the 4.5 mg/kg/day dose group. From this study, there do not appear to be any differences in PFOS in either serum or liver between the sexes.

The values of PFOS in the liver and serum should be viewed with caution. There were a very limited number, one male and one female, of animals per group. Storage of the liver samples at refrigeration temperature for long periods of time was not ideal. Although PFOS is stable, the liver itself obviously underwent some deterioration as evidenced by the separation of a liquid phase. Sample size may also have been inaccurate since the visual estimation of solid to liquid phase is not a precise measurement.

In order to determine the dose range for a six-month study, Thomford et al. (unaudited draft, 1998) conducted a 4-week range-finding study in cynomolgus monkeys. Male and female cynomolgus monkeys were administered doses of 0 (2/sex/group), 0.02 (3/sex/group), or 2.0 mg/kg/day (1/sex/group) PFOS in capsules placed directly into the stomach. The monkeys weighed 2.1- 2.4 kg at study initiation. It appears the monkeys were observed at least daily for general clinical signs and body weights were recorded twice

weekly. Hematological and clinical chemistry analyses were conducted on samples collected before the beginning of the study at day -7 (baseline values) and day 29. Additional blood samples for clinical chemistry were collected on study days 2, 7, and 14. Blood samples for serum PFOS concentrations were taken on days 7, 2, 3, 7, 14, and 29. In addition, samples from day -7 and day 29 were analyzed for levels of estradiol, estrone, estriol, thyroid stimulating hormone, triiodothyronine, and thyroxine. The study animals were terminated as scheduled at 30 days. At necropsy a sample of liver was collected from each animal for palmitoyl CoA oxidase activity analyses. Samples of liver, testes, and pancreas were collected for proliferation cell nuclear antigen evaluation. A sample of liver was also collected from each animal for PFOS concentration analysis. The following organs from control and all treated groups were examined microscopically: adrenals, eye, kidney, liver, lung, spleen, pancreas, femoral bone marrow, testes, and thymus.

None of the monkeys died before the study was ended. There were no test-related effects on clinical observations, body weight, food consumption, body temperatures, hematology, or macroscopic or microscopic pathology findings. No test-related effects were noted in the levels of estrone, estriol, thyroid stimulating hormone, and thyroxine. Cell proliferation, as measured by immunohistochemical detection of proliferating cell nuclear antigen, was not increased in the liver, testes, or pancreas of monkeys.

The monkeys in the 2.0 mg/kg/day group both showed estradiol levels that were less than their prestudy values and controls at day 29. Similarly, the monkeys in the 2.0 mg/kg/day group both showed triiodothyronine levels that were less than their prestudy values and controls at day 29. However, pre-treatment, the high-dose monkeys also had triiodothyronine values lower than controls in baseline samples as well. Since the numbers of tested animals are small and baseline levels are variable, it is not clear if these hormone level changes are treatment-related effects.

In the final study, Thomford (2002) administered PFOS to cynomolgus monkeys by oral capsule at doses of 0 (6 monkeys per sex), 0.03 (4 monkeys per sex), 0.15 (6 monkeys per sex), or 0.75 mg/kg/day (6 monkeys per sex) for 26 weeks. Two animals from the control, 0.15 and 0.75 mg/kg/day groups were assigned to a recovery group and were not treated for at least 52 weeks following the last administration of PFOS. Animals were observed twice daily for mortality and moribundity and were examined at least once daily for abnormalities and signs of toxicity; food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during weeks 26 and 52. Body weight data were recorded weekly before the start of treatment, on Days -1 and 1 and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and at specified intervals during treatment and recovery. Blood samples were also taken for hormone determinations. Samples of serum were collected at various time points during the study and sent to 3M for analysis of PFOS levels. The following organs were weighed at scheduled and unscheduled sacrifices; paired organs were weighed separately: adrenal (2), brain, epididymis (2), kidney (2), liver, ovary (2), pancreas, testis (2), and thyroid (2) with parathyroid. The following tissues were collected for histopathology: adrenals (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eyes (2), femur with bone marrow, gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mammary gland, mesenteric lymph node, ovary (2), pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, trachea, urinary bladder, uterus, and vagina. Liver specimens from the 0.15 and 0.75 mg/kg/day recovery animals were collected via biopsy and analyzed for PFOS levels.

Males weighed 3.3-3.4 kg and females weighed 2.8-2.9 kg at the beginning of the study. At the end of 26 weeks of treatment, males weighed 3.7, 3.8, 3.5, and 3.3 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. Females weighed 3.1, 3.1, 3.1 and 2.8 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. The difference between the control and the 0.75 mg/kg/day

female treatment groups was statistically significant. At the end of the recovery period, differences in weight between the control and treated animals were no longer obvious.

Two males from the 0.75 mg/kg/day group did not survive to the scheduled sacrifice. One animal died after dosing on Day 155 (Week 23). Clinical signs noted in this animal included: constricted pupils, pale gums, few, mucoid, liquid and black-colored feces, low food consumption, hypoactivity, labored respiration, dehydration, and recumbent position. In addition, the animal was cold to the touch. An enlarged liver was detected by palpation. Cause of death was determined to be pulmonary necrosis with severe acute inflammation. On day 179, the second male was sacrificed in a moribund condition. Clinical signs noted included low food consumption, excessive salivation, labored respiration, hypoactivity and ataxia. The cause of death was not determined.

Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and males and females in the 0.15 and 0.75 mg/kg/day groups had lower high density lipoprotein cholesterol during treatment. The effect on total cholesterol worsened with time. By day 182, mean total cholesterol for males and females in the high dose group were 68% and 49% lower, respectively, than levels in the control animals. The effect on high density lipoprotein was greater than that seen with cholesterol. On day 182, the mean high density lipoprotein levels were 79% and 62% lower, respectively in males and females from the high dose group than they were in male and female control animals. Males in the high dose group also had lower total bilirubin concentrations and higher serum bile acid concentrations than males in either the control or other treatment groups. The effect on total cholesterol was reversed within 5 weeks of recovery and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of recovery.

Estradiol values were lower in males given 0.75 mg/kg/day on days 62, 91, and 182 but because of variation only the day 182 value was significant. Estrone values were generally higher in the treated females on days 37, 62 and 91 but again because of variation in the data none of these values were significantly different from the controls. Except for males in the 0.15 mg/kg/day group, triiodothyronine values were significantly lower on days 91 and 182 in males and females given 0.15 and 0.75 mg/kg/day. There were other instances in which hormone values in treated groups were different from those of controls but these differences were not consistent over time or between sexes, were not clearly dose-related and did not appear to be related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in the study complicates the interpretation of the hormone data.

At terminal sacrifice, females in the 0.75 mg/kg/day dose-group had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to body weight percentages were increased in the high-dose group compared to the controls. "Mottled" livers were observed in two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had a "mottled" and large liver. Three of 4 high-dose males (including those that did not survive to scheduled sacrifice) had centrilobular or diffuse hepatocellular hypertrophy that was also observed in all high-dose females. Centrilobular or diffuse hepatocellular vacuolation occurred in 2 of 4 females and 2 of 4 males in the high-dose group.

No PFOS related lesions were observed either macroscopically or microscopically at recovery sacrifice indicating that the effects seen at terminal sacrifice may be reversible.

Serum and liver specimens collected from test animals were sent to the 3M Laboratory and analyzed for the presence of PFOS. Serum was harvested from blood that was centrifuged within one hour of collection. Liver specimens were flash frozen in liquid nitrogen. Both liver and serum samples were stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Samples were shipped frozen and on dry ice from Covance Laboratories to 3M periodically from August 1998 through March 2000 which covered the in-life phase of the study. Once received at 3M specimens were stored in freezer at either –

$55^{\circ}\text{C} \pm 10\text{-}20^{\circ}\text{C}$ or $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

During the first 26 weeks of the study a total of 550 serum specimens and 30 liver specimens were collected. Of the serum specimens, 151 were from Group 1, 99 from Group 2, 152 from Group 3 and 148 from Group 4. Eight liver samples were collected from Group 1, 8 from Group 2, 12 from Group 3 and 14 from Group 4. In the recovery Groups, 72 serum and 4 liver samples were collected from Group 1; 72 serum and 4 liver samples from Group 3 and 80 serum and 4 liver samples from Group 4.

Liver and serum samples were extracted using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to extraction. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external standard calibration.

Liver samples were homogenized in water. An aliquot of each liver homogenate and all serum samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the samples and the ion pairs were partitioned into MtBE. The extracts were evaporated until dry on a nitrogen evaporator and then were reconstituted in 1.0 mL of methanol and passed through a 0.2 μm nylon filter.

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of the fluorochemical of interest using HPLC/ES/MS/MS. Molecular ion 499, the primary ion for PFOS ($\text{C}_8\text{F}_{17}\text{SO}_3^-$) analysis, was fragmented to produce ion 99 (FSO_3^-). Ion 99 was monitored for quantitative analysis.

Although low levels of PFOS were often detected in the sera and liver of the control animals, these levels were significantly lower than those found in the low dose test animals. PFOS levels in the sera of test animals increased with dose during treatment from 21.0 ± 1.57 and 20.4 ± 2.71 $\mu\text{g}/\text{ml}$ in the Group 4 males and females respectively at the end of Week 1 to 194 ± 8.94 and 160 ± 23.1 $\mu\text{g}/\text{ml}$ in males and females respectively in Group 4 at the end of Week 27. During recovery, PFOS levels in serum samples decreased over time until they reached 41.1 ± 25.9 $\mu\text{g}/\text{ml}$ in males and 41.4 ± 1.15 $\mu\text{g}/\text{ml}$ in females from Group 4 at 79 weeks post-treatment. Control values were < LOQ (the limit of quantitation) at Week 4 in both males and females and 0.0215 ± 0.00296 and 0.0243 ± 0.00355 $\mu\text{g}/\text{ml}$ in males and females respectively at the end of Week 79. The serum values for selected weeks of treatment and recovery are shown in the table below. There were no significant differences between PFOS levels in the sera of treated males and females.

Table 2. Average PFOS Concentrations (µg/ml) in Serum of Monkeys for Selected Weeks During Treatment and Recovery

	Group 1 0.0 mg/kg/day		Group 2 0.03 mg/kg/day		Group 3 0.15 mg/kg/day		Group 4 0.75 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Week 1	<LOQ	<LOQ	0.869 ± 0.147	0.947 ± 0.110	4.60 ± 0.782	3.71 ± 0.455	21.0 ± 1.57	20.4 ± 2.71
Week 4	<LOQ	<LOQ	3.20 ± 0.577	3.40 ± 0.291	17.8 ± 1.68	16.5 ± 1.87	95.3 ± 70.4	92.7 ± 39.6
Week 16	0.0407 ± 0.0110	0.0432 ± 0.0081	11.2 ± 2.44	10.5 ± 1.90	56.2 ± 5.84	42.1 ± 4.04	189 ± 15.9	162 ± 19.3
Week 27	0.0529 ± 0.0145	0.0416 ± 0.0148	15.9 ± 5.54	11.1 ± 1.52	68.1 ± 5.75	58.5 ± 4.67	194 ± 8.93	160 ± 23.9
Week 35	0.0459 ± 0.00303	0.0723 ± 0.00352	Not Determined	Not Determined	84.5 ± 12.0	74.7 ± 9.53	181 ± 19.5	171 ± 10.1
Week 47	0.0355 ± 0.00221	0.0459 ± 0.00323	Not Determined	Not Determined	48.3 ± 3.69	42.6 ± 6.70	124 ± 25.9	98.3 ± 8.32
Week 57	0.0327 ± 0.00526	0.0445 ± 0.00385	Not Determined	Not Determined	30.2 ± 2.36	32.3 ± 1.34	78.0 ± 16.3	106 ± 3.84
Week 69	0.0406 ± 0.00313	0.0400 ± 0.00301	Not Determined	Not Determined	26.4 ± 2.59	34.5 ± 3.46	84.0 ± 52.4	75.0 ± 5.25
Week 79	0.0215 ± 0.00296	0.0243 ± 0.00355	Not Determined	Not Determined	19.1 ± 0.805	21.4 ± 2.01	41.1 ± 25.9	41.4 ± 1.15

LOQ = Lowest Observable Concentration

Liver values behaved in a manner similar to serum values and increased over time. At Week 27 mean PFOS values on an RSD basis were 22.2 ± 0.0269 in Group 1 males and 16.8 ± 0.0178 in females in Group 1; 27.0 ± 4.66 and 9.73 ± 2.15 in males and females in Group 2; 33.1 ± 19.5 in males and 21.4 ± 14.9 in females in Group 3, and 6.03 ± 23.9 in males and 5.00 ± 13.6 in females in Group 4. At Week 79 values in the liver were 71.0 ± 33.4 in males and 21.4 ± 10.8 in females in Group 4. At Week 80, values were 14.9 ± 1.38 in Group 3 males and 23.5 ± 4.98 in Group 3 females.

3.5 Carcinogenicity

The chronic toxicity and carcinogenicity of perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) have been studied in rats (3M, 2002). The results of the study show that PFOS is hepatotoxic and carcinogenic, inducing tumors of the liver, and of the thyroid and mammary glands. Based on the liver toxicity, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is 2 ppm in male rats and 5 ppm in female rats.

In this study, groups of 40-70 male and female Crl:CD (SD)IGS BR rats were given PFOS in the diets at concentrations of 0.5, 2, 5, or 20 ppm for 104 weeks. A control group was given diets containing acetone, the vehicle. A recovery group was given the test material at 20 ppm for 52 weeks and was observed till

death. Five animals/sex in the treatment groups were sacrificed during weeks 4, 14 and 53; liver samples were collected for mitochondrial activity, hepatocellular proliferation rate, and determination of palmitoyl-CoA oxidase activity. Serum and liver specimens were collected for analyses of the presence and concentration of PFOS in liver and serum during and at the end of the in-life phase of the study.

There was a significant increased trend in survival that occurred in the males that was due to significant increases in survival in mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control group. None of the other treated groups in the males revealed any significant differences in survival. No significant trend was noted in survival in females. There was a significant decrease in survival in the mid-dose (2.0 ppm) group and not in the mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control.

Males given 20 ppm had significantly lower mean body weights compared to animals in the control group during weeks 9 through 37. Females given 20 ppm had significantly lower body weights compared to animals in the control group during weeks 3 through 101. At week 105, mean body weights of the surviving males and females were not significantly different from the controls. Food consumption for males and females was similar in all treated groups compared to animals given the control material except for the high-dose females which had statistically significantly lower food consumption during weeks 2 through 44.

At the week 14 and week 53 interim sacrifice, absolute and relative liver weights were significantly increased in the males given 20 ppm. In females given 20 ppm, only the liver-to-body weight percentage was significantly increased. Treatment-related histomorphologic changes were seen in the liver in the males given 5 or 20 ppm and in the females given 20 ppm. The changes consisted of hypertrophy of hepatocytes in centrilobular areas in males and females, and midzonal to centrilobular hepatocytic vacuolation. The incidence and severity of the changes tended to be greater in the males. Dietary administration of PFOS for approximately 53 weeks was associated with mildly to moderately lower cholesterol for males and females fed 20 ppm; and mildly higher alanine aminotransferase for males fed 20 ppm. In the unscheduled sacrifices between Weeks 54 and 105, animals given 20 ppm had increased hepatocellular centrilobular hypertrophy, eosinophilic hepatocytic granules, and centrilobular hepatocytic pigment were noted. Increased hepatocellular centrilobular hypertrophy was seen in animals given 5 ppm.

At the terminal sacrifice, the livers of animals given 5 or 20 ppm exhibited a slight increase in macroscopic findings, including enlarged, mottled, diffuse darkened, or focally lightened. Hepatotoxicity, characterized by significant increases ($P < 0.05$) in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic vacuolation was noted in male and/or female rats given 5 or 20 ppm. A significant increase ($P < 0.05$) in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration was found in all the male treated groups (0.5, 2, 5, or 20 ppm); however, this lesion is believed to be due to old age of the animals and is not considered to be treatment-related.

Based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is 2 ppm in male rats and 5 ppm in female rats.

There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) at weeks 4 and 14, or by bromodeoxyuridine (BrdU) at week 53.

For neoplastic effects, a significant positive trend ($P = 0.0276$) was noted in the incidences of hepatocellular adenoma in male rats. This was due to a significant increase ($P < 0.05$) in the high-dose group (11.7%, 7/60).

over the control (0%, 0/60). A significantly increased incidence ($P<0.05$) was observed for thyroid follicular cell adenoma in the high-dose recovery group (23.1%, 9/39) when compared to the control group (5%, 3/60). There was also a slight increase in the combined thyroid follicular cell adenoma and carcinoma in the high-dose recovery group (25.6%, 10/39) as compared to that of the control group (10%, 6/60); the increase did not reach statistical significance relative to the control but did reach statistical significance relative to the high-dose group (8.5%, 5/59).

In the females, significant positive trends were observed in the incidences of hepatocellular adenoma ($P=0.0153$) and combined hepatocellular adenoma and carcinoma ($P=0.0057$). These cases were due to significant increases in the high-dose group (8.3%, 5/60, and 10%, 6/60) as compared to the control (0%, 0/60). A significant increase ($P=0.0471$) for combined thyroid follicular cell adenoma and carcinoma was observed in the mid-high (5.0 ppm) group (6%, 3/50) as compared to the control group (0%, 0/60). Except for the high-dose group (which showed a slight decrease in incidences of mammary fibroadenoma/adenoma and combined mammary fibroadenoma and carcinoma), increases in mammary tumors were observed in all treatment groups when compared to the controls. Significant increases ($P<0.05$) in mammary fibroadenoma/adenoma (60%, 30/50) and combined mammary fibroadenoma/adenoma and carcinoma (72%, 36/50) were observed in the low-dose (0.5 ppm) group as compared to the respective controls (38.3%, 23/60 and 48.3%, 29/60). The mid-dose (2.0 ppm) group also exhibited a statistically significant ($P<0.05$) increase (64.6%, 31/48) in the incidence of combined mammary fibroadenoma/adenoma/carcinoma over the control group (43.8%, 29/60). Increases in mammary tumors in the mid-high (5 ppm) dose group did not reach statistical significance relative to the control.

The carcinogenicity data of PFOS in rats are summarized in Table 3.

Table 3. Summary of carcinogenicity data of PFOS in rats.

Tumors	Tumor incidence (%)					
	Male					
Liver Hepatocellular adenoma*	0 (0/60)	0.5 ppm 6.0 (3/50)	2 ppm 6.0 (3/50)	5 ppm 2.0 (1/50)	20 ppm 11.7** (7/60)	20 ppm recovery#
Thyroid Follicular cell adenoma	5.0 (3/60)	10.2 (5/49)	8.0 (4/50)	8.2 (4/49)	6.8 (4/59)	23.1** (9/39)
Follicular cell carcinoma	5.0 (3/60)	2.0 (1/49)	2.0 (1/50)	4.1 (2/49)	1.7 (1/59)	2.6 (1/39)
Combined	10.0 (6/60)	12.2 (26/49)	10.0 (5/50)	10.2 (5/49)	8.5 (5/59)	25.6 (10/39)
Female						
Liver Hepatocellular adenoma*	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	8.3** (5/60)	
Hepatocellular carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	0 (1/50)	1.7 (1/60)	
Combined*	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	10.0** (6/60)	
Thyroid Follicular cell adenoma	0 (0/60)	0 (0/50)	0 (0/49)	4.0 (2/50)	1.7 (1/60)	
Follicular cell carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	2.0 (1/50)	0 (0/60)	
Combined	0 (0/60)	0 (0/50)	0 (0/49)	6.0** (3/50)	1.7 (1/60)	
Mammary Fibroadenoma/ adenoma	38.3 (23/60)	60.0** (30/50)	45.8 (22/48)	52.04 (26/50)	25 (15/60)	
Carcinoma	18.3 (11/60)	24.0 (12/50)	31.2 (15/48)	22.0 (11/50)	23.3 (14/60)	
Combined	48.3 (29/60)	72.0** (36/50)	64.6** (31/48)	58.0 (29/50)	40.0 (24/60)	

*Significant positive trend ($P < 0.03$).

** Significantly increased over the control ($P < 0.05$).

Recovery group; after 52 weeks of treatment.

Serum and Liver level of PFOS

Under the conditions of the studies, PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals. Detailed specimen data are presented in Table 4 and Table 5.

Table 4. Summary of PFOS Concentration-Serum (ug/mL)

Timepoint	Sex	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Group 6
							High Recovery
		Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD
Week 0	Male	<LOQ ^b (n=5)	0.907 \pm 0.0619 (n=5)	4.33 \pm 1.16 (n=5)	7.57 \pm 2.17 (n=5)	41.8 \pm 7.92 (n=5)	
	Female	0.0259 \pm 0.00663 (n = 5)	1.61 \pm 0.207 (n = 5)	6.62 \pm 0.499 (n = 5)	12.6 \pm 1.73 (n = 5)	54.0 \pm 7.34 (n = 5)	
Week 14 ^a	Male	<LOQ ^c (n = 5)	4.04 \pm 0.801 (n = 5)	17.1 \pm 1.22 (n = 5)	43.9 \pm 4.90 (n = 5)	148 \pm 13.8 (n = 5)	
	Female	2.67 \pm 4.58 (n = 5)	6.96 \pm 0.993 (n = 4d)	27.3 \pm 2.34 (n = 5)	64.4 \pm 5.48 (n = 5)	223 \pm 22.4 (n = 5)	
Week 53	Male	0.0249 \pm 0.0182 (n = 5)				146 \pm 33.5 (n = 4)	
	Female	0.395 \pm 0.777 (n = 5)				220 \pm 44.0 (n = 5)	
Day 719	Male						
	Female			20.2 \pm 13.3 (n = 9)			
Week 105	Male	0.0118 \pm 0.0104 (n = 11)	1.31 \pm 1.30 (n = 10)	7.60 \pm 8.60 (n = 17)	22.5 \pm 23.5 (n = 25)	69.3 \pm 57.9 (n = 22)	
	Female	0.0836 \pm 0.134 (n = 24)	4.35 \pm 2.78 (n = 15)		75.0 \pm 45.7 (n = 15)	233 \pm 124 (n = 25)	
Week 106	Male						2.42 \pm 5.09 (n = 10)
	Female						9.51 \pm 8.70 (n = 17)

a Not corrected for purity of the standard material.

b LOQ-Limit of Quantitation = 0.00910 pg/mL

c LOQ-Limit of Quantitation = 0.0457 pg/mL

d C92987F sample spilled during extraction, no sample remaining for analysis.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated (that the sera data are accurate to \pm 30%; liver data are accurate to \pm 50%.

Table 5. Summary of PFOS Concentration-Liver (ug/g)

Timepoint	Sex	Group 1 Control Average \pm SD	Group 2 Low Average \pm SD	Group 3 Mid Average \pm SD	Group 4 Mid-High Average \pm SD	Group 5 High Average \pm SD	Group 6 High Recovery Average \pm SD
Week 0	Male	0.104 \pm 0.0673 (n = 5)	11.0 \pm 2.31 (n = 5)	31.3 \pm 5.84 (n = 5)	47.6 \pm 12.5 (n = 5)	282 \pm 45.3 (n = 5)	
	Female	0.107 \pm 0.0486 (n = 5)	8.71 \pm 0.552 (n = 5)	25.0 \pm 6.11 (n = 5)	83.0 \pm 14.1 (n = 5)	373 \pm 44.1 (n = 5)	
Week 10	Male	0.459 \pm 0.0573 (n = 5)	23.8 \pm 3.45 (n = 5)	74.0 \pm 6.16 (n = 5)	358 \pm 28.8 (n = 5)	568 \pm 107 (n = 5)	
	Female	12.0 \pm 22.4 (n = 5)	19.2 \pm 3.77 (n = 5)	69.2 \pm 3.46 (n = 5)	370 \pm 22.3 (n = 5)	635 \pm 49.0 (n = 5)	
Week 53	Male	0.635 \pm 1.04 (n = 10)				435 \pm 96.9 (n = 9)	
	Female	0.923 \pm 1.77 (n = 10)				560 \pm 180 (n = 10)	
Day 719	Male						
	Female			55.1 \pm 31.5 (n = 9)			
Week 105	Male	0.114 \pm 0.148 (n = 11)	7.83 \pm 7.34 (n = 10)	26.4 \pm 20.4 (n = 17)	70.5 \pm 63.1 (n = 25)	189 \pm 141 (n = 22)	
	Female	0.185 \pm 0.184 (n = 24)	12.9 \pm 6.81 (n = 15)		131 \pm 61.4 (n = 15)	381 \pm 176 (n = 25)	
Week 106	Male						3.12 \pm 5.97 (n = 10)
	Female						12.9 \pm 10.4 (n = 17)

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

3.6 Developmental Toxicity

Three prenatal developmental toxicity studies of PFOS have been conducted, two studies in rats and one study in rabbits. In addition, preliminary results are available for developmental toxicity studies in rats and mice.

The first study administered four groups of 22 time-mated Sprague-Dawley rats 0, 1, 5, and 10 mg/kg/day PFOS in corn oil by gavage on gestation days (GD) 6-15 (Gortner, 1980). Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to a skeletal examination using alizarin red.

Signs of maternal toxicity consisted of significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day. No other signs of maternal toxicity were reported. Under the conditions of the study, a NOAEL of 5 mg/kg/day and a LOAEL of 10 mg/kg/day for maternal toxicity were indicated.

Developmental toxicity evident at 10 mg/kg/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and the mean numbers of viable male, female, and total fetuses, but the differences were not statistically significant. In addition, unusually high incidences of unossified, assymetrical, bipartite, and missing sternebrae were observed in all dose groups; however, these skeletal variations were also observed in control fetuses at the same rate and therefore these effects were not considered to be treatment-related. The most notable sign of developmental toxicity observed in all dose groups consisted of abnormalities of the lens of the eye, which was not seen in controls. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group. All eye abnormalities appeared to be localized to the area of the embryonal lens nucleus, although a variety of morphological appearances were present within that location. According to the authors, this abnormality appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. Under the conditions of the study, a LOAEL for developmental toxicity of 1 mg/kg/day was indicated; a developmental NOAEL could not be established.

In a second prenatal developmental toxicity study, groups of 25 pregnant Sprague-Dawley rats were administered 0, 1, 5, and 10 mg/kg/day PFOS in corn oil by gavage on gestation days (GD) 6-15 (Wetzel, 1983). Sexually mature Sprague-Dawley rats, one per sex per cage, were paired until confirmation of mating or until two weeks had elapsed. Mating was confirmed by daily vaginal examinations for the presence and viability of sperm or the presence of a copulatory plug. The day of confirmation of mating was designated as day 0 of gestation. Doses were adjusted according to the most recently recorded body weight measurements. Dams were observed twice daily for signs of mortality and moribundity and once daily for clinical signs of toxicity. Individual body weights and food consumption were recorded on GD 6, 8, 12, 16, and 20. Animals were sacrificed on GD 20 by CO₂ asphyxiation and the fetuses were delivered by cesarean section on GD 20. A gross necropsy was performed on all dams. The uterus from each female was excised, weighed and examined for the number and placement of implantation sites, number and of live and dead fetuses, number of early and late resorptions, and any abnormalities and then weighed again after the contents were removed. The ovaries were examined for the number of corpora lutea. Each female was examined by gross necropsy. Each fetus was sexed, weighed, and examined externally. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by the Wilson technique, with particular attention to the eyes, palate, and brain. The remaining fetuses were subjected to a skeletal examination that included evaluation of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles using alizarin red; bone alignment and degree of ossification were also evaluated.

Evidence of maternal toxicity, that was observed at the 5 and 10 mg/kg/day dose groups both during and following treatment and considered to be treatment-related, consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough haircoat, and bloody crust. Significant decreases in mean body weight gains during GD 6-8, 6-16, and 0-20 were also observed at the 5 and 10 mg/kg/day dose groups. These reductions were considered to be treatment-related since mean body weight gains were greater than controls during the post-exposure period (GD 16-20). Significant decreases in mean total food consumption were observed on GD 17-20 in the 10 mg/kg/day dose group, and on GD 7-16 and 0-20 in both the 5 and 10 mg/kg/day dose groups. The mean gravid uterine weight in the 10 mg/kg/day dose group was significantly lower when compared with controls. The mean terminal body weights minus the gravid uterine weights were lower in all treated groups, with significant decreases at 5 and 10 mg/kg/day. High-

dose animals also exhibited an increased incidence in gastrointestinal lesions. No significant differences were observed in pregnancy rates, number of corpora lutea, and number and placement of implantation sites among treated and control groups. Two dams in the 10 mg/kg/day dose group were found dead on GD 17. Under the conditions of the study, a NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for maternal toxicity were indicated.

Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although, none of these effects were statistically significantly different from controls. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg/day dose groups. The percent of male fetuses was 52%, 54%, and 60% for 1, 5, and 10 mg/kg/day, respectively, compared to 44% in controls. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups but not in the mid-dose group. Statistically significant increases in the incidences in the number of litters containing fetuses with visceral anomalies, delayed ossification, and skeletal variations were observed in the high dose group of 10 mg/kg/day. These included external and visceral anomalies of the cleft palate, subcutaneous edema, and cryptorchism as well as delays in skeletal ossification of the skull, pectoral girdle, rib cage, vertebral column, pelvic girdle, and limbs. Skeletal variations in the ribs and sternbrae were also observed. Under the conditions of the study, a NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for developmental toxicity were indicated.

The developmental eye abnormalities that were seen in the previous study (Gortner, 1980) were not observed in the 1983 developmental toxicity study even though the study design and doses were the same. Findings of abnormalities in eye development were initially thought to be treatment-related but later determined to be artifacts of sectioning (3M Company, 1999a).

Lau et al. (2001) administered Sprague-Dawley rats and CD-1 mice doses of 0, 1, 5 or 10 mg/kg/day PFOS in 0.5% Tween-20 by gavage beginning on gestation day 2 and continuing until term. Half of the dams were sacrificed on gestation day 21 (rats) or gestation day 17 (mice) and the remaining dams were allowed to deliver. Preliminary results are available. In rats, there was a significant reduction in maternal body weight gain at 5 and 10 mg/kg/day. Maternal serum cholesterol and triglycerides were reduced at 10 mg/kg/day, but liver weights were comparable to control. At 10 mg/kg/day, there was a reduction in fetal body weight and an increase in cleft palate and anasarca. All pups were born alive, but within 4-6 hours after birth all the pups in the 10 mg/kg/day group died, and 95% of the pups in the 5 mg/kg/day group died within 24 hours. In mice, maternal body weight was unaffected and liver weights were significantly increased at 5 and 10 mg/kg/day; serum triglycerides were reduced were elevated at 5 and 10 mg/kg/day. The incidence of fetal mortality was slightly increased at 10 mg/kg/day and mean fetal body weights were comparable to control. However, neonatal body weights were reduced during the first 3 days of life. Additional studies are underway to further elucidate the dose-response relationships and to examine the mechanism for the neonatal death.

Christian et al. (1999a) administered pregnant New Zealand White rabbits, 22 per group, doses of 0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day PFOS in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weights. The does were observed twice daily for viability, and clinical observations were recorded 1 hour prior to and after dosing during the treatment period and once daily during the post-treatment period (i.e. gestation days 20-29). Maternal body weights were recorded on gestation days 0 and 6-29; food consumption was recorded daily throughout the study. On gestation day 29, the does were euthanized; a gross necropsy of the thoracic, abdominal and pelvic viscera was conducted and the number of corpora lutea in each ovary was recorded. The uteri were examined for number and distribution of implantations, live and dead fetuses, and early and late resorptions. The fetuses were weighed, sexed and examined for external abnormalities. All fetuses were examined for visceral and skeletal abnormalities and the brain of one-half of the fetuses were free-

hand cross-sectioned and examined *in situ*.

In addition, a satellite study was conducted in which pregnant New Zealand White rabbits were administered the same doses as in the main study. The number of does was 3, 5, 3, 3 and 5 in the control, 0.1, 1.0, 2.5 and 3.75 mg/kg/day groups, respectively. The does were euthanized on gestation day 21, blood samples were collected, and the liver was weighed and sectioned. The fetuses were removed and examined for external abnormalities. Fetuses and placentae were pooled per litter. All samples were sent to the Sponsor (3M) for analysis. At this time, only the liver and serum analyses have been reported (3M Environmental Laboratory, 2001d).

Maternal toxicity was evident at doses of 1.0 mg/kg/day and above. One doe in the 2.5 mg/kg/day group and nine does in the 3.75 mg/kg/day aborted. All abortions occurred on gestation days 22-28 and were considered treatment-related by the study authors. There was a significant increase in the incidence of scant feces in the 3.75 mg/kg/day group. Scant feces were also noted in one and three does in the 1.0 and 2.5 mg/kg/day groups, respectively. Mean maternal body weight gains were significantly reduced in the 3.75 mg/kg/day group on gestation days 10-13, 13-16, 16-19 and 21-24. Mean body weight gains were also calculated for the treatment period (days 7-21), post-treatment period (days 21-29) and duration of the study (days 7-29). There was a significant reduction in mean maternal body weight gain during the treatment period in the 1.0, 2.5 and 3.75 mg/kg/day groups. Mean body weight gain for the entire study period was also significantly reduced in the 2.5 mg/kg/day group. Mean food consumption (g/kg/day) was significantly reduced in the 2.5 mg/kg/day group on gestation days 16-19, 19-21 and 21-24, as well as for the entire study period (days 7-29). Mean food consumption was significantly reduced in the 3.75 mg/kg/day group on gestation days 13-16, 16-19, 19-21 and 21-24, as well as the entire treatment period. The LOAEL for maternal toxicity was 1.0 mg/kg/day and the NOAEL was 0.1 mg/kg/day.

Developmental toxicity was evident at doses of 2.5 mg/kg/day and above. The number of corpora lutea, resorptions, live/dead fetuses, litter size and sex ratio were comparable among treated and control groups. Mean fetal body weight (male, female and sexes combined) was significantly reduced in the 2.5 and 3.75 mg/kg/day groups. There was also a significant reduction in the ossification of the sternum (litter averages) in the 2.5 and 3.75 mg/kg/day groups, and a significant reduction in the ossification of the hyoid (litter averages), metacarpals (litter averages) and pubis (litter and fetal averages) in the 3.75 mg/kg/day group. The LOAEL for developmental toxicity was 2.5 mg/kg/day and the NOAEL was 1.0 mg/kg/day.

In the satellite study of does euthanized on gestation day 21, the liver and serum analyses were reported by 3M Environmental Laboratory (2001d). All serum and liver samples (including those from untreated controls) had detectable levels of PFOS; the values are presented below:

Average Concentration of PFOS in Rabbit Liver and Serum by Dose Group

Dose group (mg/kg/day)	PFOS conc. liver (ug/g)	PFOS conc. serum (ug/ml)
0.0	0.239	0.0690
0.1	13.1	2.73
1.0	133	23.8
2.5	317	45.8
3.75	416	88.9

Qualitatively, increasing concentrations of PFOS were found in samples of liver and serum as doses of PFOS increased. The levels of PFOS are much higher in the liver than in the serum.

These values should be viewed with caution. It was stated that because radio-labeled reference material was not available, “it is not possible to verify true recovery of endogenous analyte from tissues.” Matrix spike recovery indicates the accuracy of quantitation to be $\pm 30\%$. It is also noted that liver concentrations may be biased high. The only conclusion presented in the laboratory report is that “PFOS was observed in the liver and serum of all rabbits dosed with the test article” (3M Environmental Laboratory, 2001d).

3.7 Reproductive Toxicity

A two-generation reproductive toxicity study, designed to test for the toxic effects of PFOS on reproductive function in adult animals and on developmental, learning, and reproductive effects in the offspring, was conducted in Sprague-Dawley rats (Christian et al., 1999b). Five groups of 35 rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day for six weeks prior to and during mating. Treatment in male rats continued until one day before sacrifice (approximately 22 days total); female rats were treated throughout gestation, parturition, and lactation.

F0 Generation:

Parental animals (F0) were observed twice daily for clinical signs. Body weights and food consumption values were recorded weekly during the treatment period in male rats; and weekly during mating and then daily during gestation, and on lactation days 1, 4, 7, 10, 14, and at sacrifice in female rats. Each dosage group consisted of two sets of female rats. One set consisted of the first ten female rats with confirmation of mating; this group was dosed until gestation day (GD) 10 and delivered via Caesarean-sectioning. The remaining females comprised the second set which delivered naturally. During the 21-day lactation period, the dams were evaluated for clinical signs during parturition and length of gestation, and then each litter was evaluated at least twice daily for size and pup viability at birth. Pup observations during the 21-day lactation period included physical signs, body weights, nursing behavior, surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air righting reflex. Pupil constriction was evaluated only on lactation day 21. On lactation day 4, litters were randomly culled to four male and four female pups. The remaining pups were sacrificed and necropsied. The F0 male rats were sacrificed and necropsied after the end of dosing at the time of parturition (lactation day 1). The testes, epididymides, prostate, and seminal vesicles were weighed. Evaluations of sperm number, motility, and morphology were not included in the protocol. The F0 generation females that delivered by Caesarean-section were sacrificed on GD 10 and necropsied. Pregnancy status was confirmed, the ovaries were examined for the number and distribution of corpora lutea, implantation sites were determined, and embryos were examined for viability. The F0 generation females that delivered naturally were sacrificed on lactation day (LD) 21 and necropsied. Ovaries were examined as above and the number and distribution of implantation sites was recorded. The liver from each parental rat was removed, weighed and analyzed. Blood samples were collected from 5 male rats that had mated and from 5 female rats on LD 21 for pharmacokinetic analysis; livers from the pups from the litters of these five dams were also collected for analysis.

F1 Generation:

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation. Twenty-five F1 generation rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, and 0.4 mg/kg/day beginning on LD 22 and continuing through the day before sacrifice. At 24 days of age, one rat per sex per litter in each dose group was tested in a passive avoidance paradigm. On LD 28, females were evaluated for the age of vaginal patency and on LD 34, male rats were evaluated for the age of preputial separation. One rat per sex per litter were evaluated in a water-filled M-maze on LD 70. Assignment to cohabitation within each dose group began on LD 90. Females with evidence of mating were considered to be at GD 0 and assigned to individual housing for the remainder of the dosing period. The F1 generation male rats

were sacrificed after mating, necropsied and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver naturally. Dams that delivered litters were sacrificed and necropsied on LD 21. All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups.

In the F0 generation male rats, there were no treatment-related clinical signs of toxicity, no mortality, and no effects on mating or on any of the fertility parameters evaluated in any dose group tested. Reported effects included reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dose groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4. and 1.6 mg/kg/day dose groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate were observed in F0 males at 3.2 mg/kg/day; no other organ weight changes were reported. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported.

In the F0 generation female rats, no deaths were reported at any dose level. In dams sacrificed on GD 10 for Caesarean-sectioning, there did not appear to be any effects on estrous cycling, mating and fertility parameters, the numbers of corpora lutea and implantations, or in the number of viable or non-viable embryos. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dose groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and body weight gain and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Reversible delays in reflex and physical development were observed in the F1 generation offspring. The ability to surface right was significantly delayed in the 1.6 and 3.2 mg/kg/day dose groups on LDs 3-10 (delays in the 3.2 mg/kg/day dose group were observed on LD 1, after which there were no surviving pups remaining for further observation). By the end of the observation period, however, all surviving pups in the 1.6 mg/kg/day dose group had the ability to surface right. There were no delays observed in the ability to surface right in dose groups ≤ 0.4 mg/kg/day. Similar responses were seen for pinna unfolding and eye opening. Although there were transient delays seen with these signs of physical development across all dose groups, by the end of the observation period responses in pups were similar to controls. The time of development of the acoustic startle reflex and the ability to air right were both significantly reduced in the 1.6 mg/kg/day dose group. No effects on these reflexes were observed in the low dose group of 0.1 mg/kg/day and only a transient delay (on LD 16 only) in the ability to air right was seen in the 0.4 mg/kg/day group. At the end of lactation (LD 21), all live pups in all dose groups (0, 0.1, 0.4, and 1.6 mg/kg/day) had pupil constriction response.

The most significant finding reported in the offspring was that of reduced pup viability at the two highest dose groups. The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. Statistically significant increases in the number of dams with stillborn pups were also observed at 3.2 mg/kg/day. As a result, the viability index was greatly reduced in these dose groups (0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day). The lactation index was also significantly reduced (94.6%) in the 1.6 dose group. In addition, gestation length was significantly reduced in the high-dose group and there also was a significant reduction in the number of implantation sites followed by a concomitant reduction in litter size. Statistically significant reductions in pup body weights were also observed at the two highest dose groups. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead.

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups, only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation.

Clinical observations in the F1 generation male rats appeared unremarkable. No treatment-related deaths were reported and no statistically significant differences were reported for any of the following parameters: body weights/body weight gains, average day of preputial separation; values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating or fertility parameters; necroscopic examinations; absolute or relative weights for the right or left testis, seminal vesicles, right epididymis, or prostate; and terminal body weights. The only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels.

Clinical observations for the F1 generation females were likewise unremarkable. Observations at the 0.4 mg/kg/day dose group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation. There were no statistically significant differences reported for any of the following parameters: values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or water maze performance paradigm; mating and fertility parameters; gestation index; pregnancy rates; and necroscopic examinations.

Evidence of treatment-related effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. Body weights were comparable to control levels by LD 14. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7 and 14. Mean body weights on LD21 continued to remain lower than controls, although the difference was not statistically significant (46.5 g in 0.4 mg/kg/day dose group vs. 50 g in controls). Clinical and necroscopic observations of the F2 generation pups were unremarkable. No other toxicologically significant effects were reported.

Under the conditions of the study, the NOAEL and LOAEL for both the F0 generation male and female parents are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on reductions in body weight gain and food consumption. The NOAEL for the F1 generation parental males could not be established since treatment-related reductions in absolute food consumption values were reported at the lowest dose tested, 0.1 mg/kg/day. The NOAEL and LOAEL for the F1 generation parental females are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on significant reductions in body weights and food consumption. The NOAEL and LOAEL for the F1 generation offspring are 0.4 mg/kg/day and 1.6 mg/kg/day, respectively, based on significant reductions in the number of implantation sites, litter size, pup viability, pup body weight and survival. The NOAEL and LOAEL for the F2 generation offspring are 0.1 mg/kg/day and 0.4 mg/kg/day, respectively, based on significant reductions in mean pup body weight.

Liver and sera samples were collected from the F0 and F1 animals at terminal sacrifice (after cohabitation for males and on lactation day 21 for females) and analyzed for the presence of PFOS (3M Environmental Laboratory, 1999a). The results for the F0 animals are presented below:

Dose group (mg/kg/day)	Average PFOS conc. in serum (ug/ml)	Average PFOS conc. in liver (ug/g)
0.0	female 0.0307 male 0.0244	female 0.171 male 0.665
0.1	female 5.28 male 10.5	female 14.8 male 84.9
0.4	female 18.9 male 45.4	female 58.0 male 176
1.6	female 82 male 152	female 184 male 323
3.2	female NR* male 273	female NR* male 1360

*samples not received

Qualitatively, the F0 results indicate all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group. The average PFOS concentrations in pooled liver samples from F1 animals shortly after birth were 0.0511, 6.19, 57.6, and 70.4 ug/g in the 0.0, 0.1, 0.4, and 1.6 mg/kg/day dose groups, respectively. These quantitative values for the PFOS concentration in the liver and serum should be viewed with caution. The accuracy of quantitation is $\pm 30\%$, the purity of the analytical reference substance is unknown, and there were several uncorrected dilution errors.

Two studies were then conducted to further understand the distribution of PFOS in the dam, fetus and neonate. In the first study, Sprague-Dawley rats were administered oral doses of PFOS (0.1, 0.4, 1.6, and 3.2 mg/kg/day) once daily beginning 42 days prior to cohabitation, and continued through day 14 or day 20 of presumed gestation (3M Environmental Laboratory, 2001e). Serum, urine, and feces specimens were collected from the dams before mating and at GD 7, GD 15 and GD 21; liver specimens were collected on GD21. A total of 54 pooled serum and liver specimens were collected from fetuses on GD 21. The results are shown below:

Average Results for the Analysis of Serum Samples (ug/ml)

	0 mg/kg	0.1 mg/kg	0.4 mg/kg	1.6 mg/kg	3.2 mg/kg
Day 0	0.0723	8.89	40.7	160	318
Day 7	0.126	7.82	40.9	154	105
Day 15	0.0926	8.80	41.4	156	275
Day 21	0.0714	4.24	26.2	136	155
Day 21 Fetal	0.125	9.07	34.3	101	165

Average Results for the Analysis of Liver Samples

Dose Group (mg/kg/day)	PFOS Conc. (ug/g) Female Adult	PFOS Conc. (ug/g) Fetal Liver
0	0.288	0.169
0.1	29.2	7.93
0.4	107	30.6
1.6	347	86.7
3.2	610	230

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the fetuses. PFOS was also observed in the control dams, as well as the control fetuses. As observed in the 2-generation reproductive toxicity study described above, the levels of PFOS were much higher in the liver than in the serum for the dams. The levels of PFOS remained fairly steady in the serum of the dams from GD0 – GD 15, but the levels dropped at GD21. In the GD21 fetuses, the level of PFOS in the serum was generally comparable to the level observed in the dams, whereas the level of PFOS in the fetal livers was well below that seen in the dams.

In the second study, Sprague-Dawley rats were administered oral doses of PFOS (0.1 and 1.6 mg/kg/day), once daily beginning 43 days prior to cohabitation until confirmed evidence of mating (3M Environmental Laboratory, 2001f). Urine and fecal samples were collected from the dams during the following intervals: one day prior to initiation of cohabitation to the following morning, days 6 to 7, 14 to 15, and 20 to 21 of presumed gestation and days 21-22 of lactation. Blood samples were collected from each of the dams on the day cohabitation was initiated (prior to cohabitation), GD 7, 15 and 21, and LD 14 and 22. Day 1 of lactation was defined as the day of birth. On LD 4, litters were culled to five male pups and five female pups per litter, where possible. Sera specimens were collected from pooled litter samples on LD 21. Liver specimens were collected from the dams, and the liver from each pup was collected and pooled per litter. Blood samples were collected and pooled per liter. The results are shown below:

Average Results for the Analysis of Serum Samples (ug/ml)

	0 mg/kg	0.1 mg/kg	1.6 mg/kg
GD 0	0.100	9.21	161
GD 7	0.0796	7.24	129
GD 15	0.0742	5.68	90.6
GD 21	<LLQ	2.58	39.5
LD14	0.0542	1.63	20.6
LD22	0.0492	0.979	14.1
LD 21 Pups	0.0531	1.80	27.1

Average Results for the Analysis of Liver Samples (ug/g)

	0 mg/kg	0.1 mg/kg	1.6 mg/kg
Dam	0.243	6.15	59.7
Pup	0.174	5.00	56.2

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the levels in the serum decreased with time. The levels of PFOS were much higher in the liver than in the

serum of the dams and the pups. The levels of PFOS were similar in the liver of the dams and pups, while the levels in the serum were slightly higher in the pups than in the dams.

Based on the results of the two-generation reproductive toxicity study in which significant reductions in pup viability were observed at 1.6 and 3.2 mg/kg/day, a cross-fostering study was conducted as a means of determining whether the effects observed in pups were a result of *in utero* exposure to PFOS or as a result of exposure during lactation; thus the potential for a distinction to be made between prenatal and postnatal effects following continuous maternal treatment (Christian et al., 1999c).

In this study, two groups of 25 female Sprague-Dawley rats were administered 0 and 1.6 mg/kg/day PFOS in 0.5% Tween-80 by gavage, beginning 42 days prior to mating to untreated (breeder) males, and continuing throughout gestation and into day 21 of lactation. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weight. Parental females were observed twice daily for viability and clinical observations were recorded 1 hour prior to and after dosing during the treatment period. Maternal body weights were recorded once during the acclimation period and then daily during the treatment period and at sacrifice; food consumption was also recorded once during the acclimation period and then daily during gestation and on days 1, 4, 7, 10, and 14 of lactation. During parturition, females were continually evaluated for clinical signs and also for duration of gestation, length of parturition, litter sizes, and pup viability at birth. Maternal behavior was recorded daily throughout lactation. All maternal rats were sacrificed by carbon dioxide asphyxiation on day 22 of lactation and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed; any gross lesions were preserved for future analysis. In addition, the number and distribution of implantation sites were recorded. Rats that did not deliver a litter were not included in the cross-fostering procedure and were sacrificed on lactation day 25, examined for gross lesions, and the uteri examined to confirm the presence/absence of implantation sites. Dams with no surviving pups were sacrificed after the last pup was found dead, missing, or presumed cannibalized.

Following completion of parturition, litters were immediately removed from their respective dams and placed with either a control- or PFOS-treated dam for rearing. This cross-fostering procedure resulted in four groups of 12-13 dams or pups as follows: A) control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; B) control dams with litters from control dams, i.e., negative control; C) PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure; and D) PFOS-treated dams with litters from control dams, i.e., post-natal exposure only.

On day 1 of lactation (birth), each pup was individually weighed and each litter was evaluated twice daily during lactation for viability. Pups were observed once daily for clinical signs and gross external physical anomalies. Pup body weights were recorded on days 1, 4, 7, 14, and 21 of lactation, and then at sacrifice. On day 4 of lactation, each cross-fostered litter was culled to 5 males and 5 females. On day 21 of lactation, all pups were sacrificed via decapitation and examined for gross lesions. Pups found dead or sacrificed because of moribundity were examined for gross lesions and for the cause of death or the moribund condition. The lungs, liver, and any gross lesions were collected from selected pups at various timepoints and preserved for possible future analysis. Liver samples were evaluated via electron microscopy. The method of statistical analysis consisted of calculation of averages and percentages; litter values were used where appropriate.

In addition, samples of blood, milk (including the milk-secreting glands), and liver were collected from selected maternal rats and pups (blood and liver samples were pooled per litter) at various timepoints for analysis of PFOS concentration. Only the analysis of the sera samples are available at this time.

All maternal rats survived to schedule sacrifice. Signs of clinical toxicity observed in the dams during the study period (e.g., chromorhinorrhea, scaly tail, abrasion on the head, neck, tail and/or forelimb, missing,

broken and/or misaligned incisors, and localized alopecia, among others) were not considered to be treatment-related since they also occurred in the control animals. Mean maternal body weight gains at 1.6 mg/kg/day were reduced compared to controls during pre-mating and continuing throughout gestation. Mean maternal body weights and body weight changes in the treated group were comparable to those seen in control animals during lactation. During the pre-mating period, and on into gestation and lactation, food consumption was reduced in treated animals as compared to controls. Reductions in gestation length, the average number of implantation sites, delivered sizes, and live litter size were observed in treated animals. All pregnant animals delivered live offspring.

Following cross-fostering on LD 1, live litter sizes were comparable between treated and control groups. Pup mortality was observed in two of the cross-fostered groups. On lactation days 2-4, approximately 19% of the pups in group C were either found dead or presumed cannibalized. Pup mortality was also observed in group A at a rate of 9%. In addition, on day 4 of lactation, the number of live pups, numbers of surviving pups per litter, and live litter sizes were also reduced in these two groups. Pup mortality in groups B and D during lactation days 2-4 were at 1.6% and 1.1%, respectively. Reductions in pup body weights were observed in groups A and C on day 1 of lactation. Pup body weights in group D were comparable to controls during that same period. From lactation day 4-21, pup body weights in groups A, C, and D were reduced when compared to group B (negative control), with the reductions greatest in group C. Two litters in group A and one litter on group C did not nurse. Milk analysis of the stomachs of pups found no milk in the stomachs of 57%, 100%, and 87% of the pups found dead and necropsied in groups A, C, and D, respectively. Sex ratios and the lactation index were comparable among all groups. Signs of clinical toxicity were observed in pups, but were not considered to be treatment-related since they also occurred in group B (negative control) at the same rate.

Electron microscopic examination of the liver revealed an increase in the number of peroxisomes in pups from dams treated with 1.6 mg/kg/day PFOS. No significant differences were observed between group B and the other groups following examination of pup lungs.

PFOS concentrations in the serum of untreated dams ranged from below the limit of detection (0.05 ug/ml) to 5.34 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with untreated dams, were below the limit of detection. Serum PFOS concentrations in the pups from treated dams, fostered with untreated dams, ranged from 47.6 ug/ml to 59.2 ug/ml. PFOS concentrations in the serum of treated dams ranged from 59.2 ug/ml to 157 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with treated dams, ranged from below the limit of detection to 35.7 ug/ml. Serum PFOS concentrations in the pups from treated dams, fostered with treated dams, ranged from 79.5 ug/ml to 96.9 ug/ml. These data indicate that exposure to PFOS can occur both *in utero* and via milk from treated dams (3M Environmental Laboratory, 1999b). The accuracy of quantitation for the analyses was $\pm 30\%$.

In conclusion, pups from control dams that were cross-fostered with PFOS-treated dams (post-natal exposure only) had the same low mortality rate (1.1%) as pups from control dams cross-fostered with control dams (1.6%; negative control). Mortality rates in the remaining two groups, however (i.e. control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; and PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure), were much higher at 9.6 % and 19.2%, respectively. Under the limited conditions of the study, the data appear to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse affect on pup viability. Additionally, analysis of PFOS concentration showed that PFOS was observed in the sera of F0 female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera samples taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed via lactation, but not exposed *in utero*.

Several mechanistic studies are being conducted to understand the neonatal death (3M Company, 2001c). Preliminary results indicate that reductions in serum lipids and cholesterol synthesis do not appear to play a significant role in the death of the offspring.

3.8 Human Hazard

Several occupational studies on the health effects associated with fluorochemical exposure have been conducted. The following studies have all been conducted at the 3M Decatur, Alabama plant where PFOS has been manufactured since 1991, and PFOA since 1998. The studies conducted in 2000 also included the Antwerp, Belgium plant. Only some of the studies examined a possible association between serum PFOS levels in workers and corresponding health effects. The other studies use exposure categories as surrogates for fluorochemical serum levels in workers.

Cross-sectional data from medical surveillance conducted at the plants were analyzed to determine if there were any associations between PFOS levels and hematology, clinical chemistries, and hormonal parameters (Olsen et al., 1998, 1999, 2001a). A group of male volunteers working at the Decatur plant in 1995 and 1997 and volunteers from the 2000 medical surveillance, which also included females and Antwerp employees, were analyzed.

In the 1995 and 1997 analyses, there were no consistent associations between PFOS levels in the workers and the hematology and other clinical chemistry parameters when the data from both plants were combined. Mean serum cholesterol levels remained constant or increased with increasing PFOS serum levels depending on the year; however, those employees with the highest PFOS levels had lower mean HDL values. It should be noted that age was significantly associated with higher PFOS levels. In addition, there were no associations between clinical hepatic enzyme tests and serum PFOS levels in workers.

Eleven hormone values were collected from 88 of the employees who participated in the study in 1995. The employees who participated in the hormone portion of the study were significantly different from the non-participants at both locations (younger, higher PFOS levels, smokers, and higher WBC). After adjusting for confounders, there were no significant associations between PFOS and the hormones analyzed, except estradiol; however, it seems that one employee with high PFOS measurements (12.83 ppm) and a large BMI may have influenced these results. Removal of this employee from the analyses resulted in no significant associations with estradiol.

There were several differences between the employees at the two plants, such as body mass index, alcohol consumption, and age. In addition, there were only 61 employees common to both the 1995 and 1997 cohort due to a high rate of turnover at both plants from 1996-1997. Some of the findings were not consistent over the 2 time periods. The participation rate in this voluntary biomonitoring was very low at the Decatur plant (35-40%). In addition, the employees at both sites used APFO as a surfactant (mean PFOA serum levels in 1995 were 1.46 ppm and in 1997 were 1.57 ppm). The number of years that each group worked at the 2 plants (exposure) were not provided in the analyses, although the youngest employees had the lowest levels of serum PFOS. Also, ninety-five percent of the employees across both plants and both time periods had serum PFOS levels less than 6 ppm. These limitations indicate that the results of this study should be interpreted carefully.

The results from the 2000 cross-sectional analysis were analyzed for all employees from both plant locations combined and by plant since the Antwerp and Decatur populations were significantly different from each other in several ways. Antwerp employees as compared to Decatur employees had lower PFOS serum levels (mean 0.96 ppm), were younger, had lower BMIs, worked fewer years, had higher alcohol consumption, higher mean HDL and bilirubin values, lower mean triglyceride, alkaline phosphatase, GGT,

AST, and ALT values, and mean thyroid hormone values tended to be higher. The data were also stratified by production status and gender and divided into quartiles of their serum PFOS distribution. However, the PFOS levels are different for each subgroup, making it difficult to compare the results.

Comparisons across quartiles within plant locations did not reveal many differences in thyroid, hematology, or urinalysis values. The only significant ($p < .05$) differences noted were: BUN was significantly higher in male production workers and females in the highest PFOS quartiles at the Antwerp plant, and ALT was significantly higher in the highest quartile in Decatur male production employees.

When the data were analyzed by number of employees who had values above the reference range for hepatic clinical chemistry tests and liver enzyme and bilirubin tests, a higher percentage of male Decatur production workers were in the highest PFOS quartile (2.31- 10.06 ppm) for ALT, GGT, and total liver panel than the other quartiles. Most notable were the results for ALT where 8% of employees in the lowest PFOS exposure group (Q1) and 28% in the highest exposure group (Q4) had values above the reference range, while the percentages for total liver panel (which includes alkaline phosphatase, AST, ALT, GGT, and total and direct bilirubin) were 18% and 35%, respectively. This trend was not evident in Decatur non-production employees, in Decatur females, or in any of the Antwerp employees. However, it should be noted that each sub-population had a different serum PFOS quartile distribution.

Mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly ($p < .05$) higher in Q4 (mean PFOS level 2.69, range 1.69 – 10.06 ppm) than in Q1 (mean PFOS level 0.27 ppm, range 0.04 – 0.42 ppm) for all male employees at both plants ($n = 421$). It should be noted that the number of Antwerp production employees were evenly distributed among the quartiles while this was not the case for Decatur employees. The highest number of Decatur employees was in Q4. These data were analyzed for employees who had values above the reference range for alkaline phosphatase, AST, ALT, GGT, and total liver panel. For male employees for all of these measures, the levels increased from Q1 to Q4; however, statistical significance was not reported. In Q1, 4% of the employees had values above the reference range for ALT and 6% for GGT, while 12% was reported for Q4 for both of these tests. For total liver panel, 14% of the employees had values above the reference range in Q1 as compared to 23% in Q4. The numbers of female employees with values above the reference range was very small ($n = 8$). In female employees combined for both plants ($n = 97$), alkaline phosphatase and GGT were significantly higher ($p < .05$) and total bilirubin significantly lower in Q4 than in Q1.

Thyroid results for male production employees of both plants indicated that T3 was significantly higher ($p < .05$) and THBR (T3 uptake) was significantly lower ($p < .05$) in Q4 than in Q1. The range of T3 values was very large for females; however, there were no statistically significant differences between the mean values across the quartiles.

Multivariable regression analyses were conducted to adjust for possible confounders that may affect the results of the clinical chemistry tests. The following variables were included: production job (yes or no), plant, age, BMI, cigarettes/day, drinks/day and years worked at the plant.

A positive significant ($p = .04$) association between T3 and PFOS was observed. Plant location was highly significant ($p < .0001$) in the model. BMI, cigarettes/day, alcohol/day were also significant. In the univariate analyses, Antwerp employees had higher mean T3 levels than Decatur employees overall. However, for each plant (individually) T3 values increased by quartile as PFOS serum levels increased, although the differences were not statistically significant. THBR, as well as the other thyroid hormones, were not significant in the regression analyses.

A positive statistically significant ($p = .04$) association between PFOS and cholesterol was observed. When both PFOS and PFOA were included in the model, neither were statistically significant at $p = .05$.

PFOS was not significantly associated with HDL, but triglycerides were positively associated with PFOS ($p = .01$). No significant associations were observed with PFOS in relation to alkaline phosphatase, GGT, AST or total bilirubin.

A longitudinal analysis of the above data was performed to determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of the Antwerp and Decatur facilities (Olsen et al., 2001b). The medical surveillance data from 1995, 1997, and 2000 were analyzed using multivariable regression. The plants were analyzed using 3 subcohorts that included those who participated in 2 or more medical exams between 1995 and 2000. When male employees from both plants were combined, no statistically significant ($p < .05$) associations were observed over time between PFOS and cholesterol or triglycerides. In addition, there were no significant associations between PFOS and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

There were several limitations to the 2000 cross-sectional and longitudinal studies including: 1) serum PFOS levels in these workers have been declining over time, 2) PFOS serum levels were significantly higher at the Decatur plant than at the Antwerp plant, 3) all participants were volunteers, 4) there were several consistent differences in clinical chemistry profiles and demographics between employees of the Decatur and Antwerp plants, 5) PFOA and other perfluorinated chemicals are also present in these plants, 6) plant populations cannot be compared because quartiles are different for each subgroup, and 7) only one measurement at a certain point in time was collected for each clinical chemistry test. In addition, in the longitudinal study only a small number of employees participated in all 3 sampling periods (24%), different labs and analytical techniques for PFOS were used each year, and female employees could not be analyzed because of the small number of participants.

A retrospective cohort mortality study was performed on Decatur employees to determine whether plant employees had significantly different death experiences from the general population (Mandel and Johnson, 1995). There were 1957 employees who had worked at least one year at the Decatur plant between 1961 and 1991. Seventy-four deaths were recorded, and there were no significantly elevated SMRs for all major causes of death regardless of the comparison population used (Alabama state, US, or Alabama counties). When the data were analyzed by job description (chemical department vs. film plant employees), there was a statistically significant deficit for all causes of death in both job categories.

This study had almost complete follow-up of the cohort and ascertainment of causes of death. However, since the workforce was relatively young, only 74 deaths were reported for 1,951 employees. The number of women in the study was very small (4 deaths). Additionally, PFOS serum levels were not examined in relation to mortality.

An update of this study was recently conducted to follow the cohort through December 31, 1998, thus adding 7 years of follow-up (Alexander, 2001). This cohort consisted of 2083 employees who had worked at the Decatur plant for at least 1 year. There were 145 deaths reported, almost twice that reported in the last cohort. In this study, employees were placed into 3 exposure categories based on their job descriptions: high, low, and non-exposed. Almost half of the employees (47%) were placed in the high exposure group, in which there were 65 deaths. Fourteen percent of the cohort was in the low exposure group (27 deaths), and 39% were in the non-exposed group (53 deaths).

SMRs were calculated using state of Alabama reference data. When the entire cohort was analyzed, SMRs were not elevated for most of the cancer types and for non-malignant causes. SMRs that were above 1 (cancer of the esophagus, liver, breast, urinary organs, bladder, and skin) were also elevated when the cohort was limited to any employee ever employed in a high exposure job (except breast cancer). Only 2 or 3 deaths were reported for each of these cause-specific categories and were not statistically significant,

except for bladder cancer.

Workers who were employed in high exposure jobs were 13 times more likely to die of bladder cancer than the general population of Alabama (SMR = 12.77, 95% CI = 2.63 - 37.35). This effect remained when the data were analyzed using county death rates. Three male employees in the cohort died of bladder cancer (0.12 expected). All of these workers had been employed at the Decatur plant for more than 20 years, and all of them had worked in high exposure jobs for at least 5 years (SMR = 24.49). In the previous cohort mortality study, 1 bladder cancer death was reported.

Two deaths were reported for liver cancer. One was in the low exposure group and one in the high exposure group. The SMR for workers who were employed in either high or low exposure jobs was 3.08 (95% CI = 0.37 - 11.10). Five cases of cirrhosis of the liver were reported in this cohort, 2 in the high exposure group, 1 in the low exposure group, and 2 in the non-exposed. The observed did not exceed the expected mortality experience in any of these exposure groups.

Most of the same limitations discussed above for the original cohort study apply to this update. A larger cohort was followed in the update, thus reporting twice as many deaths; however, the cohort is fairly young and the number of deaths is still small (especially for females in all categories). Death certificates were located for 96% of the cohort, but the 6 not obtained could greatly impact the results of the analyses since the number of deaths in most of the sub-cohorts was very small. In addition to fluorochemicals, workers were exposed to other chemicals in the workplace. Biological measurements of fluorochemicals were used to define jobs into exposure categories based on job descriptions; however, there is still a potential for misclassification of exposure.

The high ratio of observed bladder cancer deaths in long-time employees to those expected in the general population is troublesome. Although only 3 deaths were reported in high exposure jobs, the expected number in the general population was 0.23. It seems unlikely that this effect would be due to chance, given the magnitude of the risk estimate (12.77). Many years of follow up without another death from bladder cancer would have to occur before there would no longer be an appreciable risk. Currently, animal studies on PFOS have not reported a higher incidence of bladder cancer tumors. It is unlikely that tobacco smoking could be solely responsible for the excess in bladder cancer mortality, given the 13-fold increase. Smoking status was not determined for this cohort; however, lung cancer and other smoking-related cancers were not elevated. Therefore, it is unlikely that tobacco smoking was any higher in the employees than in the general population.

Given the limitations of this study, it is unclear whether fluorochemicals are responsible for the excess of bladder cancer deaths, or whether other carcinogens may be present in the Decatur plant. At the 3M Cottage Grove facility, where APFO and other fluorochemicals are manufactured, 5 bladder cancer deaths were reported (SMR = 1.31, 95% CI = 0.42 - 3.05). Four of these deaths occurred in employees who did not work primarily in the chemical division and therefore were assumed to have little exposure to APFO. The authors of the Decatur study report that the bladder cancer cases worked mostly in maintenance jobs or at the incinerator and wastewater treatment plant and could have been exposed to many chemicals in addition to fluorochemicals. It is important that follow up of this cohort is continued in order to gain a better understanding of the mortality experience of workers exposed to fluorochemicals.

In order to gain additional insight into the effects of fluorochemical exposure on workers' health, an "episode of care" analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals (Olsen et al., 2001c). An "episode of care" is a series of health care services provided from the start of a particular disease or condition until solution or resolution of that problem. Episodes of care were identified in employees' health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers,

procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998. Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M's employee population experience using indirect standardization techniques. A ratio of the chemical plant's observed to expected experience divided by the film plant's observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). 95% confidence intervals were calculated for each RREpC. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected).

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group had 2 ½ times more episodes of care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the reported episodes occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. Episodes of care are reported, not disease incidence; therefore, this parameter cannot be interpreted in any other manner. The data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease. In addition, many of the risk ratios for episodes of care had very wide confidence intervals. The analysis was limited to 6 years. Also, the utilization of health care services may reflect local medical practice patterns. Individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications. Episodes of care may include the same individual several times. Not all employees were included in the database, such as those on long-term disability. The analysis may be limited by the software used, which may misclassify episodes of care. The software may also assign 2 different diagnoses to the same episode. Certain services, such as lab procedures may not have been reported in the database.

4.0 Hazards to the Environment

The 3M Company have reported ecotoxicity test results on five PFOS salts: potassium, lithium, ammonium, didecyldimethylammonium and DEA (diethanolamine). The majority of testing has however been carried out on the PFOS potassium salt. Only limited data are available for the other salts, but because the salts all dissociate instantaneously at neutral pH to the PFOS ion and the appropriate counterion the potential exists for read-across. For the purposes of this review it is assumed that the toxicity of the PFOS anion will be dominant and that read-across of toxicity from the potassium salt to the other salts is acceptable as a first approximation, except for the didecyldimethylammonium salt. This is because these counterions are unlikely to be toxicologically significant compared with the contribution of the PFOS ion. Published toxicity data relating to, for example, potassium (as chloride) to fish and invertebrates show acute and chronic effect concentrations to be ≥ 100 mg/l (Dierickx and Bredael-Rozen, 1996; Biesinger and Christensen, 1972). Toxic concentrations of the PFOS potassium salt reported for fish and invertebrates in the 3M studies are however all well below 100 mg/l, suggesting that the potassium ion was not a major contributor to the overall toxicity of the substance. For the present purpose correction for the molecular weight of the counterions is considered to be unnecessary. Didecyldimethylammonium is an exception due not only to its higher molecular weight, but also because high molecular weight amines are known to have the potential for exhibiting significant toxicity to aquatic species. Only two of the studies presented for review related to the salt of this cation – acute studies with the Fathead minnow (*Pimephales promelas*) and the water flea (*Daphnia magna*). Both were judged to be unacceptable for assessing PFOS toxicity because of the use of an inappropriate method of test medium preparation. The result of the test with the fish did however suggest that the didecyldimethylammonium ion was not a source of significant toxicity in the test medium whilst the result of the test with the water flea suggested that it might have been.

Wherever possible measured exposure concentrations have been used as the basis for expressing the toxicity of the test substance. In some instances the measured concentrations were significantly below nominal. In basing effect concentrations on measured concentrations it is assumed that there is a direct relationship between PFOS concentration and toxicity.

The data summaries in Tables 7 to 18 include columns headed 'Study Standard' and 'Comments', in addition to study information required under the standard OECD column headings.

‘Study standard’ indicates the overall quality of the reported study taking into account the technical and scientific procedures employed and the information content of the test report. Five standards have been used:

- **Good** – the study fulfils all requirements in terms of method, reporting and interpretation.
- **Acceptable** – the study is of an overall acceptable standard for use in hazard and risk assessment although there are inadequacies in some elements.
- **Questionable** – there are significant inadequacies in elements of the study that raise concerns about its validity. The results should only be considered supportive of other data.
- **Unacceptable** – there are significant inadequacies in elements of the study that invalidate the data.
- **Unknown** – there is insufficient information to evaluate the standard of the study.

The column headed ‘Comments’ highlights observations on the study procedures and results that are considered relevant to the assessment. Reasons why procedures or results were considered inappropriate are also given.

The tables also contain a column headed ‘Study reference number’ that refers to the list of studies in Annex 1. A summary of the lowest acceptable effect concentrations for each trophic level is given in Annex 4.

4.1 Effects on Fish, Invertebrates and Aquatic plants (Algae and higher plants)

Acute (short-term) data were available from a total of 31 studies covering three species of freshwater fish, one species of saltwater fish, a fish acclimated to saltwater, two species of freshwater and three species of saltwater invertebrate, three species of freshwater and one species of saltwater algae and one species of freshwater higher plant. The studies have been reviewed and this has resulted in 21 being considered to be of an acceptable standard for assessing the acute (short-term) toxic hazard of PFOS to fish, invertebrates and aquatic plants.

Sub-chronic/chronic (prolonged/longer-term) data were available from a total of 8 studies covering two species of freshwater fish, one species of fresh and saltwater invertebrate and one species of freshwater unicellular algae. A review of the studies has resulted in seven being considered to be of an acceptable standard for assessing the toxic hazard of PFOS to fish and invertebrates. It should be noted that study number 25 with the Fathead minnow (*Pimephales promelas*) was considered unacceptable because of a lack information in the report. It is probable that this study was the same as that described more fully in report number 14. Study 14 was judged to be acceptable.

In addition, flow-through bioconcentration studies have been conducted in the bluegill sunfish (*Lepomis macrochirus*) and carp (*Cyprinus carpio*).

The acceptable and unacceptable studies are identified in Table 6.

Table 6. Acceptable and unacceptable studies for determining the acute and sub-chronic/chronic (prolonged/long-term) toxic hazard of PFOS to fish, invertebrates and aquatic plants (algae and higher plants)

Endpoint	Test species	Acceptable study reference numbers	Unacceptable study reference numbers
Acute (short-term) toxicity to fish (freshwater)	<i>Pimephales promelas</i>	1, 16, 28	25, 26, 27
	<i>Lepomis macrochirus</i>	20	25
	<i>Oncorhynchus mykiss</i>	31, 42	25
Acute (short-term) toxicity to fish (saltwater)	<i>Oncorhynchus mykiss</i>	30	-
	<i>Cyprinodon variegatus</i>	43	-
Sub-chronic/ chronic toxicity to fish (freshwater)	<i>Pimephales promelas</i>	8, 14	25
	<i>Lepomis macrochirus</i>	41	-
Acute (short-term) toxicity to invertebrates (freshwater)	<i>Daphnia magna</i>	3, 15, 17, 29, 33	23, 25
	<i>Unio complamatus</i>	5	-
Acute (short-term) toxicity to invertebrates (saltwater)	<i>Mysidopsis bahia</i>	4	-
	<i>Crassostrea virginica</i>	7	-
	<i>Artemia</i> sp	32	-
Sub-chronic/ chronic toxicity to invertebrates (freshwater)	<i>Daphnia magna</i>	9, 15	-
Sub-chronic/ chronic toxicity to invertebrates (saltwater)	<i>Mysidopsis bahia</i>	10	-
Toxicity to freshwater unicellular algae	<i>Selenastrum capricornutum</i> ¹	2, 13	24
	<i>Anabaena flos-aquae</i>	36	-
	<i>Navicula pelliculosa</i>	38	-
Toxicity to saltwater unicellular algae	<i>Skeletonema costatum</i>	39	-
Longer-term toxicity to freshwater unicellular algae	<i>Selenastrum capricornutum</i> ¹	13	-
Toxicity to freshwater higher plants	<i>Lemna gibba</i>	37	-

4.1.1 Fish

Acute (short-term) toxicity

The results of the acute (short-term) studies are summarized in Table 7. Three species of freshwater fish have been used for testing - *Pimephales promelas* (Fathead minnow), *Lepomis macrochirus* (Bluegill sunfish) and *Oncorhynchus mykiss* (Rainbow trout). The end point assessed in all the tests was mortality during a 96-hour exposure period.

P. promelas was the most susceptible freshwater fish species in acute tests with a lowest 96-hour LC₅₀ for PFOS lithium salt of 4.7 mg/l². *L. macrochirus*, and *O. mykiss*, were only marginally less susceptible - a 96-hour LC₅₀ value of 7.8 mg/l was determined with both species for the diethanolamine (DEA) and potassium salt respectively. A second study on *Oncorhynchus mykiss* showed an LC₅₀ of 22 mg/l again using the potassium salt. A very high 96-hour LL₅₀ value of approximately 200 mg/l determined for the didecyldimethylammonium salt of PFOS was obtained for *P. promelas* in a test on water accommodated

¹ This species has recently been renamed *Pseudokirchneriella subcapitata*.

² There are only three acute toxicity reports for Fathead minnow of acceptable or better quality, so the most sensitive value is used in this assessment.

fractions of an aqueous mixture containing the substance. However, the actual exposure concentrations of PFOS were not determined and could not be estimated in this test.

PFOS also exhibits acute toxicity to fish in saltwater. A 96-hour LC₅₀ value of 13.7 mg/l has been determined for the potassium salt in a test with *O. mykiss* acclimated to saltwater at a salinity of 30 parts per thousand. The data show saltwater acclimated *O. mykiss* to be of similar susceptibility to PFOS when compared with *O. mykiss* living in freshwater. However, in the absence of measured exposure concentrations, it should be noted that this study might have been conducted in excess of the substance's salt water solubility (2.5 to 20 mg/l, depending on salinity and purity). A further study using a saltwater fish, Sheepshead minnow (*Cyprinodon variegatus*) showed no toxicity up to the water solubility limit, indicated as 15 mg/l in the test. Loss of concentration during the test, probably due to sedimentation, indicates that this value may be above the true solubility, however.

Sub-chronic/chronic (prolonged/long-term) toxicity

The results of the sub-chronic/chronic (prolonged/long-term) studies are summarized in Table 8. Studies have been carried out with two species of freshwater fish – *Pimephales promelas* (Fathead minnow) and *Lepomis macrochirus* (Bluegill sunfish). Tests with *P. promelas* were designed to determine concentrations affecting early life-stages of the fish over exposure periods of up to 42 days. Mortality data for *L. macrochirus* were obtained from a bioconcentration study in which deaths in the treated and control groups of fish were recorded over the 62-day uptake phase of the study.

The lowest definitive no observed effect concentration (NOEC) of 0.3 mg/l was determined for *P. promelas* for the potassium salt of PFOS. This value was applicable to both survival and growth end points. This NOEC is supported by results from a bioconcentration study with *L. macrochirus* that showed no significant mortality at an exposure concentration of 0.086 mg/l over a 62-day uptake phase but 100% mortality at a concentration of 0.87 mg/l after 35 days. A NOEC of 1.0 mg/l reported for effects of PFOS potassium salt on early life-stages of *P. promelas* could not be attributed to a more specific end point because insufficient information was contained in the study report.

Bioconcentration

A flow-through bioconcentration study of PFOS in bluegill sunfish was conducted. Test concentrations of 0, 0.086 and 0.87 mg/L were used. At the 0.086 mg/L level, the fish were exposed for 62 days, while at the 0.87 mg/L level, the fish were exposed for 35 days due to excessive mortality. Fish were collected from the test chambers by random selection at 12 time points during the study. They were euthanized, blotted dry, weighed and measured. They were dissected into edible and nonedible tissue fractions and the fractions were weighed. The head, fins and viscera were considered to be nonedible tissue and the remaining tissue, including skin, was considered to be edible tissue. Whole fish concentrations were calculated from the sum of the edible and nonedible parts. Steady-state BCF values were calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different. The kinetic bioconcentration factor (BCFK), uptake rate and depuration rate were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L using BIOFAC computer software.

In this study, PFOS bioconcentrated in the tissues of bluegill sunfish. Apparent steady-state was not clearly attained for the fish exposed to 0.086 mg/L. Although the tissue residue levels of PFOS were not statistically significantly different on days 49, 56 and 62, the concentration of PFOS appeared to be still increasing up to the last day of exposure. BCFK values for edible, nonedible and whole fish tissues were calculated to be 1124, 4013, and 2796, respectively. PFOS depurated slowly. The BIOFAC estimates for

the time to reach 50% clearance for edible, nonedible, and whole fish tissues were 86, 116, and 112 days, respectively.

Kurume Laboratory (2001) conducted a flow-through bioconcentration study of PFOS in carp (*Cyprinus carpio*). The fish were checked visually and those demonstrating any abnormality were removed. The fish were reared for 8 days in a flow through system following an external disinfection. After rearing, the fish were medicated to eliminate parasites and transferred to an acclimatizing aquarium. After the second external disinfection, they were acclimatized. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 15 days in a flow through system at temperatures of $25 \pm 2^\circ \text{C}$. The fish were transferred to test tanks and reared at the same temperature in the flow through system for another 27 days. Temperature of the test water was measured with alcohol a thermometer and recorded once a day. Dissolved oxygen concentrations was measured with a dissolved oxygen probe and recorded twice a week. The pH of the test water was measured with a pH meter one a week. Temperature of test water was measured and recorded once a day. Dissolved oxygen in test water was measured and recorded twice a week. During the experimental period, the excreta of carp, dirt on test tanks, were removed once a day. The temperature ranged from 25.0 to 25.4°C; the dissolved oxygen concentration ranged from 7.9 to 8.1 mg/L; and the pH ranged from 7.6 to 7.8.

Forty carp were exposed to two concentrations of PFOS, 2 and 20 ug/L respectively for 58 days in a flow through system. The test water of each level was analyzed once before first analysis of test fish and at the same time as analysis of test fish. Analysis of test fish was performed six times at each level in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. Because the stored sample from one fish was too small for the measurement of lipid content, groups of two fish were used. Analysis of control fish was performed before the experimental starting and after the experimental completion. Six fish were taken out at each sampling time and divided into three groups, and then both were analyzed individually.

Analysis of PFOS in the test water and carp was performed using high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The test water of each level was analyzed once before first analysis of test fish and at the same time as the analysis of the test fish. Steady-state was reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within + 20% of each other. When BCFs were less than 100, it was evaluated that a steady-state had been reached after 28 days. The fish which were exposed for 61 days were separated into parts; tegument, head, viscera except liver, liver and remaining matter. BCFs were determined in all the parts.

In this study, PFOS bioconcentrated in the tissues of carp. Test concentrations of 2 and 20 ug/L were used. The fish were exposed for 58 days to makeup for excessive mortality. Bioconcentration factors were calculated to be 720 for 20 ug/L and 200 - 1500 for 2 ug/L.

Table 7. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Pimephales promelas</i> (Fathead minnow)	OECD 203 & OPPTS 850.1075 (Static)	96-hour LC ₅₀ = 9.5 96-hour NOEC = 3.3	Good	Test substance was PFOS potassium salt. Measured exposure concentrations	1
		Not noted (Static)	96-hour LC ₅₀ = 4.7 (see comments)	Acceptable	Test substance was PFOS lithium salt. Nominal exposure concentrations expressed relative to concentration of test substance (24.5% PFOS Li salt and 74.5% water). Test result divided by 4 to express 96 h LC ₅₀ in terms of PFOS Li salt concentration. Very little information on study protocol; however, result agrees reasonably well with more other studies (1, 25, 26 and 27)	16
		Not noted (Static)	96-hour LC ₅₀ = 37.6 and 51	Unknown	Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of studies cannot be judged from information supplied. However, results are consistent with other studies (1, 16, 26 and 27)	25
		Not noted (Static)	96-hour LC ₅₀ = 21	Questionable	Test substance was PFOS ammonium salt. Nominal exposure concentrations Isopropanol also present in test samples. However, results are consistent with other studies (1, 16, 25 and 27)	26
		Not noted (Static)	96-hour LC ₅₀ = 25	Questionable	Test substance was PFOS ammonium salt. Nominal exposure concentrations Isopropanol also present in test samples. Results consistent with other studies (1, 16, 25 and 26)	27

Table 7 contd. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Pimephales promelas</i> (Fathead minnow)	OECD 203 (Static)	96-hour LL_{50} = 200* 96-hour NOEL = <170* (*calculated assuming 35% of substance in mixture)	Acceptable	Test substance was a mixture of didecyldimethylammonium salt of PFOS (approximately 35%) and water with up to 5% residual perfluorochemicals. Test media were water-accommodated fractions (WAFs). Nominal exposure concentrations.	28
	<i>Lepomis macrochirus</i> (Bluegill sunfish)	OECD 203 & OPPTS 850.1075 (Static)	96-hour LC_{50} = 7.8 96-hour NOEC = 4.5 (see comments)	Acceptable	Test substance was PFOS DEA salt. Nominal exposure concentrations expressed relative to concentration of test substance (~25% PFOS DEA salt and 75% water). Results have therefore been divided by 4 to calculate the effect concentrations in terms of PFOS DEA salt concentration based on reported 96-hour LC_{50} of 31 mg/l and NOEC of 18 mg/l.	20
		Not noted (Static)	96-hour LC_{50} = 68	Unknown	Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of study cannot be judged from information supplied.	25
	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Not noted (Static)	96-hour LC_{50} = 11	Unknown	Test substance was PFOS potassium salt. Nominal exposure concentrations Standard of study cannot be judged from information supplied.	25
		Standard procedures for testing acute lethality of liquid effluents (Environment Canada)	96-hour LC_{50} = 7.8	Acceptable	Test substance was PFOS potassium salt. Sample purity not characterised Nominal exposure concentrations	31

Table 7 contd. Acute (short-term exposure) toxicity data for PFOS determined in tests with fish

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Oncorhynchus mykiss</i> (Rainbow trout)	OPPTS 850.1075 OECD 203 Static	96-hour LC ₅₀ = 22	Acceptable	Test substance was PFOS potassium salt. Sample purity 86.7%; results reported as a.i Based on measured concentrations	42
Saltwater	<i>Cyprinodon variegatus</i> (Sheepshead minnow)	OPPTS 850.1075 OECD 203 Semi-static (24 hour renewal)	96-hour LC ₅₀ >15 mg/l	Acceptable	Test substance was PFOS potassium salt. Sample purity 86.7%; results reported as a.i Based on mean of measured concentrations Concentration losses during each 24 hour period would suggest some sedimentation indicating that the water solubility may have been exceeded. Final concentrations in each 24 hour period varied between 11 – 16 mg/l	43
Saltwater	<i>Oncorhynchus mykiss</i> (Rainbow trout)	Standard procedures for testing acute lethality of liquid effluents (Environment Canada)	96-hour LC ₅₀ = 13.7	Acceptable	Test substance was PFOS potassium salt. Sample purity not characterised Test fish were acclimated to 30 parts per thousand saltwater Nominal exposure concentrations In the absence of measured exposure concentrations, it should be noted that this study might have been conducted in excess of the substance's salt water solubility (2.5 to 20 mg/l, depending on salinity and purity).	30

Table 8. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with fish

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Pimephales promelas</i> (Fathead minnow)	OECD 210 & OPPTS 850.1400 (Flow-through)	42-day NOEC _{surv.} = 0.30 42-day NOEC _{growth} = 0.30 5-day NOEC _{hatch} ≥ 4.6	Good	Test substance was PFOS potassium salt. Measured exposure concentrations. Lighting regime not described in summary. Conclusion (post-hatch survival as the most sensitive endpoint): it is possible that growth was also affected at this concentration, but the test design did not enable this to be determined.	8
		Non-standard (Flow-through)	30-day NOEC _{early life-stages} = 1	Acceptable	Test substance was PFOS potassium salt. Measured exposure concentrations. Results agree with later study Acetone present at 43 µl/l	14
		Not noted (Flow-through)	NOEC _{early life-stages} = 1	Unknown	Test substance was PFOS potassium salt. Standard of study cannot be judged from information supplied. It is however assumed that this study is the same as 14	25 (Assumed to be same study as 14)
	<i>Lepomis macrochirus</i> (Bluegill sunfish)	OECD 305 & OPPTS 850.1730	62-day NOEC _{mortality} = >0.086, <0.87	Good	Bioconcentration study Test substance was PFOS potassium salt. Measured exposure concentrations.	41

4.1.2 Invertebrates

The results of acute (short-term) and sub-chronic/chronic (prolonged/long-term) studies are summarised in Tables 9 and 10.

Acute (short-term) toxicity to freshwater species

Two species of freshwater invertebrate have been used for testing - *Daphnia magna* (the 'water flea') and *Unio complamatus* (fresh water mussel). Immobilisation/mortality during 48-hours exposure was assessed in the tests with *D. magna*. Mortality during 96-hours exposure was assessed in the test with *U. complamatus*.

A lowest acceptable 48-hour EC₅₀ value of 27 mg/l was determined for PFOS potassium salt in tests with the daphnid.¹ A 48-hour EL₅₀ value of approximately 4.0 mg/l was also determined for the didecyldimethylammonium salt of PFOS in a daphnid test on water accommodated fractions of an aqueous mixture containing the substance. However, the actual exposure concentrations of PFOS were not determined in this test and, as pointed out in the introduction to this section, it is possible that didecyldimethylammonium may have contributed to the toxicity of the test medium. These values compare with a 96-hour LC₅₀ value determined for mortality of the mussel of 59 mg/l.

Acute (short-term) toxicity to saltwater species

Three species of saltwater invertebrate have been used for testing - *Mysidopsis bahia* (Mysid shrimp), *Crassostrea virginica* (Eastern oyster) and *Artemia* sp (Brine shrimp). Mortality during 96-hours exposure was assessed in the tests with *M. bahia* and *Artemia* sp. Reduction in shell deposition was assessed over a 96-hour exposure period in the test with *C. virginica*. The test substance was PFOS potassium salt in all cases.

A 96-hour LC₅₀ value of 3.6 mg/l and an associated NOEC of 1.1 mg/l were determined in the test with the Mysid shrimp. This compares with a 96-hour EC₅₀ value of >3.0 mg/l for effects on shell deposition in the oyster and a 48-hour LC₅₀ of 8.9 mg/l for mortality of the Brine shrimp.

Sub-chronic/chronic (prolonged/long-term) toxicity to freshwater species

Two tests have been carried out on PFOS potassium salt with one species of freshwater invertebrate – the 'water flea', *Daphnia magna*. The end points assessed in the tests were survival, growth and reproduction measured over exposure periods of up to 28 days.

NOECs of 12 and 7 mg/l have been determined for *D. magna* reproduction in 21 and 28-day tests respectively. In the 21-day test the NOECs for survival and growth were also 12 mg/l, indicating that reproduction was no more sensitive than these two other end points.

¹ For *Daphnia magna* there are five 48-hour acute toxicity studies of acceptable or better quality, although one of these relates to water accommodated fractions. The remaining 48-hour EC₅₀s are 27, 58, 61 and 210 mg/l. The latter value is based on nominal exposure concentrations, but since the stock solution was prepared at a concentration that exceeded water solubility, exposure concentrations are likely to be lower than nominal. This leaves just three reports with comparable measures of toxicity, and so the most sensitive value is used in this assessment.

Sub-chronic/chronic (prolonged/long-term) toxicity to saltwater species

A test has been carried out on PFOS potassium salt with one species of saltwater invertebrate; the Mysid shrimp, *Mysidopsis bahia*. The end points assessed in the test were survival, growth and reproduction measured over an exposure period of 35 days.

The 35-day NOECs determined for survival, growth and reproduction in this test were 0.55, 0.25 and 0.25 mg/l respectively.

Table 9. Acute (short-term exposure) toxicity data for PFOS determined in tests with invertebrates

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Daphnia magna</i> (Water flea)	OECD 202 & OPPTS 850.1010 (Static)	48-hour EC ₅₀ = 61 48-hour NOEC = 33	Good	Test substance was PFOS potassium salt. Measured exposure concentrations	3
		ASTM 1981 & OECD 1981 (Static)	48-hour EC ₅₀ = 27	Acceptable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Result agrees reasonably well with those obtained in studies 3 and 23.	15
		Not noted (Static)	48-hour EC ₅₀ = 210 48-hour NOEC = 100	Acceptable	Test substance was PFOS lithium salt. Nominal exposure concentrations. EC ₅₀ is somewhat higher than previous value but stock solution was prepared at a concentration that exceeded water solubility, therefore exposure concentrations are likely to be lower than nominal.	17
		OECD 202 (Static)	24-hour EC ₅₀ = >42 48-hour EC ₅₀ = 14	Questionable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Data questionable in view of the presence of diethylene glycol butyl ether in the test material (as part of the formulation). Result is in reasonable agreement with later study (3).	23
		Not noted (Static)	48-hour EC ₅₀ = 49.2	Unknown	Test substance was PFOS potassium salt. Nominal exposure concentrations. Standard of study cannot be judged from information supplied.	25

Table 9 contd. Acute (short-term exposure) and sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with invertebrates

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
	<i>Daphnia magna</i> (Water flea)	OECD 202 (Static)	48-hour EL_{50} = 4.0 48-hour NOEL = 2.2	Acceptable	Test substance was a mixture of didecyldimethylammonium salt of PFOS (approximately 35%) and water with up to 5% residual perfluorochemicals. Test media were water-accommodated fractions (WAFs). Nominal exposure concentrations. 48-hour EL_{50} and NOEL calculated assuming 35% of substance in mixture.	29
		ISO, 1982	48-hour EC_{50} = 58	Acceptable	Test substance was PFOS potassium salt. Sample purity not characterised Nominal exposure concentrations.	33
	<i>Unio complamatus</i> (Freshwater mussel)	OECD 203, OPPTS 850.1075 & ASTM-E-729- 88a (Semi- static)	96-hour LC_{50} = 59 96-hour NOEC = 20	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	5
Saltwater	<i>Mysidopsis bahia</i> (Mysid shrimp)	OPPTS 850.1035 (Static)	96-hour LC_{50} = 3.6 96-hour NOEC = 1.1	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	7
	<i>Crassostrea virginica</i> (Eastern oyster)	OPPTS 850.1025 (Static)	96-hour EC_{50} = >3.0 96-hour NOEC = 1.9	Good	Test substance was PFOS potassium salt. Measured exposure concentrations. Solubility in seawater should have been used to set highest test concentration.	4
	<i>Artemia</i> sp. (Brine shrimp)	Draft ISO, 1981	48-hour LC_{50} = 8.9	Acceptable	Test substance was PFOS potassium salt. Sample purity not characterised Nominal exposure concentrations.	32

Table 10. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with invertebrates

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Daphnia magna</i> (Water flea)	OECD 211, OPPTS 850.1300 & ASTM 1193- 87E (Semi- static)	21-day NOEC _{repro} = 12 21-day NOEC _{surv.} = 12 21-day NOEC _{growth} = 12	Good	Test substance was PFOS potassium salt. Measured exposure concentrations. No effects on reproduction at highest concentration that had no effect on mortality.	9
		ASTM 1981 & OECD 1981 (Semi-static)	21-day EC _{50repro.} = 12 28-day NOEC _{repro} = 7 28-day EC _{50repro.} = 11	Acceptable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Result agrees well with that obtained in study 9.	15
Saltwater	<i>Mysidopsis bahia</i> (Mysid shrimp)	OPPTS 850.1350 (Flow- through)	35-day NOEC _{repro.} = 0.25 35-day NOEC _{surv.} = 0.55 35-day NOEC _{growth} = 0.25	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	10

4.1.3 Aquatic plants

The results of all the short- and longer-term studies are summarised in Tables 11 and 12 (algae) and 13 (higher plants). Note: The species *Selenastrum capricornutum* has been renamed *Pseudokirchneriella subcapitata*. For transparency, the name used in the test report has been retained in the discussion below.

Freshwater algae – Short-term

Three species of freshwater unicellular algae have been used for testing - *Selenastrum capricornutum*, *Anabaena flos-aquae* and *Navicula pelliculosa*. The end point assessed in the tests was growth measured in terms of cell density, growth rate and/or the area under the growth curve over 96-hours. Only studies that assessed toxicity by reference to effects on growth rate are considered here for the purposes of determining the toxicity of PFOS to algae. The results of study number 13 are therefore excluded because they are only expressed relative to cell numbers and cell dry weight. These results could be considered were the EC₅₀ values to be recalculated in respect of growth rate, but since algae were relatively insensitive compared with fish, this is not considered a high priority.

The lowest 96-hour EC₅₀ value for effects on growth rate of 71 mg/l was determined for *S. capricornutum*. However there are some uncertainties over the validity of this result given that exposure concentrations were not measured and diethylene glycol butyl ether was present in the test material (as part of the formulation). A 96-hour EC₅₀ value of 126 mg/l was obtained for the same species using a test protocol that was judged to meet all the criteria for acceptability of the data. The 96-hour NOECs associated with these two tests were 35 and 44 mg/l respectively. The tests with the two other species yielded 96-hour EC₅₀ values of 176 mg/l (*A. flos-aquae*) and 305 mg/l (*N. pelliculosa*) and respective NOECs of 94 and 206 mg/l.

Saltwater algae – Short-term

A 96-hour growth inhibition test has been carried out on PFOS potassium salt with *Skeletonema costatum*. The test was unable to determine a definitive 96-hour EC₅₀ value because no effects were determined at the highest dissolved PFOS concentration that could be attained under the test conditions (3.2 mg/l).

Freshwater algae – Longer-term

One species of freshwater unicellular algae has been tested - *Selenastrum capricornutum* (study reference no. 13). The test assessed effects on growth rate expressed relative to cell density over 14 days. The results from the test are not discussed further here for two reasons. Firstly, the results are expressed relative to cell density (see comments in respect of short-term tests). Secondly, there are concerns that the 14-day test period and absence of test medium renewal may have resulted in a decline in exposure concentrations over time (the test media were not analysed for PFOS concentration). The latter is suggested by comparison of the 14-day EC₅₀ value of 95 mg/l with the 96-h EC₅₀ value of 82 mg/l determined in the same test.

Freshwater higher plants

A growth inhibition test has been carried out on PFOS potassium salt with *Lemna gibba* (Duckweed). The test yielded a 7-day IC₅₀ of 108 mg/l for inhibition of frond production and a 7-day NOEC of 15.1 mg/l based on the inhibition of frond production and evidence of sub-lethal effects.

Table 11. Acute (short-term exposure) toxicity data for PFOS determined in tests with algae

Medium	Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Selenastrum capricornutum</i>	OECD 201, OPPTS 850.5400 & ASTM 1218-90E (Static)	96-hour $EC_{50}(\text{cell density}) = 71$ 96-hour $E_bC_{50}(\text{area under the curve}) = 71$ 96-hour $E_rC_{50}(\text{growth rate}) = 126$ 96-hour $NOEC(\text{growth rate, cell density, area under the growth curve}) = 44$ 72-hour $EC_{50}(\text{cell density}) = 70$ 72-hour $E_bC_{50}(\text{area under the curve}) = 74$ 72-hour $E_rC_{50}(\text{growth rate}) = 120$ 72-hour $NOEC(\text{growth rate, cell density, area under the growth curve}) = 70$	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	2
		OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23 (Static)	96-hour $EC_{50}(\text{cell density}) = 82$ 96-hour $EC_{10}(\text{cell density}) = 10$	Acceptable	Test substance was PFOS potassium salt. Result not expressed relative to growth rate. Nominal exposure concentrations. Result agrees with those obtained in studies 2 and 24.	13
		OECD 201 (Static)	96-hour $EC_{50}(\text{growth rate}) = 71$ 96-hour $NOEC(\text{growth rate}) = 35$	Questionable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Data questionable in view of the presence of diethylene glycol butyl ether in the test material (as part of the formulation).	24

	<i>Anabaena flos-aquae</i>	OPPTS 850.5400	96-hour $EC_{50(\text{growth rate})} = 176$ 96-hour $NOEC_{(\text{growth rate})} = 94$	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	36
	<i>Navicula pelliculosa</i>	OPPTS 850.5400	96-hour $EC_{50(\text{growth rate})} = 305$ 96-hour $NOEC_{(\text{growth rate})} = 206$	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	38
Saltwater	<i>Skeletonema costatum</i>	OPPTS 850.5400	96-hour $EC_{50(\text{growth rate})} = >3.2$ 96-hour $NOEC_{(\text{growth rate})} = >3.2$	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	39

Table 12. Sub-chronic/chronic (prolonged/long-term) toxicity data for PFOS determined in tests with freshwater algae

Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
<i>Selenastrum capricornutum</i>	OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23 (Static)	14-day EC ₅₀ cell density = 95 14-day NOEC _{cell density} = <26 14-day EC ₁₀ cell density = 16	Acceptable	Test substance was PFOS potassium salt. Result not expressed relative to growth rate. No analysis of exposure but result agrees with those obtained in studies 2 and 24.	13

Table 13. Acute (short-term exposure) toxicity data for PFOS determined in tests with higher plants

Medium	Species	Protocol	Result (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Lemna gibba</i> G3 (Duckweed)	OPPTS 850.4400	7-day IC ₅₀ = 108	Good	Test substance was PFOS potassium salt. Measured exposure concentrations.	37

4.2 Effects on Other Aquatic Organisms

4.2.1 Amphibians

Data summarised in Table 14 were obtained in an embryo teratogenesis assay carried out on PFOS potassium salt with *Xenopus laevis* (African clawed frog). Exposure of the embryos for 96 hours resulted in an LC₅₀ for mortality of 13.8 mg/l and an EC₅₀ for malformations of 12.1 mg/l. The minimum concentration that inhibited growth was 7.97 mg/l. and the teratogenic index was calculated, as the ratio of the 96-hour LC₅₀ to the 96-hour EC₅₀, to be 1.1. The latter value indicates that PFOS has a low potential to be a developmental hazard in this species.

Table 14. Toxicity data for PFOS determined in an embryo teratogenesis assay with the amphibian, *Xenopus laevis*

Medium	Species	Protocol	Result (mg/l)	Study Standard	Comments	Study Ref. No.
Freshwater	<i>Xenopus laevis</i> (African clawed frog)	ASTM E1439-91	96-hour $LC_{50} = 13.8$ 96-hour $EC_{50}(\text{malformations}) = 12.1$ Minimum concentration to inhibit growth = 7.97 Teratogenic index 1.1	Acceptable	Test substance was PFOS potassium salt. Measured exposure concentrations. In-life phases were not subject to GLP.	40

4.2.2 Sediment Dwelling Invertebrates

No data are available for effects on sediment dwelling invertebrates. It is possible that a predicted no effect concentration (PNEC) for sediment-dwelling invertebrates could be determined by applying equilibrium partitioning models to the data for water column organisms. However, as discussed in Annex 3, this is unlikely to be feasible at the present time.

4.2.3 Bacteria

Data summarised in Table 15 were obtained from 2 reports describing Microtox® studies (study report reference numbers 18 and 22). The Microtox® procedure assesses light output inhibition from the luminescent marine bacterium *Photobacterium phosphoreum*, following exposure to a toxicant over a short exposure period (30 minutes in the case of these two studies). Both studies are of an unacceptable standard for assessing the hazard of PFOS (lithium salt) to bacteria because of uncertainty over the true exposure concentrations and in one case because of the presence of diethylene glycol in the test sample. There are also more general concerns over the relevance of the test system and test species for determining effects for this particular group of organisms. No toxic effects were observed in either test at nominal concentrations (>250 mg/l) that were significantly in excess of the solubility of PFOS salts in saline medium (2.5 to 20 mg/l, depending on purity/salinity).

Table 15. Toxicity data for PFOS determined in Microtox tests

Species	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
<i>Photobacterium phosphoreum</i> (Microtox®)	Microbics Microtox® "BASIC"	15-minute EC ₅₀ = >250 30-minute EC ₅₀ = >250	Unacceptable	Test substance was PFOS lithium salt. Nominal exposure concentrations. Initial stock solution prepared at concentration that was likely to exceed solubility in saline water (2.5 to 20 mg/l, depending on purity/salinity) It is therefore possible that actual exposure concentrations were much lower than nominal. Result indicates probable absence of toxicity at limit of solubility.	18
		30-minute EC ₅₀ = >280	Unacceptable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Data questionable in view of use of diethylene glycol to aid dosing. Test concentrations likely to be in excess of solubility in saline medium (2.5 to 20 mg/l, depending on purity/salinity). Result is consistent with that obtained in study 18 indicating probable absence of toxicity at limit of solubility.	22

4.2.4 Activated Sludge Microorganisms

Data summarised in Table 16 were obtained from 3 reports describing activated sludge respiration inhibition studies (study report reference numbers 6, 19 and 21). Only study reference number 9 was considered to be of an acceptable standard for assessing the hazard of PFOS to activated sludge. Studies 19 and 21 had significant inadequacies in test procedures related to test conditions, duration of the exposure and/or absence of a reference substance.

The 3-hour IC_{50} value for PFOS (potassium salt) determined in the acceptable study was >905 mg/l (nominal concentration).

Table 16. Toxicity data for PFOS determined in Activated Sludge Respiration Inhibition tests

Test system	Protocol	Results (mg/l)	Study Standard	Comments	Study Ref. No.
Activated Sludge	OECD 209	3-hour IC ₅₀ = >905 (Nom.)	Good	Test substance was PFOS potassium salt. Nominal exposure concentrations of 0.9 – 905 mg/l tested. Highest test concentration gave the maximum inhibition of 38.8%.	6
	OECD 209	3-hour IC ₅₀ = >245 (as a nom. 24.5% solution in water)	Unacceptable	Test substance was PFOS potassium salt. Nominal exposure concentrations. Test temperature not controlled and fell well below requirements of standard guidelines. Respiration rates extrapolated to 20°C. Oxygenation was inadequate during the first 30 minutes of the 3-hour test. No inhibitory effect observed.	19
	Not noted	7-minute IC ₅₀ = >250 (as a nom. 25% solution in water)	Unacceptable	Test substance was PFOS DEA salt. Nominal exposure concentrations. Exposure period inadequate by current standards. No reference substance used. No inhibitory effect observed.	21

4.3 Effects on Terrestrial Organisms

4.3.1 Soil-dwelling Invertebrates

No data are available for effects on soil dwelling invertebrates. It is however possible that a predicted no effect concentration (PNEC) for soil-dwelling invertebrates could be determined by applying equilibrium partitioning models to the data for water column organisms. However, as discussed in Annex 3, this is unlikely to be feasible at the present time. It is noted that an earthworm acute toxicity and uptake study is in the planning stages, and a final report should be available in 2002.

4.3.2 Terrestrial Plants

No data are available for effects on terrestrial plants. It is however possible that a predicted no effect concentration (PNEC) for terrestrial plants could be determined by applying equilibrium partitioning models to the data for aquatic plants. However, as discussed in Annex 3, this is unlikely to be feasible at the present time. It is noted that a terrestrial plant toxicity and uptake study should be available in 2002.

4.3.3 Birds

Data summarised in Table 17 were from 2 reports describing dietary acute studies with the Mallard duck, *Anas platyrhynchos*, and the Northern Bobwhite quail, *Colinus virginianus* (study report reference numbers 11 and 12). Birds in both tests were exposed to PFOS potassium salt in their diets for 5 days and observations were made after 3 or 17 days. Both studies are considered to be of an acceptable standard for assessing the acute toxic hazard of PFOS to birds via dietary exposure.

The lowest acute dietary LC₅₀ value of 220 mg/kg of food was determined in the test with the quail. This value was approximately half that obtained in the test with the duck. The lowest NOEC of 37 mg/kg of food for effects on body weight was, in contrast, obtained in the test with the duck. Once again this value was approximately half that obtained with the other species – the quail.

It is noted that avian reproductive data will be submitted for review in 2002.

Table 17. Acute dietary toxicity data for PFOS determined in tests with birds

Test species	Protocol	Results (mg/kg of food)	Study Standard	Comments	Study Ref. No.
<i>Anas platyrhynchos</i> (Mallard duck)	OECD 205, OPPTS 850.2200 & FIFRA E 71-2	LC ₅₀ = 628 NOEC _{mortality} = 146 NOEC _{body weight} = 37	Good	Test substance was PFOS potassium salt. Measured exposure concentration in food. 5 days exposure followed by 3 or 17 days observation	11
<i>Colinus virginianus</i> (Northern Bobwhite quail)	OECD 205, OPPTS 850.2200 & FIFRA E 71-2	LC ₅₀ = 220 NOEC _{mortality} = 73 NOEC _{body weight} = 73	Good	Test substance was PFOS potassium salt. Measured exposure concentration in food. 5 days exposure followed by 3 or 17 days observation	12

4.3.4 Bees

Data summarised in Table 18 were obtained in acute oral and contact toxicity tests carried out with the Honey bee (*Apis mellifera*) on PFOS potassium salt. The studies fully meet the required standard for acceptability of the data.

The acute oral test yielded a 72-hour LD₅₀ for ingestion of PFOS of 0.40 µg/bee and a 72-hour NOEL of 0.21 µg/bee. The contact test yielded a 96-hour LD₅₀ of 4.78 µg/bee and a 96-hour NOEL of 1.93 µg/bee. The respective LD₅₀ values indicate moderate and high orders of toxicity of PFOS to bees when administered via these routes.

Table 18. Acute oral and contact toxicity data for PFOS determined in tests with Honey bees

Test species	Protocol	Results (µg/bee)	Study Standard	Comments	Study Ref. No.
<i>Apis mellifera</i> (Honey bee)	OECD 213, EPPO 170 (Oral)	72-hour LD ₅₀ = 0.40 72-hour NOEL = 0.21	Good	Test substance was PFOS potassium salt. Nominal exposure following feeding on 50% w/v sucrose.	34
<i>Apis mellifera</i> (Honey bee)	OECD 214, EPPO 170, OPPTS 850.3020 (draft) (Contact)	96-hour LD ₅₀ = 4.78 96-hour NOEL = 1.93	Good	Test substance was PFOS potassium salt. Nominal exposure following direct application of test substance dissolved in acetone to thorax.	35

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Annex 1. Ecological Studies

The following lists of study reports were contained on a 3M Fluorochemical EPA Submissions CDs dated 12th July 2000, 28th June 2001, and February 2002

12th July 2000

1. 96-Hour Static Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
2. 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)
3. 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)
4. 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
5. 96-Hour Static Acute Toxicity Test with the Freshwater Mussel (*Unio complamatus*)
6. Activated Sludge, Respiration Inhibition Test
7. 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
8. Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
9. Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)
10. Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
11. Dietary LC₅₀ Study with the Mallard
12. Dietary LC₅₀ Study with the Northern Bobwhite
13. Multi-Phase Exposure / Recovery Algal Assay Test
14. The Effects of Continuous Aqueous Exposure to 14C-78.02 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (*Pimephales promelas* / Summary of histopathological examinations of Fathead Minnow (*Pimephales promelas*) exposed to 78.02 for 30 Days
15. Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)
16. *Pimephales promelas* 96-hour Toxicity Test Data Summary. Sample FC-94-X (Li salt of PFOS)
17. 48-HR Acute Toxicity to Daphnia, *Daphnia magna*. FC-94-X (Li salt of PFOS)
18. Microbics Microtox Toxicity Test. Sample : FC-94-X (Li salt of PFOS)
19. Evaluation of FC-94-X by OECD Activated Sludge Respiration Inhibition Test #209 Review of OECD 209 and BOD/COD Test Results for FC-94- X, test data sheets (Li salt of PFOS)
20. 96-Hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)
21. Acute Toxicity to Activated Sludge (FC-99, DEA salt of PFOS)
22. Microtox data for FM-3820 (28% PFOS)
23. Acute Toxicity to *Daphnia magna* for FM-3820 (28% PFOS)
24. Toxicity to Algae (*Selenastrum capricornutum*) for FC-3820 (28% PFOS)
25. Final Comprehensive Report : FC-95
26. Data from Fathead Minnow Study on FC-93 (25% NH₄ salt of PFOS in IPA and water), 3M Environmental Lab, Aug. 2, 1974.
27. Data from Fathead Minnow Study on FC-93 (25% NH₄ salt of PFOS in IPA and water), 3M Environmental Lab, Oct. 19, 1974.
28. Acute toxicity of P3025 developmental material to Fathead minnow (*Pimephales promelas*).
29. Acute toxicity of P3025 developmental material to *Daphnia magna*.

28th June 2001

30. Acute toxicity of PFOS to Rainbow trout in saltwater
31. Acute toxicity of PFOS to Rainbow trout in freshwater
32. Acute toxicity of PFOS to *Artemia* sp.
33. Acute toxicity of PFOS to *Daphnia magna*
34. Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee
35. Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee
36. PFOS: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*)
37. PFOS: A 7-day toxicity test with Duckweed (*Lemna gibba* G3)
38. PFOS: A 96-hour toxicity test with freshwater diatom (*Navicula pelliculosa*)

- 39. PFOS: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)
- 40. PFOS: A frog embryo teratogenesis assay – *Xenopus* (FETAX)
- 41. Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill (*Lepomis macrochirus*)

February 2002

- 42. Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) in freshwater
- 43. Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*) in saltwater

Annex 2. Robust Summaries of Key Ecotoxicology Studies

Study reference number	Title
1	96-Hour Static Acute Toxicity Test with the Fathead Minnow (<i>Pimephales promelas</i>)
2	96-Hour Toxicity Test with the Freshwater Alga (<i>Selenastrum capricornutum</i>)
3	48-Hour Static Acute Toxicity Test with the Cladoceran (<i>Daphnia magna</i>)
4	96-Hour Shell Deposition Test with the Eastern Oyster (<i>Crassostrea virginica</i>)
5	96-Hour Static Acute Toxicity Test with the Freshwater Mussel (<i>Unio complamatus</i>)
6	Activated Sludge, Respiration Inhibition Test
7	96-Hour Static Acute Toxicity Test with the Saltwater Mysid (<i>Mysidopsis bahia</i>)
8	Early Life-Stage Toxicity Test with the Fathead Minnow (<i>Pimephales promelas</i>)
9	Semi-Static Life-Cycle Toxicity Test with the Cladoceran (<i>Daphnia magna</i>)
10	Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid (<i>Mysidopsis bahia</i>)
11	Dietary LC ₅₀ Study with the Mallard
12	Dietary LC ₅₀ Study with the Northern Bobwhite
13	Multi-Phase Exposure / Recovery Algal Assay Test
14	The Effects of Continuous Aqueous Exposure to 14C-78.02 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (<i>Pimephales promelas</i>) / Summary of histopathological examinations of Fathead Minnow (<i>Pimephales promelas</i>) exposed to 78.02 for 30 Days
15	Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)
16	<i>Pimephales promelas</i> 96-hour Toxicity Test Data Summary. Sample FC-94-X (Li salt of PFOS)
17	48-HR Acute Toxicity to Daphnia, <i>Daphnia magna</i> . FC-94-X (Li salt of PFOS)
20	96-Hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)
23	Acute Toxicity to <i>Daphnia magna</i> for FM-3820 (28% PFOS)
26	Data from Fathead Minnow Study on FC-93 (25% NH ₄ salt of PFOS in IPA and water), 3M Environmental Lab, Aug. 2, 1974.
27	Data from Fathead Minnow Study on FC-93 (25% NH ₄ salt of PFOS in IPA and water), 3M Environmental Lab, Oct. 19, 1974.
28	Acute toxicity of P3025 developmental material to Fathead minnow (<i>Pimephales promelas</i>).
29	Acute toxicity of P3025 developmental material to <i>Daphnia magna</i> .
30	Acute toxicity of PFOS to Rainbow trout in saltwater
31	Acute toxicity of PFOS to Rainbow trout in freshwater
32	Acute toxicity of PFOS to <i>Artemia</i> sp.
33	Acute toxicity of PFOS to <i>Daphnia magna</i>
34	Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee
35	Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee
36	PFOS: A 96-hour toxicity test with the freshwater alga (<i>Anabaena flos-aquae</i>)
37	PFOS: A 7-day toxicity test with Duckweed (<i>Lemna gibba</i> G3)
38	PFOS: A 96-hour toxicity test with freshwater diatom (<i>Navicula pelliculosa</i>)
39	PFOS: A 96-hour toxicity test with the marine diatom (<i>Skeletonema costatum</i>)
40	PFOS: A frog embryo teratogenesis assay – <i>Xenopus</i> (FETAX)
41	Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill (<i>Lepomis macrochirus</i>)
42	Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout (<i>Oncorhynchus mykiss</i>) in freshwater
43	Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow (<i>Cyprinodon variegatus</i>) in saltwater
44	Bioconcentration test of Salt (Na, K, Li) of perfluoroalkyl (C=4-12) sulfonic acid [This test was performed using Perfluorooctane sulfonic acid, potassium salt (Test substance number K-1520)] in carp

Robust Study Report Reference No. 1 – 96-Hour Static Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 203 and OPPTS 850.1075

Type: Static acute

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Pimephales promelas*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured at 0, 48, 96-hours

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: Approximately 126 days old

Length and weight: 35 (30-38) mm, 0.36 (0.21-0.49) g

Loading: 0.24 g fish/L

Pretreatment: None

Test Conditions

Dilution water: 0.45 µm filtered well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

Hardness: 131 (128-136) mg/L as CaCO₃

Alkalinity: 177 (176-178) mg/L as CaCO₃

pH: 8.3

TOC: < 1.0 mg/L

Conductivity: 311 (310-315) µmhos/cm

Stock and test solution preparation: Primary stock prepared in dilution water at 27 mg/L and mixed for ~22 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the four additional test concentrations.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.6 cm.

Number of replicates: two

Number of fish per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

7.8 – 8.8 mg/L (control exposure)

7.7 – 9.0 mg/L (28 mg/L exposure)

pH range (0 – 96 hours)

8.3 – 8.6 (control exposure)

8.4 – 8.5 (28 mg/L exposure)

Test temperature range (0 – 96 hours)

20.4 – 22.1°C (control exposure)

21.3 – 22.3 °C (28 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 3.6, 5.9, 9.9, 16, 27 mg/L

Measured concentrations: <LOQ, 3.3, 5.6, 9.5, 17, 28 mg/L

Element value: (95% confidence interval is given in brackets)

24-hour LC₅₀ = > 28 mg/L (C.I. not calculable)

48-hour LC₅₀ = > 28 mg/L (C.I. not calculable)

72-hour LC₅₀ = 27 (22 – 41) mg/L

96-hour LC₅₀ = 9.5 (8.0 – 11) mg/L

All element values based on mean measured concentrations

Statistical evaluation of mortality: Confidence limits for 24 and 48-hours could not be calculated due to lack of mortality. The 72-hour LC₅₀ value is questionable because a concentration-effect relationship was not demonstrated over a reasonable range of percent dead. The 24 and 48-hour LC₅₀ values were determined by visual interpretation. Probit was used to calculate the 72-hour LC₅₀ and Moving Average for the 96-hour LC₅₀.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.9. Samples collected at test initiation had measured values from 85.3 to 117% of nominal. Measured values for samples taken at 48 hours ranged from 86.3 to 101% of nominal. Measured values for samples taken at 96 hours ranged from 87.6 to 98.3% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L	Mean Measured Concentration mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
3.6	3.16, 3.53, 3.08, 3.22, 3.46, 3.13	3.3	92
5.9	6.05, 5.07, 5.48, 5.89, 5.70, 5.55	5.6	95
9.9	8.99, 9.47, 9.88, 9.33, 9.70, 9.52	9.5	96
16	18.2, 19.3, 15.0, 15.6, 14.8, 16.2	17	106
27	28.5, 28.5, 27.0, 27.8, 26.8, 26.6	28	104

Biological observations after 96-hours: Fish in the negative control and the 3.3 mg/L exposure concentration appeared normal. Some or all of the surviving fish were observed to be swimming erratically (4/16 in 5.6 mg/L exposure, 10/10 in 9.5 mg/L, 4/4 in 17 mg/L) at test termination.

Cumulative percent mortality:

Mean Measured Test Concentration mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
3.3	0	0	0	0
5.6	0	0	0	20
9.5	0	0	0	50
17	0	0	15	80
28	0	0	50	100

Lowest concentration causing 100% mortality: 28 mg/L**Mortality of controls:** None**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour LC₅₀ for fathead minnow was determined to be 9.5 mg/L with a 95% confidence interval of 8.0 –11 mg/L. The 96-hour no mortality and no effects concentration was 3.3 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 1**REFERENCES**

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 2 – 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 201, OPPTS 850.5400, ASTM 1218-90E

Test: Static acute

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Selenastrum capricornutum*

Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured at 0, 72, 96-hours

Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate

Exposure period: 96-hours

Start date: 4/12/99

End date: 4/16/99

Analytical monitoring: Test concentrations measured at 0, 72 and 96-hours.

Test organisms laboratory culture: Algae cultures had been actively growing in freshwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Test temperature range: 23.6- 25.8°C

Growth medium: ASTM Standard Guide1218-90E, 1990

Compound	Nominal concentration	Units
MgCl ₂ 6H ₂ O	12.16	mg/l
CaCl ₂ 2H ₂ O	4.40	mg/l
H ₃ BO ₃	0.1856	mg/l
MnCl ₂ 4H ₂ O	0.416	mg/l
ZnCl ₂	3.28	µg/l
FeCl ₂ 6H ₂ O	0.1598	mg/l
CoCl ₂ 6H ₂ O)	1.428	µg/l
Na ₂ MoO ₄ 2H ₂ O	7.26	µg/l
CuCl ₂ 2H ₂ O	0.012	µg/l
Na ₂ EDTA2H ₂ O	0.300	mg/l
NaNO ₃	25.50	mg/l
MgSO ₄ 7H ₂ O	14.70	mg/l
K ₂ HPO ₄	1.044	mg/l

NaHCO ₃	15.0	mg/l
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Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl and the medium was sterilized by filtration (0.22µm) prior to use.

Stock and test solution preparation: A primary stock solution was prepared in algal medium at a concentration of 183 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with algal medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

Exposure vessels: Sterile 250 mL polycarbonate Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at 100 rpm

Number of replicates: three

Initial algal cell loading: 1.0×10^4 cells/mL

Number of concentrations: six plus a negative control plus an abiotic control at the highest concentration tested

Water chemistry:

pH range (0- 96 hours)

7.5 - 8.1 (control exposure)

7.4 - 7.5 (179 mg/L exposure)

Test temperature range (0- 96 hours)

23.6 - 25.8°C

Light levels (0- 96 hours)

3870 - 4610 lux from continuous cool-white fluorescent lighting

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 5.7, 11, 23, 46, 91, 183 mg/L plus 183 mg/L abiotic control.

Measured concentrations: <LOQ, 5.5, 11, 21, 44, 86, 179, 169 mg/L

Element value: (95% confidence interval is given in brackets)

24-hour EC₅₀ (cell density) = 163 (74 -191) mg/L

24-hour EbC₅₀ (area under curve) = 122 (19 -176) mg/L

24-hour EC₅₀ (growth rate) = 136 (30 -204) mg/L

48-hour EC₅₀ (cell density) = 81 (72 - 90) mg/L

48-hour EbC₅₀ (area under curve) = 84 (67 - 146) mg/L

48-hour ErC₅₀ (growth rate) = 142 (107 - 185) mg/L

72-hour EC₁₀ (cell density) = 37 (<O - 64) mg/L

72-hour EbC₁₀ (area under curve) = 46 (<O - 56) mg/L

72-hour ErC₁₀ (growth rate) = 53 (23 - 64) mg/L

72-hour EC₅₀ (cell density) = 70 (44 - 78) mg/L

72-hour EbC₅₀ (area under curve) = 74 (55 - 82) mg/L

72-hour ErC₅₀ (growth rate) = 120 (103 - 132) mg/L

72-hour EC₉₀ (cell density) = 153 (130 - 165) mg/L

72-hour EbC₉₀ (area under curve) = 165 (145 - 176) mg/L

72-hour ErC₉₀ (growth rate) = >179 mg/L (C.I. not calculable)

96-hour EC₁₀ (cell density) = 49 (43 - 50) mg/L

96-hour EbC₁₀ (area under curve) = 49 (40 - 50) mg/L

96-hour ErC₁₀ (growth rate) = 59 (54 - 63) mg/L

96-hour EC₅₀ (cell density) = 71 (66 - 73) mg/L
 96-hour EbC₅₀ (area under curve) = 71 (67- 74) mg/L
 96-hour ErC₅₀ (growth rate) = 126 (115 - 138) mg/L
 96-hour EC₉₀ (cell density) = 137 (105 - 153) mg/L
 96-hour EbC₉₀ (area under curve) = 145 (125 - 155) mg/L
 96-hour ErC₉₀ (growth rate) = >179 mg/L (C.I. not calculable)
 72-hour NOEC (growth rate, cell density, area under the curve) = 44 mg/L
 96-hour NOEC (growth rate, cell density, area under the curve) = 44 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using "The SAS System for Windows", Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits at 72 and 96 hours. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilks's test and Bartlett's test, respectively. The treatment groups were then compared to the control using Dunnett's test. Results of the statistical analyses were used to determine the NOEC values.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.1. Samples collected at test initiation had measured values from 82.0 to 98.1% of nominal. Measured values for samples taken at 72 hours ranged from 91.7 to 105% of nominal. Measured values for samples taken at 96 hours ranged from 90.3 to 102% of nominal. For the abiotic controls, measured values for samples taken at 72-hours ranged from 81.9 to 105% of nominal and for samples taken at 96-hours, 90.3 to 103% of nominal.

Summary of analytical chemistry data:

Nominal test concentration, mg/l	Measured values at 0, 72, and 96-hours respectively, mg/l	Mean measured concentration, mg/l	Percent nominal
Negative control	All <LOQ	<LOQ	-
5.7	4.73, 6.04, 5.84	5.5	96
11	10.7, 11.2, 12.1	11	100
23	19.8, 23.1, 20.7	21	91
46	42.7, 41.9, 46.3	44	96
91	83.3, 86.0, 88.3	86	95
183	179, 186, 172	179	98
183 (abiotic)	Not analyzed, 150, 188	169	92

Control response: satisfactory

Biological observations after 96-hours:

Mean measured concentration, mg/l	Mean number of cells per ml	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative control	2,740,00	-	-	-
5.5	3,040,000	-11	-8.5	-1.9
11	2,880,000	-5.1	-3.3	-0.84
21	3,240,000	-18	-13	-3.0
44	3,080,000	-12	-5.3	-2.0
86	626,667	77	75	27
179	33,667	99	98	79

Observations: After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the negative control or any test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative control, although a few cells appeared enlarged in the 86 and 179 mg/L treatment groups.

Reversibility of Growth Inhibition: The 179 mg/L treatment group was maximally inhibited after 96-hours. Aliquots of the test solution were diluted with algal medium and cultured for five days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC₅₀ and 95% confidence interval for *Selenastrum capricornutum* was determined using three calculation methods. By cell density, it was 71 (66 - 73) mg/L, by area under the growth curve it was 71 (67 - 74) mg/L and by growth rate 126 (115 - 138) mg/L. The 96-hour NOEC was determined by Dunnett's procedure ($p < 0.05$) to be 44 mg/L using all three methods. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 3 - 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 202 and OPPTS 850.1010

Test type: Static acute

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Daphnia magna*

Analytical monitoring: PFOS measured at 0, 24, 48-hours

Statistical methods: EC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test daphnid source: Obtained from cultures maintained by Wildlife International Ltd., Easton, MD. Identification of the original brood stock was verified by the Academy of Natural Sciences, Philadelphia, PA., USA

Test daphnid age at study initiation: < 24-hours

Test conditions

Dilution water: 0.45 µm filtered well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

Hardness: 132 (128-136) mg/L as CaCO₃

Alkalinity: 178 (176-178) mg/L as CaCO₃

pH: 8.3 (8.2-8.3)

TOC: < 1.0 mg/L

Conductivity: 313 (310-315) µmhos/cm

Ca/Mg ratio: 35/13.5

Na/K ratio: 21.3/6.62

Lighting: Colortone® 50 fluorescent lights, intensity approximately 359 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

Stock and test solutions preparation: A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for ~19.5 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

Exposure vessels: 250 mL plastic beakers containing 240 mL of test solution. The approximate depth of test solution was 6.4 cm.

Number of replicates: two

Number of daphnids per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 48 hours):

8.6 – 8.9 mg/L (control exposure)

8.6 – 9.1 mg/L (91 mg/L exposure)
pH range (0 – 48 hours)

8.2 – 8.5 (control exposure)
 8.5 – 8.6 (91 mg/L exposure)

Test temperature range (0 – 48 hours)

19.5 – 20.2°C (control exposure)
 19.3 – 20.1°C (91 mg/L exposure)

Element basis: mortality and immobilization

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 12, 20, 33, 55, 91 mg/L

Measured concentrations: <LOQ, 11, 20, 33, 56, 91 mg/L

Element value: (95% confidence interval is given in brackets)

24-hour EC₁₀ = 82 (81-83) mg/L
 24-hour EC₅₀ = >91 mg/L (C.I. not calculable)
 24-hour EC₉₀ = >91 mg/L (C.I. not calculable)
 48-hour EC₁₀ = 53 (<11->91) mg/L
 48-hour EC₅₀ = 61 (33-91) mg/L
 48-hour EC₉₀ = 63 (<11->91) mg/L

All element values based on mean measured concentrations

Statistical evaluation: The EC₅₀ values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan. The EC₁₀ and EC₉₀ values were calculated when possible using the Bruce-Versteeg method because there were less than two concentrations with partial mortality or immobility.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.58 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 96.2. Samples collected at test initiation had measured values from 85.5 to 112% of nominal. Measured values for samples taken at 24 hours ranged from 92.2 to 115% of nominal. Measured values for samples taken at 48 hours ranged from 91.6 to 106% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration mg/L	Measured duplicated values at 0, 24, and 48-hours respectively, mg/L	Mean Measured Concentration mg/L	Percent Of Nominal
Negative Control	All < LOQ	<LOQ	-
12	10.5, 10.6, 11.5, 12.5, 10.9, 12.0	11	92
20	17.2, 18.1, 22.8, 21.6, 21.4, 18.8	20	100
33	30.2, 34.1, 34.0, 36.1, 31.3, 34.0	33	100
55	50.5, 49.9, 57.0, 63.0, 56.8, 56.4	56	102
91	87.6, 102, 90.1, 84.4, 88.7, 92.4	91	100

Biological observations after 48-hours: Daphnids in the negative control, the 11 and the 20 mg/L

treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. Five percent mortality was observed at 48-hours in the negative control. The effects noted in this study were mortality; no immobilization was noted at any test concentration.

Cumulative percent mortality:

Mean Measured Test Concentration mg/L	24-hours	48-hours
Negative Control	0	5
11	0	0
20	0	0
33	0	0
56	0	35
91	35	100

Control response: satisfactory

CONCLUSIONS

The potassium perfluorooctanesulfonate 48-hour EC₅₀ for *Daphnia magna* was determined to be 61 mg/L with a 95% confidence interval of 33-91 mg/L. The 48-hour no immobilization and no observed effect concentration was 33 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 4 - 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1025

Type: Static acute

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Crassostrea virginica*

Supplier: P. Cummins Oyster Company, Inc., Baltimore, MD, USA

Shell grinding: Prior to test initiation, recently deposited shell at the rounded (ventral) end was removed using a small electric grinder. Care was taken to remove the shell rim uniformly to produce a smooth, rounded, blunt profile.

Analytical monitoring: PFOS measured at 0,48,96-hours

Exposure period: 96-hours

Statistical methods: Shell growth inhibition was calculated for each treatment group as the percent reduction in shell growth relative to mean shell growth in the negative control. The EC₅₀ value was estimated by visual inspection of shell growth inhibition data. The shell growth data was evaluated for normality and homogeneity of variances using the Chi-Square test and Bartlett's test, respectively. Dunnett's test was used to identify treatment groups that had a statistically significant (≤ 0.05) reduction in shell growth as compared to the control.

Test oyster age: unknown

Length: 33.8 (27.8-41.5) mm,

Pretreatment: None

Test conditions:

Dilution water: Natural seawater diluted to a salinity of 20 ‰ with well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

salinity: 21 (20-21) ‰

pH: 8.1 (8.0-8.2)

TOC: < 1.0 mg/L

Stock and test solution preparation: Primary stock prepared in dilution water at 9.1mg/L and mixed for ~24 hours prior to use. After mixing, primary stock solution appeared clear and colorless with some white particulate material suspended throughout the solution. It was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless. Due to the relatively low solubility of PFOS in natural seawater, the highest concentration attainable with this matrix is approximately 3.3mg/L.

Concentrations dosing rate: Once

Exposure vessels: 52L polyethylene aquaria containing approximately 40L of test solution; water depth approximately 21 cm. Each chamber was continuously stirred to circulate the supplemental

algae diet using an electric paddle mixer.

Feeding: Algal cells (*Thalassiosira pseudonana*, *Skeletonema* sp., *Chaetoceros* sp., and *Isochrysis* sp.) were provided to supplement naturally occurring algae and to maximize oyster growth rates during the test.

Number of replicates: one

Number of oysters per replicate: twenty

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0- 96 hours):

6.6 - 7.6mg/L (control exposure)

6.1 - 7.7mg/L (3.0 mg/L exposure).

pH range (0- 96 hours):

7.6 - 8.1 (control exposure)

7.6 - 8.1 (3.0 mg/L exposure)

Test temperature range (0- 96 hours):

22.2 - 22.3 °C (control exposure)

21.8 - 22.7 °C (3.0 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 1.2, 2.0, 3.3, 5.5, 9.1 mg/L

Measured concentrations: <LOQ, 0.36, 0.40, 1.3, 1.9, 3.0 mg/L

Element value: (95% confidence interval is given in brackets)

96-hour EC₅₀ = >3.0 mg/L (C.I. not calculable)

96-hour NOEC = 1.9 mg/L

All element values based on mean measured concentrations

Statistical evaluation of shell growth:

EC₅₀ values could not be calculated due to insufficient shell growth inhibition at the highest attainable concentration.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. Samples collected at test initiation had measured values from 28 to 46% of nominal. Measured values for samples taken at 48-hours ranged from 15 to 41 % of nominal. Measured values for samples taken at 96-hours ranged from <LOQ to 52% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/l	Measured Duplicate Values at 0, 48 and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/l	Percent of Nominal
Negative Control	All <LOQ	<LOQ	-
1.2	0.331, 0.353, 0.341, 0.429, <LOQ, <LOQ	0.36	30
2.0	0/622, 0.633, 0.299,	0.40	20

	0.313, 0.249, 0.257		
3.3	1.36, 1.15, 0.924, 0.878, 1.58, 1.72	1.3	39
5.5	2.42, 2.53, 2.02, 2.24, 1.45, 0.970	1.9	35
9.1	3.39, 3.44, 3.01*, 3.74, 3.57, 1.99, 2.19	3.0	33

*3 replicates analyzed at time 0

Biological observations after 96-hours: Oysters in the negative control and all PFOS treatment groups appeared normal and healthy throughout the exposure period.

Shell deposition and shell growth Inhibition at test termination:

Mean Measured Concentration, mg/l	Shell Deposition Mean \pm SD, mm	Percent Inhibition in Shell Growth
Negative Control	2.67 \pm 0.824	-
0.36	2.50 \pm 0.933	6.4
0.40	2.40 \pm 0.820	10
1.3	2.51 \pm 0.919	6.0
1.9	2.13 \pm 0.804	20
3.0	1.91 \pm 0.591	28

Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC₅₀ for the Eastern Oyster was determined to be > 3.0 mg/L, the highest concentration tested and the practical limit of solubility in unfiltered seawater. The 96-hour no effect concentration was 1.9 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 5/3100

Robust Study Report Reference No. 5 - 96-Hour Static Acute Toxicity Test with the Freshwater Mussel (*Unio complamatus*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: The study was conducted using a protocol based on procedures outlined in U.S. Environmental Protection Agency Series 850 – Ecological Effects Guidelines, OPPTS Number 850.1075 ; OECD 203: *Fish, Acute Toxicity Test*; and ASTM Standard E729-88a, *Standard Guide for Conducting Toxicity Tests with Fishes, Macroinvertebrates and Amphibians*.

Test type: Semi-static Renewal

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Unio complamatus*

Analytical monitoring: Test substance concentrations measured by LCMS at 0, 48, 96-hours

Statistical methods: LC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test organism source: Obtained from Carolina Biological Supply Company, Burlington, North Carolina, USA. Carolina collected from the wild.

Test organism age at study initiation: Unknown

Test Conditions

Dilution water: 0.45 µm filtered well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

Hardness: 126 (120-132) mg/L as CaCO₃

Alkalinity: 174 (170-178) mg/L as CaCO₃

pH: 8.3 (8.1-8.5)

TOC: < 1.0 mg/L

Conductivity: 321 (310-330) µmhos/cm

Ca/Mg ratio: 35/13.5

Na/K ratio: 21.3/6.62

Lighting: Colortone® 50 fluorescent lights, intensity approximately 369 lux. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

Stock and test solutions preparation: A primary stock solution was prepared in dilution water at 91 mg/L. It was mixed for approximately 24 hours prior to use. After mixing, the primary stock was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

Exposure vessels: 25 liter polyethylene aquaria containing approximately 20 L of test solution. The approximate depth of test solution was 23.2 cm.

Number of replicates: two

Number of test organisms per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

5.8 – 8.5 mg/L (control exposure)

5.0 – 8.6 mg/L (79 mg/L exposure)

pH range (0 – 96 hours):

8.0 – 8.4 (control exposure)

7.9 – 8.5 (79 mg/L exposure)

Test temperature range (0 – 96 hours):

21.4– 21.8°C (control exposure)

21.8 – 23.7 °C (79 mg/L exposure)

Element Basis: Mortality. Mussels with open shells and not responding to gentle prodding were considered dead. The number of individuals exhibiting clinical signs of toxicity or abnormal behavior also were evaluated.

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: <LOQ, 5.7, 11, 23, 46, 91 mg/L

Measured concentrations: <LOQ, 5.3, 12, 20, 41, 79 mg/L

Element value: (95% confidence interval is given in brackets)

96-hour LC₅₀ = 59 mg/L (51-68 mg/L)

Statistical evaluation: The LC₅₀ values and 95% confidence intervals were calculated when possible by probit analysis, the moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7%. Samples collected at test initiation had measured values from 73.7% to 96.0% of nominal. Measured values for samples taken at 48 hours ranged from 81.2 to 98.9% of nominal. Measured values for samples taken at 96 hours ranged from 88.5 to 130% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration , mg/L	Measured duplicated values at 0, 48, and 96-hours respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
5.7	5.47, 4.93, 5.18, 5.70, 5.24, 5.26	5.3	93
11	11.4, 10.1, 11.2, 10.5, 10.9, 15.4	12	109
23	19.0, 16.8, 18.7, 18.7, 22.9, 22.4	20	87
46	37.2, 40.6, 37.1, 39.5, 48.2, 40.5	41	89
91	69.0, 74.7, 81.3, 77.6, 88.2, 85.7	79	87

Biological observations after 96-hours: Mussels in the negative control, the 5.3, 12 and the 20 mg/L treatments appeared healthy and normal throughout the test with no mortality or overt clinical signs of toxicity. Five percent mortality was observed at 96-hours in the 41 mg/L treatment and 90% mortality was observed in the 79 mg/L treatment. No abnormal behavior was noted in these concentrations.

Cumulative percent mortality:

Mean Measured Test Concentration mg/L	24 Hours	48 Hours	72 Hours	96 Hours
Negative Control	0	0	0	0
5.3	0	0	0	0
12	0	0	0	0
20	0	0	0	0
41	0	0	0	5
79	30	40	50	90

Control response: Satisfactory**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour LC₅₀ for the Freshwater Mussel, *Unio complamatus* was determined to be 59 mg/L with a 95% confidence interval of 51-68 mg/L. The 96-hour no mortality concentration was 20 mg/L.

Submitter: 3M Corporation, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA**DATA QUALITY**

Reliability: Klimisch ranking 1.**REFERENCES**

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 6 – Activated Sludge, Respiration Inhibition Test

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 209

Test type: Static acute

GLP: Yes

Year Completed: Study completed 1999. Report completed 2000

Analytical monitoring: Dissolved oxygen concentrations.

Statistical methods: Probit analysis using the computer software of C.E. Stephan.

Test organism source. Activated sludge collected from the Prospect Bay Wastewater Treatment Facility, Grasonville, Maryland, USA.

Test conditions

Dilution water: NANOpure®

Synthetic Sewage:

1 liter municipal water
16.0 g peptone
11.0 g meat extract
3.0 g urea
0.7 g NaCl
0.4 g CaCl₂ 2H₂O
0.2 g MgSO₄ 7H₂O
2.8 g K₂HPO₄

Reference and test solution preparation: A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL of 1N NaOH, diluted to 30 mL with NANOpure® water, then brought to the point of incipient precipitation with 1 NH₂SO₄, and diluted to 1 L with NANOpure4® water. The pH of the reference solution was measured to be 7.18. PFOS was added directly to test vessels rather than volumetric addition of a stock solution. This method was deemed appropriate based on the observed solubility of the test substance in water.

Test vessels: Mixtures were prepared and aerated in 500 mL Erlenmeyer flasks and then transferred into 300 mL Biochemical Oxygen Demand (BOD) bottles

Number of concentrations: 7 plus 3 reference controls and 2 Blank controls

Temperature: 19-21°C

Total Suspended Solids and pH for sludge on day of testing: 4380 mg/L and 7.87 respectively.

Element Basis: Respiration inhibition as determined by dissolved oxygen concentration.

Method Remarks: Stock solutions of PFOS that were prepared at nominal concentration of approximately 500 and 1 000 mg/L in NANOpure® water contained test material that was not in solution after 20-minutes of sonication. Therefore, direct weight addition was employed to administer PFOS to the test system.

Test mixtures were prepared at 15-minute intervals and aerated until the contact time of the test substance with the activated sludge was three hours. After 3-hours of contact time, dissolved oxygen was measured over a period of up to 10-minutes.

RESULTS

Nominal concentrations: Two blank controls, three reference substance controls, 0.90, 2.7, 9.0, 27, 90, 271, 905 mg/L test material solutions.

Statistical Analyses: EC₅₀ values were calculated for the reference material by probit analysis using the computer software of C.E. Stephan. An EC₅₀ value could not be calculated for the test substance.

Analytical Methodology: Analysis of DO concentrations in all test solutions were performed at Wildlife International Ltd. using a YSI Model 50B Dissolved Oxygen Meter. Dissolved oxygen readings were recorded every 10 seconds for 10 minutes or until the dissolved oxygen dropped below 1.0 mg/L

Respiration Rates and Percent Inhibitions

Treatment	Respiration Rate mg O ₂ /L/hour	Percent Inhibition
Control 1	39.6	NA
Control 2	41.1	NA
3,5-dichlorophenol 3mg/L	31.1	22.9
3,5-dichlorophenol 15 mg/L	14.6	63.8
3,5-dichlorophenol 50 mg/L	5.1	87.4
Test substance 0.90 mg/L	38.9	3.6
Test substance 2.7 mg/L	35.1	13.0
Test substance 9.0 mg/L	33.5	17.0
Test substance 27 mg/L	37.9	6.1
Test substance 90 mg/L	32.7	19.0
Test substance 271 mg/L	28.1	30.4
Test substance 905 mg/L	24.7	38.8

Control response: satisfactory

CONCLUSIONS

The test substance exhibited a maximum inhibitory effect of 38% upon respiration at a nominal test substance concentration of 905 mg/L. The EC₅₀ (respiration inhibition) is therefore greater than the solubility of the test substance.

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

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

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

ENV/JM/RD(2002)17/FINAL

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 7 - 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, 1H-HMR, 19F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1035

Type: Static acute

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Mysidopsis bahia*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured at 0, 48, 96-hours

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: < 24-hours old

Pretreatment: None

Test Conditions:

Dilution water: Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

Dilution water chemistry (during the 4-week period immediately preceding the test):

Salinity: 20 (20-20) ‰

pH: 8.2 (8.1-8.2)

TOC: < 1.0 mg/L

Stock and test solution preparation: Primary stock prepared at 8.2 mg/L and mixed for ~22 hours prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the four additional test concentrations. All test solutions appeared clear and colorless.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 2L polyethylene aquaria containing approximately 1000mL of test solution; water depth approximately 6.6 cm.

Number of replicates: two

Number of mysids per replicate: ten

Number of concentrations: five plus a negative control

Feeding: Live brine shrimp nauplii daily

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

6.8 – 7.4 mg/L (control exposure)

6.8 – 7.3 mg/L (5.4 mg/L exposure)

pH range (0 – 96 hours):

8.1 – 8.2 (control exposure)
 8.1 – 8.2 (5.4 mg/L exposure)
Test temperature range (0 – 96 hours):
 24.2 – 25.4°C (control exposure)
 23.8 – 24.5°C (5.4 mg/L exposure)
Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 1.1, 1.8, 3.0, 4.9, 8.2 mg/L

Measured concentrations: <LOQ, 0.57, 1.1, 1.9, 3.0, 5.4 mg/L

Element value: (95% confidence interval is given in brackets)

24-hour LC₅₀ = > 5.4 mg/L (CI not calculable)

48-hour LC₅₀ = > 5.4 mg/L C.I. not calculable)

72-hour LC₅₀ = 4.4 (3.6-6.2) mg/L

96-hour LC₅₀ = 3.6 (3.0-4.6) mg/L

All element values based on mean measured concentrations

Statistical evaluation of mortality: LC₅₀ values could not be calculated for 24 and 48-hours of exposure due to the lack on an adequate concentration-response pattern. The probit method was used to evaluate mortality at 72 and 96 hours.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctane sulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.115 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.4. Samples collected at test initiation had measured values from 52.4 to 70.7% of nominal. Measured values for samples taken at 48 hours ranged from 43.5 to 71.0% of nominal. Measured values for samples taken at 96 hours ranged from 35.5 to 71.1% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured duplicate values at 0, 48, and 96-hours respectively, mg/L	Mean Measured Concentration mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
1.1	0.575, 0.622, 0.605, 0.640, 0.391, 0.580	0.57	52
1.8	1.12, 1.19, 1.10, 1.09, 1.04, 1.13	1.1	61
3.0	1.92, 1.99, 1.92, 1.91, 1.79, 1.91	1.9	63
4.9	3.05, 2.66, 2.96, 3.35, 3.11, 3.11	3.0	61
8.2	5.82, 5.78, 3.58, 5.85, 5.22, 5.86	5.4	66

Biological observations after 96-hours: Mysids in the negative control, and the 0.57 and 1.1 mg/L (mean measured concentrations) treatment groups appeared normal and healthy during the test.

Cumulative percent mortality:

Mean Measured Test Concentration, mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
0.57	0	0	0	0
1.1	0	0	0	0
1.9	0	0	5	10
3.0	5	15	30	40
5.4	15	45	60	75

Mortality of controls: None**CONCLUSIONS**

The potassium perfluorooctanesulfonate 96-hour LC_{50} for saltwater mysids was determined to be 3.6 mg/L with a 95% confidence interval of 3.0 – 4.6 mg/L. The 96-hour no mortality and NOEC concentration was 1.1 mg/L.

Submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA**DATA QUALITY**

Reliability: Klimisch ranking = 1**REFERENCES**

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 8 - Early Life-Stage Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OECD 210, OPPTS 850.1400

Type: Flow-through chronic

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Pimephales promelas*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured on days 0, 4, 7, 14, 21, 28, 35, 42, and 47

Exposure period: 47 days

Statistical methods: Discrete-variable data were analyzed using 2 x 2 contingency tables to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the negative control group. All continuous-variable data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means.

Test fish age: eggs < 24-hours old at test initiation

Pretreatment: None

Test Conditions

Dilution water: 0.45 µm filtered well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

Hardness: 126 (124-128) mg/L as CaCO₃

Alkalinity: 172 (170-172) mg/L as CaCO₃

pH: 8.2 (8.2-8.3)

TOC: < 1.0 mg/L

Conductivity: 321 (315-330) µmhos/cm

Stock and test solution preparation: Primary stock prepared in dilution water at 88.4 mg/L and mixed until all test substance dissolved prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 44.2, 22.1, 11.0, 5.52, and 2.76 mg/L. Stock solutions were prepared every three to four days during the test. The six stocks were injected into the diluter mixing chambers (at a rate of 6.0 mL/minute) where they were mixed with dilution water (at a rate of 116 mL/minute) to achieve the desired test concentrations.

Flow through rate: Approximately six volume additions of test water every 24-hours

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 9L glass aquaria filled with approximately 7 L of test solution with a depth of approximately 17 cm. Embryo incubation cups were constructed from glass cylinders approximately 50 mm in diameter with 425 µm nylon screen mesh attached to the bottom with

silicone sealant. The cups were suspended in the water column of each 9L glass aquarium and attached to a rocker arm with a reciprocating motion of approximately 2 rpm.

Number of replicates: four

Number of fish per replicate: twenty

Number of concentrations: six plus a negative control

Feeding: Live brine shrimp nauplii. Fed 3 times per day during the first 7 days post-hatch. On days 8 through 40 post-hatch, fed 3 times daily on weekdays and 2 times daily on weekends. Not fed for at least 48 hours prior to the termination of test to allow for gut clearance prior to weight measurements.

Water chemistry during the study:

Dissolved oxygen range (0 – 47 days):

7.6 – 8.2 mg/L (control exposure)

7.6 – 8.2 mg/L (1.2 mg/L exposure)

pH range (0 – 47 days):

8.0 – 8.4 (control exposure)

8.0 – 8.4 (1.2 mg/L exposure)

Test temperature range (0 – 47 days):

24.4 – 24.7°C (control exposure)

24.3 – 24.7°C (1.2 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 0.14, 0.29, 0.57, 1.1, 2.3, 4.6 mg/L

Measured concentrations: <LOQ, 0.15, 0.30, 0.60, 1.2, 2.4, 4.6 mg/L

Element value: 5-day hatchability NOEC = 4.6 mg/L
 42-day post-hatch survival NOEC = 0.30 mg/L
 42-day post-hatch growth NOEC = 0.30 mg/L
 42-day post-hatch survival LOEC = 0.60 mg/L

All element values based on mean measured concentrations

Statistical evaluation of mortality: The statistical difference for growth at concentrations equal to and higher than 0.60 mg/L was not evaluated due to a significant effect on survival. No statistically significant difference between the negative control and the highest concentration tested was seen for hatchability.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctane sulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 102. Samples collected at pre-test ranged from 91.4 to 105% of nominal. Samples at test initiation had measured values from 95.5 to 114% of nominal. Measured values for samples taken at test termination ranged from 95.2 to 111% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured duplicate values at 0, 4, 7, 14, 21, 28, 35, 42, and 47 Days respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	-	-
0.14	0.147, 0.160, 0.141, 0.140, 0.144, 0.148, 0.134, 0.135, 0.153, 0.143, 0.160, 0.158, 0.179, 0.173, 0.157, 0.160, 0.147, 0.155	0.15	107
0.29	0.287, 0.277, 0.270, 0.289, 0.292, 0.296, 0.269, 0.266, 0.307, 0.315, 0.343, 0.341, 0.311, 0.325, 0.319, 0.313, 0.296, 0.276	0.30	103
0.57	0.571, 0.576, 0.619, 0.659, 0.597, 0.642, 0.539, 0.535, 0.608, 0.580, 0.639, 0.617, 0.646, 0.644, 0.575, 0.576, 0.545, 0.543	0.60	105
1.1	1.14, 1.13, 1.21, 1.25, 1.13, 1.23, 1.03, 1.10, 1.19, 1.24, 1.30, 1.31, 1.30, 1.31, 1.14, 1.19, 1.13, 1.09	1.2	109
2.3	2.21, 2.27, 2.52, 2.46, 2.43, 2.38, fish all dead at Day 7	2.4	104
4.6	4.56, 4.40, 4.79, 4.79, 4.46, 4.76, fish all dead at Day 7	4.6	100

Biological Observations

Hatching success and time to hatch: All viable fathead minnow embryos hatched on Day 4 or 5. There were no apparent differences between the time to hatch in the negative control and the PFOS treatment groups.

Survival: All fish surviving to test termination appeared normal with no overt signs of sublethal toxicity. Fish which did not survive generally appeared to be swimming erratically prior to death.

Growth: Fish exposed to PFOS at concentrations of 0.15 or 0.30 mg/L for 42 days post-hatch showed no statistically significant reduction in total length, wet weight or dry weight in comparison to the negative control.

Hatchability

Mean Measured Concentration mg/L	Number of Eggs Exposed	Number Hatched, Day 3	Number Hatched, Day 4	Number Hatched, Day 5	Total Number Hatched	Percent Hatching Success
Negative Control	80	0	20	54	74	93
0.15	80	0	18	58	76	95
0.3	80	0	14	58	72	90
0.6	80	0	28	48	76	95
1.2	80	0	25	49	74	93
2.4	80	0	16	59	75	94
4.6	80	0	14	60	74	93

Larval Survival

Mean Measured Concentration, mg/L	Percent Survival, Day 42
Negative Control	88
0.15	79
0.3	81
0.6	66
1.2	5.4
2.4	0
4.6	0

Growth

Mean Measured Concentration, mg/L	Number of Surviving Larvae	Total Length Mean + SD, mm	Wet Weight Mean + SD, mg	Dry Weight Mean + SD, mg
Negative Control	65	26.5 + 0.721	158 + 9.10	32.5 + 1.20
0.15	60	26.6 + 0.208	160 + 3.10	33.3 + 0.900
0.30	58	26.6 + 0.813	167 + 11.9	34.2 + 2.70
0.60	50	26.5 + 0.399	166 + 11.3	33.5 + 2.70
1.2	4	26.7 + 2.02	185 + 33.8	35.4 + 6.66
2.4	0	-	-	-
4.6	0	-	-	-

CONCLUSIONS

Fathead minnows exposed to potassium perfluorooctanesulfonate at concentrations ≤ 0.30 mg/L for 42 days post-hatch showed no statistically significant reductions in time to hatch, hatching success, survival or growth. The most sensitive endpoint in this study was post-hatch survival.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 9 - Semi-Static Life-Cycle Toxicity Test with the Cladoceran (*Daphnia magna*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1300, OECD Guideline 211, and ASTM Standard E 1193-87.

Type: Semi-Static Life-Cycle Toxicity

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Daphnia magna*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured on days 0, 2, 11, 14, 18, and 21.

Exposure period: 21 days

Statistical methods: Survival data was evaluated on first-generation daphnids, the number of live young and the length and dry weight of the surviving first-generation daphnids. Survival data were analyzed using Fisher's exact test. Reproduction and growth (length and dry weight) data were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test was used to identify treatment groups that were statistically significant in comparison to the negative control ($p \leq 0.05$). All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

Test organism age: < 24-hours old at test initiation

Pretreatment: None

Test Conditions

Dilution water: 0.45 µm filtered well water passed through a UV sterilizer to remove microorganisms and fine particles

Dilution water chemistry (during the 4-week period immediately preceding the test):

Hardness: 124 (120-128) mg/L as CaCO₃

Alkalinity: 169 (164-172) mg/L as CaCO₃

pH: 8.2 (8.0-8.3)

TOC: < 1.0 mg/L

Conductivity: 329 (315-340) µmhos/cm

Ca/Mg ratio: 35/13.5

Na/K ratio: 21.3/6.62

Stock and test solution preparation: Primary stock solution was prepared in dilution water at 46 mg/L. It was stirred until all test substance was dissolved prior to use. After mixing, the primary stock solution was proportionally diluted with UV sterilized dilution water to prepare five additional stock solutions at nominal concentrations of 1.4, 2.9, 5.7, 11, and 23 mg/L. All test solutions appeared clear and colorless.

Renewal rate: Every Monday, Wednesday and Friday.

Exposure vessels: 250-mL plastic beakers containing approximately 200 mL test solution. The depth was approximately 5 cm.

Number of replicates: 10

Number of test organisms per replicate: 1

Number of concentrations: 6 plus a negative control

Feeding: Each test chamber was fed 0.3 mL of YCT (a mixture of yeast, Cerophyll®, and trout chow at 1800 mg TSS/L) and 0.60 mL of *Selenastrum capricornutum* (3.5×10^7 cells/mL) once daily.

Lighting: Colortone® 50 fluorescent lights. Intensity ranged from 329 - 383 lux at the water surface. Photoperiod of 16-hours light, 8-hours dark with a 30-minute transition period.

Water chemistry of new and old solutions during the study:

Dissolved oxygen range (0 – 21 days):

8.3 – 8.9 mg/L (negative control exposure)

8.3 – 9.0 mg/L (12 mg/L exposure)

8.4 – 8.9 mg/L* (48 mg/L exposure)

pH range (0 – 21 days):

8.1 – 8.4 (negative control exposure)

8.2 – 8.5 (12 mg/L exposure)

8.4 – 8.5* (48 mg/L exposure)

Test temperature range (0 – 21 days):

19.4 – 20.1°C (negative control exposure)

19.4 – 20.1°C (12 mg/L exposure)

19.4 – 19.5 °C* (48 mg/L exposure)

* (Measurements discontinued at Day 3 due to 100% mortality).

Element basis: Survival, reproduction and growth. Effect concentrations based on survival.

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 1.4, 2.9, 5.7, 11, 23, 46 mg/L

Measured concentrations: <LOQ, 1.5, 2.9, 5.6, 12, 24, 48 mg/L

Element value: 21-day NOEC = 12 mg/L

21-day LOEC = 24 mg/L

21-day MATC = 17 mg/L

2nd generation acute survival NOEC = 12 mg/L

All element values based on mean measured concentrations.

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for the test substance was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.458 mg/L in this study. The mean procedural recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Measured values of new samples ranged from 94 to 121% of nominal. Measured values from the old solutions ranged from 90 to 108% of nominal values. PFOS was stable throughout the renewal periods.

Summary of analytical chemistry data:

Nominal Test Concentration mg/L	Measured duplicate values at 0, 2, 11, 14, 18, and 21 Days respectively, mg/L	Mean Measured Concentration mg/L	Percent of Nominal
Negative Control	All < LOQ	-	-
1.4	1.78, 1.72, 1.58, 1.56, 1.38, 1.47, 1.36, 1.32, 1.38, 1.43, 1.50, 1.45	1.5	107
2.9	3.20, 3.05, 3.01, 3.07, 2.75, 2.77, 2.85, 2.71, 2.79, 2.81, 2.81, 2.82	2.9	100
5.7	5.97, 5.87, 5.65, 5.72, 5.63, 5.59, 5.36, 5.39, 5.58, 5.75, 5.24, 5.37	5.6	98
11	11.5, 11.5, 11.6, 11.8, 11.3, 11.3, 11.2, 11.6, 11.8, 11.6, 11.5, 11.3	12	109
23	24.2, 23.1, 24.0, 24.6, 22.8, 22.5, 23.6, 23.1, 24.8, 25.0, all daphnids dead after 18-days exposure	24	104
46	47.3, 48.0, 49.1, 49.4, all daphnids dead after 2-days exposure	48	104

NOTE: Mean measured concentrations were determined from new (renewal solutions) and corresponding old solutions during each week of the test. Days 0, 11, and 18 are “new” and days 2, 24, and 21 are “old”.

Biological Observations

Survival: All surviving first generation daphnids appeared normal at test termination. Survival in the 24 and 48 mg/L treatments was statistically significantly different from the negative control group.

Reproduction: Daphnids in the control and treatment groups ≤ 12 mg/L started producing neonates on Day 9. The Bonferroni t-test showed that reproduction was not significantly reduced in any treatment group ≤ 12 mg/L ($p > 0.05$). The 24 and 48 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant effect on survival.

Growth: The Bonferroni t-test showed that mean length and dry weight in the treatment groups ≤ 12 mg/L were not significantly reduced in comparison to the negative control ($p > 0.05$).

Second Generation Acute Exposure: After 48-hours of exposure, survival in the negative control was 95%. Survival in the 1.5, 2.9, 5.6, 12, and 24 mg/L treatment groups was 100, 100, 100, 90, and 0% respectively. Survival in the 24 mg/L treatment group was significantly different from the negative control ($p \leq 0.05$).

Summary of Percent Mortality

Mean Measured Concentration, mg/L	Day 7	Day 14	Day 21
Negative Control	0	0	0
1.5	0	0	10
2.9	0	0	10
5.6	0	0	10
12	0	10	10
24	70	90	100
48	100	100	100

Second Generation Mortality

Mean Measured Concentrations, mg/L	Total Number Exposed	Number Alive after 48-hours	Cumulative Percent Dead
Negative Control	20	19	5
1.5	20	20	0
2.9	20	20	0
5.6	20	20	0
12	20	18	10
24	8	0	100

Summary of Length and Dry Weight of Surviving Individually-Exposed First-Generation Daphnids

Mean Measured Concentration, mg/L	Number of Surviving Daphnids	Total Length, Mean + SD, mm	Dry Weight, Mean + SD, mg
Negative Control	10	4.65 + 0.111	0.695 + 0.100
1.5	9	4.66 + 0.118	0.669 + 0.0623
2.9	9	4.62 + 0.100	0.724 + 0.110
5.6	9	4.61 + 0.124	0.727 + 0.0665
12	9	4.59 + 0.102	0.723 + 0.0661
24	0	--	--
48	0	--	--

Reproduction

Mean Measured Concentration, mg/L	Number of Surviving Daphnids	Mean Live Young/ Surviving Adult Daphnid (+SD)	First Day of Reproduction	Total Number of Dead / Immobile Neonates	Total Number of Aborted Eggs
Negative Control	10	122 + 19.2	9	0	0
1.5	9	142 + 24.7	9	0	0
2.9	9	136 + 17.9	9	0	0
5.6	9	132 + 19.5	9	0	0
12	9	119 + 26.5	9	1	0
24	0	--	11	10	0
48	0	--	None	--	--

CONCLUSIONS

There were no adverse effects on survival, reproduction or growth of *Daphnia magna* exposed to the test substance at concentration ≤ 12 mg/L for 21 days. *Daphnia magna* exposed to 24 and 48 mg/L had significantly reduced survival.

Author and/or submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

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

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 10 - Flow-through Life-Cycle Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.1350

Type: Flow-through chronic

GLP: Yes

Year completed: Study completed 1999. Report completed 2000

Species: *Mysidopsis bahia*

Supplier: In-house cultures, Wildlife International, Ltd., Easton, MD, USA

Analytical monitoring: PFOS measured on days 0, 7, 14, 21, 28, and 35

Exposure period: 35 days

Statistical methods: Survival data was evaluated (prior to pairing and after pairing) using 2 x 2 contingency tables to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the negative control group. All continuous-variable data (reproduction and growth) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test. Analysis of variance and Dunnett's test were used to evaluate differences between treatment and control means. All statistical tests were performed using a personal computer with SPSS/PC Version 2.0 or "TOXSTAT Release 3.5" statistical software.

Test mysids age: < 24-hours old at test initiation

Pretreatment: None

Test Conditions: Natural seawater diluted to 20‰ with well water, 0.45µm filtered.

Dilution water chemistry (during the 4-week period immediately preceding the test):

Salinity: 20 (20-20) ‰

TOC: < 1.0 mg/L

Stock and test solution preparation: Primary stock prepared at 0.0895 mg/L and mixed for approximately 24 hours prior to use. After mixing, the primary stock solution was proportionally diluted with dilution water to prepare five additional stock solutions at concentrations of 0.0447, 0.0224, 0.0112, 0.00559, and 0.00280 mg/L. The six stocks were injected into the diluter mixing chambers (at a rate of 4.60 mL/minute) where they were mixed with dilution water (at a rate of 150 mL/minute) to achieve the desired test concentrations.

Flow through rate: Approximately eleven volume additions of test water every 24 hours

Stability of the test chemical solutions: Extremely stable

Exposure vessels: Prior to pairing, mysids placed in glass beakers with nylon mesh screen attached to two holes on opposite sides. After reaching sexual maturity, pairs placed in glass petri dishes with sides of nylon mesh screen attached with silicone adhesive. Both pre-pairing and post-pairing exposure vessels were placed in 9L glass aquaria filled with approximately 5 L of test solution. The depth was approximately 6.2 cm prior to pairing and 5.5 cm after pairing.

The test chambers for the second generation exposure were 2L beakers with 1L of test solution which was dipped out of a test chamber from the appropriate treatment group.

Number of replicates: four

Number of concentrations: six plus a negative control

Number of fish per replicate: Fifteen juveniles before pairing, 5 pairs (10 adults) when possible after pairing.

Feeding: Fed live brine shrimp nauplii 3 or four times per day. Not fed the last day of the test.

Water chemistry during the study:

Dissolved oxygen range (0 – 35 days):

6.0 – 6.4 mg/L (control exposure)

5.9 – 6.3 mg/L (1.3 mg/L exposure)

pH range (0 – 35 days):

8.2 – 8.4 (control exposure)

8.3 – 8.4 (1.3 mg/L exposure)

Test temperature range (0 – 35 days):

24.5 – 25.2°C (control exposure)

24.4 – 25.1°C (1.3 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Negative control, 0.086, 0.17, 0.34, 0.69, 1.4, 2.7 mg/L

Measured concentrations: <LOQ, 0.057, 0.12, 0.25, 0.55, 1.3, 2.6 mg/L

Element value: 20-day survival (pre-pairing) NOEC = 0.55 mg/L

35-day (post-pairing) survival NOEC = 0.55 mg/L

35-day reproduction NOEC = 0.25 mg/L

35-day growth NOEC = 0.25 mg/L

35-day reprod & growth LOEC = 0.55 mg/L

2nd generation acute survival NOEC = 0.55 mg/L (highest concentration tested)

All element values based on mean measured concentrations

Analytical methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.0458 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 92.8. Samples collected at pretest ranged from 57.4 to 99.3% of nominal. Samples at test initiation had measured values from 67.1 to 103% of nominal. Measured values for samples taken at test termination ranged from 59.8 to 90.0% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured duplicate values at 0, 7, 14, 21, 28, 35, Days respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	-	-
0.086	0.0694, 0.0578, 0.0478, 0.0619, 0.0606, 0.0614, 0.0554, 0.0509, 0.0515, 0.0569, 0.0580, 0.0514	0.057	66
0.17	0.125, 0.114, 0.0778, 0.125, 0.124, 0.127, 0.0970, 0.112, 0.122, 0.128, 0.124, 0.119	0.12	71
0.34	0.289, 0.286, 0.231, 0.197, 0.276, 0.253, 0.227, 0.212, 0.262, 0.271, 0.278, 0.251	0.25	74
0.69	0.562, 0.659, 0.581, 0.450, 0.543, 0.542, 0.516, 0.528, 0.529, 0.544, 0.556, 0.583	0.55	80
1.4	1.23, 1.32, 1.13, 1.20, 1.35, 1.27, 1.23, 1.15, 1.39, 1.39, 1.26, 1.20	1.3	93
2.7	2.56, 2.79, 2.58, 2.30, 2.54, 2.69, all mysids dead after 14-days exposure	2.6	96

Biological observations

Survival: All surviving mysids appeared normal. Survival in the 1.3 and 2.6 mg/L treatments were statistically significantly different from the negative control group.

Reproduction: The day of first brood release in this study was Day 22. Dunnett's test showed that reproduction was significantly reduced in the 0.55 mg/L treatment group when compared to the negative control ($p < 0.05$). The 1.3 and 2.6 mg/L treatment groups were not included in the statistical analysis of the reproduction data due to a statistically significant difference in survival.

Growth: Mysids exposed to PFOS at concentrations < 0.25 mg/L showed no statistically significant reductions in length or dry weight ($p < 0.05$).

Second Generation Acute Exposure: Survival in all PFOS treatment groups was > 95% and was not statistically different from the controls. All surviving mysids in the second-generation exposure appeared normal with no overt signs of toxicity.

Percent Survival

Mean Measured Concentration, mg/L	Juvenile Pre-Pairing Survival, Day 20	Adult Post- Pairing Survival, Day35
Negative Control	78	92
0.057	92	96
0.12	75	90
0.25	82	97
0.55	83	95
1.3	32	57
2.6	0	-

Second Generation Survival

Mean Measured Concentrations, mg/L	Total Number Exposed	Number Alive after 96-hours	Percent Survival
Negative Control	71	68	96
0.057	65	63	97
0.12	83	79	95
0.25	62	59	95
0.55	13	13	100

Adult Mysid Growth

Mean Measured Concentration, mg/L	Number of Surviving Mysids/Number Exposed	Total Length, Mean \pm SD, mm	Dry Weight, Mean \pm SD, mg
Negative Control	36/39	6.43 \pm 0.0634	0.634 \pm 0.0510
0.057	44/46	6.43 \pm 0.0729	0.599 \pm 0.0276
0.12	36/40	6.56 \pm 0.105	0.641 \pm 0.0241
0.25	36/37	6.40 \pm 0.0548	0.622 \pm 0.0227
0.55	35/37	6.14 \pm 0.0794	0.562 \pm 0.00624
1.3	8/14	5.85 \pm 0.178	0.436 \pm 0.0441

Reproduction

Mean Measured Concentration, mg/L	Replicate	Number of Reproductive Days	Number of Young	Mean Number of Young/ Reproductive Day	Overall Mean \pm SD
Negative control	A	70	18	0.257	0.315 \pm 0.0925
	B	53	14	0.264	
	C	70	20	0.286	
	D	42	19	0.452	
0.57	A	60	17	0.283	0.261 \pm 0.0873
	B	70	14	0.200	
	C	70	13	0.186	
	D	56	21	0.375	
0.12	A	70	21	0.300	0.361 \pm 0.101
	B	46	22	0.478	
	C	54	22	0.407	
	D	70	18	0.257	
0.25	A	70	19	0.271	0.252 \pm 0.0723
	B	56	12	0.214	
	C	61	21	0.344	
	D	56	10	0.179	
0.55	A	54	3	0.0556	0.0559 \pm 0.0376
	B	56	6	0.107	
	C	70	3	0.0429	
	D	56	1	0.0179	

1.3	A	22	0	-	-
	B	14	0	-	
	C	0	0	-	
	D	11	0	-	

CONCLUSIONS

There were no statistically significant effects on survival, reproduction or growth of mysid shrimp exposed to potassium perfluorooctanesulfonate at concentrations ≤ 0.25 mg/L for 35 days. Reproduction, length and dry weight were the most sensitive biological endpoints in this study. Second generation mysids exposed to PFOS during a static 96-hour exposure showed no adverse effects.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 7/9/01

Robust Study Report Reference No. 11 – Dietary LC₅₀ Study with the Mallard duck

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.2200, OECD 206, and FIFRA Subdivision E. Section 71-2

Type: Dietary acute

GLP: Yes

Year completed: 2000

Species: *Anas platyrhynchos*

Supplier: Whistling Wings, Inc., Hanover, IL, USA

Analytical monitoring: PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.

Test phases: Acclimation - 9 days
Exposure - 5 days
Post-exposure observation - 3 or 17 days

Statistical methods: LC₅₀ values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.

Test bird age: 10 days

Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors In batteries of thermostatically controlled brooding pens. Floor space of each pen measured approximately 62 x 90 cm. Ceiling height was approximately 25.5 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

Identification: Each group of birds Identified by pen number and test concentration. Individuals identified by wing bands.

Number of replicates: Six for controls, two for each treatment group

Number of ducks per replicate: five

Number of concentrations: Eight plus a negative control

Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration

Ingredients	Percent
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Form (Fermatco) ¹	0.25
Salt iodized	0.10

¹Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix

Vitamin or Mineral	Amount Per Ton
Vitamin D ₃	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 g
Niacin	40 g
Pantothenic Acid	10 g
Vitamin B ₁₂	8 mg
Folic Acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 g
Thiamine	1.2 g
Vitamin E	20,000 I.U.
Vitamin K (Menadione dimethylpyrimidinol bisulfite)	5.8 g
Manganese	102 g
Zinc	47 g
Copper	6.8 g
Iodine	1.5 g
Iron	51 g
Selenium	182 g

Prophylaxis: None

Brooding compartment mean temperature: $38 \pm 2^{\circ}\text{C}$

Ambient room mean temperature: $25.2 \pm 0.7^{\circ}\text{C}$

Average relative humidity: $53 \pm 18\%$

Photoperiod: Sixteen hours light per day

Lighting: fluorescent lights which closely approximate noon-day sunlight; average approximately 207 lux.

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the 9.1 ppm and six from the lowest and highest concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Two verification samples from the remaining concentrations and one from the control were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Negative control, 9.1, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm

Measured concentrations: <LOQ, 9.8, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm

Element value: Dietary LC₅₀ = 628 (448 - 958) ppm
 No mortality concentration 146 ppm
 NOEC (body weight gain) 36.6 ppm

All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102 - 108% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 94 - 130% of nominal.

Summary of analytical chemistry data

Homogeneity in Avian diet

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
9.1	9.52, 9.70, 9.79, 8.09, 10.9, 10.5	9.8	108
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9	19.6	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in avian diet

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	46.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293-	274, 307	291	99
586	550, 523	537	92

Ambient stability in avian diet

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	
9.8	12.3, 11.0	11.7	119
19.5	18.2, 19.7	19.0	97
40.2	47.9, 56.8	52.4	130
74.5	77.6, 77.9	77.8	104
174	167, 160	164	94.3
291	297, 293	295	101
537	552, 530	541	101
1196	1150, 1122	1136	95

Biological observations

Survival and clinical observations: No mortalities occurred in the control group, and all birds were normal in appearance and behavior throughout the test. The first deaths occurred on day 4 in the 1171 ppm treatment. Mortality occurred through Day 8 in all dose groups ≥ 293 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations ≤ 146 ppm. Birds at all concentrations ≥ 293 ppm displayed signs of toxicity including reduced reaction to stimuli (sound and motion), loss of coordination, ruffled appearance, lethargy and lower limb weakness. Birds at the 1171 ppm level also displayed prostrate posture, depression and convulsions through Day 8. Recovery with normal appearance and behavior was noted from Day 9 through test termination

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations ≤ 36.6 ppm. During the Day 8-15 and Day 15-22 post-exposure periods, body weight gain appeared comparable among all groups. There was a statistically significant ($p < 0.05$) reduction in weight gain at the 9.1 ppm level for the Day 0-5 and Day 5-8 periods. However, differences from the control group at the 9.1 ppm level appear to be due to a lower mean Day 0 body weight for the 9.1 ppm level, and were not dose responsive. Therefore, these differences were not considered treatment related. Marked, treatment-related, concentration responsive effects on body weight was noted in concentrations > 73.2 ppm for Days 0-5; Day 5-8 post-exposure weight gain continued to be reduced at concentrations ≥ 293 ppm.

Feed Consumption: When compared to the control group, there was a marked reduction in feed consumption in the treatment groups ≥ 293 ppm throughout the study.

Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, empty crops, and empty gastrointestinal tracts. These necropsy findings were considered to be treatment related.

Percent Cumulative Mortality

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
9.1	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0

73.2	0	0	0	0	0	0	0	0
146	0	0	0	0	0	0	0	0
293	0	0	0	0	0	0	10	20
586	0	0	0	0	10	20	30	30
1171	0	0	0	20	60	80	90	90

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

Bodyweight (grams)

Nominal Concentration, ppm	Exposure Period		Recovery Period				
	Mean Body Weight, Day 0	Mean Body Weight Change Day 0-5	Mean Body Weight Change Day 5-8	Mean Body Weight Change Day 8-15	Mean Body Weight Change Day 15-22	Mean Total Body Weight Change Day 8-22	Mean Body Weight Day 22
Negative Control	135	144	101	230	183	413	823
9.1	119	108	91	230	198	427	773
18.3	146	131	100	241	186	427	811
36.6	147	128	100	243	208	451	828
73.2	143	117	82	216	203	418	782
146	143	100	89	232	124	356	688
293	129	32	57	256	234	490	701
586	144	-6	36	221	219	439	613
1171	147	-37	1531	198	251	449	634

Mean average feed consumption

Nominal Concentration, ppm	Grams food/bird/day Days 0-5	Grams food/bird/day Days 6-8	Grams food/bird/day Days 8-15	Grams food/bird/day Days 15-22
Negative Control	92	125	171	180
9.1	73	117	172	198
18.3	91	132	186	204
36.6	94	125	165	179
73.2	77	101	148	173
146	105	159	159	164
293	44	63	109	132
586	36	55	114	143
1171	22	25	106	154

Gross pathological observations from Birds that died in study

Finding	293ppm N = 2	586ppm N = 3	1171ppm N = 9
Crop empty	0	2	7
Emaciated	1	1	4
G.I.Tract, primarily empty	1	1	1
Gizzard contents bile stained	0	2	4
Gizzard, empty	0	0	1
Intestinal contents, black and tar-like	0	0	1
Keel prominent	0	0	2

Kidneys, pale	0	0	1
Loss of muscle mass	2	2	5
Spleen, grey	0	0	1
Spleen, small and pale	1	1	2
Spleen, pale	0	2	3
Thin	1	1	4

CONCLUSIONS

The dietary LC₅₀ value for Mallard Duck exposed to PFOS was determined to be 628 ppm with a 95% confidence interval of 448 to 958 ppm. The slope of the concentration-response curve was 3.67 and the chi-square value was 2.13. The no mortality concentration was 146 ppm. Based upon reductions in body weight gain at the 73.2 ppm test concentration, the no observed effect concentration was 36.6 ppm.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

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

OTHER

Last changed: 5/1/00

Robust Study Report Reference No. 12 – Dietary LC₅₀ Study with the Northern Bobwhite quail

TEST SUBSTANCE

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

Remarks field: The test substance is a white powder. Sample was taken from 3M lot number 217. Sample was stored under ambient conditions prior to testing. Purity determined to be 90.49% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.2200, OECD 205, and FIFRA Subdivision E. Section 71-2

Type: Dietary acute

GLP: Yes

Year completed: 1999 (Test), 2000 (Report)

Species: *Colinus virginianus*

Supplier: Wildlife International Ltd. Production Flock, Easton, Maryland, USA

Analytical monitoring: Test substance concentration in standards and samples were determined by reversed-phase HPLC and mass spectroscopy. PFOS measured on Day 0 for homogeneity in feed and verification, and Day 5 for stability.

Test phases: Acclimation – 10 days
Exposure – 5 days
Post-exposure observation – 3 or 17 days

Statistical methods: LC₅₀ values calculated by probit analysis using the computer software of C.E. Stephan. Body weight data were compared by Dunnett's test using TOXSTAT software. No statistical analyses were applied to feed consumption data.

Test bird age: 10 days

Pretreatment: None

Test conditions:

Housing and environmental conditions: Indoors in batteries of thermostatically controlled brooding pens. Each pen's floor space measured approximately 72 x 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

Identification: Each group of birds Identified by pen number and test concentration. Individuals Identified by leg bands.

Number of replicates: Six for controls, two for each treatment group

Number of bobwhite per replicate: five

Number of concentrations: seven plus a negative control

Feed and water: Game bird ration formulated as below, water from the town of Easton public water supply. Both provided ad libitum during acclimation and testing.

Game Bird Ration

Ingredients	Percent
Fine Corn Meal	44.83
Soy Bean Meal, 48% Protein	30.65
Wheat Midds	6.50
Protein Base	6.00
Agway Special, 60% Protein	4.00
Alfalfa Meal, 20% Protein	3.00
Dried Whey	2.50
Ground Limestone	0.90
Eastman CalPhos	0.60
Methionine Premix + Liquid	0.35
Vitamin and Mineral Premix (see below)	0.32
GL Form (Fermatco) ¹	0.25
Salt iodized	0.10

¹Fermentation by-products (source of unidentified growth factors)

Vitamin and Mineral Premix

Vitamin or Mineral	Amount Per Ton
Vitamin D ₃	2,000,000 I.C.U.
Vitamin A	7,000,000 I.U.
Riboflavin	6 g
Niacin	40 g
Pantothenic Acid	10 g
Vitamin B ₁₂	8 mg
Folic Acid	600 mg
Biotin	64 mg
Pyridoxine	1.2 g
Thiamine	1.2 g
Vitamin E	20,000 I.U.
Vitamin K (Menadione dimethylpyrimidinol bisulfite)	5.8 g
Manganese	102 g
Zinc	47 g
Copper	6.8 g
Iodine	1.5 g
Iron	51 g
Selenium	182 g

Prophylaxis: None

Brooding compartment mean temperature: $38 \pm 2^{\circ}\text{C}$

Ambient room mean temperature: $27.3 \pm 1.2^{\circ}\text{C}$ -

Average relative humidity: $31 \pm 14\%$

Photoperiod: Sixteen hours light per day

Lighting: fluorescent lights which closely approximate noon-day sunlight; average of approximately 139 lux of illumination

Test diet preparation: Test substance mixed directly into the ration by means of a Hobart mixer. No

carrier was used.

Diet sampling: Homogeneity of the test substance in the diet evaluated by collecting six samples from the, 18.3 ppm concentration and six from the 1171 ppm concentration. Samples collected from the top, middle, and bottom of the left and right sections of the mixing vessel. These samples also served as the verification samples for these concentrations. Verification samples of the other treatment groups (two samples from each) and the control (one sample) were collected at preparation on Day 0. Stability samples were collected at the end of the exposure period (Day 5) from the control (one sample) and each treatment group (two samples each).

RESULTS

Nominal concentrations: Negative control, 18.3, 36.6, 73.2, 146, 293, 586, and 1171 ppm

Measured concentrations: <LOQ, 19.5, 40.2, 74.5, 174, 291, 537, and 1196 ppm

Element value: Dietary LC₅₀ = 220 (164 - 289) ppm
No mortality concentration = 73.2 ppm
NOEC (body weight gain) = 73.2 ppm

All element values based on nominal concentrations

Analytical Methodology: Diet samples were extracted with methanol. Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 1.15 ppm in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 94.7. Samples collected for determination of homogeneity in diet ranged from 102-107% of nominal. Samples collected for verification in diet had measured values from 92 to 119% of nominal. Measured values for ambient stability samples taken at Day 5 ranged from 101 – 122% of nominal.

Summary of analytical chemistry data

Homogeneity in avian diet

Nominal Test Concentration, ppm	Measured Values at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
18.3	18.5, 23.4, 18.3, 17.3, 19.4, 19.9	19.5	107
1171	1239, 1221, 1118, 1301, 1163, 1133	1196	102

Verification in avian diet

Nominal Test Concentration, ppm	Measured Duplicate Concentrations at Day 0, ppm	Mean Measured Concentration, ppm	Percent of Nominal
Negative Control	< LOQ	-	-
36.6	46.7, 34.6	40.2	110
73.2	77.8, 71.2	74.5	102
146	176, 172	174	119
293	274, 307	291	99
586	550, 523	537	92

Ambient stability in avian diet

Day 0 Mean Measured Concentration, ppm	Measured Duplicate Concentrations at Day 5, ppm	Mean Measured Concentration, ppm	Mean Percent of Day 0
Negative Control	< LOQ	-	
19.5	19.2, 19.9	19.6	101
40.2	44.4, 53.8	49.1	122
74.5	76.4, 77.9	77.2	104
174	177, 174	176	101
291	318, 315	317	109
537	560, 665	613	114
1196	1260, 1187	1224	102

Biological observations

Mortalities and clinical observations: One incidental mortality occurred in the control group as a result of a broken leg on the morning of Day 5. It was subsequently euthanized on Day 6. Two other birds in the control group were intermittently noted with foot lesions associated with cage mate aggression. Otherwise, all control birds were observed to be normal in appearance and behavior throughout the test.

The first treatment-related mortalities occurred on Day 3 in the 586 and 1171 ppm treatment groups. Mortality occurred through Day 8 in all dose groups ≥ 146 ppm with some of the deaths being during the post-exposure period. There were no treatment-related mortalities or overt signs of toxicity at concentrations ≤ 73.2 ppm.

There was 11 % mortality in the 146 ppm treatment group, and two additional birds displayed clinical signs of toxicity (wing droop). All other birds in this test group displayed normal appearance and behavior for the duration of the test. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 80% mortality (occurring on Days 5, 6, and 7) for birds in the 293 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, wing droop, loss of coordination, lower limb weakness and convulsions. Recovery with normal appearance and behavior occurred on Day 9 to test termination.

There was 100% mortality (occurring from Day 3 through Day 7) for birds in the 586 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, lower limb rigidity, prostrate posture, and convulsions.

There was 100% mortality (occurring from Day 2 (noted on Day 3 for Day 2 afternoon) through Day 4) for birds in the 1171 ppm treatment group. Signs of toxicity observed prior to death included a ruffled appearance, reduced reaction to stimuli (sound and motion), lethargy, depression, wing droop, loss of coordination, lower limb weakness, and lower limb rigidity.

Body weight gain: When compared to the control group, there were no apparent treatment related effects on body weight among the birds in concentrations ≤ 73.2 ppm. During Days 0-5 statistically significant reductions in body weight gain or body weight loss occurred in the 146, 293, and 586 ppm treatment groups. Body weight effects could not be determined for test

organisms in the 1171 ppm group due to total mortality.

Feed Consumption: No apparent treatment related effects were noted for feed consumption for birds in concentrations ≤ 146 ppm. Reduced feed consumption was noted for birds in treatment groups ≥ 293 ppm from Days 0-5. No treatment-related effects on feed consumption in any of the surviving treatment groups during the Day 6-8 post-exposure period were observed.

Gross Necropsy: All birds that died during the study, half of those surviving at Day 8 and the rest at test termination were subjected to a gross necropsy. Necropsy results for birds found dead were similar, including thin condition, loss of muscle mass, altered spleen color, autolysis of tissues and pale organs. These necropsy findings were considered to be treatment related. The single bird euthanized from the 293 ppm treatment was found to have treatment related necropsy findings. Necropsy results for all other birds euthanized on Day 8 and Day 22 were unremarkable.

% Cumulative Mortality

Nominal Concentration, ppm	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8*
Negative Control	0	0	0	0	0	0	0	0
18.3	0	0	0	0	0	0	0	0
36.6	0	0	0	0	0	0	0	0
73.2	0	0	0	0	0	0	0	0
146	0	0	10**	0	0	0	10	10
293	0	0	0	0	20	40	80	80
586	0	0	10	20	50	80	100	100
1171	0	0	30	100	100	100	100	100

*No mortalities occurred in any of the treatment levels from Day 8 to Day 22

**Bird euthanized on day 3 after sustaining a broken leg.

Body Weight (grams)

Nominal Concentration (ppm)	Exposure Period		Recovery Period				
	Mean Body Weight, Day 0	Mean Body Weight Change Day 0-5	Mean Body Weight Change Day 5-8	Mean Body Weight Change Day 8-15	Mean Body Weight Change Day 15-22	Mean Total Body Weight Change, Day 8-22	Mean Body Weight, Day 22
Negative Control	20	+10	+8	+23	+22	45	82
18.3	21	+11	+9	+24	+23	47	87
36.6	20	+11	+8	+26	+24	50	89
73.2	20	+9	+7	+24	+20	44	79
146	20	+7*	+6**	+24	+21	45	79
293	20	-2**	-1**	+14	+20	34	55
586	20	-4**	-	-	-	--	-
1171	20	-	-	-	-	-	-

Note: numbers may not add manually due to rounding. Values for 293 ppm treatment group are impacted by the fact that only one bird remained in that group after day 8.

*Statistically different from the control group at $p < 0.05$ (Dunnett's t-test)

**Statistically different from the control group at $p < 0.01$ (Dunnett's t-test)

(-) = no data available due to mortality

Mean average feed consumption

Nominal Concentration, ppm	Grams Feed/Bird/Day Days 0-5	Grams Feed/Bird/Day Days 6-8	Grams Feed/Bird/Day Days 8-15	Grams Feed/Bird/Day Days 15-22
Negative Control	9	10	9	13
18.3	9	11	10	12
36.6	8	12	14	15
73.2	10	13	13	15
146	9	10	11	14
293	5	9	8	-
586	6	19	-	-
1171	4	-	-	-

(-) = No data available due to mortality.

Gross Pathological Observations from Birds that Died In Study

Finding	Male, Female, and Undetermined (ppm)				
	Control N = 1	146 N = 2	293 N = 8	586 N = 10	1171 N = 10
Abdominal cavity, some autolysis	0	0	2	2	4
Abdominal cavity, autolysis throughout	0	0	0	1	1
Crop, empty	0	0	2	5	2
Emaciated	0	0	2	5	8
Fractured leg	1	1	0	0	0
G.I.Tract, empty	0	0	1	1	0
Gizzard contents bile stained	0	0	2	5	1
Heart, anterior portion mottled white color	0	0	1	0	0
Heart, pale .	0	0	0	2	1
Intestinal contents tar-like	0	0	0	2	0
Keel, prominent	0	0	1	3	10
Kidneys, pale	0	0	0	2	0
Liver, pale and mottled	0	1	0	0	0
Loss of muscle mass	0	0	4	7	9
Muscular-skeletal, pale	0	1	0	0	0
Small in stature	0	0	3	0	0
Spleen, black	0	0	0	1	0
Spleen, dark	0	0	0	0	2
Spleen, grey	0	0	0	1	0
Spleen, grey-brown	0	0	0	0	1
Spleen, pale	0	0	1	0	1
Spleen, small	0	0	0	0	1
Spleen, small and pale	0	0	0	3	0
Thin	0	0	0	4	2
Not remarkable	0	0	1	0	0

CONCLUSIONS

The dietary LC₅₀ value for Northern Bobwhite exposed to perfluorooctanesulfonate was determined to be 220 ppm with a 95% confidence Interval of 164 to 289 ppm. The slope of the concentration response curve was 7.005 and the chi-square value was 0.023. The no mortality concentration was 73.2 ppm. Based upon treatment related mortality, signs of toxicity and effects upon body weight gain at the 146 ppm test concentration, the no observed effect concentration was 73.2 ppm.

Author and/or submitter: 3M Corporation, Environmental Laboratory, P.O. Box 33331, St.-Paul, Minnesota, 55133, USA

DATA QUALITY

Reliability: Klimisch ranking = 1.

REFERENCES

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

OTHER

Last changed: 5/3/00

Robust Report Reference No. 13 - Multi-Phase Exposure / Recovery Algal Assay Test**TEST SUBSTANCE**

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

Remarks field: The test substance is a white powder (3M Lot 583) of uncharacterized purity. The following summary is abbreviated due to the fact that this study has been superseded by a more recent test.

METHOD

Method: Test protocol utilized was modified after those described by USEPA - 600/9-78-018, 1978; ASTM-E-35.23, 1981; OECD 201,1979. ASTM STP #667

Type: Semi-chronic

GLP: No

Year completed: 1981

Species: *Selenastrum capricornutum*

RESULTS

EC₅₀ Values, mg/L (95% confidence interval is given in brackets)

Exposure (contact) Days	Cell-Dry Weight	Cell-Count
4	115 (18 - 65)	82 (No Conf. Limits)
7	122 (41 - 366)	99 (19 - 398)
10	128 (46 - 372)	98 (16 - 431)
14	146 (33 - 350)	95 (12 - 455)

Remarks: The statistical program used was questionable.

DATA QUALITY

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

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 1981.

OTHER

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

Last changed: 5/3/00

Robust Study Report Reference No. 14 - The Effects of Continuous Aqueous Exposure to 14C-78.02 on Hatchability of Eggs and Growth and Survival of Fry of Fathead Minnow (*Pimephales promelas* / Summary of histopathological examinations of Fathead Minnow (*Pimephales promelas*) exposed to 78.02 for 30 Days

TEST SUBSTANCE

Identity: Potassium perfluorooctanesulfonate; may also be referred to as 14C-78.02, PFOS or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test substance is a white powder. Sample was radiolabeled. Sample purity was not characterized.

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

METHOD

Method: Method was developed by E G & G, Bionomic and closely followed those presented in the "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish", U.S. EPA, 1972.

Type: Flow-through chronic

GLP: No

Year completed: 1978

Species: *Pimephales promelas*

RESULTS

30-Day NOEC: 1 mg/L

30-Day LOEC: 1.9 mg/L

30-Day MATC: >1 mg/L and <1.9 mg/L

DATA QUALITY

Reliability: Klimisch ranking = 2. This study satisfied criteria for quality testing at the time performed, but the analytical methodology was questionable.

REFERENCES

This study was conducted at E G & G, Bionomics, Aquatic Toxicology Laboratory in Wareham, Massachusetts at the request of the 3M Company.

OTHER

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

Last changed: 5/3/00

Robust Study Report Reference No. 15 - Effect of Potassium Perfluorooctanesulfonate on Survival, etc. (Daphnid reproduction)**TEST SUBSTANCE**

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

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

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

METHOD

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

Type: Acute static and Chronic renewal

GLP: No

Year completed: 1984

Species: *Daphnia magna*

RESULTS (95% confidence interval is given in brackets)

48-hour EC₅₀ (immobilization): 27 (25-28) mg/L
 14-day EC₅₀ (reproduction)*: 14.7(12-18) mg/L
 21-day EC₅₀ (reproduction)*: 12.4(11-14) mg/L
 28-day EC₅₀ (reproduction)*: 11.4(10-13) mg/L
 28-day NOEC**: 7 mg/L
 14, 21 and 28-day MATC: 11.2 mg/L

*Cumulative (Young/Adult)

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

DATA QUALITY

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

REFERENCES

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

OTHER

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

Last changed: 5/3/00

**Robust Study Report Reference No. 16 - *Pimephales promelas* 96-hour Toxicity Test Data Summary.
Sample FC-94-X (Li salt of PFOS)**

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Lithium salt; may also be referred to as PFOS Li salt, FC-94, or FC-94-X. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, lithium salt, CAS # 29457-72-5)

Remarks: Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Not noted.

Type: Static acute

GLP: No

Year completed: 1994

Species: *Pimephales promelas*

Supplier: Aquatic Biosystems Inc., Fort Collins, CO, USA

Analytical monitoring: pH and DO content

Exposure period: 96-hours

Statistical methods: LC₅₀ values calculated by Trimmed Spearman - Karber.

Test fish age: 79 days.

Length and weight: Average length = 2.1 + 0.3cm Average weight = 0.069 ± 0.03 g

Loading: 0.69 g fish / L

Pretreatment: None

Test Conditions

Dilution water: Carbon filtered well water

Dilution water chemistry:

pH: 8.4

DO: 8.1 mg/L

Stock and test solution preparation: A primary stock solution was prepared in dilution water to yield a test sample concentration of 400 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water to make 1 L of solution per concentration.

Stability of the test chemical solutions: Not noted.

Exposure vessels: 2 L glass beakers

Number of replicates: two.

Number of fish per replicate: ten

Number of concentrations: six plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

6.0-7.2 mg/L (control exposure)

4.8-7.9 mg/L (56.0 mg/L exposure)

pH range (0 – 96 hours):

8.0-8.4 (control exposure)

8.0-8.4 (56.0 mg/L exposure)

Test temperature range (0 – 96 hours):

19.2-19.5°C

RESULTS

Nominal concentrations: Negative control, 3.2, 5.6, 10.0, 18.0, 32.0, 56.0 mg/L

Element value (95% confidence interval is given in brackets):

96-hour LC_{50} = 19 mg/L (16-24)

Mortality of controls: None

Remarks: Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

CONCLUSIONS

The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 96-hour LC_{50} for fathead minnow of 19 mg/L. This value must be divided by 4 in order to express the result in terms of the concentration of PFOS.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 3/25/94.

OTHER

Last changed: 7/9/01

Robust Study Report Reference No. 17 - 48-hour Acute Toxicity to Daphnia, *Daphnia magna*. FC-94-X (Li salt of PFOS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Lithium salt; may also be referred to as PFOS Li salt, FC-94, or FC-94-X. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, lithium salt, CAS # 29457-72-5)

Remarks: The test sample is a mixture of the test substance in water (approximately 24.5% test substance and 75.5% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Not noted.

Test type: Static acute

GLP: No

Year completed: 1994

Species: *Daphnia magna*

Analytical monitoring: pH and DO content

Statistical methods: EC₅₀ values calculated using Trimmed Spearman-Kärber method

Test daphnid source: Obtained from U.S. EPA-NETAC, Duluth, Minnesota, USA.

Test daphnid age at study initiation: < 24-hours

Test Conditions

Dilution water: Carbon-filtered well water

Dilution water chemistry:

pH: 8.4

DO: 8.6 mg/L

Stock and test solutions preparation: A primary stock solution was prepared in dilution water to yield a test sample concentration of 1000 mg/L. All test solutions were made by diluting the appropriate amount of stock solution with dilution water to make 50 mL of solution per concentration.

Exposure vessels: 100 mL glass beakers containing 50 mL of test solution.

Number of replicates: 4

Number of daphnids per replicate: 5

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen at test termination:

7.0 mg/L (control exposure)

7.8 mg/L (1000 mg/L exposure)

pH at test termination:

8.6 (control exposure)

8.6 (1000 mg/L exposure)

Test temperature range (0 – 48 hours) 20.1-21.0 C

Element basis: mortality and immobilization

RESULTS

Nominal concentrations: Negative control, 100, 180, 320, 560, 1000 mg/L

Element value: (95% confidence interval is given in brackets)

24-hour EC_{50} = 330 (290-370) mg/L

48-hour EC_{50} = 210 (190-230) mg/L

48-hour NOEC = 100 mg/L

Statistical Evaluation: The EC_{50} values and 95% confidence intervals were calculated using the Trimmed Spearman-Kärber method with trim set to 0%.

Mortality of controls: None

Remarks: Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

CONCLUSIONS

The test sample containing 24.5% Perfluorooctanesulfonate, Lithium salt exhibited a 48-hour EC_{50} for *Daphnia magna* of 210 mg/L. This value must be divided by 4 in order to express the result in terms of the concentration of PFOS.

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

DATA QUALITY

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for: determination of the test substance concentration in the test solutions; and determination of the sample purity.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, Lab Request number M1018, 2/10/94.

OTHER

Last changed: 7/9/01

Robust Study Report Reference No. 20 - 96-hour Acute Toxicity Test on Bluegill Sunfish (FC-99, DEA salt of PFOS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, DEA salt; may also be referred to as PFOS DEA salt, FC-99, or 3M Sample No. 2. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1), CAS # 70225-14-8)

Remarks: Test sample is a mixture of the test substance in water (approximately 25% test substance and 75% water). All values reported relate to this mixture. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. Standard Methods.

Type: Static acute

GLP: Yes

Year completed: 1979

Species: *Lepomis macrochirus*

Supplier: Osage Catfisheries, Inc. in Osage Beach, Missouri, USA.

Analytical monitoring: pH and DO / ammonia content

Exposure period: 96-hours

Statistical methods: Probit analysis.

Test fish age: Not noted.

Length and weight: Average length = 28.6 ± 2.17 mm.
Average weight = 0.60 ± 0.15 g

Loading: 0.2 g fish / L

Pretreatment: None

Test Conditions

Dilution water: Laboratory well water

Dilution water chemistry:

Dissolved oxygen: 9.3 mg/L

Hardness: 255 mg/L as CaCO₃

Alkalinity: 368 mg/L as CaCO₃

pH: 7.8

Conductivity: 50 µmhos/cm

Stock and test solution preparation: Primary stock prepared in deionized water at a concentration of 150 mg/mL. The test concentrations were prepared by transferring appropriate aliquots of the stock standard directly to the test chambers. The test solutions were noted to foam when stirring in toxicant aliquots. Test concentrations were prepared based on total sample, not on percent concentration of the test substance in the test sample.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted

Exposure vessels: 40 liter glass aquaria containing 30L of test solution.

Number of replicates: one

Number of fish per replicate: ten

Number of concentrations: six plus a negative control

Water chemistry during the study:**Dissolved oxygen range** (0 – 96 hours):

6.0 - 8.4 mg/L (control exposure)

5.8 - 8.3 mg/L (18 mg/L exposure)

pH range (0 – 96 hours):

8.2 - 8.3 (control exposure)

8.3 - 8.3 (18 mg/L exposure)

Test temperature: Temperature held constant at 22°C through use of a water bath for test vessels.**RESULTS****Nominal concentrations:** Negative control, 18, 37, 75, 160, 320, 650 mg/L**Element value:** (95% confidence interval is given in brackets)24-hour LC₅₀ = 460 (370-580) mg/L48-hour LC₅₀ = 370 (290-470) mg/L96-hour LC₅₀ = 31 (22-43) mg/L

96-hour NOEC = 18 mg/L (C.I. not calculated)

All element values based on nominal concentrations

Statistical evaluation of mortality:Probit analysis was used to calculate LC₅₀ values and the corresponding confidence limits.**Quality Check for Test Organism Health:**

The bluegill sunfish were challenged with a reference compound, Antimycin A. The observed 96-hour LC₅₀ and 95% confidence limits (C.I.) were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition.

Cumulative percent mortality:

Nominal Test Concentration mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
18	0	0	0	0
37	0	0	10	80
75	0	0	30	90
160	0	0	70	100
320	0	20	100	100
650	100	100	100	100

CONCLUSIONS

The test sample 96-hour LC₅₀ for bluegill sunfish was determined to be 31 mg/L with a 95% confidence interval of 22-43 mg/L. The 96-hour no observed effect concentration was 18 mg/L. These values must be divided by 4 in order to express the results in terms of the concentration of PFOS.

Submitter: 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN 55133, USA.**DATA QUALITY**

Reliability: Klimisch ranking 2. This study, while well conducted, lacks analytical data for determination of the test substance concentration in the test solutions and determination of the sample purity. There were

also gaps in the measurement of water quality criteria for a number of the concentrations at given time intervals.

REFERENCES

This study was conducted by Analytical BioChemistry Laboratories, Inc. of Columbia, Missouri on behalf of the 3M Company.

OTHER

Last changed: 5/2/00

Robust Study Report Reference No. 23 – Acute toxicity to Aquatic Invertebrates (e.g. Daphnia)**TEST SUBSTANCE**

Identity: Potassium perfluorooctanesulfonate; may also be referred to as PFOS or FC-95 or as part of the mixed product FM-3820 (see Remarks). (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: The test sample is FM-3820, a mixture of the test substance in diethylene glycol butyl ether and water (approximately 24-28% test substance in diethylene glycol butyl ether and water). Calculations were made to adjust test values using the upper limit concentration of the test substance (28%) in the test sample and no adjustment was made for the presence of the diethylene glycol butyl ether or water when noted below. These calculations assumed that all toxicity was due to the presence of the Perfluorooctanesulfonate substance.

METHOD

Method: OECD 202

Test type: Static acute

GLP: Yes

Year completed: 1991

Species: *Daphnia magna*

Analytical monitoring: DO, pH, Conductivity, and temperature were monitored daily.

Statistical methods: EC₅₀ values calculated, when possible by standard statistical techniques (Stephan, 1983)

Test daphnid source: Obtained from cultures maintained by EnviroSystems Division, Resource Analysts, Inc., Hampton, NH, USA.

Test daphnid age at study initiation: < 24-hours

Test Conditions

Dilution water: Well water from wells at EnviroSystems in Hampton, New Hampshire.

Dilution water chemistry:

pH: 7.8*

Conductivity: 1200 µmhos/cm*

TOC: <2.0 mg/L

*Values measured at time of test.

Lighting: Cool white fluorescent lights, intensity 23 µE/s/m². Photoperiod of 16-hours light, 8-hours dark. No transition period noted.

Stock and test solutions preparation: A primary stock solution was prepared in dilution water at 1000 mg/L. The primary stock was proportionally diluted with dilution water to prepare the five test concentrations.

Exposure vessels: 250 mL plastic beakers containing 200 mL of test solution. The approximate depth of test solution was 6 cm.

Number of replicates: Four

Number of daphnids per replicate: Five

Number of concentrations: Five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 48 hours):

8.2 – 8.5 mg/L (control exposure)

8.1 – 8.5 mg/L (150 mg/L exposure)

pH range (0 – 48 hours):

7.8 – 8.6 (control exposure)

7.8 – 8.6 (150 mg/L exposure)

Test temperature range (0 – 48 hours):

20.8 – 21.0°C (control exposure)

20.7 – 20.9°C (150 mg/L exposure)

Conductivity range (0 – 48 hours):

1200 – 1300 µmhos/cm (control exposure)

1200 – 1300 µmhos/cm (150 mg/L exposure)

Element basis: mortalityRESULTS

Nominal concentrations: Negative control, 25, 40, 60, 100, 150 mg/L**Element values:** 24-hour EC₅₀ = >150 mg/L (C.I. not calculable)48-hour EC₅₀ = 49 (43-56) mg/L**Perfluorooctanesulfonate concentration adjusted element value:**24-hour EC₅₀ = >42 mg/L48-hour EC₅₀ = 14 mg/L

All element values based on nominal concentrations

Biological observations: Ninety five percent survival occurred in the control exposure. The number of surviving organisms and the occurrence of sub-lethal effects and immobilization or other sublethal effects were determined visually and recorded initially and after 24 and 48 hours.**Cumulative percent mortality:**

Nominal Test Concentration mg/L	24-hours	48-hours
Neg. Control	0	5
25	0	0
40	5	25
60	0	25
100	0	100
150	20	100

Control response: SatisfactoryCONCLUSIONS

The test substance 48-hour EC₅₀ for *Daphnia magna* was determined to be 49 mg/L with a 95% confidence interval of 43-56 mg/L. If you assume all toxicity of the mixture is due to the Perfluorooctanesulfonate, the adjusted 48-hour EC₅₀ value is 14 mg/L (49 mg/L x 0.28).

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

Reliability: Klimisch ranking = 3. The study lacks analytical measurement of test substance

concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data are for a mixture and toxicity cannot be positively attributed to PFOS as the diethylene glycol butyl ether could also contribute to the toxicity. The basic water quality parameters (hardness, alkalinity and calcium/magnesium ratio) were not included in the final report.

REFERENCES

This study was conducted at EnviroSystems Division, Resource Analysts, Incorporated, Hampton, NH, USA at the request of the 3M Company.

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 26 – Acute toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH₄⁺ salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, ammonium salt, CAS # 29081-56-9)

Remarks: Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Not noted.

Type: Static acute

GLP: No

Year completed: 1974

Species: *Pimephales promelas*

Supplier: Not noted.

Analytical monitoring: pH and DO content

Exposure period: 96-hours

Statistical methods: Plotted LC₅₀

Test fish age: Not noted.

Length and weight: Average length = 2 inches, Average weight = 1.5 g

Loading: Not noted.

Pretreatment: Not noted

Test Conditions

Dilution water: carbon filtered city of St. Paul, MN water

Dilution water chemistry: Not noted.

Stock and test solution preparation: Not noted.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted.

Exposure vessels: Not noted.

Number of replicates: One.

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

5.0-5.9 mg/L (control exposure)

4.2-5.0 mg/L (100 mg/L exposure)

pH range (0 – 96 hours):

7.0-7.1 (control exposure)

7.0-7.2 7.0-7.1 (100 mg/L exposure)

Test temperature range (0 – 96 hours):

21 – 22°C (70-72°F)

RESULTS

Nominal concentrations: Negative control, 10, 25, 50, 75, 100 mg/L

Element value: 96-hour LC_{50} = 85 mg/L (C.I. not determined)

Mortality of controls: None

Remarks: 95% confidence limits were not calculated for this material. Additionally, testing was conducted on the mixture of the test substance in 20% isopropanol and 55% water. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvent or what portion of the toxicity can be contributed to the Perfluorooctanesulfonate, ammonium salt.

CONCLUSIONS

The test sample containing 25% Perfluorooctanesulfonate, ammonium salt exhibited a 96-hour LC_{50} for fathead minnow of 85 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 3.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 7/29/74 to 8/2/74.

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 27 – Acute toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate, Ammonium salt; may also be referred to as PFOS NH₄⁺ salt or FC-93. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, ammonium salt, CAS # 29081-56-9)

Remarks: Test sample was taken from 3M production lot #1. The test sample is a mixture of the test substance in isopropanol and water (25% test substance, 20% isopropanol, 55% water). No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: Not noted.

Type: Static acute

GLP: No

Year completed: 1974

Species: *Pimephales promelas*

Supplier: Not noted.

Analytical monitoring: pH and DO content

Exposure period: 96-hours

Statistical methods: Plotted LC₅₀.

Test fish age: Not noted.

Length and weight: Average length = 2 inches, Average weight = 1.5 g

Loading: Not noted.

Pretreatment: Not noted

Test Conditions

Dilution water: Carbon filtered city of St. Paul, MN water

Dilution water chemistry: Not noted.

Stock and test solution preparation: Not noted.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted.

Exposure vessels: Not noted.

Number of replicates: One.

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

4.5-5.7 mg/L (control exposure)

3.8-5.0 mg/L (125 mg/L exposure)

Not recorded at highest conc. (150 mg/L) due to 100% mortality.

pH range (0 – 96 hours):

7.0-7.0 (control exposure)

7.0-7.0 (125 mg/L exposure)

Not recorded at highest conc. (150 mg/L) due to 100% mortality.

Test temperature range (0 – 96 hours):

20 – 21°C (69-70 °F)

RESULTS

Nominal concentrations: Negative control, 50, 75, 100, 125, 150 mg/L

Element value: 96-hour LC_{50} = 100 mg/L (C.I. not determined)

Mortality of controls: None

Remarks: 95% confidence limits were not calculated for this material. Additionally, testing was conducted on the mixture of the test substance in 20% isopropanol and 55% water. The value reported applies to that mixture and not the test substance. No attempt was made to determine the impact of the presence of the organic solvent or what portion of the toxicity can be contributed to the Perfluorooctanesulfonate, ammonium salt.

CONCLUSIONS

The test sample containing 25% Perfluorooctanesulfonate, ammonium salt exhibited a 96-hour LC_{50} for fathead minnow of 100 mg/L

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

DATA QUALITY

Reliability: Klimisch ranking 3.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, 10/15/74 to 10/19/74.

OTHER

Last changed: 5/3/00

Robust Study Report Reference No. 28 – Acute toxicity of P3025 Developmental Material to Fathead minnow (*Pimephales promelas*)

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

Remarks: The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: OECD 203

Type: Static acute

GLP: No

Year completed: 1996

Species: *Pimephales promelas*

Supplier: Not noted.

Analytical monitoring: DO, pH, temperature, and conductivity were monitored daily.

Exposure period: 96-hours

Statistical methods: LL₅₀ values calculated using the Trimmed Spearman-Kärber method. The NOEL was calculated using Fisher's Exact tests.

Test fish age: Not given.

Length and weight: Average length = 11.3 mm, Average weight = 7.8 mg

Loading: 0.26 g/L

Pretreatment: None

Test Conditions

Dilution water: Dechlorinated City of Duluth, MN tap water. Water was aerated for 24-hours prior to use in the test.

Dilution water chemistry:

Hardness: 48 mg/L as CaCO₃

pH: 8.08

Lighting: Cool-white fluorescent bulbs. Photoperiod of 16-hours light, 8-hours dark used. No transition period noted.

Stock and test solution preparation: Water accommodated fractions. Test solutions were prepared individually for each test replicate concentration by mass addition of vigorously shaken test substance in 4 L of dilution water. The solutions were vigorously stirred for 21-hours (vortex 1/2 to 1/3 solution depth). The aqueous phase was siphoned from the vessel at mid-depth.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Not noted.

Exposure vessels: 4-L glass jars containing 3-L of test solution. The jars were sealed with Teflon-lined lids fitted with stoppers to accommodate oxygen flushing of headspace.

Number of replicates: two

Number of fish per replicate: ten

Number of concentrations: three plus a negative control

Water chemistry during the study:

Dissolved oxygen range: (0 – 96 hours):

9.1 – 14.6 mg/L (control exposure)

8.7 – 18.2 mg/L (700 mg/L exposure)

pH range: (0 – 96 hours):

7.80 – 8.08 (control exposure)

7.78 – 7.99 (700 mg/L exposure)

Test temperature range (0 – 96 hours): 20.8 – 20.9°C

Conductivity range (0 – 96 hours):

128 – 142 µmhos/cm (control exposure)

118 – 154 µmhos/cm (700 mg/L exposure)

Remarks: Oxygen was added to the headspace in the jars before sealing initially and at each observation period. The dissolved oxygen concentrations were super-saturated in the test vessels, particularly in the 700 mg/L exposure concentration.

RESULTS

Nominal loading concentrations: Negative control, 400, 700, 1,000 mg/L.

Element value: (95% confidence interval is given in brackets)

24-hour LL₅₀ = 618 (568 - 673) mg/L

48-hour LL₅₀ = 607 (554 - 664) mg/L

72-hour LL₅₀ = 595 (551 - 643) mg/L

96-hour LL₅₀ = 562 (523 - 604) mg/L

96-hour NOEL = <490 mg/L

All element values based on nominal concentrations.

Biological observations after 96-hours: No mortality or abnormal behavior observed in the negative control during the test. Mortality was observed in the remaining exposure concentrations. Surfacing was observed in half of the fish at the 700 mg/L exposure concentration at 24-hours, and 2 fish were quiescent at 96-hours. No abnormal behavior was observed in the 400 mg/L exposure concentration.

Cumulative percent mortality:

Nominal Loading Test Concentration, mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
490	10	15	15	25
700	75	75	80	90
1,000	100	100	100	100

Lowest concentration causing 100% mortality: 1,000 mg/L

Mortality of controls: None

Remarks: Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.

CONCLUSIONS

The test sample 96-hour LL₅₀ for fathead minnow was determined to be 562 mg/L with a 95% confidence interval of 523 – 604 mg/L. The 96-hour no observed effects level (NOEL) was <490 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone. Also, supersaturation of the test solutions with oxygen could also have contributed to the toxicity.

REFERENCES

This study was conducted at AScI Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

Last changed: 5/24/00

Robust Study Report Reference No. 29 – Acute toxicity of P3025 Developmental Material *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, didecyldimethylammonium salt; may also be referred to as Fluoroalkyl ammonium derivative. [1-Decaminium, N-decyl-N,N-dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid (1:1), CAS # 251099-16-8]

Remarks: The 3M production lot number was Lot 1. The test sample is L-14394 referred to by the test laboratory as P3025. The sample was labeled F-11615, Lot 1. The test sample is a mixture of the test substance in water (approximately 30-40% test substance, 60-70% water, and 0-5% of residual perfluorochemicals). All values reported relate to this mixture. The test sample appears to be a 2-phase dispersion (clear liquid with opaque solid) which rapidly separates after agitation. No calculations were made to adjust for the actual concentration of the test substance in the test sample.

METHOD

Method: OECD 202

Test type: Static acute

GLP: No

Year Completed: 1996

Species: *Daphnia magna*

Analytical monitoring: DO, pH, temperature and conductivity were monitored daily.

Statistical methods: EL₅₀ values calculated using Trimmed Spearman-Kärber method. NOEL value calculated using Steel's Many-One Rank test.

Test daphnid source: Obtained from cultures maintained by ASci Corporation, Duluth, MN.

Test daphnid age at study initiation: < 24-hours

Test Conditions:

Dilution water: Dechlorinated City of Duluth, MN tap water. Water was aerated for 24-hours prior to use in the test.

Dilution water chemistry:

Hardness: 44 mg/L as CaCO₃

pH: 8.04

Lighting: Cool-white fluorescent bulbs. Photoperiod of 16-hours light, 8-hours dark. No transition period noted.

Stock and test solutions preparation: Water-accommodated fractions. Test solutions were prepared individually for each concentration by mass addition of vigorously shaken test substance in 1 L of dilution water. The solutions were vigorously stirred for 23-hours (vortex 1/2 to 1/3 solution depth). The aqueous phase was siphoned from the vessel at mid-depth after settling for 1-hour.

Exposure vessels: 250 mL borosilicate glass beakers containing 200 mL of test solution. The solutions were kept covered during the test.

Number of replicates: Four

Number of daphnids per replicate: Five

Number of concentrations: Five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 48 hours):

8.6 – 9.1 mg/L (control exposure)

8.0 – 8.8 mg/L (50 mg/L exposure)

pH range (0 – 48 hours):

8.04 – 8.11 (control exposure)

7.92 – 8.00 (50 mg/L exposure)

Test temperature range (0 – 48 hours):

20.9 – 21.0°C

Conductivity range (0 – 48 hours):

142 – 155 µmhos/cm (control exposure)

120 – 124 µmhos/cm (50 mg/L exposure)

Element basis: mortality and immobilizationRESULTS

Nominal loading concentrations: Negative control, 3.13, 6.25, 12.5, 25, 50 mg/L**Element value:** (95% confidence interval is given in brackets)24-hour EL_{50} = 27.0 (18.7-39.0) mg/L48-hour EL_{50} = 11.3 (9.6-13.2) mg/L

48-hour NOEL = 6.25 mg/L

All element values based on nominal concentrations.

Statistical Evaluation: The EL_{50} values and 95% confidence intervals were calculated by the Trimmed Spearman-Kärber method. The NOEL was calculated using Steel's Many-One Rank test using the TOXSTAT statistical software Version 3.2, University of Wyoming.**Biological observations:** Daphnids in the negative control, and the 3.13 and 6.25 mg/L treatments appeared healthy and normal throughout the test with no mortality, immobility or overt clinical signs of toxicity. The effects noted in this study were mortality; no immobilization was noted at any test concentration. The number of surviving organisms were determined visually and recorded initially and after 24 and 48 hours.**Cumulative percent mortality:**

Nominal Loading Test Concentration mg/L	24-hours	48-hours
Negative Control	0	0
3.13	0	0
6.25	0	0
12.5	5	70
25	50	95
50	75	100

Control response: satisfactory**Remarks:** Values reported are for the test sample. No calculations were made to adjust for the concentration of the test substance in the test sample.CONCLUSIONS

The test substance 48-hour EL_{50} for *Daphnia magna* was determined to be 11.3 mg/L with a 95% confidence interval of 9.6-13.2 mg/L. The 48-hour no observed effect level (NOEL) was 6.25 mg/L.**Submitter:** 3M Company, Environmental Laboratory P.O. Box 33331 St. Paul, MN, 55133, USA.

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, data is for a mixture and toxicity cannot be positively attributed to didecyldimethylammonium Perfluorooctylsulfonate salt alone.

REFERENCES

This study was conducted at ASci Corporation, Environmental Testing Division, Duluth, MN, at the request of the 3M Company.

OTHER

Last changed: 5/24/00

Robust Study Report Reference No. 30 - Acute toxicity of PFOS to Rainbow trout in saltwater

TEST SUBSTANCE

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

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

METHOD

Method: Standard procedures for Testing Acute Lethality of Liquid Effluents (Environment Canada, 1980)

Type: Acute static, renewal after 48-hours

GLP: No

Year completed: 1985

Species: Rainbow Trout, *Salmo gairdneri*

Fish source: Rainbow Springs, Thamesford

Fish age at test initiation: not noted

Fish acclimation to salt water: fish gradually acclimated to increasing salinity; held at 30 parts per thousand salinity 8 days prior to test initiation.

Exposure period: 96-hours

Analytical monitoring: Dissolved oxygen, pH, conductivity

Statistical methods: Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC₅₀.

Test conditions:

Dilution water: Mississauga dechlorinated tap water amended with calcium, magnesium, sodium, and chloride to obtain 30 parts per thousand salinity

Dilution water chemistry (initial):

pH: 7.4 - 8.0

D.O.: 8.6 – 9.0 mg/L

Conductivity: > 20,000 µmhos/cm

Stock solution preparation: 1000 mg/L

Exposure vessels: Not noted; solution volume 35 L

Number of replicates: 2 tests – run 4 days apart, not replicated

Number of organisms/vessel: 6

Loading: 0.75 g/L

Number of concentrations: 4 plus a blank control

Water chemistry during the studies:

Dissolved oxygen ranges

8.1 – 10.3 (control)

8.8 – 10.1 (30 mg/L)

pH ranges

7.6 – 8.2 (control)

7.3 – 8.0 (30 mg/L)

Test temperature (0 – 48 hours): 15°C

Photoperiod: 12-hours light, 12-hours dark

Element basis: mortality

RESULTS

Nominal concentrations: 5, 10, 20, 30 mg/L

Element values (95% confidence interval) calculated per replicate:

96-hour LC₅₀ = 13.7 (10.7 – 17.7) mg/L

96-hour LC₅₀ = 13.7 (10.7 – 17.8) mg/L

Mortality of controls: 17% (1/6 in both studies)

DATA QUALITY

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

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 7/19/01

Robust Study Report Reference No. 31 - Acute toxicity of PFOS to Rainbow trout in freshwater

TEST SUBSTANCE

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

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

METHOD

Method: Standard procedures for Testing Acute Lethality of Liquid Effluents (Environment Canada, 1980)

Type: Acute, renewal after 48-hours

GLP: No

Year completed: 1985

Species: Rainbow Trout

Fish source: Rainbow Springs, Thamesford

Fish age at test initiation: not noted

Exposure period: 96-hours

Analytical monitoring: Dissolved oxygen, pH, conductivity

Statistical methods: Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC₅₀.

Test conditions:

Dilution water: Mississauga dechlorinated tap water

Dilution water chemistry (initial):

pH: 7.5 - 8.5

D.O.: 9.0 – 10.4 mg/L

Stock solution preparation: 1000 mg/L; noted as cloudy

Exposure vessels: Not noted; solution volume 35 L

Number of replicates: 2 tests – run one week apart, not replicated

Number of organisms/vessel: 6

Loading: 0.72 g/L

Number of concentrations: 5 plus a blank control, 4 plus a blank control

Water chemistry during the studies:

Dissolved oxygen ranges

8.2 – 10.4 (control)

8.0 – 9.5 (30 mg/L)

pH ranges

7.4 – 8.3 (control)

7.5 – 8.7 (30 mg/L)

Conductivity range:

270 – 380 µmhos/cm

Test temperature (0 – 48 hours): 15°C

Element basis: mortality

RESULTS

Element values (95% confidence interval) calculated per replicate:

96-hour LC₅₀ = 7.8 (6.2 – 9.8) mg/L

96-hour LC_{50} = 9.9 (7.5 – 13.4) mg/L

Mortality of controls: None

DATA QUALITY

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

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 7/19/01

Robust Study Report Reference No. 32 - Acute toxicity of PFOS to *Artemia* sp.

TEST SUBSTANCE

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

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

METHOD

Method: Draft International Standards Organization (Vanhaecke and Persoone, 1981)

Type: Acute static

GLP: No

Year completed: 1985

Species: *Artemia* sp.

Artemia source: Salt lake Brine Shrimp Inc.

Artemia age at test initiation: naupuli < 24 hours old.

Exposure period: 48-hours

Analytical monitoring: Dissolved oxygen, pH, conductivity

Statistical methods: Not noted. Element values were calculated for each replicate series, but not combined for the whole study. A cumulative mortality-concentration plot was used to estimate the LC₅₀.

Test conditions:

Dilution water: 30 parts per thousand NaCl solution

Dilution water chemistry (initial):

pH: 8.0 – 8.2

D.O.: > 6 mg/L

Stock solution preparation: 1000 mg/L; noted as cloudy

Exposure vessels: Not noted; solution volume 10 mL

Number of replicates: 3

Number of organisms/replicate: 10

Number of concentrations: 6 plus a blank control

Water chemistry during the study:

Dissolved oxygen ranges (test and control):

> 6.0 mg/L

pH (test and control)

8.0 – 8.2

Test temperature range (0 – 48 hours): 21 - 21°C

Element basis: mortality

RESULTS

Nominal concentrations: 1, 2, 3, 5, 10, 20 mg/L

Element values (95% confidence interval) calculated per replicate:

48-hour EC₅₀ = 9.4 (7.4 – 12.1) mg/L

48-hour EC₅₀ = 9.4 (7.3 – 12.2) mg/L

48-hour EC₅₀ = 8.9 (6.7 – 11.9) mg/L

Mortality of controls: None

DATA QUALITY

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

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 6/12/01

Robust Study Report Reference No. 33 - Acute toxicity of PFOS to *Daphnia magna*

TEST SUBSTANCE

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

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

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

METHOD

Method: International Standards Organization (1982)

Type: Acute static

GLP: No

Year completed: 1985

Species: *Daphnia magna*

RESULTS

Nominal concentrations: 10, 20, 30, 50, 100 mg/L

Number of replicates: 2

Element values (95% confidence interval) calculated per replicate

48-hour EC_{50} = 58 (46 – 72) mg/L

48-hour EC_{50} = 67 (48 – 92) mg/L

DATA QUALITY

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

REFERENCES

This study was conducted by Beak Consultants Limited, Mississauga, Ontario, Canada for Panarctic Oils Ltd, Calgary, Alberta, Canada.

OTHER

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

Last changed: 5/26/01

Robust Study Report Reference No. 34 - Perfluorooctanesulfonate, Potassium salt (PFOS): An acute oral toxicity study with the Honey bee

TEST SUBSTANCE

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

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

METHOD

Method: OECD Guideline 213, EPPO Guideline 170

Test type: Acute Oral

GLP: Yes

Year Completed: 2001

Species: *Apis mellifera* L.

Analytical monitoring: None – nominal concentrations

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

Test honey bee age at study initiation: Young adult

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

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

Test conditions

Humidity: 65% + 5%

Temperature: 25 + 2°C

Lighting: Conducted in darkness

Stock and test solutions preparation:

Test substance: Initial stock solution prepared in analytical grade acetone to a final concentration of 47.8 µg PFOS/µL (nominal concentration). Final test concentrations prepared from dilutions of this solution with 50% w/v sucrose. Resulting acetone concentration was 5%.

Reference toxicant: Primary stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Secondary stock solutions were made by diluting the primary stock solution in deionized water containing 1 g/L Triton X-100. Final test concentrations prepared from dilutions of these solutions with 50% w/v sucrose.

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

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

Feeding: During the first four hours of the test, bees provided with 50% w/v aqueous sucrose solutions containing the appropriate PFOS dose. After 4-hours, dosed sucrose removed, and bees provided with 50% w/v aqueous sucrose solutions, continuously available through the end

of the exposure period.

Number of replicates: Three

Number of bees per replicate: Ten

Negative control: 50% w/v sucrose

Solvent control: 50% w/v sucrose plus 5% acetone

Reference substance: Dimethoate

Reference substance control: Triton X-100

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

Dose administration: The bees were anaesthetized with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the petri dish cage (drones were discarded). Each group of 10 bees was offered 0.2 mL of a given test concentration or control solution. The dose was measured into a small, pre-weighed, glass feeder within the cage using a variable volume pipette. This volume of solution is equivalent to 20 µL per bee.

Dose frequency: Once, for 4 hours of exposure

Dose calculation: Feeders were weighed after removal from the cages to determine the dose consumed per bee.

Element basis: Mortality

RESULTS

Nominal concentrations: Negative control (sucrose only), acetone + sucrose control, 0.205, 0.450, 0.991, 2.17, 4.78 µg/bee

Element value and 95% confidence interval:

24-hour LD₅₀ = 0.72 (0.60 – 0.85) µg/bee

48-hour LD₅₀ = 0.46 (0.32 – 0.55) µg/bee

72-hour LD₅₀ = 0.40 (0.33 – 0.48) µg/bee

72-hour NOEL = 0.21 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test (p<0.05)

Biological observations: There was significant mortality at all doses above a mean intake of 0.21 µg/bee with a steep dose response between mean intakes of 0.45 and 2.2 µg/bee.

Cumulative percent mortality:

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	3.3	3.3	3.3
0.205	0	0	6.7	10
0.450	0	20	50	60
0.991	0	70	93	97
2.17	6.7	100	100	100
4.78	30	100	100	100

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

Nominal Test Conc., µg/bee	4-hours	24-hours	48-hours	72-hours
Negative Control	0	0	0	0
Solvent Control	0	0	0	0
0.205	0	0	0	0
0.450	0	3.3 (S)	3.3 (K)	3.3 (K)
0.991	0	0	0	0
2.17	0	0	0	0
4.78	10 (K)	0	0	0

Control response: satisfactory

Reference toxicant response: satisfactory – dimethoate 72-hour LD₅₀ = 0.11 µg/bee

CONCLUSIONS

The potassium perfluorooctanesulfonate 72-hour oral LD₅₀ for the honey bee was determined to be 0.40 µg/bee with a 95% confidence interval of 0.33 – 0.48. The 72-hour no observed effect level was 0.21 µg/bee. The dose response was steep between a mean uptake of 0.45 and 2.2 µg/bee.

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

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

OTHER

Last changed: 5/1/01

Robust Study Report Reference No. 35 - Perfluorooctanesulfonate, Potassium salt (PFOS): An acute contact toxicity study with the Honey bee

TEST SUBSTANCE

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

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

METHOD

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

Test type: Acute Contact

GLP: Yes

Year Completed: 2001

Species: *Apis mellifera* L.

Analytical monitoring: None – nominal concentrations

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

Test honey bee age at study initiation: Young adult

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

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

Test conditions

Humidity: 65% + 5%

Temperature: 25 + 2°C

Lighting: Conducted in darkness

Stock and test solutions preparation:

Test substance: Stock solution prepared in analytical grade acetone to a final concentration of 90.4 µg PFOS/µL (nominal concentration). Final test concentrations prepared in acetone from dilutions of this solution. **Reference toxicant:** Stock solution of dimethoate was prepared in deionized water containing 1 g/L Triton X-100 to a final concentration of 3.0 µg/µL. Final test concentrations prepared from dilutions of this solution.

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

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

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

Number of replicates: Three

Number of bees per replicate: Ten

Negative control: Undosed

Solvent control: Acetone

Reference substance: Dimethoate

Reference substance control: Triton X-100

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

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

Dose frequency: Once

Element basis: Mortality

RESULTS

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

Element value and 95% confidence interval:

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

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

72-hour LD₅₀ = 6.0 (4.7 – 7.6) µg/bee

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

96-hour NOEL = 1.93 µg/bee

All element values based on nominal concentrations

Statistical Evaluation: Probit mortality plotted against the logarithm of dose using the contract laboratory Probit 1 package. A least-squares regression (Finney 1971) was fitted to these. The NOELs were estimated using Student's t-test (p<0.05)

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

Cumulative percent mortality:

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

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

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

Control response: satisfactory

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

CONCLUSIONS

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

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

DATA QUALITY

Reliability: Klimisch ranking 1

REFERENCES

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

OTHER

Last changed: 5/1/01

Robust Study Report Reference No. 36 - PFOS: A 96-hour toxicity test with the freshwater alga (*Anabaena flos-aquae*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400

Test: Acute static

GLP: Yes

Year completed: 2001

Species: *Anabaena flos-aquae*

Source: Originally from UTEX – The Culture Collection of Algae at the university of Texas at Austin, and maintained in culture medium at Wildlife International Ltd., Easton, MD

Analytical monitoring: PFOS measured at 0, 72, 96-hours

Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate

Exposure period: 96-hours

Start date: 1/28/00

End date: 6/5/00

Test organisms laboratory culture: Algae cultures had been actively growing in algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water. Solutions were then diluted in purified well water to prepare final growth media.

Test Conditions:

Freshwater Algal medium

Compound	Nominal Concentration	Units
MgCl ₂ .6H ₂ O	12.16	mg/L
CaCl ₂ .2H ₂ O	4.40	mg/L
H ₃ BO ₃	0.1856	mg/L
MnCl ₂ .4H ₂ O	0.416	mg/L
ZnCl ₂	3.28	µg/L
FeCl ₃ .6H ₂ O	0.1598	mg/L
CoCl ₂ .6H ₂ O	1.428	µg/L
Na ₂ MoO ₄ .2H ₂ O	7.26	µg/L
CuCl ₂ .2H ₂ O	0.012	µg/L
Na ₂ EDTA.2H ₂ O	0.300	mg/L
NaNO ₃	25.5	mg/L
MgSO ₄ .7H ₂ O	14.7	mg/L
K ₂ HPO ₄	1.044	mg/L
NaHCO ₃	15.0	mg/L

Dilution water source: The pH of the medium was adjusted to 7.5 ± 0.1 and it was sterilized by

filtration (0.22µm) prior to use.

Test solution preparations: Individual test solutions were prepared in algal medium at each of the six nominal concentrations. The solutions were stirred with magnetic stir plates for approximately 18 hours. The final test solutions appeared clear and colorless.

Exposure vessels: Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at 100 rpm

Number of replicates: six (including 3 for analysis of exposure concentration).

Initial algal cell loading: 1.0×10^4 cells/mL

Cell counts method: hemacytometer and microscope

Number of concentrations: six plus a negative control plus an abiotic control at the highest concentration tested

Water chemistry: pH range (0 – 96 hours)

7.4 – 7.6 (control exposure)

7.4 – 7.4 (329 mg/L exposure)

Test temperature range (0 – 96 hours)

22.8 – 23.8°C

Light levels: (0 – 96 hours)

1990 – 2310 lux from cool-white fluorescent lighting

Photoperiod: 24-hours light

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 37.9, 58.6, 88.8, 139, 216, 331 mg/L plus 331 abiotic replicate

Measured concentrations: <LOQ, 37.9, 63.9, 93.8, 143, 235, 329 mg/L; abiotic replicate = 349 mg/L

Element values (95% confidence interval):

24-hour EC₅₀ (cell density) = 105 mg/L (C.I. not calculable)

24-hour EbC₅₀ (area under curve) = 90 (40 - 150) mg/L

24-hour ErC₅₀ (growth rate) = 94 (33 - 145) mg/L

48-hour EC₅₀ (cell density) = 117 mg/L (C.I. not calculable)

48-hour EbC₅₀ (area under curve) = 103 mg/L (C.I. not calculable)

48-hour ErC₅₀ (growth rate) = 128 mg/L (C.I. not calculable)

72-hour EC₁₀ (cell density) = 43 (34 – 84) mg/L

72-hour EbC₁₀ (area under curve) = <38 mg/L (C.I. not calculable)

72-hour ErC₁₀ (growth rate) = 82 (49 - 116) mg/L

72-hour EC₅₀ (cell density) = 120 (92 - 139) mg/L

72-hour EbC₅₀ (area under curve) = 116 (49 - 142) mg/L

72-hour ErC₅₀ (growth rate) = 174 (146 - 208) mg/L

72-hour EC₉₀ (cell density) = 224 (193 - 275) mg/L

72-hour EbC₉₀ (area under curve) = 204 (134 - 226) mg/L

72-hour ErC₉₀ (growth rate) = 275 (162 - 330) mg/L

96-hour EC₁₀ (cell density) = 82 (29 - 123) mg/L

96-hour EbC₁₀ (area under curve) = 56 (26 - 107) mg/L

96-hour ErC₁₀ (growth rate) = 109 (84 - 125) mg/L

96-hour EC₅₀ (cell density) = 131 (106 - 142) mg/L

96-hour EbC₅₀ (area under curve) = 124 (104 - 138) mg/L

96-hour ErC₅₀ (growth rate) = 176 (169 - 181) mg/L

96-hour EC₉₀ (cell density) = 213 (203 - 219) mg/L

96-hour EbC₉₀ (area under curve) = 209 (197 - 218) mg/L
 96-hour ErC₉₀ (growth rate) = 225 (220 - 235) mg/L
 72-hour NOAEC (cell density, area under curve): 37.9 mg/L
 72-hour NOAEC (growth rate): 93.8 mg/L
 96-hour NOAEC (cell density, growth rate): 93.8 mg/L
 96-hour NOAEC (area under curve): 63.9 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. The EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits were calculated by linear interpolation with treatment response and exposure concentration data using TOXSTAT Version 3.5. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality and homogeneity of variances using the Shapiro-Wilk’s test and Levene’s test, respectively. Where the data were normally distributed with equal variances, the treatment groups were compared to the control using Dunnett’s test. In the one instance where data were not normally distributed, the non-parametric Kruskal-Wallis test was used. Results of the statistical analyses were used to determine the NOAEC values.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.80 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 103%. Samples collected at test initiation had measured values from 100 to 112% of nominal. The measured values for the samples taken at 72-hours were 99.0 - 110% of nominal. The measured values for the samples taken at 96-hours were 99.0 - 109% of nominal. For the abiotic replicate, the measured value for the sample taken at 72-hours was 103% of nominal and for the sample taken at 96-hours, 107% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
37.9	37.9, 38.2, 37.6	37.9	100
58.6	65.6, 62.7, 63.4	63.9	109
88.8	94.3, 97.4, 89.8	93.8	106
139	142, 146, 142	143	103
216	230, 238, 236	235	109
331	331, 328, 329	329	99.4
331 (abiotic)	Not analyzed, 342, 356	349	105

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	569,167	-	-	-
37.9	605,000	-6.3	3.0	-1.3
63.9	585,833	-2.9	13	-0.97
93.8	492,500	13	24*	3.6
143	228,833	60*	66*	22*
235	2,333	100*	99*	100*
329	8,500	99*	100*	96*

*Indicates a significant difference from the negative control using the appropriate statistical test ($p < 0.05$)

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any treatment group. In addition, there were no noticeable changes in cell morphology when compared to the negative control.

Reversibility of Growth Inhibition: Aliquots of the 235 and 329 mg/L test solutions were diluted with algal medium and cultured for nine days after the exposure phase of the study concluded. Based on the increase in growth observed by Day 9 of the recovery phase, the effect on algal growth was algistatic at a concentration of 235 mg/L. However, no algal cells were detected during the recovery phase in the 329 mg/L treatment, indicating that PFOS was algicidal at that concentration.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC_{50} and 95% confidence interval for *Anabaena flos-aquae* was determined using three calculation methods. By cell density, it was 131 (106 – 142) mg/L, by area under the growth curve it was 124 (104 – 138) mg/L and by growth rate 176 (169 – 181) mg/L. The 96-hour NOAEC values were determined to be 63.9 mg/L using the area under the growth curve, and 93.8 mg/L with the cell density and growth rate calculation method. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. PFOS was determined to be algistatic at a concentration of 235 mg/L and algicidal at 329 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 7/19/01

Robust Study Report Reference No. 37 - PFOS: A 7-day toxicity test with Duckweed (*Lemna gibba* G3)**TEST SUBSTANCE**

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

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

METHOD

Method: OPPTS 850.4400

Test: Static acute

GLP: Yes

Year completed: 2001

Species: *Lemna gibba* G3

Source: Originally from The United States Department of Agriculture. Maintained in culture medium at Wildlife International Ltd., Easton, MD

Analytical monitoring: Test concentrations measured at 0, 3, 5, and 7-days

Element basis: Number of fronds

Exposure period: 7-days

Start date: 3/3/00

End date: 3/10/00

Test organisms laboratory culture: Duckweed cultures had been actively growing in freshwater medium (20X AAP) for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Test temperature range: 24.2 – 25.2°C

Light levels: 5000 + 750 lux from continuous warm-white fluorescent lighting

Growth medium: USEPA OPPTS 850.4400 20X AAP, 1996

Compound	Nominal Concentration	Units
MgCl ₂ ·6H ₂ O	243.2	mg/L
CaCl ₂ ·2H ₂ O	88.0	mg/L
H ₃ BO ₃	3.712	mg/L
MnCl ₂ ·4H ₂ O	8.32	mg/L
ZnCl ₂	65.6	µg/L
FeCl ₃ ·6H ₂ O	3.196	mg/L
CoCl ₂ ·6H ₂ O	28.56	µg/L
Na ₂ MoO ₄ ·2H ₂ O	145.2	µg/L
CuCl ₂ ·2H ₂ O	0.240	µg/L
Na ₂ EDTA·2H ₂ O	6.00	mg/L
NaNO ₃	510	mg/L
MgSO ₄ ·7H ₂ O	294	mg/L
K ₂ HPO ₄	20.88	mg/L
NaHCO ₃	300	mg/L

The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl.

Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl and the medium was sterilized by filtration (0.22 μ m) prior to use.

Stock and test solution preparation: A primary stock solution was prepared in duckweed medium at a concentration of 351 mg/L. The primary stock solution was stirred with a magnetic stir plate for approximately 24 hours. After mixing, the primary stock solution was proportionally diluted with duckweed medium to prepare the five additional test concentrations. All final test solutions appeared clear and colorless.

Exposure vessels: 250 mL plastic beakers containing 100 mL test solution, each covered with a disposable petri dish lid.

Agitation: None

Number of replicates: three plus 2 additional replicates for analytical sampling on Days 3 and 5

Initial loading: 5 plants/replicate, 15 fronds/replicate

Number of concentrations: six plus a negative control plus abiotic controls at the highest concentration tested

Water chemistry: pH range (0 – 96 hours)

7.9 – 8.9 (control exposure)

8.4 – 8.7 (230 mg/L exposure)

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at Days 0, 3, 5, and 7.

RESULTS

Nominal concentrations: Negative control, 11, 22, 43.9, 87.9, 176, and 351 mg/L plus 351 mg/L abiotic control.

Measured concentrations: <LOQ, 7.74, 15.1, 31.9, 62.5, 147, 230 mg/L; abiotic control = 231 mg/L

Element value and 95% confidence interval (based on frond number):

3-day IC₁₀: 101 mg/L (C.I. not calculable)

3-day IC₅₀: > 230 mg/L (C.I. not calculable)

3-day IC₉₀: > 230 mg/L (C.I. not calculable)

5-day IC₁₀: 30.7 mg/L (13.3 – 142 mg/L)

5-day IC₅₀: 182 mg/L (89.1 – 240 mg/L)

5-day IC₉₀: > 230 mg/L (C.I. not calculable)

7-day IC₁₀: 22.1 mg/L (13.3 – 26.0 mg/L)

7-day IC₅₀: 108 mg/L (45.7 – 144 mg/L)

7-day IC₉₀: > 230 mg/L (C.I. not calculable)

7-day NOAEC (number of fronds): 15.1 mg/L

All element values based on mean measured concentrations

Statistical methods: Mean plant and frond numbers, percent inhibition values and the percentages of necrotic, chlorotic and dead fronds were calculated using “Microsoft Excel Version 5.0”, while statistical analyses were conducted using “TOXSTAT Version 3.5”. Percent inhibition values were calculated for each treatment group as the percent reduction in mean frond number relative to mean frond number in the control replicates. The IC₁₀, IC₅₀, and IC₉₀ values and 95% confidence intervals were determined, when possible, using linear interpolation with frond number and exposure concentration data. The percentages of dead, chlorotic and necrotic fronds also were calculated relative to the total number of fronds in each test chamber. The frond number data was evaluated for normality and homogeneity of variances ($p = 0.05$)

using the Shapiro-Wilks' and Levene's tests, respectively. The data were normally distributed and the variances were homogeneous, thus statistically significant differences between the control and treatment groups were identified using ANOVA and Dunnett's test. Results of the statistical analyses, as well as an evaluation of the concentration-response pattern and other observations of effects were used in the determination of the no -observed-adverse-effect-concentration (NOAEC).

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). Samples were centrifuged as necessary prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 104%. Samples collected at test initiation had measured values from 64.2 to 82.6% of nominal. Measured values for samples taken at Day 3 ranged from 67.3 to 83.3% of nominal. Measured values for samples taken at Day 5 ranged from 65.4 to 85.4% of nominal. Samples collected at test termination (Day 7) ranged from 63.9 to 83.8% of nominal. For the abiotic controls, measured values for samples taken at Day 3, Day 5, and Day 7 ranged from 64.2 – 66.9% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at Days 0, 3, 5, and 7, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
11	7.57, 8.35, 7.47, 7.55	7.74	70
22	15.2, 15.4, 14.6, 15.2	15.1	69
43.9	32.2, 31.9, 31.8, 31.7	31.9	73
87.9	63.5, 63.1, 61.5, 61.8	62.5	71
176	145, 146, 150, 147	147	84
351	226, 237, 232, 224	230	66
351 (abiotic)	not analyzed, 225, 235, 232	231	66

Biological observations after 7-Days:

Counts

Mean Measured Concentration, mg/L	Mean Number of Plants	Mean Number of Fronds	Percent Inhibition via Frond Number
Negative Control	19	197	-
7.74	18	177	10
15.1	20	219	-11
31.9	14	151*	24
62.5	11	134*	32
147	15	69*	65
230	17	37*	81

*Statistically significant difference ($p < 0.05$) from the negative control using ANOVA and Dunnett's Test.

Effects

Mean Measured Concentration, mg/L	Mean Dead Fronds, %	Mean Chlorotic Fronds, %	Mean Necrotic Fronds, %
Negative Control	0	0	0
7.74	0	0	0
15.1	0	1.1	0
31.9	0	0	0.23
62.5	0	0.9	0.61
147	1.0	11	4.5
230	3.8	9.4	19

Control response: satisfactory. Plants appeared healthy and exhibited normal growth throughout the test with the exception of one necrotic frond observed on Day 3 and Day 5 of the test.

Observations: Duckweed exposed to 147 and 230 mg PFOS/L exhibited a dose-responsive increase in the incidence of dead, chlorotic or necrotic fronds during the test. By Day 7, all treatment groups > 31.9 gm/L showed evidence of sublethal effects, including root destruction and/or a cupping of the plant downward on the water surface.

CONCLUSIONS

The potassium perfluorooctanesulfonate 7-Day IC₅₀ and 95% confidence interval for duckweed was determined to be 108 (45.7 – 144) mg/L. The 7-Day NOAEC, based on the inhibition of frond production and evidence of sub-lethal effects, was 15.1 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 7/19/01

Robust Study Report Reference No. 38 - PFOS: A 96-hour toxicity test with freshwater diatom (*Navicula pelliculosa*)

TEST SUBSTANCE

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

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

METHOD

Method: OPPTS 850.5400

Test: Acute static

GLP: Yes

Year completed: 2001

Species: *Navicula pelliculosa*

Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at Wildlife International Ltd., Easton, MD

Analytical monitoring: PFOS measured at 0, 72, 96-hours

Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate

Exposure period: 96-hours

Start date: 2/25/00

End date: 2/29/00

Test organisms laboratory culture: Algae cultures had been actively growing in freshwater algal culture medium with silica and selenium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water.

Test Conditions:

Growth medium

Compound	Nominal Concentration	Units
MgCl ₂ ·6H ₂ O	12.16	mg/L
CaCl ₂ ·2H ₂ O	4.40	mg/L
H ₃ BO ₃	0.1856	mg/L
MnCl ₂ ·4H ₂ O	0.416	mg/L
ZnCl ₂	3.28	µg/L
FeCl ₃ ·6H ₂ O	0.1598	mg/L
CoCl ₂ ·6H ₂ O	1.428	µg/L
Na ₂ MoO ₄ ·2H ₂ O	7.26	µg/L
CuCl ₂ ·2H ₂ O	0.012	µg/L
Na ₂ EDTA·2H ₂ O	0.300	mg/L
NaNO ₃	25.50	mg/L
MgSO ₄ ·7H ₂ O	14.70	mg/L
K ₂ HPO ₄	1.044	mg/L
NaHCO ₃	15.0	mg/L
Na ₂ SiO ₃ ·9H ₂ O	20.0	mg/L
Na ₂ SeO ₃ ·5H ₂ O	0.010	mg/L

Dilution water source: Wildlife International Ltd. well water purified by reverse osmosis. The test medium was prepared by adding the appropriate volumes of stock nutrient solutions to purified well water. The pH of the medium was adjusted to 7.5 ± 0.1 using 10% HCl and 0.1 N NaOH. The medium was sterilized by filtration (0.22µm) prior to use.

Test solution preparation: A primary stock solution was not prepared for this study. Individual test solutions were prepared in algal medium at each of the seven nominal concentrations. The individual test solutions were stirred with a magnetic stir plate for approximately 24 hours. All final test solutions appeared clear and colorless.

Exposure vessels: Sterile 250 mL plastic Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at ~100 rpm

Number of replicates: three.

Initial algal cell loading: 1.0×10^4 cells/mL

Number of concentrations: seven plus a negative control plus an abiotic control at the highest concentration tested

Water chemistry: pH range (0 – 96 hours)

7.5 – 8.6 (control exposure)

7.5 – 7.7 (335 mg/L exposure)

Test temperature range (0 – 96 hours)

23.1 – 24.6°C

Light levels: (0 – 96 hours)

3910 – 4510 lux from continuous cool-white fluorescent lighting

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 61.5, 81.3, 110, 147, 198, 264, 347 mg/L plus 347 mg/L abiotic control.

Measured concentrations: <LOQ, 62.3, 83.2, 111, 150, 206, 266, 335 mg/L; abiotic control = 339 mg/L

Element value (95% confidence interval):

24-hour EC₅₀ (cell density) = 281 (214 - 312) mg/L

24-hour EbC₅₀ (area under curve) = 262 (205 - 308) mg/L

24-hour ErC₅₀ (growth rate) = 279 (212 - 306) mg/L

48-hour EC₅₀ (cell density) = 261 (219 - 306) mg/L

48-hour EbC₅₀ (area under curve) = 259 (227 - 303) mg/L

48-hour ErC₅₀ (growth rate) = 294 (271 - 307) mg/L

72-hour EC₁₀ (cell density) = <62.3 (C.I. not calculable) mg/L

72-hour EbC₁₀ (area under curve) = <62.3 (C.I. not calculable) mg/L

72-hour ErC₁₀ (growth rate) = 221 (190 - 252) mg/L

72-hour EC₅₀ (cell density) = 242 (200 - 276) mg/L

72-hour EbC₅₀ (area under curve) = 246 (210 - 277) mg/L

72-hour ErC₅₀ (growth rate) = 295 (288 - 305) mg/L

72-hour EC₉₀ (cell density) = 317 (306 - 326) mg/L

72-hour EbC₉₀ (area under curve) = 318 (307 - 325) mg/L

72-hour ErC₉₀ (growth rate) = 335 (323 - 335) mg/L

96-hour EC₁₀ (cell density) = <62.3 (C.I. not calculable) mg/L

96-hour EbC₁₀ (area under curve) = <62.3 (C.I. not calculable) mg/L

96-hour ErC₁₀ (growth rate) = 243 (209 - 295) mg/L

96-hour EC₅₀ (cell density) = 263 (217 - 299) mg/L

96-hour EbC₅₀ (area under curve) = 252 (220 - 285) mg/L
 96-hour ErC₅₀ (growth rate) = 305 (295 - 316) mg/L
 96-hour EC₉₀ (cell density) = 322 (310 - 328) mg/L
 96-hour EbC₉₀ (area under curve) = 319 (308 - 326) mg/L
 96-hour ErC₉₀ (growth rate) = >335 mg/L (C.I. not calculable)
 72-hour NOAEC (cell density, area under the curve): <62.3 mg/L
 72-hour NOAEC (growth rate): 206 mg/L
 96-hour NOAEC (cell density): 150 mg/L
 96-hour NOAEC (area under the curve): <62.3 mg/L
 96-hour NOAEC (growth rate): 206 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. These values were then analyzed by linear interpolation using TOXSTAT Version 3.5 to estimate the EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were also evaluated for normality and homogeneity of variances using the Shapiro-Wilkes’s test and Levene’s test, respectively. The treatment groups were then compared to the control using Dunnett’s test. Results of the statistical analyses were used to determine the NOAEC values.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 4.39 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 106% of nominal. Measured values for samples taken at 72 hours ranged from 98.5 to 106% of nominal. Measured values for samples taken at 96 hours ranged from 94.8 to 101% of nominal. For the abiotic controls, the measured value for the sample taken at 72 hours was 98.2% of nominal and for the sample taken at 96 hours, 96.8% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
61.5	62.3, 63.6, 61.1	62.3	101
81.3	83.8, 84.7, 81.0	83.2	102
110	109, 113, 110	111	101
147	147, 154, 149	150	102
198	209, 209, 199	206	104
264	271, 268, 258	266	101
347	334, 342, 329	335	96.5
347 (abiotic)	Not analyzed, 341, 336	339	97.7

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	2,726,667	-	-	-
62.3	2,366,667	13	24*	2.5
83.2	2,366,667	13	22*	2.7
111	2,373,333	13	24*	2.5
150	2,473,333	9.3	16	1.7
206	2,093,333	23*	24*	4.7
266	1,330,000	51*	58*	13*
335	35,333	99*	99*	79*

*Indicates a significant difference from the negative control using Dunnett's test ($p < 0.05$)

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the negative control or any test treatment group. In addition, there were no noticeable changes in cell color or morphology when compared to the negative control, although at 72 and 96 hours of exposure a few cells in the 335 mg/L treatment group appeared small in comparison to the control.

Reversibility of Growth Inhibition: The 335 mg/L treatment group was maximally inhibited after 96-hours. The treatment group was diluted to a concentration of the test substance that would not inhibit growth and exposed for 7 days. Based on the growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour EC_{50} and 95% confidence interval for *Navicula pelliculosa* was determined using three calculation methods. By cell density, it was 263 (217 - 299) mg/L, by area under the growth curve it was 252 (220 - 285) mg/L and by growth rate 305 (295 - 316) mg/L. The 96-hour NOAEC was determined by Dunnett's procedure ($p < 0.05$) to be 150 mg/L using cell density, <62.3 mg/L when using area under the curve and 206 mg/L by growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls. This test substance was determined to be algistatic.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 6/19/01

Robust Study Report Reference No. 39 - PFOS: A 96-hour toxicity test with the marine diatom (*Skeletonema costatum*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: OPPTS 850.5400

Test: Acute static

GLP: Yes

Year completed: 2001

Species: *Skeletonema costatum*

Source: Originally from The Culture Collection of Algae and Protozoa, Dunstaffnage marine Laboratory at Oban Argyll, Scotland, and maintained in culture medium at Wildlife International Ltd., Easton, MD

Analytical monitoring: PFOS measured at 0, 72, 96-hours

Element basis: Reported three ways: number of cells/ml, area under the growth curve and growth rate

Exposure period: 96-hours

Start date: 5/19/00

End date: 5/23/00

Analytical monitoring: Test concentrations measured at 0, 72, and 96-hours.

Test organisms laboratory culture: Algae cultures had been actively growing in saltwater algal culture medium for at least two weeks prior to test initiation. Stock nutrient solutions were prepared by adding reagent-grade chemicals to reverse osmosis-purified well water. Solutions were then diluted in artificial saltwater to prepare final growth media.

Test Conditions:

Algal saltwater medium

Compound	Nominal Concentration	Units
FeCl ₃ ·6H ₂ O	0.72	mg/L
MnCl ₂ ·4H ₂ O	2.16	mg/L
ZnSO ₄ ·7H ₂ O	0.675	mg/L
CuSO ₄ ·5H ₂ O	2.36	µg/L
CoCl ₂ ·6H ₂ O	6.06	µg/L
H ₃ BO ₃	17.1	mg/L
Na ₂ EDTA·2H ₂ O	15.0	mg/L
K ₃ PO ₄	3.0	mg/L
NaNO ₃	50.0	mg/L
Na ₂ SiO ₃ ·9H ₂ O	20.0	mg/L
Thiamine Hydrochloride	0.25	mg/L
Biotin	0.05	µg/L
B12	0.5	µg/L

Dilution water source: The stock nutrient solutions were prepared by adding the appropriate volumes reagent-grade chemicals to Wildlife International Ltd. well water purified by reverse osmosis. The algal medium was prepared by adding appropriate volumes of the stock nutrient solutions to artificial saltwater at 30 ppt salinity. The pH of the medium was 8.1 and it was sterilized by filtration (0.22µm) prior to use.

Test solution preparation: A single test solution (3.46 mg/L) was prepared for this study in algal saltwater medium. The solution was sonicated for approximately 30 minutes and was stirred with a magnetic stir plate for approximately 43 hours. The final test solution appeared clear and colorless.

Exposure vessels: Sterile 250 mL glass Erlenmeyer flasks plugged with foam stoppers containing 100 mL of test solution.

Agitation: Shaken continuously at 100 rpm

Number of replicates: six.

Initial algal cell loading: 7.7×10^4 cells/mL

Number of concentrations: one plus a negative control plus an abiotic control at the highest concentration tested

Water chemistry: pH range (0 – 96 hours)

8.0 – 8.4 (control exposure)

8.0 – 8.4 (3.20 mg/L exposure)

Test temperature range (0 – 96 hours)

20.2 – 21.4°C

Light levels: (0 – 96 hours)

3880 – 4710 lux from cool-white fluorescent lighting

Photoperiod: 14-hours light and 10 hours dark

Method of calculating mean measured concentrations: arithmetic mean obtained using results obtained at 0-hours, 72-hours and 96-hours

RESULTS

Nominal concentrations: Negative control, 3.46 mg/L plus 3.46 mg/L abiotic control. This is apparently the highest concentration of PFOS attainable in this saltwater algal media.

Measured concentrations: <LOQ, 3.20 mg/L; abiotic control = 3.18 mg/L

Element value (95% confidence interval):

72 and 96-hour EC₁₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

24, 48, 72, and 96-hour EC₅₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72 and 96-hour EC₉₀ via cell density, area under the curve and growth rate: > 3.20 mg/L (C.I. not calculable)

72-hour NOAEC (cell density, area under the curve, growth rate): 3.20 mg/L

96-hour NOAEC (cell density, area under the curve, growth rate): 3.20 mg/L

All element values based on mean measured concentrations

Statistical methods: Cell densities, area under the growth curve values, growth rates and percent inhibition values were calculated using “The SAS System for Windows”, Release 6.12. The EC₁₀, EC₅₀, and EC₉₀ values and 95% confidence limits could not be calculated using statistical methods. Cell densities, areas under the growth curve and growth rates at 72 and 96 hours were evaluated for normality using the Shapiro-Wilk’s test and for equality of variance using an F-test. The treatment groups were then compared to the control using ANOVA and a 2-sample t-test.

Results of the statistical analyses were used to determine the NOAEC values.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). The 72 and 96-hour samples were centrifuged approximately 10 minutes at approximately 2000 rpm prior to analysis. When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.480 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 108%. Samples collected at test initiation had measured values from 96.2 to 88.5% of nominal. The measured value for the sample taken at 72 hours was 92.2% of nominal. The measured value for the sample taken at 96 hours was 91.2% of nominal. For the abiotic control, the measured value for the sample taken at 72 hours was 97.1% of nominal and for the sample taken at 96 hours, 86.8% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Values at 0, 72, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
3.46	3.26, 3.19, 3.15	3.20	92.5
3.46 (abiotic)	Not analyzed, 3.36, 3.00	3.18	91.9

Biological observations after 96-hours:

Mean Measured Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Area Under the Curve	Percent Inhibition via Growth Rate
Negative Control	2,481,667	-	-	-
3.20	2,601,667	-4.8	-7.3	-1.3

Control response: satisfactory

Observations: After 96 hours of exposure, there were no signs of aggregation or adherence of the algae to the flasks in the treatment group. However there were signs of adherence to the test chamber in the negative control group. There were no noticeable changes in cell morphology when compared to the negative control.

Reversibility of Growth Inhibition: After 96-hours of exposure, there was no significant inhibition of growth in the highest concentration tested (3.20 mg/L). Therefore, a recovery phase was not conducted.

CONCLUSIONS

A single concentration of potassium perfluorooctanesulfonate was evaluated for toxicity to *Skeletonema costatum*. This mean measured concentration, 3.20 mg/L, was the highest concentration attainable in this algal media. The 96-hour EC₅₀ and 95% confidence interval for *Skeletonema costatum*, as determined by cell density, area under the growth curve, and by growth rate was found to be > 3.20 mg/L. The 96-hour NOAEC was determined by ANOVA and a 2-sample t-test to be 3.20 mg/L calculated using cell density, area under the curve and growth rate. No signs of cell aggregation or adherence were noted in any of the test solutions or the controls.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 7/19/01

Robust Study Report Reference No. 40 - PFOS: A frog embryo teratogenesis assay – *Xenopus* (FETAX)**TEST SUBSTANCE**

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS, U2723 or FC-95. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt, CAS # 2795-39-3)

Remarks: Sample obtained from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ¹H-HMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD

Method: ASTM E1439-91

Test type: Static renewal

GLP: In-life phase – no; stock solution preparation and measurement of test concentrations - yes

Year completed: 2001

Number of studies: 3

	Study 1	Study 2	Study 3
Start date:	5/15/00	5/22/00	5/22/00
End date:	5/19/00	5/26/00	5/26/00

(Study 2 and 3 set up concurrently with common stock solutions)

Analytical monitoring: PFOS measured at 0 and 96-hours

Species: *Xenopus laevis*

Source: Breeding colonies at the University of Maryland Wye Research and Education Center (UMD/WREC), Queenstown, Maryland.

Test organisms laboratory culture: Mating pairs were bred in the dark in 23.5 ± 0.5°C UMD/WREC non-chlorinated well water at ~ 70 day intervals by injecting 400 and 800 I. U. of human chorionic gonadotropin (HCG) in the dorsal lymph sac of the males and females, respectively. Amplexus occurred 4-6 hours after injecting HCG; egg deposition occurred 9-12 hours following HCG injection.

Age at test initiation: Embryos; normal stage 8 blastula to normal stage 11 gastrula

Loading: 25 embryos/10 mL

Pretreatment: Embryos de-jelled in a 2% L -cysteine solution, then rinsed and re-suspended in FETAX solution prior to introduction to test chambers.

Element basis: mortality, malformations (via the atlas of Bantle et al., 1991), growth

Exposure period: 96-hours

Test Conditions (all 3 studies):

Dilution water: ASTM (1998) FETAX solution

Test temperature: 24.0 ± 0.2°C

Light levels: 60-85 foot candle fluorescent lights

Photoperiod: 12-hour light: 12-hour dark

Stock and test solution preparation: A primary stock solution was prepared in FETAX medium (supplied by UM-WREC) by Wildlife International, Ltd. at 48 mg PFOS/L. The primary stock solution was mixed by sonication and stirring. After mixing, the primary stock solution was proportionally diluted with FETAX medium to prepare the six test concentrations. The six test concentration solutions were delivered to UM-WREC prior to the start of each study.

Reference substance: 6-aminonicotinamide

Stock and reference substance solution preparation: as outlined in the ASTM (1998) protocol

Exposure vessels: Covered 60 mm glass Petri dishes containing 10 mL test solution

Number of replicates: controls – 4, treatments – 2

Number of embryos per replicate: 25

Number of concentrations: six plus a negative control plus an abiotic control at the highest concentration tested, plus two reference substance concentrations.

Renewal frequency: every 24 hours

Stability of the test chemical solutions: Extremely stable

Water chemistry during all 3 studies:

pH range (0 – 96 hours)

7.1 – 7.7 (control exposure)

7.0 – 7.6 (24 mg/L nominal exposure)

Dissolved oxygen range (0-96 hours)

7.3 – 8.4 mg/L (control exposure)

7.0 – 8.5 mg/L (24 mg/L nominal exposure)

Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal PFOS concentrations: Negative control, 1.82, 3.07, 5.19, 8.64, 14.4 and 24.0 mg/L plus 24.0 mg/L abiotic control.

Nominal 6-aminonicotinamide concentrations: 5.5 and 2500 mg/L

Mean measured PFOS concentrations:

Study 1: <LOQ, 2.00, 2.83, 4.73, 7.90, 14.7, 24.6 mg/L;

abiotic control = 23.7 mg/L

Study 2: < LOQ, 1.91, 3.04, 4.82, 7.97, 13.3, 23.1 mg/L;

abiotic control = 23.9 mg/L

Study 3: < LOQ, 1.93, 3.27, 5.25, 8.26, 14.0, 23.9 mg/L;

abiotic control = 24.1 mg/L

PFOS element values and 95% confidence intervals, mg/L

Study Number	96-Hr LC ₅₀	96-Hr EC ₅₀	Minimum conc. to Inhibit Growth (MCIG)	Teratogenic Index (TI)
1	13.8 (12.4 - 15.3)	12.1 (10.0 – 14.6)	Not calculable	1.1
2	17.6 (15.5 – 20.0)	17.6 (13.5 – 22.9)	7.97	1.0
3	15.3 (13.1 – 17.8)	16.8 (12.4 – 22.8)	8.26	0.9

All element values based on mean measured concentrations

Statistical methods: The Trimmed Spearman-Kärber statistical procedure was used to determine the 96-hour LC₅₀ for mortality and 96-hour EC₅₀ for malformations. The MCIG was determined by Bonferroni's T-Test. All statistical tests were performed using Toxstat (WEST and Gulley, 1994). A minimum probability level of 0.05 was used. The teratogenic index (TI) was calculated by dividing the LC₅₀ by the EC₅₀.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd., Easton, MD using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the

basis of individual isomeric components. The LOQ (limit of quantitation) was 0.240 mg/L in these studies. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 97.2%. Samples collected at test initiation had measured values from 112 to 141% of nominal in the first study, and in the second and third studies, from 95.8 to 117% of nominal. Measured values for samples taken at 96-hours ranged from 54.7 to 98.6% of nominal in the first study and 80.7 to 112% of nominal in the second and third studies. The samples from the abiotic 24.0 mg/L treatment group was comparable to samples from the 24.0 mg/L treatment group with the embryos present.

Summary of analytical chemistry data:

Study 1

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
1.82	2.58, 1.42	2.00	110
3.07	3.94, 1.72	2.83	92.2
5.19	6.62, 2.84	4.73	91.1
8.64	10.7, 5.09	7.90	91.4
14.4	18.5, 10.8	14.7	102
24.0	26.9, 22.3	24.6	103
24.0 (abiotic)	not analyzed, 23.7	23.7	98.6

Study 2

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
1.82	1.77, 2.04	1.91	105
3.07	3.59, 2.49	3.04	99.0
5.19	5.45, 4.18	4.82	92.9
8.64	8.43, 7.51	7.97	92.2
14.4	14.5, 12.1	13.3	92.4
24.0	23.0, 23.1	23.1	96.3
24.0 (abiotic)	not analyzed, 23.9	23.9	99.6

Study 3

Nominal Test Concentration, mg/L	Measured Values at 0 and 96-hours Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	
1.82	1.77, 2.08	1.93	106
3.07	3.59, 2.94	3.27	107
5.19	5.45, 5.05	5.25	101
8.64	8.43, 8.09	8.26	95.6
14.4	14.5, 13.5	14.0	97.2
24.0	23.0, 24.7	23.9	99.6
24.0 (abiotic)	not analyzed, 24.1	24.1	100

Biological observations after 96-hours:**Mortality and Malformations**

Nominal Concentration*, mg/L	Test 1		Test 2		Test 3	
	Percent Mortality	Percent Malformations	Percent Mortality	Percent Malformations	Percent Mortality	Percent Malformations
Negative Control	1.0	4.0	1.0	4.0	0	2.0
1.82	2.0	8.2	0	8.0	0	6.0
3.07	4.0	15	10	4.4	0	4.0
5.19	10	22	8.0	11	0	6.0
8.64	12	25	10	20	14	14
14.4	38	65	30	37	44	39
24.0	100	-	70	67	78	73

*Nominal concentrations used for ease of comparison table

Malformations: The most common types of malformations noted were improper gut coiling, edema, notochord abnormalities and facial abnormalities.

Growth – Mean length (mm) after 96-hours Exposure

Nominal Concentration*, mg/L	Test 1	Test 2	Test 3
Negative Control	8.59	8.88	9.47
1.82	8.29	8.45	9.10
3.07	8.80	8.57	9.28
5.19	8.51	8.72	9.28
8.64	8.71	7.93**	8.51**
14.4	8.08	7.51**	8.11**
24.0	- (total mortality)	7.39**	7.80**

*Nominal concentrations used for ease of comparison table **Significantly different at alpha = 0.05 (Bonferroni T-Test)

Control response: satisfactory.

Reference substance response: satisfactory at low concentration (5.5 mg/L). Did not meet ASTM (1998) criteria for high concentration (2,500 mg/L). However, results obtained at high concentration were consistent and not at variance with previous experience in this testing laboratory.

Observations: Majority of embryo mortality appeared to be caused by the gut coiling through the body wall at the two highest test concentrations.

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC₅₀ range for FETAX was determined to be 13.8 – 17.6 mg/L. The 96-hour EC₅₀ range was 12.1– 17.6 mg/L. The range for Minimum Concentration to Inhibit Growth (MCIG) was 7.97 to >14.7 mg/L. The Teratogenic Index (TI) was found to be 0.9 – 1.1. This TI range indicates that potassium perfluorooctanesulfonate has a low potential to be a developmental hazard.

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

DATA QUALITY

Reliability: Klimisch ranking = 2

Although these were well-conducted studies, the in-life phases were not conducted in accordance with Good Laboratory Practices.

REFERENCES

These studies were conducted at the University of Maryland Wye Research and Education Center (UM-WREC) in Queenstown, Maryland and at Wildlife International Ltd., Easton, MD at the request of the 3M Company. Lab Request number U2723

OTHER

Last changed: 7/19/01

Robust Study Report Reference No. 41 - Perfluorooctanesulfonate, Potassium salt (PFOS): A flow-through bioconcentration test with the Bluegill (*Lepomis macrochirus*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance is a white powder. Purity determined to be 86.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analyses techniques.

METHOD:

Method/guideline followed: US EPA OPPTS 850.1730 and OECD 305

Type: Flow-through exposure with flow-through depuration phase.

GLP (Y/N): Yes

Year: 2001

Species: Bluegill (*Lepomis macrochirus*)

Supplier: Osage Catfisheries, Inc., Osage Beach, Missouri

Length and weight at test termination: Mean length = 62 mm, range 56-66 mm Mean weight = 2.70 g, range 2.03 – 3.32 g

Loading: 0.48 g fish/L/day (based on initial loading of 90 fish per tank, using mean fish weight at the end of the study and volume of water that passed through test chamber in 24-hours).

Fish age: Approximately 7 months at test initiation

Analytical monitoring: Concentration of PFOS in water and fish.

Pretreatment: None

Number of concentrations: Two plus a negative control

Test concentrations (mean measured): Negative control, 0.086 and 0.87 mg/L

Uptake period: 62-days (0.086 mg/L exposure)
35-days (0.87 mg/L exposure – this exposure ended after 35-days due to fish mortality)

Depuration period: 56-days (0.086 mg/L exposure)
None (0.087 mg/L exposure)

Test conditions:

Dilution water: Moderately-hard well water

Dilution water chemistry:

Specific conductance: 313 (310 – 315 µmhos/cm)

Hardness: 130 (128 – 132 mg/L)

Alkalinity: 178 (176 – 178)

pH: 8.1 (8.0 – 8.2)

Measured during the 4 -week period immediately preceding the test.

Stock and test solution preparation: Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless.

Diluter flow rate: Approx. 6.3 volume additions per 24-hours

Exposure vessels: 104 L stainless steel aquaria filled with approximately 80 L solution.

Number of replicates: None – one vessel per concentration

Number of fish per vessel: 90

Diet: Flake food, Ziegler Brothers, Inc., Gardners, PA

Water chemistry ranges during the study:

	Neg. Control	0.086 mg/L	0.87 mg/L
Dissolved oxygen, mg/L:	6.8 – 8.6	6.8 – 8.6	6.4 – 8.2
Temperature, °C:	21.8 – 22.0	21.7 – 22.0	21.7 – 21.9
pH:	7.9 – 8.2	7.9 – 8.2	7.9 – 8.2

Photoperiod: 16 hours light and 8 hours dark with a 30-minute transition period.

Light intensity: 278 lux at surface of the negative control vessel at test initiation

Collection of tissue samples: Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed and measured. Fish then rinsed with dilution water, blotted dry again and dissected into edible and non-edible tissue fractions. The fractions were individually weighed. The head, fins and viscera were considered to be non-edible tissue. The remaining tissue, including skin was considered to be edible tissue.

Statistical methods: Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different ($p > 0.05$). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, data was transformed in an attempt to correct the data. Mean tissue concentrations were then compared using ANOVA and Dunnett's test.

The kinetic bioconcentration factor (BCFK), uptake rate (k_1) and depuration rate (k_2) were calculated for the edible, nonedible and whole fish exposed to 0.086 mg/L PFOS using BIOFAC computer software. BIOFAC is a nonlinear parameter estimate routine which estimates rate constants from a set of sequential time-concentration data. These rate constants were then used to calculate a BCFK ($BCFK = K / K_{12}$).

RESULTS

Nominal concentrations: Negative control, 0.1 and 1.0 mg/L

Mean measured concentrations: < 0.05, 0.086 and 0.87 mg/L

Bioconcentration factors (BCF):

0.086 mg/L apparent steady-state BCF

Edible	Non-edible	Whole Fish
484	1124	856

0.87 mg/L (study ended prior to achieving steady-state) BCF:

Edible	Non-edible	Whole Fish
136	386	278

BIOFAC Estimates (using 0.086 mg/L exposure)

	Edible	Non-edible	Whole Fish
BCFK:	1866	4312	3614
Time to reach 50% clearance:	146 days	133 days	152 days

PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Non-edible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	0.167, 0.155, 0.144, 0.182	0.415, 0.519, 0.417, 0.497	0.293, 0.351, 0.286, 0.363
1	0.734, 0.726, 0.631, 0.806	1.68, 1.85, 1.72, 2.07	1.26, 1.34, 1.29, 1.53
3	1.73, 2.07, 2.03, 2.11	4.59, 5.50, 5.47, 5.97	3.21, 4.04, 4.18, 4.38
7	3.73, 4.25, 4.73, 6.25	10.2, 10.6, 11.9, 15.2	7.33, 7.66, 8.73, 11.4
14	11.4, 9.07, 13.7, 12.6	27.3, 23.2, 35.3, 32.6	20.2, 16.9, 26.0, 24.6
21	11.7, 12.0, 12.9, 10.6	33.3, 22.7, 24.6, 24.4	23.3, 18.4, 19.8, 18.5
28	18.3, 13.7, 23.9, 23.1	49.4, 40.7, 65.3, 57.9	35.3, 29.2, 45.4, 44.1
35	22.6, 27.7, 23.8, 20.6	67.1, 73.3, 62.0, 59.1	46.3, 53.8, 46.6, 40.9
42	27.6, 25.3, 21.2, 27.6	64.0, 68.1, 54.4, 79.6	50.1, 49.4, 40.9, 56.3
49	33.3, 36.2, 39.0, 30.6	85.0, 95.1, 93.1, 77.7	62.8, 69.6, 70.8, 57.4
56	48.3, 38.9, 44.1, 38.3	122, 94.2, 73.2, 106	90.6, 71.6, 63.3, 74.8
62	42.4, 66.2, 42.2, 39.2	101, 112, 105, 96.4	77.0, 92.7, 79.6, 73.1
Depuration Day			
14	48.5, 31.8, 31.6, 42.0	124, 79.4, 81.8, 113	90.3, 60.4, 61.6, 85.3
28	26.0, 33.3, 38.7, 55.8	85.7, 95.1, 85.7, 94.8	58.2, 70.1, 68.1, 81.1
42	24.1, 31.2, 30.0, 33.0	71.7, 80.6, 78.3, 82.1	51.4, 61.4, 61.0, 62.2
56	21.1, 37.6, 32.9, 31.2	57.7, 80.3, 85.4, 84.4	41.6, 66.5, 65.8, 62.1

PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L

Values are from 4 individual fish at each sample period.

Uptake Day	Edible Tissue, mg/kg	Non-edible Tissue, mg/kg	Whole Fish Conc., mg/kg
0 (4-hours)	1.46, 1.48, 1.19, 1.39	3.52, 4.37, 4.22, 4.06	2.71, 3.08, 2.84, 2.89
1	4.68, 6.59, 5.56, 5.64	11.1, 14.2, 13.3, 12.1	8.00, 10.9, 10.2, 9.47
3	17.3, 15.8, 19.0, 20.8	39.3, 42.0, 43.8, 51.8	30.5, 30.7, 34.5, 39.1
7	42.0, 44.0, 57.7, 46.8	100, 102, 102, 120	74.9, 77.0, 85.3, 89.8
14	87.1, 81.6, 90.7, 73.3	177, 207, 245, 214	141, 157, 180, 158
21	79.4, 117, 104, 102	201, 278, 246, 229	146, 210, 185, 172
28 ⁽¹⁾	102, 131, 107, 133	289, 372, 320, 361	205, 267, 232, 263

⁽¹⁾ Sampling of fish stopped after Uptake Day 28 due to mortality.

Test organism mortality:

Negative control: None during the uptake phase (62 days) or depuration phase (35 days)

0.086 mg/L exposure: One fish died after 49 days and one after 59 days of exposure in the uptake phase, none during the depuration phase.

0.87 mg/L exposure: Mortality first noted on Day 9 and continued through Day 35 of the uptake phase at which time all of the fish had either died or had been sampled

Analytical methodology: Analyses of test solutions and fish tissues were performed at Wildlife

International, Ltd. Water samples were diluted and analyzed by HPLC with single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

CONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. Apparent steady-state was attained on Day 49 for the fish exposed to 0.086 mg a.i./L. Although Day 49, 56 and 62 tissue residues were not statistically significantly different, PFOS concentrations appeared to be still increasing during this time. Apparent steady-state BCF values for edible, non-edible and whole fish tissues were calculated to be 484, 1124, and 859, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, non-edible and whole fish tissues were 146, 133 and 152 days, respectively.

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

This study was conducted at Wildlife International, Ltd., Easton, MD at the request of the 3M Company, Lab Request number U2723.

OTHER

Last changed: 7/19/01

Robust Study Report Reference No. 42 - Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) in freshwater

TEST SUBSTANCE

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

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

METHOD

Method: OPPTS 850.1075 and OECD 203

Type: Static acute

GLP: Yes

Date completed: Study completed 2001, report completed 2002

Species: *Oncorhynchus mykiss*

Supplier: Thomas Fish Company, Anderson, CA

Analytical monitoring: PFOS measured at 0, 48, 96-hours

Exposure period: 96-hours

Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: juveniles

Average Total Length and weight: 3.6 (3.4–4.0) cm, 0.34 (0.25-0.47) g

Loading: 0.23 g fish/L

Pretreatment: None

Test conditions:

Dilution water: 0.45 µm filtered moderately hard well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

hardness: 130 (128-132) mg/L as CaCO₃

alkalinity: 177 (176-178) mg/L as CaCO₃

pH: 8.3 (8.2 – 8.4)

TOC: Not given

Conductivity: 311 (310-315) µmhos/cm

Stock and test solution preparation: Primary stock prepared in dilution water at 150 mg/L and mixed for ~23 hours prior to use.

After mixing, primary stock solution was proportionally diluted with dilution water to prepare the five test concentrations.

Concentrations dosing rate: Once

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approx 17.5 cm.

Number of replicates: two

Number of fish per replicate: ten

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours) :9.4 – 10.7 mg/L (control exposure)
9.2 – 10.8 mg/L (50 mg/L exposure)

pH range (0 – 96 hours)
8.1 – 8.4 (control exposure)
8.2 – 8.4 (50 mg/L exposure)

Test temperature range (0 – 96 hours)
12.1 – 12.6°C (control exposure)
11.8 – 12.9°C (50 mg/L exposure)

- Method of calculating mean measured concentrations: arithmetic mean

RESULTS

Nominal concentrations: Bk control, 3.1, 6.3, 13, 25, 50 (exposure), 50 (abiotic) mg/L

Measured concentrations: <LOQ, 3.0, 6.3, 13, 25, 50, 52 mg/L

Element value: 24-hour LC50 = > 50 mg/L (C.I. not calculable)
48-hour LC50 = > 50 mg/L (C.I. not calculable)
72-hour LC50 = > 50 mg/L (C.I. not calculable)
96-hour LC50 = 22 (18 - 27) mg/L
All element values based on mean measured concentrations

Statistical Evaluation of Mortality:

Element values and confidence limits for 24, 48, and 72-hours could not be calculated due to lack of mortality. Probit Analysis was used to calculate the 96-hour LC50.

Analytical Methodology: Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used.

No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 0.200 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 101. Samples collected at test initiation had measured values from 93.2 to 103% of nominal. Measured values for the biotic samples taken at 48-hours ranged from 93.6 to 103% of nominal, while abiotic samples ranged from 105 to 106% of nominal. Measured values for biotic samples taken at 96-hours ranged from 91.4 to 105% of nominal, while the abiotic samples were 102% of nominal.

Summary of analytical chemistry data:

Nominal Test Concentration, Mg/L	Measured Duplicate Values at 0, 48, and 96-hours, Respectively, mg/L	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
3.1	3.15, 3.02, 2.90, 3.01, 2.83, 2.97	3.0	97
6.3	6.22, 6.21, 6.16, 6.43, 6.15, 6.60	6.3	100
13	13.2, 12.1, 12.7, 12.3, 13.1, 12.6	13	100
25	25.0, 25.7, 24.3, 25.7, 25.7, 26.2	25	100
50	49.7, 49.8, 51.1, 51.5, 49.6, 50.8	50	100
50 (abiotic) (1)	53.1, 52.6, 50.9, 51.0	52	104

(1) Samples taken at 48 and 96-hours only

Biological observations after 96-hours:

Fish in the negative control and the 3.0 and 6.3 mg/L exposure concentration appeared normal with no mortalities or overt signs of toxicity. All surviving fish in the 13 and 25 mg/L exposures appeared normal with no overt signs of toxicity after 96-hours.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Neg. Control	0	0	0	0
3.0	0	0	0	0
6.3	0	0	0	0
13	0	0	0	20
25	0	0	0	50
50	0	5	35	100

Lowest concentration causing 100% mortality: 50 mg/L

Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC50 for rainbow trout was determined to be 22 mg/L with a 95% confidence interval of 18 –27 mg/L. The 96-hour no mortality and no effects concentration was 6.3 mg/L.

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 1/24/02

Robust Study Report Reference No. 43 - Perfluorooctanesulfonate, Potassium salt (PFOS): 96-Hour Semi-Static Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*) in saltwater

TEST SUBSTANCE

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

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

METHOD

Method: OPPTS 850.1075
Type: Static renewal
GLP: Yes
Date completed: Study completed 2001, report completed 2002
Species: *Cyprinodon variegatus*
Supplier: Aquatic BioSystems, Inc., Fort Collins, CO
Analytical monitoring: PFOS measured at initiation, prior to and after renewal at 24, 48, and 72 hours and at test termination (96-hours)
Exposure period: 96-hours
Statistical methods: The use of a single test concentration (at water solubility) precluded the statistical calculation of LC50 values.
Test fish age: Juveniles
Average Total Length and weight: 3.0 (2.4–3.5) cm, 0.44 (0.21-0.66) g
Loading: 0.29 g fish/L
Pretreatment: None

Test conditions:

Dilution water: Natural seawater, filtered and diluted to a salinity of approximately 20 parts per thousand with well water

Dilution water chemistry (during the 4-week period immediately preceding the test):

Salinity: 20 (20 – 20) parts per thousand
pH: 8.2 (8.1 – 8.3)

Stock and test solution preparation:

Primary stock prepared in methanol at 40 mg/L, sonicated for approximately 20 minutes and inverted to mix prior to use. After mixing, primary stock solution was proportionally diluted with dilution water to prepare the one test concentration. Each solution was stirred with a stainless steel whisk for approximately one minute. All test solutions appeared clear and colorless.

Solvent: Methanol
Solvent concentration (treatment and solvent control groups): 0.5 mL/L

Concentrations dosing rate: Daily static renewal

Stability of the test chemical solutions: Extremely stable

Exposure vessels: 25L polyethylene aquaria containing approximately 15L of test solution; water depth approximately 17.1 cm.

Number of replicates: three (biotic), two (abiotic)

Number of fish per replicate: ten

Number of concentrations: One plus a negative and a solvent control, and an abiotic solution.

Water chemistry during the study:

Dissolved oxygen range	(0 – 96 hours): 2.8 – 7.4 mg/L (negative control exposure) 1.7 – 7.6 mg/L (solvent control exposure) 1.6 – 7.6 mg/L (15 mg/L exposure)
pH range	(0 – 96 hours) 7.9 – 8.3 (negative control exposure) 7.9 – 8.3 (solvent control exposure) 7.9 – 8.3 (15 mg/L exposure)
Test temperature range	(0 – 96 hours) 21.9 – 22.6°C (negative control exposure) 22.1 – 22.9°C (solvent control exposure) 22.2 – 23.1°C (15 mg/L exposure)

- **Method of calculating mean measured concentrations:** arithmetic mean

RESULTS

Nominal concentrations: Bk control, solvent control, 20 mg/L (biotic), 20 mg/L (abiotic)

Measured concentrations: <LOQ, <LOQ, 15, 13 mg/L

Element value: 24-hour LC50 = >15 mg/L (C.I. not calculable)
48-hour LC50 = >15 mg/L (C.I. not calculable)
72-hour LC50 = >15 mg/L (C.I. not calculable)
96-hour LC50 = >15 mg/L (C.I. not calculable)

All element values based on mean measured concentrations

Statistical Evaluation of Mortality:

Element values and confidence interval could not be calculated due to lack of mortality.

Analytical Methodology:

Analyses of test solutions were performed at Wildlife International Ltd. using high performance liquid chromatography with mass spectrometric detection (HPLC/MS). When determining the concentration of the test substance in the test solutions, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ (limit of quantitation) was 5.00 mg/L in this study. The mean percent recovery of matrix fortifications analyzed concurrently during sample analysis was 99.2. Samples collected in exposure vessels at test initiation had measured values from 78.6 to 82.0% of nominal. New samples collected at Hours 24, 48 and 72 had measured concentrations from 74.9 to 81.2 and 86.9 to 92.9, and 82.9 to 87.1% of

nominal, respectively. Old samples collected at Hours 24, 48, and 72 had measured concentrations ranging from 66.6 to 76.2, 56.4 to 66.9, and 75.8 to 89.9% of nominal, respectively. Mean measured concentrations of PFOS in samples collected at test termination were 55.6 to 68.4% of nominal. The measured concentrations of PFOS from the abiotic treatment group were slightly lower than those from the exposure treatment group. This may have been due to increased deposition of test substance at the limit of solubility, which could have resulted from the absence of the natural mixing action that was provided by the movement of the fish in the exposure treatment group.

Summary of analytical chemistry data:

Nominal Test Concentration, mg/L	Measured Replicate Values of old and new Test Solutions Respectively, mg/L (1)	Mean Measured Concentration, mg/L	Percent of Nominal
Negative Control	All < LOQ	<LOQ	-
Solvent Control	All < LOQ	<LOQ	-
20 ⁽²⁾	16.4, 15.7, 16.0; 15.2, 13.3, 15.2; 16.2, 15.7, 15.0; 13.4, 11.3, 12.7; 17.4, 18.2, 18.6; 15.2, 18.0, 16.2; 17.0, 16.6, 17.4; 13.7, 11.1, 13.4	15	75
20 (3) (abiotic)	10.9, 9.22; 16.1, 16.6; 9.62, 9.69; 16.6, 17.5; 9.01, 9.25; 17.7, 15.9; 9.84, 9.22	13	65

(1) Replicate samples are listed in this order: Day 0 (new), 24-hours (old), 24-hours (new), 48-hours (old), 48-hours (new), 72-hours (old), 72-hours (new), 96-hours (old)

(2) Triplicate samples

(3) Day 0 not measured, Duplicate samples

Biological observations after 96-hours:

Fish in the negative control and solvent control appeared healthy and normal throughout the exposure period. No mortalities appeared in the 15 mg/L treatment group during the study. However, upon transfer to the new test solution at approximately 48 and 72 hours, some fish were observed swimming erratically and turning a dark color. The fish appeared normal within approximately two hours after transfer, although one fish still appeared to be discolored at test termination.

Cumulative percent mortality:

Mean Measured Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Negative Control	0	0	0	0
Solvent control	0	0	0	0
15	0	0	0	0

Lowest concentration causing 100% mortality: none – mortality limit apparently greater than solubility limit.

Mortality of controls: None

CONCLUSIONS

The potassium perfluorooctanesulfonate 96-hour LC50 for sheepshead minnow was determined to be >15 mg/L, the limit of solubility in this study. The 96-hour no mortality concentration was 15 mg/L and no observed effects concentration was <15 mg/L mg/L (1 fish out of 30 was discolored at 96-hours).

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

DATA QUALITY

Reliability: Klimisch ranking = 1

REFERENCES

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

OTHER

Last changed: 1/24/02

Robust Study Report Number 44. Bioconcentration test of Salt (Na, K, Li) of perfluoroalkyl (C=4-12) sulfonic acid [This test was performed using Perfluorooctane sulfonic acid, potassium salt (Test substance number K-1520)] in carp

TEST SUBSTANCE

Identity: K-1520. Perfluorooctane sulfonic acid, potassium salt. Lot number A37626B

Supplier: Kishida Chemical Co., Ltd.

Remarks: Test substance number K-1520. The test substance is a white powder. Purity determined to be 100%

METHOD

Method/guideline followed: Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body” stipulated in the “Test Method for New Chemical Substance” July 13, 1974, Revised October 8, 1998, No. 5, Planning and Coordination Bureau, Environmental Agency; No.615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare; and No. 392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan), and Bioconcentration : Flow-through Fish test (Guideline 305, June 14, 1996)” in the OECD Guidelines for Testing of Chemicals.

Type: Flow-through system

GLP (Y/N): Yes

Year: October 13, 2000 – February 16, 2001

Species: Carp (*Cyprinus carpio*)

Supplier: Fukuokaken yabegawa fisherman’s cooperative association (Address: 193-1 Yamauchi, Yame-shi, Fukuoka 834-0012, Japan)

Length and weight at test termination:

Mean length = 6.4 – 9.6

Mean weight = No weight recorded

Loading: 2 and 20 ug/L respectively

Fish Age: Yearling fish

Analytical monitoring: High-performance liquid chromatography-Mass spectrometry

Pretreatment: The fish were checked visually in the receiving and those demonstrating any abnormality were removed. The fish were reared for 8 days in a flow thorough system following an external disinfection. After rearing, the fish were medicated to eliminate parasites and transferred to an acclimatizing aquarium. After the second external disinfection, they were acclimatized. The fish demonstrating any abnormality during this period were removed and the remainder of the fish were reared for 15 days in a flow through system at temperatures of $25 \pm 2^{\circ}$ C. The fish were transferred to test tanks and reared at the same temperature in the flow through system for another 27 days.

The fish were starved for 24 hours before sampling.

Number of concentrations: Two plus a negative control

Test concentration: (mean measured): Negative control, 2 and 20 ug/L

Uptake period: 58 days

Depuration period: 37 days

Test conditions:

Dilution water: Groundwater from the premises of Kurume Laboratory

Dilution water chemistry:

Specific conductance: Not recorded

Hardness: 111 mg/L

Alkalinity: 96.1

pH: 7.6 to 7.8

Dissolved Oxygen: 7.9 to 8.1

Temperature: 25.0 to 25.8°C

Stock and test solution preparation: Based on preliminary test results for the 96 hour LC50 value and analytical detection limits, test concentrations of the test substance were decided as follows. The control was set as a blank test. Level 1 was 20 ug/L and Level 2 was 2 ug/L. The test substance was dissolved with ion-exchanged water to prepare 16 and 1.6 mg/L stock solutions.

Diluter flow rate: 2 mL/min for stock solution and 1600 mL/min for dilution water; 2307 liters/day for test water were supplied.

Exposure Vessels: 100 liter tank

Number of replicates: None

Number of fish per vessel: 40

Diet: Nippon Formula Feed Mfg. Co., Ltd.

Water chemistry ranges during the study:

	Neg. Control	2 ug/L	20 ug/L
Dissolved Oxygen:	8.0 – 8.1 mg/L	8.0 – 8.1 mg/L	7.9 – 8.1 mg/L
Temperature°C:	25.1 – 25.4°C	25.5 – 25.8°C	25.0 – 25.4°C
pH:	7.6 – 7.8	7.6 – 7.8	7.6 – 7.8

Photoperiod: Artificial light of white fluorescent lamp (14 hrs./day)

Light intensity: Artificial light of white fluorescent lamp

Collection of tissue samples: Analysis of test fish was performed six times at each level in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. Analysis of control fish was performed before the experimental starting and after the experimental completion. Six fish were taken out at each sampling time and divided into three groups, and then both were analyzed individually. Because the stored sample taken out from one fish was too small for the measurement of lipid content, a group of two fish was employed. The fish were separated into parts; tegument, head viscera except liver, liver and remaining matter were weighted separately. The tegument consisted of the skin except head, scales, fin, alimentary canal or gills. The viscera consisted of internal organs except alimentary canal.

Statistical methods: Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different.

RESULTS

Nominal concentrations: Negative control,

Mean measured concentrations: 2 and 20 ug/L

Bioconcentration factors (BCF):

PFOS Concentration in test water:

Conc.	After 1 Day	After 7 Days	After 14 Days	After 21 Days	After 28 Days	After 43 Days	After 58 Days	Average (STD)
20 ug/L	15.1	14.5	15.7	16.5	17.7	15.4	16.8	16 + 1.12
2 ug/L	1.78	1.76	1.87	1.93	1.89	1.92	2.01	1.88 + 0.087

PFOS BCFs of Carp Exposed to 2 and 20 ug/L:

Conc.	After 7 Days	After 14 Days	After 21 Days	After 28 Days	After 43 Days	After 58 Days
20 ug/L	260	440	300	690	750	720

2 ug/L	240	520	410	860	890	1300
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Analysis in parts of test fish

Level 1 (20 ug/L) BCF

	Parts									
	Tegument		Head		Viscera		Liver		Remainder Parts	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Concentration (ng/g)	22800	16700	23400	17500	36000	45300	37800	32000	6260	5380
BCF	1400	1000	1400	1100	2200	2700	2300	1900	380	320

Level 2 (2 ug/L) BCF

	Parts									
	Tegument		Head		Viscera		Liver		Remainder Parts	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Concentration (ng/g)	5490	4750	5600	4730	9900	7410	9190	7650	1810	1390
BCF	2800	2400	2900	2400	5100	3800	4700	3900	930	720

Time to reach 50% clearance: 49 days at level 1 and 152 days at level 2

Test organism mortality:

Negative control: None documented.

Level 1 (20 ug/L): None documented.

Level 2 (2 ug/L): None documented.

Analytical methodology:

Analysis of PFOS in the test water and carp was performed using high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The test water of each level was analyzed once before first analysis of test fish and at the same time as the analysis of the test fish. Steady state was reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within + 20% of each other. When BCFs were less than 100, it was evaluated that a steady-state had been reached after 28 days.

Recoveries in water and fish tissues were 89.3% and 90.6% respectively. Analytical results were corrected for procedural recovery.

CONCLUSIONS

In this study, PFOS bioconcentrated in the tissues of carp. Test concentrations of 2 and 20 ug/L were used. The fish were exposed for 58 days to makeup for excessive mortality. Bioconcentration factors were calculated to be 720 for 20 ug/L and 200 - 1500 for 2 ug/L.

REFERENCES

Kurume Laboratory (2001). Chemicals Evaluation and Research Institute, Japan. Test number: 51520

Annex 3. Application of Equilibrium Partitioning Models to Determining Effect Concentrations for PFOS Salts in Soil and Sediment

The review of the complete set of ecotoxicity test reports presented in section 2.2.1, 2.3.1 and 2.3.2 has highlighted the absence of data describing the toxicity of PFOS salts to sediment and soil-dwelling organisms and terrestrial plants. Since tests with aquatic invertebrates and plants have demonstrated toxicity of PFOS salts, and there is generally a correlation between toxicity to aquatic organisms living in the water column and toxicity to sediment- and soil-dwelling organisms. There is as a consequence a need to consider the possibility of deriving effect concentrations for PFOS in soil and sediment to fill the data gaps based on data that already exist for aquatic organisms. One approach to deriving such data is to use equilibrium partitioning models.

Equilibrium partitioning models are used in two ways:

- To calculate soil/sediment pore water concentration from overall soil/sediment concentration
- To calculate $PNEC_{\text{soil/sediment}}$ from $PNEC_{\text{water}}$.

In both cases, the equilibrium constant for distribution between water and soil/sediment, $K_{\text{psoil/sediment}}$, is needed. For many substances, $K_{\text{psoil/sediment}}$ is replaced by K_{oc} , which is equivalent to assuming that only the organic components of soil or sediment are important in affecting the equilibrium. However, for an anionic surfactant, such as PFOS, it is very likely that interaction with the inorganic substrate will also be important (Salloum et al, 2000), as has been demonstrated in a recent study with PFOS (Ellefson). Therefore K_{p} values for an appropriate soil or sediment would have to be used. It should also be noted that salinity affects the solubility of PFOS; the high ionic content of interstitial water could also affect the adsorption behaviour of PFOS. Furthermore, it is uncertain over what time-scale equilibrium would be achieved (Ellefson), and as a consequence the extent of adsorption may be dependent upon the concentration of PFOS in the aqueous phase.

Whilst for all substances extrapolation of PNEC from aquatic data to the terrestrial or sediment compartment is subject to uncertainty, that uncertainty is compounded when the mode of uptake is different for organisms present in different environmental compartments. The mode of toxic action and the mechanism of uptake of surfactants are complex. Therefore the use of equilibrium partition models to obtain $PNEC_{\text{soil}}$ or $PNEC_{\text{sediment}}$ is subject to considerable doubt.

Should any laboratory soil or sediment organism test results become available then there is a further complication in extrapolating from these results to PNEC values appropriate to these compartments. The normal method of extrapolation is to apply a correction that takes into account the organic matter content of the matrix. For substances that adsorb preferentially to inorganic matter it would be necessary to correct for both the type of inorganic matrix, the composition of the aqueous phase and the concentration of the substance. This is unlikely to be straightforward.

A search of the open literature was carried out in order to assess the environmental fate and behaviour properties of substances with similar chemistry and properties to PFOS. Published papers are relevant to the consideration of equilibrium partitioning as a relevant model for PFOS environmental fate. Several papers were located and the abstracts are summarised below. It is beyond the scope of this work to investigate the papers in detail but it can be noted that the technical quality of the work, as with all open literature, has already been peer reviewed (although not subject to GLP audit). The findings of these papers do not preclude the possibility of applying equilibrium partitioning to PFOS but do highlight the technical difficulties that might be encountered.

Conclusion

It is concluded that on the basis of the presently available data for PFOS that equilibrium partitioning theory cannot be applied to determine either concentrations in interstitial water of soil and sediments or PNEC values for soil and sediments for the following reasons:

- the nature of the adsorption process cannot be assumed to be linearly dependent upon concentration;
- the adsorption is likely to be highly dependent upon soil composition, particularly the inorganic component; and
- the rate at which equilibrium might be achieved is unknown

REFERENCES

Salloum, M.J., Dudas, M.J., McGill, W.B. and Murphy, S.M. (2000). Surfactant sorption to soil and geologic samples with varying mineralogical and chemical properties. *Environ. Toxicol. Chem.* 19(10), 2436-2442.

Soil Adsorption/Desorption Study of Potassium Perfluorooctanesulfonate (PFOS), Mark E. Ellefson, 3M Laboratory Report No: E00-13 11

Review of open literature

Report title	Summary of abstract	Author(s)	Journal
Terrestrial risk assessments for linear alkylbenzenesulfonate (LAS) in sludge-amended soils	Reviews investigations of factors affecting fate of LAS in the terrestrial environment. Refers to precipitating effect of calcium/magnesium ions in addition to primary sorption. Sorbed form is noted as being different to the commercially available form (for which data were available).	DeWolfe, Watze; Feijtel, Tom	Chemosphere, vol 36, no. 6, pp 1319-1343, 1998
Effect of sediment organic-carbon on the toxicity of a surfactant to <i>Hyalella azteca</i>	Investigation of the effects of sediment organic carbon levels on the sorption and toxicity to <i>Hyalella azteca</i> of anionic surfactants, specifically ABS (alkylbenzene sulfonate). Studies indicated that higher levels of organic carbon led to higher apparent sorption coefficients. A higher sediment concentration was required to elicit the toxic response at higher organic carbon levels.	Cano, M.L.; Dyer, S.C.; Decarvalho, A.J.	Environ. Toxicol. Chem., vol 15, no. 8, pp 1411-1417, 1996
Anionic surfactant transport characteristics in unsaturated soil	Investigations showed that for an alkyl ether sulfate and a linear alkylbenzenesulfonate, soil-surfactant interactions were reversible and that equilibrium conditions were quickly achieved.	Allred, Barry; Brown, Glenn O.	Soil Sci., vol 161, no. 7, pp 415-425. 1996

Annex 4. Summary of the Lowest Acceptable Effect Concentrations

Endpoint	Species	Protocol	Result (mg/l)	
Freshwater fish - acute toxicity	<i>Pimephales promelas</i> (Fathead minnow)	Not given	96-hour LC ₅₀	4.7
Freshwater fish - chronic toxicity	<i>Pimephales promelas</i> (Fathead minnow)	OECD 210 & OPPTS 850.1400	42-day NOEC _{surv/growth}	0.30
Invertebrate - acute toxicity (Freshwater)	<i>Daphnia magna</i> (Water flea)	ASTM 1981 & OECD 1981	48-hour EC ₅₀	27
Invertebrate - acute toxicity (Salt water)	<i>Mysidopsis bahia</i> (Mysid shrimp)	OPPTS 850.1035	96-hour LC ₅₀	3.6
Invertebrate - chronic toxicity (Freshwater)	<i>Daphnia magna</i> (Water flea)	ASTM 1981 & OECD 1981 (Semi-static)	28-day NOEC _{repro}	7
Invertebrate - chronic toxicity (Salt water)	<i>Mysidopsis bahia</i> (Mysid shrimp)	OPPTS 850.1350	35-day NOEC _{repro/growth}	0.25
Aquatic plants – growth inhibition of freshwater algae - Short-term exposure	<i>Selenastrum capricornutum</i> (now <i>Pseudokirchneriella subcapitata</i>)	OECD 201, OPPTS 850.5400 & ASTM 1218-90E	96-hour EC ₅₀ cell density 96-hour E _b C ₅₀ area under the curve 96-hour E _r C ₅₀ growth rate 96-hour NOEC _{growth rate, cell density, area under the growth curve} 72-hour EC ₅₀ cell density 72-hour E _b C ₅₀ area under the curve 72-hour E _r C ₅₀ growth rate 72-hour NOEC _{growth rate, cell density, area under the growth curve}	71 71 126 44 70 74 120 70
Aquatic plants – growth inhibition of Saltwater algae – Short-term exposure	<i>Skeletonema costatum</i>	OPPTS 850.5400	96-hour EC ₅₀ growth rate 96-hour NOEC _{growth rate}	>3.2 >3.2
Aquatic plants – growth inhibition of freshwater algae - Longer-term exposure	<i>Selenastrum capricornutum</i> (now <i>Pseudokirchneriella subcapitata</i>)	OECD 201, US EPA 600/9-78-018 & ASTM-E-35.23	14-day EC ₅₀ cell density 14-day NOEC _{cell density} 14-day EC ₁₀ cell density	95 <26 16
Freshwater higher plants – growth inhibition	<i>Lemna gibba</i>	OPPTS 850.4400	7-day IC ₅₀	108
Amphibians – embryo survival, growth and development	<i>Xenopus laevis</i> (African clawed frog)	ASTM E1439-91	96-hour LC ₅₀ 96-hour EC ₅₀ malformations Minimum concentration to inhibit growth	13.8 12.1 7.97
Sewage treatment organisms	Activated sludge	OECD 209	3-hour IC ₅₀ respiration inhibition	>905

Endpoint	Species	Protocol	Result (mg/kg of food)	
Dietary toxicity to birds	<i>Anas platyrhynchos</i> (Mallard duck)	OECD 205, OPPTS 850.2200 & FIFRA E 71-2	LC ₅₀ NOEC _{mortality} NOEC _{body weight}	628 146 37
	<i>Colinus virginianus</i> (Northern Bobwhite quail)	OECD 205, OPPTS 850.2200 & FIFRA E 71-2	LC ₅₀ NOEC _{mortality} NOEC _{body weight}	220 73 73

Endpoint	Species	Protocol	Result (µg/bee)	
Oral toxicity to bees	<i>Apis mellifera</i> (Honey bee)	OECD 213, EPPO 170	72-hour LD ₅₀ 72-hour NOEL	0.40 0.21
Contact toxicity to bees		OECD 214, EPPO 170, OPPTS 850.3020 (Contact)	96-hour LD ₅₀ 96-hour NOEL	4.78 1.93

Annex 5. Robust Summaries for Physical Chemical Properties and Environmental Fate Studies

VAPOR PRESSURE

Title: Impinger Studies of Volatility of FC-95 and FC-143

TEST SUBSTANCE

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

Remarks: The 3M production lot number was not noted. The test sample is a white powder of uncharacterized purity.

METHOD

Method: Internal

GLP: No

Year study performed: 1993

Remarks: Details outlined in the results section. As described below, there are a number of methodological concerns with this study. In addition, the document submitted is a combination of two reports. The first report contained Parts I and II, and a second report contained Experiment # 3. In addition, the first report contains portions of two versions. As neither of the first report versions is dated, it is unknown which is the final version. **Part I Procedure:** A mixture of 10 ppm FC-95 (PFOS) and 10 ppm FC-143 (PFOA) was prepared in water or water/isopropanol solutions of the polar-organic compounds listed in the table below. Aliquots of each solution were analyzed in triplicate for fluorochemical content by LC-thermospray mass spectrometry both before and after bubbling 280 liters of air through them. The flow rate of the air passing through the stock solutions was 1 L/min. The solutions were kept in an ice bath during bubbling. The concentrations before and after bubbling were compared after adjusting for volume lost during bubbling. **Part II Procedure:** Air was passed through an apparatus containing dry test material and then through glass wool at room temperature to a chain of impingers. A 50:50 propanol:water solution containing 500 ppm ammonium acetate was used in the impingers to catch any volatilized PFOS. All impingers were in ice water. **Experiment #3 Procedure:** The same study as in Part II was conducted in duplicate at 90°C.

RESULTS

Vapor Pressure Value: Part II- 8.7×10^{-8} torr, Experiment # 3- 1.2×10^{-7} torr

Temperature °C: 90°C in experiment # 3

Decomposition: not stated

Part I- Analysis before and after passing 280 liters of air through various stock solutions.**Results:**

Solution	Original PFOS Conc., ppm	% PFOS retained
500 ppm Tetrabutylammonium hydroxide	10.0	90
500 ppm Ammonium acetate	10.0	71
503 ppm Laurylpyridinium chloride	10.0	90
500 ppm N-Alkyldimethylbenzyl-ammonium chloride	10.0	100
500 ppm Cetyltrimethylammonium bromide	10.0	95
505 ppm Tallowtrimethylammonium chloride	10.0	93
500 ppm Dicocodimethylammonium chloride	10.0	84
Water/1-Propanol (50:50)	10.0	89
Water/1-Propanol (50:50)	0	0
500 ppm Ammonium acetate in water/1-propanol (50:50)	0.10	96
500 ppm Ammonium acetate in water/1-propanol (50:50)	0.20	101
500 ppm Ammonium acetate in water/1-propanol (50:50)	0.40	90
500 ppm Ammonium acetate in water/1-propanol (50:50)	0.80	95
500 ppm Ammonium acetate in water/1-propanol (50:50)	2.0	96

Remarks: These findings appear to suggest a small loss of PFOS. However, in comments dated 12/7/93, Dr. Edwin Tucker of the Chemistry Department at the University of Oklahoma indicates that it is very unlikely that these fluorochemicals were removed by bubbling air through water due to their vapor pressures, which are very low. Tucker thought more likely mechanisms for loss from the solution phase were concentration of the surfactants in foam and loss from the bubbled solutions as foam or micro-droplets.

Part II- Analysis of impinger ammonium acetate solutions.

Results: No test material was found to be present in either the first or second impinger. This indicates that any test material transported from the solids to air and then into the ammonium acetate solutions in the impingers is below the detection limit. The calculated maximum pressure was 8.7×10^{-8} torr.

Remarks: In the report, the maximum vapor pressure calculations section contains errors. Equation 1 should use the value 0.625:g, not 0.625:g/mL. In addition, the “maximum” vapor pressure calculated was erroneously called “minimum” vapor pressure in the text below both equations 5 and 7. Dr. Edwin Tucker of the Chemistry Department at the University of Oklahoma states that the experimental conditions do not provide firm evidence that the number is reasonable. There is no evidence that vapor pressure equilibrium was attained between the solid and the flowing gas.

Experiment # 3- Measuring Vapor Pressure at 90°C

Results: The report concludes that PFOS has a measurable vapor pressure at this temperature. The minimum vapor pressure for the test material was purported to be 1.2×10^{-7} torr at 90°C. It was considered a minimum because the impinger trains may not have caught all of the fluorochemical that had been volatilized.

Remarks: There are reasons to consider this a questionable result. No notation was made about the sensitivity of the analytical measurements in this study, but the quantification limit in the analysis in Part II was 0.625 µg. The concentration range from impinger train 1 in Experiment # 3 was 0.07-0.075 µg. Impinger concentrations ranged from 0-0.18 µg in impinger train 2. These concentrations are near or below the quantification limit reported in Part II.

CONCLUSIONS

No reliable conclusions can be made based on this study. The general observation is that this compound has very low volatility or a very low vapor pressure under ambient conditions.

Remarks: none

DATA QUALITY

Reliabilities: Klimisch ranking 3. There is no information on the validity of the test method for determining volatility of the test substance. This study lacks characterization of the purity of the test substance. There is no information on the validity of the analysis method.

REFERENCE

3M Environmental Laboratory. 1993. 3M Lab Request Number L3306, 3M Company, St. Paul, MN.

WATER SOLUBILITY STUDY

Title: Solubility of PFOS in water

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analysis techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840.

GLP: Yes

Year study performed: 2001

Remarks: Water source is ASTM Type I water, Millipore. The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 225 rpm at 30°C for 24, 48, or 72 hours followed by 24-hours of equilibration at 24-25°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

RESULTS

Value (mg/L) at temperature °C: 680 mg/L at 24-25°C

Description of solubility: Slightly soluble

pH value and concentration at temperature °C: not stated

pKa value at 25°C: not stated

Remarks: The 24-, 48-, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations.

CONCLUSIONS

The overall mean solubility concentration of the test substance in pure water was 680 mg/L.

Remarks: none

DATA QUALITY

Reliabilities: Klimisch ranking 1

REFERENCE

Ellefson, M. 2001c. Solubility of PFOS in Water. 3M Company, 3M Environmental Laboratory, Project Number E00-1716.

WATER SOLUBILITY STUDY

Title: Solubility of PFOS in Natural Seawater and an Aqueous Solution of 3.5% Sodium Chloride

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analysis techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840

GLP: Yes

Year study performed: 2001

Remarks: The definitive test consisted of placing an excess amount of test substance with the appropriate water in centrifuge tubes. The tubes were vortexed and shaken at 150 rpm at 30°C for 24, 48, and 72 hours followed by 24 hours of equilibration at 22-24°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

Water Sources: Natural Seawater = Ocean Scientific, lot # LN 58, salinity = 3.5%

Sodium Chloride = EM Science, 99% pure, mixed with ASTM Type 1 water to achieve salinity of 3.5%

RESULTS

Value (mg/L) at temperature °C: Natural Seawater: 12.4 mg/L at 22-23°C; 3.5% NaCl Solution: 20.0 mg/l at 22-24°C

Description of solubility: Slightly soluble

pH value and concentration at temperature °C: not stated

pKa value at 25°C: not stated

Remarks: The 24-, 48-, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentrations for the natural seawater. The 24-hour values were not included in the mean solubility of the sodium chloride calculation because when the coefficient of variation was calculated for all of the replicate analyses for that day, it was >15%.

CONCLUSIONS

The overall mean solubility concentration of the test substance in natural seawater was 12.4 mg/L. In a 3.5% NaCl solution it was 20.0 mg/L. PFOS solubility decreases with increasing ionic strength of the medium.

Remarks: none

DATA QUALITY

Reliabilities: Klimisch ranking 1

Remarks: none

REFERENCE

Ellefson, M. 2001a. Solubility of PFOS in Natural Seawater and an Aqueous Solution of 3.5% Sodium Chloride. 3M Company, 3M Environmental Laboratory, Lab Project Number E00-1716.

SOLUBILITY STUDY

Title: Solubility of PFOS in Octanol

TEST SUBSTANCE

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

Remarks: The test substance is a white powder. The sample was recrystallized from a production lot of FC-95, and assigned a test, control, and reference number TCR 00017-046. The purity was determined to be 97.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR and elemental analysis techniques.

METHOD

Method: Based on OECD 105, OPPTS 830.7840

GLP: Yes

Year study performed: 2001

Remarks: Octanol Source: Aldrich

Screen Test: Lot JU0873804, 99+%

Definitive Test: Lot 06238CI, 99.9+% HPLC Grade

The definitive test consisted of placing ~0.010 g test substance with ~10 mL octanol in centrifuge tubes. The tubes were shaken at ~150 rpm at ~30°C for 24, 48, or 72 hours followed by 24 hours of equilibration at 22-23°C. Following equilibration, samples were centrifuged and the supernatant was analyzed by high performance liquid chromatography with mass spectrometric detection (LCMS).

RESULTS

Value (mg/L) at temperature °C:

24 hours: 56.9 mg/L

48 hours: 55.7 mg/L

72 hours: 55.4 mg/L

Mean solubility of PFOS in octanol = 56.0 mg/L

Description of solubility: not stated

pH value and concentration at temperature °C: not stated

pKa value at 25°C: not stated

Remarks: The 24, 48, and 72-hour solubility concentrations were averaged to obtain the overall mean solubility concentration.

CONCLUSIONS

The overall mean solubility concentration of the test substance in pure octanol was 56.0 mg/L.

Submitters' Remarks: Typically, the Column Elution Method is recommended for use with substances with solubility screening results of <10 mg/L. However, the shake flask method was utilized in this study because of the difficulty in obtaining tubing compatible with octanol and the possible explosion hazard posed by possible leaks of a flammable solvent in an incubator.

DATA QUALITY

Reliabilities: Klimisch ranking 1

Remarks: none

REFERENCE

Ellefson, M. 2001b. Solubility of PFOS in Octanol. 3M Company, 3M Environmental Laboratory, Laboratory Project Number E00-1716.

SOIL ADSORPTION

Title: Pilot Study on Soil Adsorption

TEST SUBSTANCE

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

Remarks: The sample was radiolabeled (isotope and labeling position not specified). The 3M production lot number was not noted. The test substance was a white powder of uncharacterized purity.

METHOD

Method/guideline followed: The study explored the use of autoradiography of Thin Layer Chromatography Plates for determining the soil mobility of PFOS. This method was developed by 3M while looking for alternatives for studying the adsorption/desorption properties of the test substance.

GLP (Y/N): No

Year study performed: 1978

Statistical methods: None

Temperature: Not indicated

Remarks: Little detail was available regarding the study method.

RESULTS

Results: The study used TLC greenhouse soil plates. The radiolabeled spot due to PFOS was too faint to be visualized.

CONCLUSIONS

No conclusion could be reached in this study.

DATA QUALITY

Reliability: Klimisch ranking 3. These studies lacked sufficient detail regarding methodology. Isotope specific activity and chemical/radiochemical purity of the test substance were not provided. The method for radiosynthesis was also not provided. The analytical methodology lacked validation and a means of identifying and quantifying potential degradation products.

REFERENCES

Boyd, S.A. 1993. Review of Technical Notebook. Soil Thin Layer Chromatography. Number 48277, p30. Michigan State University.
Mendel, A. 1978. Soil Thin Layer Chromatography—FC-95, FC-143, FM-3422. Excerpt from 3M Technical Notebook. October 13, 1978. Number 48277, p30. Project Number 9970612600.

BIODEGRADATION STUDY

Title: Determination of Methylene Blue Active Substance - FC-95

TEST SUBSTANCE

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

Remarks: The 3M production lot was 158. The test substance was a white powder of uncharacterized purity.

METHOD

Method/guideline followed: EPA Method 425.1

Test Type: Methylene Blue Active Substance

GLP (Y/N): No

Year study performed: 1989

Contact time units: Not indicated

Inoculum: Not indicated

RESULTS

Degradation % after time: Not indicated

Results:

3M Lab Request Number	MBAS Result
G1512-1	835,000 mg/kg
G1827-2	831,000 mg/kg
G1828-2	730,000 mg/kg

Kinetic (for sample, positive and negative controls): Not indicated

Breakdown products (yes/no): Not indicated

Remarks: None

CONCLUSIONS

Reviewer's remarks: The test indicated that the test sample exhibited high activity in this MBAS study.

DATA QUALITY

Reliability: Klimisch ranking 3. This study lacked information regarding methodology and testing

parameters. Test substance purity within the test sample was not sufficiently characterized. The sample description from Twin City Testing was “liquid”, but FC-95 was a solid material. This may indicate that the FC-95 was sent in a solution and that the actual MBAS may be higher than the values cited.

REFERENCE

Determination of Methylene Blue Active Substance - FC-95. 1989. Pace Analytical and Twin City Testing. Minneapolis, Minnesota—at the request of the 3M Company, 3M Lab Request numbers: G1512, G1827, and G1828.

SOIL ADSORPTION

Title: Soil Adsorption/Desorption Study of Potassium Perfluorooctane Sulfonate (PFOS)

TEST SUBSTANCE

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

Remarks: The test substance was a white powder. The sample was recrystallized from a production lot of FC-95. The purity was determined to be 97.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR, and elemental analysis techniques.

METHOD

Method/guideline followed: OECD 106

GLP (Y/N): Yes

Year study performed: 2001

Statistical methods: Statistical analysis and plotting of the data was done according to OECD Method 106 using Microsoft Excel.

Temperature: Room temperature (19-30°C)

Stock and test solution preparation: The test concentrations and conditions were determined in a preliminary experiment. For the definitive experiment, test solutions were made by diluting a stock solution of unradiolabeled perfluorooctanesulfonate to a final test substance concentration of approximately 0.5 mg/L in aqueous 0.01 M CaCl₂.

Soil Characteristics

Soil Class	Clay	Loam	Clay Loam	Sandy Loam	River Sediment	Domestic Sludge
Source	Agvise Laboratories, Northwood, ND	Agvise Laboratories, Northwood, ND	Agvise Laboratories, Northwood, ND	Agvise Laboratories, Northwood, ND	Agvise Laboratories, Northwood, ND	NIST, from Denver, CO POTW
Lot Number	00-2407	00-2404	00-2405	99-2564	00-2046	2781
Physical Description	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	1.00 mm air-dried, 0-6" deep	200 mesh, oven-dried, sterilized
% Organic Carbon	2.6%	4.9%	2.6%	2.8%	1.3%	Not analyzed
% Sand	16%	39%	21%	58%	39%	Not analyzed
% Silt	22%	50%	46%	22%	42%	Not analyzed
% Clay	62%	11%	33%	20%	19%	Not analyzed
CEC (meq/100g)	54.5	23.9	24.7	23.3	17.5	Not analyzed
pH in 0.01 M CaCl ₂	7.2	7.4	6.0	7.8 ¹	7.7	Not analyzed

¹Value is for pH in water, not pH in 0.1 M CaCl₂

Test Conditions:

Adsorption kinetics: Replicate study samples containing the soils (or sediments or sludges) were equilibrated by shaking for at least 12 hours at room temperature with 0.01M CaCl₂. Study samples were dosed with the test substance at approximately 0.5 mg/L and placed on an orbital shaker. Replicate sets of these study samples were removed at designated time points throughout a 48-hour time period. Study samples were then prepared and analyzed for the target analyte. The adsorption kinetics were determined using this data. The last set of study samples (48-hour) were saved and used for the desorption kinetics portion of the method.

Desorption kinetics (one concentration): After the adsorption kinetics experiment, the 48-hour study samples were centrifuged and the aqueous phase removed. The volume of solution removed was replaced by an equal volume of 0.01 M CaCl₂ without test substance. The new mixture was agitated until the desorption equilibrium was reached. During a 48-hour period, at defined time intervals, small aliquots of the aqueous phase were removed and analyzed for the target analyte. The desorption kinetics were determined using this data.

RESULTS

Adsorption Kinetics of PFOS, 1:5 Soil:Solution Ratio, 48-hour Time Point

Soil Type	Average Distribution Coefficient, K_d , L/g	Percentage of Organic Carbon in Soil	Average Organic Carbon normalized Adsorption Coefficient, K_{oc} , L/g
Clay	0.0183	2.6	70.4
Clay Loam	0.00972	2.6	37.4
Sandy Loam	0.0353	2.8	126
River Sediment	0.00742	1.3	57.1
Domestic Sludge	<0.120	Not available	Not calculable

All matrices adsorbed the test substance strongly. The sludge demonstrated very strong adsorption (>96%) and PFOS was not detected in the extracts. The data indicated that adsorption occurred within the first few hours of exposure and the test substance concentration did not vary significantly after 16 hours.

Apparent Desorption Kinetics of PFOS, 1:5 Soil:Solution Ratio, 48-hour Time Point

Soil Type	Desorption Coefficient, K_{des} , L/g
Barnes Loam	0.0000471
Clay Loam	0.0000158
Clay	0.0000349
River Sediment	0.0000100
Domestic Sludge	<0.000237

The test substance was poorly desorbed from the soil/sediment/sludge matrices during the 48-hour study period. The river sediment displayed the most desorption at 39% after 48 hours. The sludge samples did not desorb a detectable amount of test substance. Desorption that did occur was accomplished rather quickly; after the 8-hour time point the test substance concentration did not vary significantly.

Adsorption Isotherms

Soil Type	Log K_F^{ads}	$K_F^{ads(1)}$	Regression constant, $1/n$	Regression Constant, n
Clay	-1.2515	0.0560	0.884	1.13
Clay Loam	-1.3762	0.0421	0.841	1.19
Sandy Loam	-1.0369	0.0919	0.829	1.21
River Sediment	-2.0261	0.0094	0.989	1.01
Domestic Sludge	-1.246	0.0568	1.2581	0.795

(1) Freundlich adsorption coefficient

Desorption Isotherms

Soil Type	Log K_F^{des}	$K_F^{\text{des}} (1)$	Regression Constant, 1/n	Regression Constant, n
Clay	-0.653	0.222	0.935	1.07
Clay Loam	-1.084	0.082	0.954	1.05
Sandy Loam	-0.981	0.104	1.01	0.988
River Sediment	-1.41	0.039	1.02	0.984
Domestic Sludge	1.47	29.5	0.327	3.06

(1) Freundlich desorption coefficient

Freundlich adsorption isotherms were used to relate the amount of test substance adsorbed on the soil to the amount present in the aqueous solution at equilibrium. The values calculated for the regression constant indicate that the data obtained for the test substance over two orders of magnitude were slightly non-linear.

CONCLUSIONS

Perfluorooctanesulfonate (PFOS) appeared to adsorb strongly to all of the soil/sediment/sludge matrices tested. PFOS would not be considered to be qualitatively mobile as per OECD Guideline 106 (1/21/00) as the K_d values are >1 mL/g. The test substance, once adsorbed, does not desorb readily, even when extracted with an organic solvent. In either case, adsorption or desorption, an equilibrium is achieved in less than 24 hours, with substantial adsorption ($>50\%$) occurring in some of the time 0 samples after approximately 1 minute of contact. The test substance exhibited low mobility in all of the adsorbants tested. The shape of the PFOS adsorption isotherm (H-type) indicated a very strong chemical/adsorbent interaction. Because PFOS is a strong acid, it likely forms strong bonds with soils, sludge, and sediment via the mechanism of chemisorption.

DATA QUALITY

Reliability: Klimisch ranking 1.

REFERENCES

Ellefson, M.E. 2001d. Soil Adsorption/Desorption Study of Potassium Perfluorooctanesulfonate (PFOS). 3M Technical Report. Project Number E00-1311, Completion date June 4, 2001.

BIOCONCENTRATION (PARTITION COEFFICIENT)

Title: Kow (Solubility in Water, Natural Seawater, An Aqueous Solution of 3.5% Sodium Chloride, and n-Octanol with Subsequent Calculation of the n-Octanol Water Partition Coefficient (Kow) of PFOS for each of the Aqueous Matrices)

TEST SUBSTANCE

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

Remarks: The test substance was a white powder. The sample was recrystallized from a production lot of FC-95, and assigned the internal reference number of TCR-00017-046. Purity was determined to be 97.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR, and elemental analysis techniques.

METHOD

Method/guideline followed: Calculated from n-octanol solubility and water solubility according to OPPTS 830.7550 and OECD 107.

Test Type: n-Octanol/Water partition coefficient (at saturation)

GLP (Y/N): Yes

Year study performed: 2001

Test temperature: n-Octanol solubility value at 23-24°C
Water solubility value at 24-25°C

Remarks: The physical properties of PFOS did not allow a determination of the partition coefficient by the shake flask method per guidance provided in the OPPTS and OECD guidelines. Therefore, this study did not bring the two phases (n-octanol and water) into contact with PFOS at the same time, and this testing reflected the partition coefficient for the subject material at saturation only. The n-Octanol/Water partition coefficient was calculated by dividing the solubility of PFOS in n-octanol by the solubility in water and expressing it as the logarithmic value.

RESULTS

The calculated log Kow for PFOS was determined to be -1.08 at saturation (log(56 mg/L in n-octanol/680 mg/L in water)).

CONCLUSIONS

Remarks: No conclusions could be derived from this information. It applied only to a saturated system, which would not likely exist in the environment.

DATA QUALITY

Reliability: Klimisch ranking 1

Remarks: The solubility studies were conducted properly. However, the applicability of this data point is limited. Application of this value in a risk assessment has limited or no value as it only applies to saturated systems.

REFERENCES

Kow (Solubility in Water, Natural Seawater, An Aqueous Solution of 3.5% Sodium Chloride, and n-Octanol with Subsequent Calculation of the n-Octanol Water Partition Coefficient (Kow) of PFOS for each of the Aqueous Matrices). 2001. 3M Company. St. Paul, Minnesota. Environmental Laboratory Project Number E00-1716.

BIODEGRADATION STUDY

Title: Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) II. Aerobic Soil Biodegradation

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt) CAS #2795-39-3

Remarks: White powder, 86.9% purity, also called PFOS or FC-95

METHOD

Method/guideline followed (experimental/calculated): Method was designed by Springborn Laboratories, Inc.

Test Type (aerobic/anaerobic): Aerobic

GLP (Y/N): No

Year study performed: 2000

Contact time/units: 20 weeks

Inoculum: The inoculum sources were soils collected from a hardwood forest in Hanson, MA, a pine forest in Onset, MA, and a river bank in Bridgewater, MA; and sediments collected from brackish sites below the Wareham, MA wastewater treatment plant outfall and from the Narrows area in Wareham, MA. Biomass was determined on day 83 by both the fumigation/extraction and standard plate count methods and was reported as 17.4 mg C/100 g soil and 6×10^5 cells/g, respectively.

Remarks: Soil and sediment samples were air-dried, 2.0 mm-sieved, and mixed together in equal dry weight portions. A nutrient mixture was prepared by combining a sterile potting soil extract, a trace mineral solution, a yeast extract, and reagent water. The soil/sediment mixture was adjusted to 75% of the water holding capacity using the above nutrient mixture. Soil moisture was monitored weekly during the study, and adjusted using reagent water as needed. The nominal test concentration was approximately 21.2 mg/kg. The incubation temperature was $22 \pm 3^\circ\text{C}$, and the test was conducted in the dark. Information on test solution agitation was not provided. Test vessels were 40-mL I-Chem glass vials with silicone/Teflon-lined septum screw caps containing 10 g (dry weight) soil/sediment mixture. Each test flask received all components at test initiation. Samples were taken at days 7, 14, 21, 28, 35, 42, 49, 56, and 63. Entire samples were extracted with methanol via accelerated solvent extraction. Extracts passed through a 0.2 μm nylon filter prior to analysis. Samples were diluted as necessary in methanol and analyzed via LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, which is approximately twice the water solubility of PFOS. No day 0 samples were taken to determine starting concentrations.

RESULTS

Degradation % after time: Not specified

Results: Essentially no PFOS metabolism occurred during the study.

Kinetic (for sample, positive and negative controls): Not specified

Breakdown products (yes/no): None indicated

Remarks: None

CONCLUSIONS

Remarks: PFOS is recalcitrant in the activated soil/sediment system.

DATA QUALITY

Reliability: Klimisch ranking = 2. Study not conducted according to Good Laboratory Practices. The stock solution used to dose the systems was prepared at twice the water solubility of PFOS. No Day 0 samples were taken to determine starting concentrations.

REFERENCE

The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

BIODEGRADATION STUDY

Title: Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) III. Anaerobic Sludge Biodegradation

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt) CAS #2795-39-3

Remarks: White powder, 86.9% purity, also called PFOS or FC-95

METHOD

Method/guideline followed (experimental/calculated): Method was designed by Springborn Laboratories, Inc.

Test Type (aerobic/anaerobic): Anaerobic

GLP (Y/N): No

Year study performed: 2000

Contact time/units: 56 days

Inoculum: The inoculum source was an anaerobic digester at the Rockland, MD wastewater treatment plant.

Remarks: A mixture containing dried sludge extract (from rotating biological contactor wastewater treatment, Bridgewater, MA), unspecified OECD mineral media, and resazurin indicator served as the test medium. The media were prepared under nitrogen purge to exclude oxygen. The nominal test concentration was approximately 20.8 mg/L, and loading in test vessels was 300 mL anaerobic sludge per liter of medium. The test temperature was 35°C, and the test was conducted in the dark. Information on test solution agitation was not provided. Twenty 160-mL serum bottles containing 100 mL of test solution (purged with nitrogen after filling) with crimped butyl rubber tops served as test flasks. Each flask received all components at test initiation. Samples were taken from the test bottle on days 7, 14, 21, 28, 35, 42, 49, and 56, and from inoculum control bottles on days 7 and 56. For analysis, an aliquot was removed from each bottle and centrifuged. Both the supernatant and the solid biomass portion were analyzed via LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, approximately twice the water solubility of PFOS. No day 0 samples were taken to determine starting concentrations.

RESULTS

Degradation % after time: Not specified

Results: No apparent PFOS biodegradation occurred over the 56-day period.

Kinetic (for sample, positive and negative controls): Not specified

Breakdown products (yes/no): None indicated

Remarks: None

CONCLUSIONS

Remarks: PFOS is recalcitrant in the anaerobic system.

DATA QUALITY

Reliability: Klimisch ranking = 2. Study not conducted according to Good Laboratory Practices. The stock solution used to dose the systems was prepared at twice the water solubility. No Day 0 samples were taken to determine starting concentrations.

REFERENCE

The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 10/31/00. Lab Project number E01-0434.

BIODEGRADATION STUDY

Title: Microbial Metabolism (Biodegradation) studies of Perfluorooctane Sulfonate (PFOS) IV. Pure Culture Study

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt) CAS #2795-39-3

Remarks: White powder, 86.9% purity, also called PFOS or FC-95

METHOD

Method/guideline followed (experimental/calculated): Two study types were used (Pure Culture and Closed Vial Headspace) and Springborn Laboratories, Inc./Betts et al., 1974 is cited.

Test Type (aerobic/anaerobic): Aerobic

GLP (Y/N): No

Year study performed: 2000

Pure Culture Studies

Contact time/units: 7 days

Inoculum: Four separate pure cultures from the American Type Culture Collection (ATCC) were tested:

Cunninghamella echinulata var. *echinulata* (fungi, ATCC #9244)

Mucor circinelloides f. *griseocyanus* (fungi, ATCC#1207a)

Phanerochaete chrysosporium (fungi, ATCC #24725)

Streptomyces griseus (actinomycete, ATCC #13273)

Remarks: The test medium was soybean grits-glucose (SGG) and test vessel loading was 6 mL of Stage II cultures into 60 mL media, with 24 hours of agitation on a shaker table at 250 rpm prior to the addition of PFOS. The nominal test concentration was approximately 20.9 mg/L. The test temperature was 26°C. The test vessel type was not noted. Each test flask received all necessary components at test initiation. All work utilized strict aseptic technique until harvest at day 7. Samples were taken on days 0 and 7. Analysis of the broth and cells collected via centrifugation was performed by LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,060 mg/L, approximately twice the water solubility of PFOS.

Degradation % after time: Not specified

Results: The studies with *Cunninghamella*, *Mucor*, and *Streptomyces* did not provide any indication of biotransformation of PFOS. Possible biotransformation of PFOS (90% mass balance) by *Phanaerochaete* was noted, and closed vial studies were performed to confirm this.

Kinetic (for sample, positive and negative controls): Not specified

Breakdown products (yes/no): None indicated

Remarks: None

Closed Vial Headspace Study

Contact time/units: 3 days

Inoculum: *Phanerochaete chrysosporium* (fungi, ATCC #24725)

Remarks: The test medium was soybean grits-glucose (SGG) medium at 1/10 and 1/100 strength plus resazurin. The test concentration was 0.2 mg/L. The incubation temperature was 26°C, and a shaker table in an environmental chamber at 250 rpm was used in incubation. Sterile 22-mL vials served as test vessels. Initial inoculum test vessel loading details were not noted. Each test flask received all necessary components at test initiation. Headspace was purged with oxygen and vials were immediately crimped. Samples were taken once on day 3. Analysis of the broth and cells collected via centrifugation was performed by LC/MS. The stock solution used to dose the biodegradation test systems was prepared at a concentration of 1,011 mg/L, approximately twice the water solubility of PFOS. No initial measured concentrations were taken in this closed vial study. Difficulties were encountered in maintaining aerobicity and only 3 days of exposure were maintained.

RESULTS

Degradation % after time: Not specified

Results: The results indicated no significant biotransformation of PFOS by *Phanaerochaete* fungi.

Kinetic (for sample, positive and negative controls): Not specified

Breakdown products (yes/no): None indicated

Remarks: None

CONCLUSIONS

Remarks: It did not appear that the four species were capable of metabolizing PFOS.

DATA QUALITY

Reliability: Klimisch ranking = 2. The stock solution used to dose the systems was prepared at twice the water solubility. There were no initial measured concentrations taken in the closed vial study. These studies were not conducted in accordance with Good Laboratory Practices.

REFERENCE

The study was conducted at Springborn Laboratories, Inc., Wareham, Massachusetts, at the request of the 3M Company. Report completed 11/3/00. Lab Project number E01-0434.

BIODEGRADATION STUDY

Title: The 18-day aerobic biodegradation study of perfluorooctanesulfonyl-based chemistries.

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or $C_8F_{17}SO_3^-K^+$. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS# 2795-39-3)

Remarks: PFOS is a white powder. The original 3M product lot number was not noted. The PFOS was HPLC purified and assigned 3M standard identification #TCR-00017-046. Upon receipt at the testing laboratory, the test article was given a test, control, and reference (TCR) number CA-TCR02-014. The submitter suggests that an Interim Certificate of Analysis reports the purity to be 97.9%. All results in the study were calculated assuming 100% purity. However, in the study report, the purity is noted as 86.4%. No explanation of this discrepancy was given.

METHOD

Method/guideline followed: Based on EPA guidelines OPPTS 835.3200

Test Type: Aerobic

GLP: No

Year study performed: 2001

Contact time: 18 days

Inoculum: Activated sludge collected 7/31/00 from the aeration basin at the Metro Wastewater Treatment Plant, St. Paul, MN. The MLSS was determined to be 2,280 mg/L when first collected. The MLSS was stored at 4 °C for approximately 5 weeks prior to being used for this study. The sludge was allowed to settle and the solids used for inoculum. The settled sludge constituted approximately 20% of the volume (~200 mL) of the MLSS used.

Test Medium: Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Methanol (1 mL per liter) was added per liter of mineral medium. Fifty mL of settled sludge was added per liter of mineral salts medium. Mineral medium plus sludge was prepared 9/7/00, while fresh medium without sludge (abiotic controls) was prepared 8/10/00.

Remarks: Three types of samples were prepared for this study: blank sludge controls (mineral medium, inoculum), abiotic controls (mineral medium, PFOS), and test substance samples (mineral medium, inoculum, PFOS). The test vessels, sterile 125 mL Nalgene polycarbonate culture flasks containing 25 mL of media, were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

The test concentration used was 2.455 mg/L. The samples were agitated at ~200 rpm at a temperature of 25 ± 3 °C. The test vessels were spiked with 6 µL of a 10,230 mg/L solution of PFOS in methanol yielding 2.455 mg/L. Sampling was done on days 0 and 18. The day 0 test vessels were prepared and immediately placed in a freezer that was maintained at -20 °C until analyzed. After 18 days, the test vessels were removed from the incubator and frozen until final sample preparation by solid phase extraction (SPE). Following thawing, test vessel contents were adjusted to 1% acetic acid and then passed through a

conditioned SEP-VAC C18 6cc SPE cartridge. Methanol was then added to the emptied culture flask, shaken vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative-ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to PFOS, the additional compounds quantified are specified below. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

Compound Name	Acronym	Chemical Formula
2-(N-ethyl Perfluorooctane sulfonamido) ethyl alcohol	N-EtFOSE Alcohol	$C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$
2-(N-ethyl Perfluorooctane sulfonamido) acetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$
2-(Perfluorooctane sulfonamido) acetic acid	M556	$C_8F_{17}SO_2NH(CH_2COOH)$
N-Ethyl perfluorooctane sulfonamide	N-EtFOSA	$C_8F_{17}SO_2NH(C_2H_5)$
Perfluorooctane sulfinate, potassium salt	PFOSulfinate	$C_8F_{17}SO_2^-K^+$
Perfluorooctanoate, ammonium salt	PFOA	$C_8F_{15}COO^-NH_4^+$
Perfluorooctane sulfonamide	FOSA	$C_8F_{17}SO_2NH_2$

No reference substance was used. However, when the results from an EtFOSE alcohol study conducted at the same time are compared to the previous EtFOSE alcohol 35-day study, the viability of the microbial inoculum is confirmed.

RESULTS

Degradation % after time: 0

Results: After 18 days, the analytical results demonstrate that after exposure to municipal wastewater treatment sludge, 2.455 mg/L PFOS was not measurably degraded biotically or abiotically. Mass balance for PFOS test vessels was excellent and ranged from 104-108%.

Breakdown products: No

Remarks: None

CONCLUSIONS

No loss of PFOS was demonstrated. Mass balance was 104-108%. The results from this study confirm the results from other aerobic biodegradation studies of PFOS.

Submitters' Remarks: The submitters suggested a Klimisch data quality ranking of 2 because the study was conducted as a non-GLP study, but with the understanding that good data quality objectives be met.

REFERENCE

Lange, C. 2001a. The 18-day aerobic biodegradation study of perfluorooctanesulfonyl-based chemistries. Pace Analytical Services, Inc. Minneapolis, MN. 3M Company. Minneapolis, MN.

STABILITY IN WATER STUDY

Title: Hydrolysis Reactions of Perfluorooctane Sulfonate (PFOS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS-potassium salt, 1-perfluorooctanesulfonic acid-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluorooctanesulfonate-potassium salt (CAS #2795-39-3).

Remarks: 3M production lot number was 171. Test substance is a light-colored powder at 25 °C.

METHOD

Method/guideline followed: Based on OPPTS: 835.2110.

Test Type: Hydrolysis as a function of pH

GLP (Y/N): No

Year study performed: 2001

Test sample preparation: Test solutions consisted of 1.0 mL buffered aqueous solutions at 6 pH levels (1.5, 3.0, 5.0, 7.0, 9.0, 11.0). The resulting PFOS concentration in all test samples (sample triplicates and matrix spike samples) was approximately 500 µg/L. Samples were shielded from light during incubation at 50 °C for periods of 0 to 49 days. Control samples and blanks addressed potential non-hydrolytic degradation routes.

Analytical Procedures: Samples were analyzed by quantitative HPLC/MS.

Remarks: This study was conducted at 50 °C in order to facilitate hydrolysis. Rates derived at 50 °C were extrapolated to 25 °C by dividing by a factor of 10, which is valid for reactions with Arrhenius heats of activation near 18 kcal/mole.

RESULTS

Degradation %: Not applicable; no degradation was reported.

Half-life ($t_{1/2}$): ≥ 41 years

Breakdown products (yes/no): No

Remarks: The analytical results indicate no degradation of PFOS or dependence on pH. The mean and standard deviation of all observed PFOS concentrations, pooled over the 6 observed pH levels, indicate that the pseudo-first order hydrolytic half-life of PFOS is greater than 41 years.

CONCLUSIONS

The analytical results indicate no degradation of PFOS or dependence on pH. The study indicates that the hydrolytic half-life of PFOS in water is greater than 41 years.

Submitters' Remarks: The authors assigned a Klimisch ranking of 2 for the reliability of this study. The authors also noted that the study was well-conducted, but not under GLP.

Reviewer's Remarks: None

REFERENCE

Hatfield, T. 2001a. Hydrolysis Reactions of Perfluorooctane Sulfonate (PFOS). 3M Environmental Laboratory and Pace Analytical Services. Lab request number W1878. Minneapolis, MN.

BIODEGRADATION STUDY

Title: The 35-day aerobic biodegradation study of PFOS

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate; may also be referred to as PFOS or $C_8F_{17}SO_3^-K^+$. (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt, CAS# 2795-39-3)

Remarks: PFOS is a white powder. The original 3M production lot number was not noted. The PFOS was HPLC purified. An Interim Certificate of Analysis reports the purity to be 97.9%. All results in this report are based on a purity of 86.4%, however. The lower purity value was associated with a standard the laboratory was using during this study.

METHOD

Method/guideline followed: Based on EPA Guidelines OPPTS 835.3200, OPPTS 835.3210, OPPTS 835.5045.

Test Type: Aerobic

GLP: No

Year study performed: 2001

Contact time: 35 days

Inoculum: Activated sludge collected 9/18/00 from the aeration basin at the Metro Wastewater Treatment Plant, St. Paul, MN. The suspended solids were allowed to settle for approximately 2 days at 4EC and the settled sludge, approximately 20% of the volume, was used to prepare cultures for the biodegradation study. The mixed liquor suspended solids, MLSS, was not noted.

Test medium: Test flasks were prepared using a mineral salts medium defined in EPA Guideline OPPTS 835.3200. Fifty mL of settled sludge was added per liter of mineral salts medium.

Remarks: Three types of samples were prepared for this test: blank sludge controls (mineral medium, inoculum), abiotic controls (mineral medium, PFOS), and test substance samples (mineral medium, inoculum, PFOS).

Test vessels, sterile 125 mL Nalgene polycarbonate culture flasks containing 25 mL media, were set in duplicate. Additional quality control samples (blanks) were prepared and analyzed as appropriate.

The test concentration used was 2.582 mg/L. The samples were agitated at 200 rpm at 25 ± 3 °C. Test vessels were spiked 6 µL of 10,760 mg/L solution of PFOS in methanol yielding a 2.582 mg/L PFOS solution. Samples were taken on days 0, 2, 5, 7, 14, and 35.

The day zero test vessels were prepared and immediately placed in a freezer that was maintained at -20 ± 7 °C. Upon removal from the incubator, test vessels on other days were either immediately frozen, or prepared by solid phase extraction (SPE). Following thawing, if needed, test vessel contents were adjusted to 1% acetic acid and then passed through a conditioned SEP-VAC C18 6cc SPE cartridge containing a plug of quartz wool to deter plugging. Methanol was then added to the emptied culture flask, shaken

vigorously and then passed through the SPE cartridge to extract adsorbed analytes. A second methanol wash was then collected separately for analysis to ensure quantitative extraction.

Quantitative analysis was conducted on an HP1100 high performance liquid chromatograph with mass spectrometer detector (HPLC/MSD) system. The MSD was operated in electrospray ionization in negative-ion mode using selected-ion monitoring (SIM) for quantitation.

In addition to the parent, PFOS, the compounds below were quantified. In the case of the compounds that are potassium or ammonia salts, only the concentration of the fluorochemical anion was quantified and reported.

Compound Name	Acronym	Chemical Formula
2-(N-ethyl Perfluorooctane sulfonamido) ethyl alcohol	N-EtFOSE Alcohol	$C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$
2-(N-ethyl Perfluorooctane sulfonamido) acetic acid	N-EtFOSAA	$C_8F_{17}SO_2N(C_2H_5)(CH_2COOH)$
2-(Perfluorooctane sulfonamido) acetic acid	M556	$C_8F_{17}SO_2NH(CH_2COOH)$
N-Ethyl perfluorooctane sulfonamide	N-EtFOSA	$C_8F_{17}SO_2NH(C_2H_5)$
Perfluorooctane sulfinate, potassium salt	PFOSulfinate	$C_8F_{17}SO_2^-K^+$
Perfluorooctanoate, ammonium salt	PFOA	$C_8F_{15}COO^-NH_4^+$
Perfluorooctane sulfonamide	FOSA	$C_8F_{17}SO_2NH_2$

RESULTS

Degradation % after time: 0

Results: The analytical results demonstrate that when exposed to municipal wastewater treatment sludge for 35 days, the 2.582 mg/L PFOS samples generated no quantifiable degradation products. PFOS was recovered at 2.553 ± 0.102 mg/L in the pooled study samples and at 2.653 ± 0.083 mg/L in the pooled abiotic samples. The measured concentration of PFOS was always $100 \pm 7\%$ of the expected concentration.

Breakdown products: No

Remarks: None

CONCLUSIONS

The six-sample point screening study established that PFOS is not biodegraded by the microbial populations of the municipal waste treatment inoculum used under the conditions tested.

Submitters' remarks: The submitters' assigned a data quality rating of 2 for this study since it was conducted as a non-GLP study, but with the understanding that good data quality objectives be met.

No sample matrix spikes were included in this study. However, PFOS was recovered at expected concentrations, and previous duplicated results obtained during a related project showed excellent PFOS recoveries from sludge using the same extraction method.

A series of positive controls were not run with this study. However, the sludge used in this study was also used for the preparation of samples in other biodegradation studies, and in some of those studies, preliminary results demonstrated positive biological activity for degradation of test samples.

REFERENCE

Lange, C. 2001b. The 35-day aerobic biodegradation study of PFOS. Pace Analytical Services, Inc. Minneapolis, MN. 3M Company. Minneapolis, MN.

PHOTODEGRADATION STUDY

Title: Screening studies on the aqueous photolytic degradation of potassium perfluorooctane sulfonate (PFOS)

TEST SUBSTANCE

Identity: Perfluorooctanesulfonate-potassium salt. May also be referred to as: PFOS, PFOS-potassium salt, 1-perfluorooctanesulfonic-potassium salt, or 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoropotassium salt.

Remarks: 3M production lot number 171. The test substance is a light-colored powder at 25 °C. The purity was determined to be 86.4% by LC/MS, ¹H-NMR, ¹⁹F-NMR, and elemental techniques.

METHOD

Method/guideline followed: Based on OPPTS: 835.5270 and OECD Draft Document "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis," August 2000.

Test Type: Direct and indirect photolysis

GLP (Y/N): No

Year study performed: 2001

Light Source: Suntest CPS+ or Suntest XLS+ lamp

Light Spectrum (nm): 290-800 nm

Relative Intensity based on Intensity of Sunlight: 680 w/m²

Spectrum of substance (max lambda, max epsilon and epsilon 295): A UV/Vis spectrum of a saturated aqueous solution of PFOS was recorded between 190 and 110 nm.

Test sample preparation: Aliquots of PFOS were added to 3 separate sets of VOA screw cap vials (exposed, unexposed, and control vials) containing 5 mL of appropriate matrix. Test vials were placed in the photoreactor. Control vials were wrapped in aluminum foil, sealed in a plastic bag, and placed in the photoreactor.

Analytical Procedures: Samples were analyzed by quantitative LC/MS and GC/MS techniques.

Remarks: The duration of the study was 67-167 hours. The test media used were water, H₂O₂/water (1:1 molar equivalent), Fe₂O₃/water (Fe³⁺ at 24X molar excess), Fe₂O₃/water with H₂O₂, and commercial (Aldrich) humic material prepared as in OPPTS 835.5270. All tests included a series of unexposed controls (kept in the dark) for the evaluation of any degradation reactions occurring without the presence of light. Solvent, matrix, and control blanks and spikes were tested under each condition.

RESULTS

Concentration of Substance: Not stated

Temperature EC: 25 ± 3

Direct photolysis: No decomposition was observed.

Indirect photolysis: Data obtained from the Fe_2O_3 matrix samples (with and without H_2O_2) were pooled to provide sufficient data to estimate the minimum half-life. The mean standard deviation of these data indicate that the minimum environmental half-life of PFOS due to indirect photolysis at 25 °C is greater than 3.7 years.

Breakdown products (yes/no): No

Remarks: No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. Direct photolytic decomposition of PFOS was not observed based on loss of starting material, nor were any of the predicted degradation products detected above their limits of quantitation.

CONCLUSIONS

No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. The mean and standard deviation of the observed PFOS concentrations in an aqueous $\text{Fe}_2\text{O}_3/\text{H}_2\text{O}_2$ matrix indicate that the indirect photolytic half-life of PFOS at 25 °C is greater than 3.7 years.

Submitters' Remarks: The authors assigned a Klimisch ranking of 2 for the data quality of this study.

Reviewer's Remarks: None

REFERENCE

Hatfield, T. 2001b. Screening studies on the aqueous photolytic degradation of potassium perfluorooctane sulfonate (PFOS). 3M Environmental Laboratory. 3M Company, St. Paul, MN. Report number W2775.

BIOCONCENTRATION IN FISH

Title: Perfluorooctanesulfonate, potassium salt (PFOS): A flow-through bioconcentration test with bluegill (*Lepomis macrochirus*)

TEST SUBSTANCE

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

Remarks: Sample from 3M production lot number 217. The test substance was a white powder. The purity was determined to be 86.9% by LC/MS, ¹H-NMR, ¹⁹F-NMR, and elemental analysis techniques.

METHODS

Method/guideline followed: USEPA OPPTS 850.1730 and OECD 305

Test type: Flow-through exposure with flow-through depuration phase (flow rate: approximately 6 volume additions per 24 hours)

GLP: Yes

Year study performed: 2001

Species: Bluegill sunfish (*Lepomis macrochirus*); mean length = 62 mm (56-66 mm); mean weight: 2.70 g (2.03-3.32 g); age ~ 7 months old

Supplier: Osage Catfisheries, Inc. Osage Beach, Missouri

Concentrations tested: 0, 0.086, and 0.87 mg/L

Uptake period: 62 days (0.086 mg/L exposure); 35 days (0.087 mg/L exposure– this exposure ended after 35 days due to fish mortality)

Depuration period: 56 days (0.086 mg/L exposure); None (0.87 mg/L exposure)

Analytical monitoring: Analyses of test solutions and fish tissues were performed at Wildlife International, Ltd. Water samples were diluted and analyzed by HPLC with a single quadrupole mass spectrometric detection. Tissue samples were homogenized, extracted, diluted, and analyzed by HPLC with triple quadrupole mass spectrometric detection. When determining the concentration of the test substance in the samples, the same and most prominent peak response for perfluorooctanesulfonate was used. No attempt was made to quantify on the basis of individual isomeric components. The LOQ was 0.05 mg/L for water in this study. For tissue samples, the LOQ was calculated on an individual basis for each sample since each entire submitted sample, of differing weight, was extracted without an adjustment to constant weight.

Recovery was excellent in both water and fish tissues, ranging from 84.9 to 122% of fortification levels. Analytical results were not corrected for procedural recovery.

Collection of tissue samples: Fish were collected from test chambers by random selection at 12 time points during the 62-day uptake phase. They were euthanized, blotted dry, weighed, and measured. Fish

were then rinsed with dilution water, blotted dry again, and dissected into edible and nonedible fractions. The fractions were individually weighed. The head, fins, and viscera were considered to be nonedible tissue. The remaining tissue including skin was considered to be edible tissue.

Statistical methods: Whole fish concentrations were calculated based on the sum of the edible and nonedible parts. Steady-state bioconcentration BCF values calculated from the tissue concentrations at apparent steady-state divided by the mean water concentration. Tissue concentrations were considered to be at apparent steady-state if 3 or more consecutive sets of tissue concentrations were not significantly different ($p > 0.05$). Tissue concentrations were evaluated for normality and homogeneity of variance using the Shapiro-Wilk's test and Bartlett's test, respectively. If the data did not meet the assumptions, data were transformed in an attempt to correct the data. Mean tissue concentrations were then compared using ANOVA and Dunnett's test.

The kinetic bioconcentration factor (BCFK), uptake rate (k_1), and depuration rate (k_2) were calculated for the edible, nonedible, and whole fish exposed to 0.086 mg/L PFOS using BIOFAC computer software. BIOFAC is a nonlinear parameter estimate routine, which estimates rate constants from a set of sequential time-concentration data. These rate constants were then used to calculate a BCFK ($BCFK = k_1/k_2$).

Test conditions: Stainless steel aquaria (104 L) filled with approximately 80 L solution were used as exposure vessels. One vessel per concentration was used. The loading rate was 0.48 g fish/L/day (90 fish per vessel). The fish were fed flake food (Ziegler Brothers, Inc., Gardners, PA) and given 16 hours of light and 8 hours of dark with a 30 minute transition period. The light intensity was 278 lux at the surface of the negative control vessel at test initiation.

Two stock solutions were prepared at 10 and 100 mg a.i./L. Stock solutions were stirred with an electric top-down mixer to aid in the solubilization of the test substance. After mixing, the stocks appeared clear and colorless. Stocks were prepared at approximately weekly intervals during the uptake phase. Stocks were injected into the diluter mixing chambers at a rate of 3.5 mL/minute where they were mixed with dilution water at a rate of 350 mL/minute to achieve the desired test concentrations. All final test solutions appeared clear and colorless. The dilution water was moderately-hard well water with specific conductance of 313 umhos/cm, hardness of 130 mg/L, alkalinity of 178, and pH of 8.1. During the test, the dissolved oxygen levels ranged from 6.4-8.6 mg/L, the temperature ranged from 21.7 to 22.0 °C, and the pH ranged from 7.9 to 8.2.

Remarks: None

RESULTS

Bioconcentration factors (BCF):

0.086 mg/L apparent steady-state BCF:

Edible: 484

Nonedible: 1124

Whole fish: 856

0.87 mg/L(study ended prior to achieving steady-state) BCF:

Edible: 136

Nonedible: 386

Whole fish: 278

BIOFAC Estimates (using 0.086 mg/L exposure):

	<u>Edible</u>	<u>Nonedible</u>	<u>Whole fish</u>
BCFK:	1866	4312	3614
Time to reach 50% clearance:	146 days	133 days	152 days

PFOS Concentrations in Tissues of Bluegill Exposed to 0.086 mg/L:

(Values are from 4 individual fish at each sample period.)

Uptake day	Edible tissue, mg/kg	Nonedible tissue, mg/kg	Whole fish conc., mg/kg
0 (4 hours)	0.167, 0.155, 0.144, 0.182	0.415, 0.519, 0.417, 0.497	0.293, 0.351, 0.286, 0.363
1	0.734, 0.726, 0.631, 0.806	1.68, 1.85, 1.72, 2.07	1.26, 1.34, 1.29, 1.53
3	1.73, 2.07, 2.03, 2.11	4.59, 5.50, 5.47, 5.97	3.21, 4.04, 4.18, 4.38
7	3.73, 4.25, 4.73, 6.25	10.2, 10.6, 11.9, 15.2	7.33, 7.66, 8.73, 11.4
14	11.4, 9.07, 13.7, 12.6	27.3, 23.2, 35.3, 32.6	20.2, 16.9, 26.0, 24.6
21	11.7, 12.0, 12.9, 10.6	33.3, 22.7, 24.6, 24.4	23.3, 18.4, 19.8, 18.5
28	18.3, 13.7, 23.9, 23.1	49.4, 40.7, 65.3, 57.9	35.3, 29.2, 45.4, 44.1
35	22.6, 27.7, 23.8, 20.6	67.1, 73.3, 62.0, 59.1	46.3, 53.8, 46.6, 40.9
42	27.6, 25.3, 21.2, 27.6	64.0, 68.1, 54.4, 79.6	50.1, 49.4, 40.9, 56.3
49	33.3, 36.2, 39.0, 30.6	85.0, 95.1, 93.1, 77.7	62.8, 69.6, 70.8, 57.4
56	48.3, 38.9, 44.1, 38.3	122, 94.2, 73.2, 106	90.6, 71.6, 63.3, 74.8
62	42.4, 66.2, 42.2, 39.2	101, 112, 105, 96.4	77.0, 92.7, 79.6, 73.1
Depuration day			
14	48.5, 31.8, 31.6, 42.0	124, 79.4, 81.8, 113	90.3, 60.4, 61.6, 85.3
28	26.0, 33.3, 38.7, 55.8	85.7, 95.1, 85.7, 94.8	58.2, 70.1, 68.1, 81.1
42	24.1, 31.2, 30.0, 33.0	71.7, 80.6, 78.3, 82.1	51.4, 61.4, 61.0, 62.2
56	21.1, 37.6, 32.9, 31.2	57.7, 80.3, 85.4, 84.4	41.6, 66.5, 65.8, 62.1

PFOS Concentrations in Tissues of Bluegill Exposed to 0.87 mg/L:

(Values are from 4 individual fish at each sample period.)

Uptake day	Edible tissue, mg/kg	Nonedible tissue, mg/kg	Whole fish conc., mg/kg
0 (4 hours)	1.46, 1.48, 1.19, 1.39	3.52, 4.37, 4.22, 4.06	2.71, 3.08, 2.84, 2.89
1	4.68, 6.59, 5.56, 5.64	11.1, 14.2, 13.3, 12.1	8.00, 10.9, 10.2, 9.47
3	17.3, 15.8, 19.0, 20.8	39.3, 42.0, 43.8, 51.8	30.5, 30.7, 34.5, 39.1
7	42.0, 44.0, 57.7, 46.8	100, 102, 102, 120	74.9, 77.0, 85.3, 89.8
14	87.1, 81.6, 90.7, 73.3	177, 207, 245, 214	141, 157, 180, 158
21	79.4, 117, 104, 102	201, 278, 246, 229	146, 210, 185, 172
28 ¹	102, 131, 107, 133	289, 372, 320, 361	205, 267, 232, 263

¹Sampling of fish stopped after Uptake day 28 due to mortality.

Remarks: Test organism mortality was none in the negative control (during both the uptake and depuration phases). At 0.086 mg/L, one fish died after 49 days and one died after 59 days. None died during the depuration phase. At 0.87 mg/L, mortality was first noted on day 9 and continued through day 35 of the uptake phase, at which time all fish either died or had been sampled.

Was control response satisfactory: Yes

Statistical results: None

CONCLUSIONS

PFOS bioconcentrated in the tissues of bluegill sunfish during this study. Apparent steady-state was attained on Day 49 for the fish exposed to 0.086 mg a.i./L. Although Day 49, 56, and 62 tissue residues were not statistically significantly different, PFOS concentrations appeared to be still increasing during this time. Apparent steady-state BCF values for edible, nonedible, and whole fish tissues were calculated to be 484, 1124, and 859, respectively.

PFOS depurated slowly. The BIOFAC estimates for the time to reach 50% clearance for edible, nonedible, and whole fish tissues were 146, 133, and 152 days, respectively.

Submitters' remarks: The authors assigned this study a Klimisch data reliability ranking of 1.

Reviewers' remarks: None

REFERENCE

Drottar, K., VanHoven, R., and H. Krueger. 2001. Perfluorooctanesulfonate, potassium salt (PFOS): A flow-through bioconcentration test with bluegill (*Lepomis macrochirus*). Wildlife International, Limited. Project number 454A-134. 3M Company. St. Paul, MN.

Annex 6. Robust Summaries of Toxicology and Human Biomonitoring Studies

PHARMACOKINETIC STUDIES

Title: Absorption of FC-95-14C in Rats after a Single Oral Dose (1979)

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS 2795-39-3

Remarks: FC-95-14C (carbon-14 label alpha to sulfur atom, Riker Isotope Inventory Number 442). The specific activity is 0.459 +/- 0.008 uCi/mg. Thin-layer and column chromatography showed the FC-95-14C to be at least 99% radiochemically pure. The FC-95-14C was found to be suitable for metabolism studies. (Synthesis described in Johnson and Behr, 1979).

METHOD

Method/guideline followed: NA

Test type: *in vivo*

Species/strain/cell type or line: rat, Charles River CD

Sex: male

Age and body weight range of animals used: 8 weeks, bw mean 285 g (range 243-315)

Number of animals/sex/dose: 24

Route of administration: oral

Vehicle: 0.9% NaCL solution containing 1.2 mg FC-95-14C/2.0 ml

Doses: 4.2 mg/kg average, single dose

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

red blood cells, plasma, urine, feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and carcass

Statistical methods used: mean, log mean concentration versus time least squares line

Method remarks:

Rats were conditioned to individual metal metabolism cages for 24 hours prior to dosing. Rats were allowed free access to Purina Ground Chow and water before and after dosing. Each non-fasted rat was weighed immediately before being given a single oral dose of FC-95-14C. The dosing solution was prepared by adding ~200 mg of FC-95-14C to 0.9% NaCl, shaking for one half hour at moderate speed in a mechanical shaker, and centrifuging. The supernatant was removed and used for dosing solution. The carbon-14 content of the dosing solution was determined by direct counting. The dose was delivered with a 2.0 cc glass syringe (Trylon) fitted with a stainless steel intubation tube. Recovery of total carbon-14 from series of FC-95-14C spiked, blank biological samples was used to adjust the recovery of the test samples to account for label loss during the experimental manipulations.

Groups of three rats were sacrificed by exsanguination at 1, 2, 6, 12, 24, 48, 96, and 144 hours post dose. Rats were anesthetized with diethyl ether and blood was drawn from the descending aorta of each rat and immediately transferred to a heparinized tube. Plasma was prepared promptly by centrifugation. In addition to plasma and red blood cells, total urine, total feces, spleen, digestive tract plus contents (esophagus, stomach, small intestine, large intestine, and colon), and remainder of carcass were saved from each of the three rats in the 24 and 48 hours post dose groups for carbon-14 analysis.

RESULTS

Detailed results:

After a single oral dose of FC-95-14C (mean dose, 4.2 mg/kg) in solution to groups of three male rats, at least 95% of the total carbon-14 is systemically absorbed at 24 hours. The half-life for elimination of total carbon-14 from plasma is 7.5 days.

The digestive tract and contents contained on the average, 3.45% of the dose. The mean fecal excretion is 1.55% of the dose at 24 hours and 3.24% at 48 hours. At 24 hours, the mean sum of total carbon-14 in feces and digestive tract plus contents is 5% of the dose. Some of this 5% likely represents systemically absorbed carbon-14 present either in the digestive tract tissues or in the digestive tract contents as a result of excretion. The data from the 48 hour post dose group of rats are consistent with the 24 hour post dose data. Thus, at least 95% of the FC-95-14C dose was absorbed from solution after administration to non-fasted rats. The major portion of the radioactivity recovered was found in the carcass. The carcass data are not as reliable as the other tissue data since large volume homogenates were necessary and homogeneity of sample aliquots was difficult to assure. There is some excretion of total carbon-14 in urine (1-2%/day). The spleens from the 24 hour and 48 hour post dose rats were analyzed for total carbon-14 content, and the percent of the dose in the whole organ was ~0.2%. The concentrations of total carbon-14 in red blood cells and plasma were compared. The mean ratio of red blood cell to plasma concentration at 24 and 48 hours is 0.25 and 0.39, respectively. Thus, at 24 and 48 hours after a single oral dose of FC-95-14C, there is no selective retention of carbon-14 in red blood cells.

The half-life of elimination from plasma was determined by analysis of plasma samples from groups of three rats at 1, 2, 6, 12, 24, 48, 96, and 144 hours after a single oral dose of FC-95-14C. The log of mean concentration versus time for these data was plotted. The least squares line through the individual points from 24 to 144 hours for these data fits the equation: $C_p = 15.65e^{(-0.00387t)}$ where C_p is plasma concentration. The half-life of elimination from plasma is 179 hours (7.5 days). Thus, elimination from plasma of total carbon-14 after a single oral dose of FC-95-14C is slow.

Metabolites measured: none

CONCLUSIONS

agree

REFERENCE

Absorption of FC-95-14C in Rats after a Single Oral Dose. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN. Project No. 890310200. Johnson, JD, Gibson, SJ, and Ober, RF, October 26, 1979.

Title: Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95-14C (1979)

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS 2795-39-3

Remarks: FC-95-14C (carbon-14 label alpha to sulfur atom, Riker Isotope Inventory Number 442). The specific activity is 0.459 +- 0.008 uCi/mg. Thin-layer and column chromatography showed the FC-95-14C to be at least 99% radiochemically pure. The FC-95-14C was found to be suitable for metabolism studies. (Synthesis described in Johnson and Behr, 1979).

METHOD

Method/guideline followed: NA

Test type: *in vivo*

Species/strain/cell type or line: rat, Charles River CD

Sex: male

Age and body weight range of animals used: 8 weeks, bw mean 288 g (range 262-303)

Number of animals/sex/dose: 6

Route of administration: iv, via tail vein

Vehicle: 0.9% NaCL solution containing 1.2 mg FC-95-14C/2.0 ml

Doses: 4.2 mg/kg average, single dose

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

urine, feces, liver, plasma, kidney, lung, spleen, bone marrow, adrenals, skin, testes, muscle, fat, eye, brain

Statistical methods used: mean, standard deviation

Method remarks:

Rats were conditioned to individual metal metabolism cages for 24 hours prior to dosing. The rats were allowed free access to Purina Ground Chow and water before and after dosing. Each rat was weighed, anesthetized with diethyl ether, then given a single iv dose using a 3.0 cc disposable plastic syringe fitted with a 26 gauge 1/2" needle. Urine and feces were collected at intervals for each of the six rats for 89 days. At 89 days post dose, the rats were anesthetized with diethyl ether; blood was drawn from the descending aorta, animals were sacrificed by exsanguination, and tissue samples were collected.

RESULTS

Detailed results:

By 89 days post dose, mean urinary excretion was 30.2+-1.5% of total C-14 administered. Mean cumulative fecal excretion was 12.6+-1.2%. The authors note that radioactive content in feces was too low to measure after 64 days. At day 89, mean tissue C-14 concentrations above one ug FC-95-14C

equivalents/g were as follows: liver, 20.6; plasma, 2.2; kidney, 1.1; and lung, 1.1. Other tissues such as muscle, skin, bone marrow, and spleen had concentrations ranging from 0.2 to 0.6 ug/g. There was a difference in C-14 content of subcutaneous fat (0.2 ug/g) and abdominal fat (≤ 0.08 ug/g). Very little C-14 was found in whole eye (0.16 ug/g) and no detectable C-14 was found in brain. Only liver and plasma contained a substantial percentage of dose at 89 days post dose, 25.21% and 2.81%, respectively. The low levels of radioactivity found for kidney, lung, testes, and spleen are due in part to blood still contained in these organs when homogenized.

Mean Excretion of Total Carbon-14 in Urine Over Time

Collection Period (Days)	Percent Dose During Period
0-0.5	0.91
0.5-1	0.77
1-2	1.21
2-3	1.03
3-4	0.93
4-5	0.83
5-6	0.71
6-7	0.76
7-8	0.75
8-9	0.68
9-10	0.68
10-11	0.59
11-12	0.58
12-13	0.59
13-14	0.55
14-15	0.54
15-16	0.51
16-17	0.48
17-18	0.43
18-19	0.39
19-21	0.84
21-23	0.78
23-25	0.66
25-27	0.68
27-29	0.68
29-32	0.86
32-36	1.05
36-40	0.99
40-43	0.75
43-47	0.92
47-50	0.68
50-54	0.78
54-57	0.61
57-61	0.79
61-69	1.50
69-78	1.64
78-89	2.08
Total	30.2

Mean Excretion of Total Carbon-14 in Feces Over Time

Collection Period (Days)	Percent Dose During Period
0-0.5	0.049
0.5-1	0.842
1-2	0.795
2-3	0.649
3-4	0.656
4-5	0.577
5-6	0.510
6-7	0.588
7-8	0.482
8-9	0.421
9-10	0.387
10-11	0.370
11-12	0.296
12-13	0.310
13-14	0.281
14-15	0.276
15-16	0.272
16-17	0.187
17-18	0.163
18-19	0.129
19-21	0.311
21-23	0.302
23-25	0.262
25-27	0.208
27-29	0.202
29-32	0.223
32-36	0.526
36-50	1.530
50-64*	0.833
Total	12.6

*The radioactive content of the feces was too low to measure after 64 days.

Metabolites measured: none.

CONCLUSIONS

agree

REFERENCE

Extent and Route of Excretion and Tissue Distribution of Total Carbon-14 in Rats after a Single Intravenous Dose of FC-95- 14 C. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN. Johnson, JD, Gibson, SJ, and Ober, RE , December 28, 1979.

Title: Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium [14C]Perfluorooctanesulfonate (1984)

TEST SUBSTANCE

Identity:

Potassium perfluorooctanesulfonate (14C-PFOS)

Ammonium perfluorooctanoate (14C-PFO)

Remarks:

14C-PFOS: sp act 0.46 uCi/mg, radiochemical purity >99%,

14C label in PFOS is adjacent to sulfur

14C-PFO: sp act 0.51 uCi/mg, radiochemical purity >98%

METHOD

Method/guideline followed: NA

Test type: *in vivo*

Species/strain/cell type or line: rat, Charles River CD

Sex: male

Age and body weight range of animals used: 12 weeks, 300-342 g

Number of animals/sex/dose: 5

Route of administration: iv

Vehicle: 0.9% NaCl, 2 ml/rat

Doses:

Potassium [14C]Perfluorooctanesulfonate (PFOS): 3.4 mg/kg mean, single dose, 0.56 mg/ml

PFOS control animals: 3.5 mg/kg mean

Ammonium [14C]Perfluorooctanoate (PFO): 13.3 mg/kg mean, single dose, 2.1 mg/ml

PFO control animals: 13.5 mg/kg mean

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

Urine, plasma, red blood cells, liver

Statistical methods used: mean, standard deviation, Student's t test

Method remarks:

Rats were housed in individual stainless-steel metabolism cages and fasted with free access to water for 24 hrs prior to receiving the fluorochemicals. The radiolabeled compounds were administered as single intravenous doses (lateral tail vein). Two ml of dosing solution was administered to each rat. Ten rats were dosed with each compound. Five rats from each group were fed cholestyramine (dried and ground resin Z-620), 4% in feed (Purina Lab Chow), for 14 days after administration of PFO and for 21 days after administration of PFOS. Control rats were administered radiolabeled fluorochemical but were not treated with cholestyramine. In order to allow comparison of the radiometric results on an absolute basis, the

radiolabel doses were not adjusted for individual body weights. Urine and feces samples were collected at intervals for individual rats in each group until 14 days after 14C-PFO administration and 21 days after 14C-PFOS administration. At these times, rats were anesthetized with diethyl ether and exsanguinated by drawing blood from the descending aorta. Plasma and red blood cells were prepared promptly by centrifugation. Liver was collected as the whole organ and stored frozen until analysis.

RESULTS

Detailed results:

After 21 days of cholestyramine treatment, the mean percentage of 14C-PFOS dose eliminated via feces (75.8 \pm 5.0) was 9.5-fold the mean percentage of dose eliminated via feces by control rats (8.0 \pm 0.8). After adjustment for the amount of carbon-14 excreted in urine (18% for controls and 5% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 19% for cholestyramine-treated rats and 74% for control rats. After 14C-PFOS administration, the mean liver carbon-14 content at 21 days represents 11% and 40% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 21 days of cholestyramine treatment.

After 14 days of cholestyramine treatment, the mean percentage of 14C-PFO dose eliminated via feces (43.2 \pm 5.5) was 9.8-fold the mean percentage of dose eliminated via feces by control rats (4.4 \pm 1.0). After adjustment for the amount of carbon-14 excreted in urine (67% for controls and 41% for cholestyramine-treated), the amounts of carbon-14 remaining to be excreted are 16% for cholestyramine-treated rats and 28% for control rats. After 14C-PFO administration, the mean liver carbon-14 content at 14 days represents 4% and 8% of the dose for cholestyramine-treated and control rats, respectively. Mean plasma and red blood cell carbon-14 concentrations are significantly lower after 14 days of cholestyramine treatment.

Carbon-14 Concentration (expressed as ug eq/g tissue or ml fluid)

Treatment Group	Liver	Plasma	Red Blood Cells
14C-PFOS			
Cholestyramine	9.4 \pm 1.6*	0.9 \pm 0.1*	0.3 \pm 0.1*
Control	35.6 \pm 5.6	6.9 \pm 0.6	1.8 \pm 0.4
14C-PFO			
Cholestyramine	12.1 \pm 2.1*	5.1 \pm 1.7*	1.8 \pm 0.7*
Control	22.3 \pm 6.2	14.7 \pm 6.8	4.2 \pm 2.4

*Significantly different from control values (p<0.05)

The authors conclude that the high concentration of 14C-PFOS or 14C-PFO in liver at 2 to 3 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 by nearly 10-fold suggest that there is a considerable enterohepatic circulation of 14C-PFOS and 14C-PFO.

Metabolites measured: none

CONCLUSIONS

agree

REFERENCE

Johnson, J. D., Gibson, SJ, and Ober, RE (1984). Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [14C]Perfluorooctanoate or Potassium [14C]Perfluorooctanesulfonate. *Fundamental and Applied Toxicology* 4, pages 972-976.

See also Johnson, J. D., Gibson, SJ, and Ober RE (1984). Enhanced elimination of FC-95-14C and FC-143-14C in rats with cholestyramine treatment. Project No. 8900310200, Riker Laboratories, Inc. St. Paul, MN.

Title:

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats, Analytical Laboratory Report, Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine, and Feces Samples

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, potassium salt, CAS 2795-39-3

Remarks: Purity 86.9%, Lot # 217

METHOD

Method/guideline followed:

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions noted on page 3 of report.

Test type: *in vivo*

Species/strain/cell type or line: rat/Sprague-Dawley/pregnant Crl:CD(R)BR VAF/Plus(R)

Sex: F0: female, F1: both

Age and body weight range of animals used: 60 days, 200-225 g

Number of animals/sex/dose: F0: 16, F1: 5 male and 5 female pups/litter

Route of administration: oral

Vehicle: 0.5% Tween(R) 80 in R.O. deionized water, dosage volume 5 ml/kg

Doses: 0 (vehicle), 0.1, 0.4, 1.6, and 3.2 mg/kg/day in volume of 5 ml/kg, once daily beginning 42 days prior to cohabitation, and continued through day 14 or day 20 of presumed gestation. Only the F0 females were dosed.

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

F0 urine, feces, serum, liver. F1 liver and serum.

Statistical methods used: mean and standard deviation.

Method remarks: Serum, urine, and feces specimens were collected from adult female rats (F0 dams) before mating and at gestation day (GD) 7, GD 15 and GD 21. Liver specimens were collected from F0 dams at termination of the study (GD 21). A total of 54 pooled serum and liver specimens were collected from fetuses on GD 21. Specimens were sent to the 3M Environmental Laboratory and the contract labs to be analyzed for PFOS.

RESULTS

Detailed results:**Average Results for the Analysis of Serum Samples (ug/ml)**

	0 mg/kg	0.1 mg/kg	0.4 mg/kg	1.6 mg/kg	3.2 mg/kg
Day 0 dam	0.0723	8.89	40.7	160	318
Day 7 dam	0.126	7.82	40.9	154	105
Day 15 dam	0.0926	8.80	41.4	156	275
Day 21 dam	0.0714	4.24	26.2	136	155
Day 21 Fetal	0.125	9.07	34.3	101	165

Average Results for the Analysis of Liver Samples (GD 21)

Dose Group (mg/kg/day)	PFOS Conc. (ug/g) Female Adult (F0)	PFOS Conc. (ug/g) Fetal Liver (F1)
0	0.288	0.169
0.1	29.2	7.93
0.4	107	30.6
1.6	347	86.7
3.2	610	230

Average Results for the Analysis of Urine Samples from F0 Dams

	0 mg/kg	0.1 mg/kg	0.4 mg/kg	1.6 mg/kg	3.2 mg/kg
Day 0	<LOQ	0.0497	0.302	0.959	1.53
Day 7	<LOQ	0.0620	0.308	1.10	1.60
Day 15	0.00905	0.0685	0.526	0.622	0.563
Day 21	0.0194	0.0574	0.555	2.71	1.61

Average Results for the Analysis of Feces Samples from F0 Dams

	0 mg/kg	0.1 mg/kg	0.4 mg/kg	1.6 mg/kg	3.2 mg/kg
Day 0	0.0380	0.499	2.42	10.3	23.9
Day 7	0.0155	0.490	2.16	9.19	33.0
Day 15	0.0322	0.662	2.93	11.1	29.5
Day 21	0.0342	0.416	2.39	9.94	20.1

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the fetuses. PFOS was also observed in the control dams, as well as the control fetuses. On sacrifice on GD21, the levels of PFOS were much higher in the liver than in the serum for the dams. The levels of PFOS remained fairly steady in the serum of the dams from GD0 – GD 15, but the levels dropped at GD21. In the GD21 fetuses, the level of PFOS in the serum was generally comparable to the level observed in the dams, whereas the level of PFOS in the fetal livers was well below that seen in the dams.

Results Remarks:

As stated in the report. "It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to 50% or greater."

Metabolites measured: none

CONCLUSIONS

Conclusions: Under the conditions of the present studies, PFOS was observed in the livers, urine, feces and sera of all female rats dosed with PFOS during the in-life phase of the study. Additionally, PFOS was observed in fetal liver and serum taken during gestation from the same group of female rats.

Agree

REFERENCE

Study Title: Oral (Gavage) Pharmacokinetic Study of PFOS in Rats
Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine, and Feces Samples
3M Medical Department Study: T-6295.12, Argus In-Life Study: #418-013, FACT TOX-110, 3M Laboratory Request No. U2849, 3M Environmental Laboratory, May 4, 2001.

Title:

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, Analytical Laboratory Report, Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of CrI:CDBR VAF/Plus ® Rats Exposed to PFOS via Gavage

TEST SUBSTANCE

Identity: Perfluorooctylsulfonate, potassium salt (FC-95), CAS 2795-39-3

Remarks: Purity 86.9%, Lot # 217

METHOD

Method/guideline followed:

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions noted on page 3 of report. It does not appear these exceptions would significantly impact the results or conclusions. The analytical phase completed at the 3M Environmental Laboratory was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Test type: *in vivo*

Species/strain/cell type or line: rat/Sprague-Dawley/pregnant CrI:CD(R)BR VAF/Plus ®

Sex: F0: female, F1: both

Age and body weight range of animals used: 65 days, 192-231 g

Number of animals/sex/dose: F0: 8, F1: 5 male and 5 female pups/litter

Route of administration: oral

Vehicle: 0.5% Tween(R) 80 in R.O. deionized water, dosage volume 5 ml/kg

Doses: 0 (vehicle), 0.1 and 1.6 mg/kg/day in volume of 5 ml/kg (0.00, 0.02, and 0.32 mg/mL), once daily beginning 43 days prior to cohabitation until confirmed evidence of mating. Only the F0 females were dosed.

Excretion routes, body fluids, and tissues monitored and/or sampled during study:

F0 urine, feces, serum, liver. F1 liver and serum.

Statistical methods used: means, standard deviations, and percentages

Method remarks:

Rat dams were exposed to PFOS via gavage prior to and during mating. Exposure to PFOS was halted on the first day of presumed gestation. Pups were not directly exposed to PFOS, but may have been exposed *in utero* and during lactation. Male rats of the same source and strain were used only as breeders and were not administered the test article or considered part of the test system.

Urine and fecal samples were collected from F0 female rats for the following intervals: one day prior to initiation of cohabitation to the following morning, days 6 to 7, 14 to 15, and 20 to 21 of presumed

gestation (DG 6 to 7, 14 to 15, and 20 to 21), and days of lactation (DL) 21 to 22. Blood samples were collected from each of the maternal rats on the day cohabitation was initiated (prior to cohabitation), DG 7, 15 and 21, and DL 14 and 22. Day 1 of lactation was defined as the day of birth. On DL 4, litters were culled to five male pups and five female pups per litter, where possible. Sera specimens were collected from pooled litter samples on DL 21. On DL 22, all surviving Generation F0 and Generation F1 animals assigned to the study were sacrificed, and a liver specimen was collected from each animal. The liver from each pup was collected and pooled per litter. Blood samples were collected and pooled per liter. Urine, fecal, serum and liver samples were shipped to the Sponsor for analysis. On days 1 to 4 of the 43-day pre-mating period, F0 female rats received 25% greater dose due to an incorrect calculated amount of test substance in vehicle.

RESULTS

Detailed results:

Average Results for the Analysis of Sera Samples (PFOS Conc. ug/ml)

	DG0	DG7	DG15	DG21	DL14	DL21	DL22
0.0 mg/kg F0	0.100	0.0796	0.0742	<LLQ	0.0542	NS	0.0492
0.0 mg/kg F1	NS	NS	NS	NS	NS	0.0531	NS
0.1 mg/kg F0	9.21	7.24	5.68	2.58	1.63	NS	0.979
0.1 mg/kg F1	NS	NS	NS	NS	NS	1.80	NS
1.6 mg/kg F0	161	129	90.6	39.5	20.6	NS	14.1
1.6 mg/kg F1	NS	NS	NS	NS	NS	27.1	NS

Average Results for the Analysis of Liver Samples (PFOS Conc. ug/g)

	DL22
0.0 mg/kg F0	0.243
0.0 mg/kg F1	0.174
0.1 mg/kg F0	6.15
0.1 mg/kg F1	5.00
1.6 mg/kg F0	59.7
1.6 mg/kg F1	56.2

Average Results for the Analysis of Urine Samples (PFOS Conc. ug/ml)

	DG0	DG6/7	DG14/15	DG20/21	DL21/22
0.0 mg/kg F0	0.00819	0.0100	0.00685	0.00614	<LOQ
0.1 mg/kg F0	0.0905	0.0307	0.0327	0.0231	0.00555
1.6 mg/kg F0	2.11	0.888	0.613	0.340	0.0334

Average Results for the Analysis of Feces Samples (PFOS Conc. ug/g)

	DG0	DG6/7	DG14/15	DG20/21	DL21/22
0.0 mg/kg F0	ND	ND	ND	ND	ND
0.1 mg/kg F0	0.601	0.399	0.294	0.119	0.0522
1.6 mg/kg F0	10.9	8.39	4.83	2.06	0.387

In general, there was a dose-related increase in the levels of PFOS in the liver and serum of the dams and the levels in the serum decreased with time. The levels of PFOS were much higher in the liver than in the serum of the dams and the pups. The levels of PFOS were similar in the liver of the dams and pups, while the levels in the serum were slightly higher in the pups than in the dams.

Results Remarks:

As stated in the report. "It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to 50% or greater."

Metabolites measured: none

CONCLUSIONS

Statement of Conclusion

Under the conditions of the present study, perfluorooctanesulfonate was observed in all sample types of all Generation 0 test system animals dosed with the test substance during the in-life phase of the study, and in all sample types of their offspring (Generation F1).

Agree

REFERENCE

Study Title: Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats Analytical Laboratory
Report Title: Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of CrI:CDBR VAF/Plus ® Rats Exposed to PFOS via Gavage, 3M Environmental Laboratory Report No. FACT TOX-111, Laboratory Request No. U2994, 3M Ref. No. T-6295.14, Argus In-Life Study 418-015, May 4, 2001.

Title: Half-Life Study of PFOS in Serum, 2000

TEST SUBSTANCE

Identity: PFOS

METHOD

Study design: PFOS half-life study on retired workers from the Decatur, Alabama plant

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the half-life of PFOS in retired perfluorochemical production workers.

Study period: Nov. 1998 to 2003

Setting: N/A

Total population: 27 retirees from the Decatur, Alabama and Cottage Grove, Minnesota plants

Subject selection criteria: The participants volunteered for this study

Total # of subjects in study: 18 males

Comparison population: N/A

Participation rate: Unknown

Subject description: Retirees ranged in age from 55-74 years, worked in the plant for an average of 28 years, average time from retirement to start of study was 30 months (range: 5-130 months).

Health effects studied: N/A

Data collection methods: Blood sera samples collected every 6 months

Details on data collection: No information was provided as to how the blood was drawn, stored, etc.

Exposure period: Unknown.

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Medians and ranges calculated.

Other methodological information: Half-lives were calculated assuming a one-compartment model. A log-linear relationship was used to estimate the serum fluorochemical elimination half-life in participating

retirees. In this log linear relationship, the slope of the line is related to the elimination constant via the equation $\text{slope} = -k_{el}(2.303)$. Once the elimination constant is calculated, the half-life is determined using the relationship $t_{1/2} = 0.693/k_{el}$.

If 3 data points were not available for any of the subjects and if there was a lack of fit to the model, that retiree was not included in the analyses. Eighteen participants met these requirements.

RESULTS

Describe results: The median serum half-life of PFOS was 270 days, with a range of 139 to 640 days. It should be noted that the difference in serum PFOS levels between retirees was quite large (0.2 - 2.0 ppm).

Study strengths and weaknesses: For most of the participants not included in the analysis, the second measurement was higher than the first. Therefore, the data did not fit the model and they were excluded. Although this may justify not including those participants in the analysis, it is an indication of the many limitations of the data. It is stated in the report that neither age nor number of months retired was associated with the serum PFOS half-life calculations; however, this statement is not supported with any data in the report. In addition, no individual data were provided in the report and the relationship between number of years exposed in the workplace and PFOS levels and half-life were excluded. Retirees were excluded from these calculations if their second measurement was higher than the first. It is unknown why this occurred, but the exclusion of those retirees introduces bias to the results. Also, elimination of PFOS occurs via urine and feces; however, these measurements were not taken. Therefore, it cannot be determined that the half-life suggested by the preliminary results reported here represents a true elimination half-life from the body. Finally, the effect of continued non-occupational, low-level exposure on the half-life is unknown.

Research sponsors: 3M Environmental Lab

Consistency of results: These results are not consistent with the first study in which 3 retirees were followed over a 5-year period. In that study, the estimated half-life was 3 to 4 years. There is no explanation in this current report as to why there is such a large difference in the results or why none of the retirees in the current study didn't fall within the range of the first study.

CONCLUSIONS

N/A

REFERENCE

Determination of Serum Half-Lives of Several Fluorochemicals, June 8, 2000, 3M Company. FYI-0700-1378, 8(e) Supplemental Submission, 8EHQ-0373/0374.

ACUTE TOXICITY STUDIES

Title: An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. 1979

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: Dust, PFOS (T-2306 CoC). Purity not specified.

METHOD

Method/guideline followed: Similar to OECD 403

GLP: N, no QA/QC indicated

Year study performed: 1979

Species/Strain: Rat/Sprague-Dawley

Sex (Males/females/both): Both

No. of animals/sex/dose: 5/sex/group

Route of Administration: Inhalation

Remarks: Concentrations of 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09, 45.97 mg/l PFOS were administered to eight test groups. A Wright dust-feed mechanism with dry air at a flow rate of 12 to 16 liters per minute was used to administer the PFOS dust. Rats were exposed for 1 hour. The test group rats weighed 201-299 g at study initiation. The control group rats weighed 203-263 g at study initiation. The control rats were exposed to dry air at a flow rate of 12 liters per minute. All other protocols were the same as the test group rats. The rats were observed for abnormal signs prior to exposure, at 15-minute intervals during the 1-hour exposure, at removal from the exposure chamber, hourly for four hours after exposure, and daily thereafter for 14 days. Individual bodyweights were recorded on Day 0 (prior to exposure), Day 1, Day 2, Day 4, Day 7, and Day 14. It is reported that all animals dying spontaneously were necropsied as soon as possible after death. Blood samples were collected on Day 14 from all surviving animals, but analyses were not provided.

RESULTS

$LC_{50} = 5.2$ (4.4 – 6.4) mg/l, (95% confidence limits); referenced method of Litchfield and Wilcoxon

Number of deaths at each dose level (by sex):

0.0 mg/l: 0/10; 1.89 mg/l: 0/10; 2.06 mg/l: 1/10; 4.88 mg/l: 2/10; 6.49 mg/l: 8/10;
7.05 mg/l: 8/10; 24.09 mg/l: 10/10 (authors did not provide summary by sex)

Remarks: The highest dose group, 45.97 mg/l, was not used in the LC_{50} calculations and terminated on Day 2. At that point, only 5 animals survived and blood samples were taken at termination. The 13.9 mg/l group was also terminated early (Day 1) because of a mechanical problem during exposure. These animals

were also not used in the LC₅₀ determination.

In the 24.09 mg/l exposure group, all animals died by Day 6. At 7.05 and 6.49 mg/l there was 80% mortality with last deaths at Day 10. At 4.88, 2.86, and 1.89 mg/l there was 20%, 10%, and 0% mortality, respectively. At 2.86 mg/l, deaths occurred on Day 7 and 10. At 1.89 mg/l, one death occurred on Day 12. The rats in all these groups showed signs of toxicity including emaciation, red material around the nose or other nasal discharge, yellow material around the anogenital region, dry rales or other breathing disturbances, and general poor condition. Abnormal in-life observations were reported to be less frequent in the lower exposure groups.

The most common abnormality was discoloration of the liver and lung. Discoloration of the lung was also observed in control rats and therefore may not be treatment related. Therefore, the most significant treatment-related abnormality was varying degrees of discoloration of the liver. Among animals that died prematurely, decreased body weight, discoloration of the lung, and discoloration and distention of the small intestine were also observed.

CONCLUSIONS

LC50 = 5.2 (4.4 – 6.4) mg/l, (95% confidence limits). Only conclusion provided; seems reasonable with available data

REFERENCE

Rusch, G.M., W.E. Rinehart and C.A. Bozak. 1979. An Acute Inhalation Toxicity Study of T-2306 CoC in the Rat. Project No. 78-7185, Bio/dynamics Inc.

Title: Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD₅₀) Study in Rats. 1978.

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: FC-95. Purity not specified.

METHOD

Method/guideline followed: Similar to OECD 401

GLP (Y/N): N, no QA/QC indicated

Year study performed: 1978

Species/Strain: Rat/Charles River CD

Sex (Males/females/both): both

Number of animals/sex/dose: 5/sex/dose

Vehicle: 20% acetone/80% corn oil

Route of Administration: gavage

Remarks: Levels of 100, 215, 464, and 1000 mg/kg PFOS were tested. All dose levels were administered as volumes of 10ml/kg body weight. The rats weighed 172-212 g at the beginning of the study immediately prior to dosing and weights were recorded at Day 7 and Day 14. The rats were observed for abnormal signs during the four hours after exposure, and daily thereafter for 14 days. It is reported that all animals dying spontaneously were grossly necropsied, as well as all rats that survived to the end of the 14-day study.

RESULTS

LD50: 251 (199-318) mg/kg, (95% confidence limits); 3 references for statistical tables are given.

Number of deaths at each dose level (by sex): 100 mg/kg: 0/5 males, 0/5 females;
215 mg/kg: 2/5 males, 1/5 females; 464 mg/kg: 5/5 males, 5/5 females;
1000 mg/kg: 5/5 males, 5/5 females

Remarks: All rats in the 464 and 1000 mg/kg dose groups died before the end of the study. Three animals in the 215 mg/kg group died prematurely. It appears signs of toxicity most frequently observed included: hypoactivity, decreased limb tone, and ataxia. At necropsy observations included: yellow-stained urogenital region, stomach distention and signs of irritation of the glandular mucosa, and lung congestion. No differences between sexes were noted.

LD50 male rats: 233 (160-339) mg/kg (95% confidence limits)

LD50 female rats: 271 (200-369) mg/kg (95% confidence limits)

CONCLUSIONS

None specified beyond LD50

REFERENCE

Dean, W.P., D.C. Jessup, G. Thompson, G. Romig, and D. Powell. 1978. Fluorad Fluorochemical Surfactant FC-95 Acute Oral Toxicity (LD₅₀) Study in Rats. Study No. 137-083, International Research and Development Corporation. (Includes Acute Oral Toxicity Study in Rats with T-2297 CoC. Project No. 78-1433A, Biosearch, Inc.) .

SKIN IRRITATION

Title: Eye and Skin Irritation Report on Sample T-1117. 1974

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: FC-95, Sample T-1117. Purity not specified.

METHOD

Note pH of test material: **Not specified**

Method/Guideline followed: **Not specified**

Test Type: *in vivo*

Species/strain/cell type: **Rabbits/ albino**

Sex (males/females/both): **Not specified**

Number of animals/sex/dose: **6 total**

Total dose: **appears to be 1.0 gram, 0.5g placed on each of 2 prepared test sites (intact-wet, abraded-wet); total dose not specified**

Vehicle: **None? Not specified**

Length of time test material is in contact with animal/cell: **72 hr**

Grading scale: **Separate scores for erythema formation and edema formation are summed.**

Reference source not provided.

Remarks: Six albino rabbits had their hair clipped from their backs and flanks, and five tenths of one gram (0.5 g) of test material was placed on abraded-wet or intact-wet prepared test sites, then covered with gauze patches. After 24 hours and 72 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale.

RESULTS

Results: In all cases it is reported the primary skin irritation scores were 0; which indicates no reddening or swelling detected.

Primary irritation score: zero

Remarks: No indication of reliability. No QA/QC. No effects reported.

CONCLUSIONS

No irritation. Inadequate information is presented in report to evaluate quality of study and validity of conclusion.

REFERENCE

J. A. Biesemeier and D.L. Harris. 1974. Eye and Skin Irritation Report on Sample T-1117. Project No.4102871, WARF Institute Inc.

EYE IRRITATION

Title: Eye and Skin Irritation Report on Sample T-1117. 1974

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS No.: 2795-39-3
Remarks: T-1117. Purity not specified.

METHOD

Note pH of test material: Not specified
Method/Guideline followed: Not specified
Test Type: *in vivo*
Species/strain/cell type or line: Rabbit/New Zealand White
Sex (males/females/both): Not specified
Number of animals/sex/dose: 6/single dose

Total dose: it appears 0.1 gram, (protocol states either 0.1 ml or 0.1g and T-1117 is reported to be a solid {FC-95} by 3M), total dose not specified

Length of time test material is in contact with animal/cell: could be 72 hours,
But at 1 hr observation and at each scoring after "any accumulated discharge or residue of test material was flushed from the eye."

Observation period: 1hr, 24 hr, 48 hr, 72 hr
Scoring method used: Not specified or referenced - "The reaction to the test material was read according to the scale of scoring for damage to the cornea, iris, and the bulbar and palpebral conjunctivae..."

Remarks: Rabbits were placed in collars so they could not rub their eyes. One tenth of a gram (0.1 g) of the test substance was instilled in one eye, the other eye was left untreated as a control. It is reported that the reaction to the test material was read against a scale of damage to the cornea, iris, and the bulbar and palpebral conjunctivae at 1, 24, 48, and 72 hours after treatment. The scale criteria are not presented or referenced. Each time the eyes were scored, any accumulated discharge or residue of test material was flushed from the eye. It appears that scores were maximal at 1 hour and 24 hours after treatment then decreased over the rest of the study.

RESULTS

Corrosive: no

Irritation score: Only total scores provided.
1 hr: 8.00; 24 hr: 9.33; 48 hr: 3.33; 72 hr: zero

Tool used to assess score: Not specified

Description of lesions: none

Remarks: Inadequate description and discussion in report. Scores appear reduced in all rabbits over time. Decreases were noted at 48hrs and at 72hrs values were zero.

CONCLUSIONS

Only conclusion provided in study is that test substance is irritating to eyes. Inadequate information is presented in report to evaluate quality of study and validity of conclusion.

REFERENCE

J. A. Biesemeier and D.L. Harris. 1974. Eye and Skin Irritation Report on Sample T-1117. Project No. 4102871, WARF Institute Inc.

GENETIC TOXICITY STUDIES

Title: MUTAGENICITY EVALUATION OF T-2014 CoC IN THE AMES
SALMONELLA/MICROSOME PLATE TEST, 1978

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS #2795-39-3

Remarks: T-2014 CoC, FC-95, Purity not specified.

METHOD

Method/Guideline followed: Ames, 1975

Test type: Reverse mutation

Test system: Bacteria; Yeast

GLP: N

Year study performed: 1977

Species/Strain/cell-type/cell line: *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA09,
Saccharomyces cerevisiae D4

Metabolic activation: 0.1 ± .05 ml S9 homogenate of Aroclor 1254 induced Sprague Dawley rat liver

Concentrations tested: 0.01 µg/plate, 1.0 µg/plate, 10.0 µg/plate, 100 µg/plate, 500 µg/plate nonactivated;
0.1 µg/plate, 1.0 µg/plate, 10.0 µg/plate, 100 µg/plate, 500 µg/plate activated

Statistical methods used: None

Remarks: There were no significant protocol variations. (1) For the time when the test was done (1977) a single plate per concentration was routine; (2) the negative control was the solvent DMSO; the positive controls were chosen according to strain being tested and activation condition and included ethyl methanesulfonate, quinoline hydroxide, nitrofluorene, 2-anthramine; and 2-dimethylnitrosamine (3) a limited repeat study was done with strain TA100 both with and without activation because the testing laboratory believed that there was some evidence of mutagenicity with this strain. The doses tested without activation were 100 µg/plate, 500 µg/plate, and 1000 µg/plate and 500 µg/plate, 1000 µg/plate, and 2000 µg/plate with activation. However, a review of the data shows that was originally thought to be mutagenicity was within the normal variation of the assay. The repeat was inadequate because the doses tested were too high and too toxic to shed any light on possible mutagenic activity; there were no signs of mutagenicity in any of the other strains tested. The test with *Saccharomyces* was also negative. (4) criteria to evaluate results were as follows: dose-response over 3 concentrations with lowest increase equal to 3X the solvent control for TA1535, TA1537 and TA1538. Dose-response over 3 concentrations with lowest increase equal to 3X background for TA100 and 2x-3X background for TA98 and D4.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative with and without activation

Cytotoxic concentration: 1000 µg/ml both with and without activation.

Statistical results: No statistics performed.

Remarks: None

CONCLUSIONS

Author's conclusions are accurate but for the wrong reasons. The test chemical is negative not because it was negative on repeat testing but because what was taken as mutagenicity in the first test was within normal variation of the assay.

REFERENCE

Litton Bionetics, Inc. Kensington, Maryland 20795 1978. Mutagenicity Evaluation of T-2014 CoC in the Ames Salmonella/Microsome Plate Test. Final Report. Submitted to: 3M Company, Saint Paul, Minnesota 55101

Title: *SALMONELLA – ESCHERICHIA COLI*/MAMMALIAN-MICROSOME REVERSE MUTATION ASSAY WITH PFOS, 1999

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS #2795-39-3

Remarks: FC-95, T-6295, purity not specified.

METHOD

Method/Guideline followed: Ames et al., 1975; Green and Muriel, 1976; Maron and Ames, 1983

Test type: Reverse mutation

Test system: Bacterial

GLP: Y

Year study performed: 1999

Species/Strain/cell-type/cell line: *Salmonella typhimurium* TA1535, TA100, TA98, TA1537
Escherichia coli WP2uvrA

Metabolic activation: 0.1 ml S9 liver homogenate from Aroclor 1254 induced Sprague-Dawley rats

Concentrations tested: *S. typhimurium*: 33.3 µg/plate, 100 µg/plate, 333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000 µg/plate with activation and 0.333 µg/plate, 1.00 µg/plate, 3.33 µg/plate, 10.0 µg/plate, 33.3 µg/plate, 100 µg/plate, 333 µg/plate, 1,000 µg/plate and 5,000 µg/plate without activation.

E. coli: 33.3 µg/plate, 100 µg/plate, 333 µg/plate, 1,000 µg/plate, 3,330 µg/plate, and 5,000 µg/plate both with and without activation.

Statistical methods used: None

Remarks: There were no significant protocol deviations. (1) There were 3 plates per test concentration and control; the positive controls were strain and activation condition specific and included benzo[a]pyrene, 2-nitrofluorene, 2-aminoanthracene, sodium azide, ICR-191 and 4-nitroquinoline-N-oxide. The vehicle control was DMSO; (2) the solvent was DMSO; (3) the assay was not repeated. (4) For the test article to be considered positive in strains TA98, TA100 and WP2uvrA, there had to be at least a 2-fold increase in the mean revertants per plate over that of the appropriate vehicle control. The increase had to be accompanied by a dose response to increasing concentrations of the test article. For strains TA1535 and TA1537 there had to be at least a 3-fold increase in the mean revertants per plate over that of the appropriate vehicle control. The increase had to be accompanied by a dose response to increasing concentrations of the test article.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): PFOS was not

genotoxic when tested either with or without metabolic activation.

Cytotoxic concentration: Cytotoxicity was noted at 5000 µg/plate without metabolic activation. This cytotoxicity was evidenced by a slight reduction in the bacterial lawn.

Statistical results: Results were not evaluated statistically.

Remarks: There were no test-specific confounding factors. Mutation frequencies were within the range of the vehicle controls.

CONCLUSIONS

Author's conclusions are that PFOS is negative in this assay. This is accurate.

REFERENCE

Mecchi, M.S. 1999. *Salmonella – Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay with PFOS. Covance Laboratories Inc. (Covance) Vienna, Virginia 22182 Final Report Covance Study No.: 20784-0-409. Submitted to: 3M Corporate Toxicology St. Paul, Minnesota 55144-1000

Title: CHROMOSOMAL ABERRATIONS IN HUMAN WHOLE BLOOD LYMPHOCYTES WITH PFOS, 1999

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS #2795-39-3

Remarks: FC-95, purity not specified.

METHOD

Method/Guideline followed: Galloway, 1994

Test type: *In vitro* cytogenetics

Test system: Human cells in culture

GLP: Y

Year study performed: 1999

Species/Strain/cell-type/cell line: Human lymphocytes

Metabolic activation: Aroclor 1254 induced rat liver S9 homogenate, 15.0 µL/ml, plus NADP and isocitric acid.

Concentrations tested: 12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 199 µg/ml, 249 µg/ml, 299 µg/ml, 349 µg/ml, 449 µg/ml, 599 µg/ml without activation.

12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 199 µg/ml, 249 µg/ml, 349 µg/ml, 449 µg/ml with activation

Statistical methods used: Cochran-Armitage test for linear trend; Fisher's Exact Test

Remarks: There were no significant protocol deviations. (1) Each concentration was tested in replicate; each replicate was considered an independent unit. The negative control for the nonactivation assay was DMSO at 10 µl/ml, which was the highest concentration used in the test cultures; in the activation assay it was DMSO plus the S9 mix; the positive control was mitomycin C for the nonactivation assay and cyclophosphamide for the activation assay. Three concentrations of each positive control were tested. Cultures were exposed to chemical for 3 hours and harvested 22 hours later. One hundred metaphases from each replicate of the useable treatment cultures and the solvent and one dose of the positive control were used; mitotic index was evaluated by analysing the number of mitotic cells in at least 1000 cells per culture; (2) the solvent for the chemical was DMSO; (3) there was no follow up study done although in a study such as this where there are negative results after 3 hours incubation with a 22 hour harvest time a second study with a continuous exposure of 22 hours for the nonactivated portion of the assay is recommended. (4) The test article would have been considered positive if there had been a significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations at one or more concentrations. The test article was considered negative because there was no significant increase observed in the number of cells with chromosomal aberrations at any concentration tested.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative both with and without activation.

Cytotoxic concentration: 299 µg/ml without metabolic activation and 199 µg/ml with activation were the first cytotoxic concentrations tested as evidenced by a reduction in mitotic index.

Statistical results: Negative

Remarks: Mitotic index was reduced 38%, 8% 15%, 15%, 12%, 19%, 24%, 69% and 92% in cultures treated with 12.5 µg/ml, 24.9 µg/ml, 49.7 µg/ml, 99.3 µg/ml, 149 µg/ml, 249 µg/ml, 299 µg/ml, 149 µg/ml and 449 µg/ml without activation. Aberrations were analysed from cultures treated 199 µg/ml, 249 µg/ml, 299 µg/ml, and 349 µg/ml.

With metabolic activation, mitotic index was reduced by 12%, 41%, 71%, and 53% in cultures treated with 49.7 µg/ml, 199 µg/ml, 249 µg/ml, and 299 µg/ml. Aberrations were analysed from cultures treated with 99.3 µg/ml, 149 µg/ml, 199 µg/ml, and 299 µg/ml. Only 27 and 4 metaphases were available for analysis from cultures treated with 299 µg/ml.

CONCLUSIONS

Author's conclusions are that PFOS does not cause mutation in human lymphocytes; this is correct as stated.

REFERENCE

Murli, H. 1999. Chromosomal Aberrations in Human Whole Blood Lymphocytes with PFOS. Covance Laboratories Inc. (Covance) Final Report. Covance Study No.: 20784-0-449. Submitted to: 3M Corporate Toxicology, St. Paul Minnesota 55144-1000.

Title: Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS, 1999

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS #2795-39-3

Remarks: T-6295, purity not specified.

METHOD

Method/Guideline followed: Williams, 1977; Williams, 1980; Butterworth et al., 1987

Test type: Unscheduled DNA Synthesis in Mammalian Cells in Culture

Test system: Primary cells in culture

GLP: Y

Year study performed: 1999

Species/Strain/cell-type/cell line: Primary hepatocytes from a Fischer 344 rat male rat.

Metabolic activation: None

Concentrations tested: 15 concentrations between 0.025 µg/ml and 4000 µg/ml. Six, 0.5 µg/ml, 1.0 µg/ml, 2.5 µg/ml, 5 µg/ml, 10.0 µg/ml and 25.0 µg/ml, chosen for evaluation based upon cytotoxicity.

Statistical methods used: None

Remarks: There were no significant protocol deviations. (1) Triplicate cultures on coverslips were incubated for 19.6-20.0 hours, then the assay was terminated and ³H-thymidine added to the cultures for 30 minutes after which the cells were fixed, dried over night, coverslips were mounted on slides, dipped in emulsion and stored for 6 days at 2-8° C after which the emulsions were developed, fixed and stained. 150 cells per dose were read (50 from each coverslip) and the mean net nuclear grain count determined. (2) The solvent for the assay was DMSO; (3) there was no follow-up repeat study; (4) the positive control was 2-AAF; (5) for a treatment to be considered positive, there must be an increase in the mean net nuclear grain count to at least 5 grains per nucleus above the concurrent vehicle control value, and/or an increase in the number of nuclei with five or more net grains such that the percentage of these nuclei in test cultures is 10% above the percentage seen in the vehicle control cultures. The positive control satisfied both of these criteria.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative

Cytotoxic concentration: Excessive cytotoxicity at and at 50.0 µg/ml; weak cytotoxicity at 25.0 µg/ml. Cell morphology was suitable for analysis at and below 25.0 µg/ml.

Statistical results: The results were not evaluated statistically.

Remarks: There were no test-specific confounding factors.

CONCLUSIONS

The author concludes that PFOS is negative in this assay. This is accurate.

REFERENCE

Cifone M.A. 1999. Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures with PFOS. Covance Laboratories Inc. Vienna, VA 22182 Final Report. Covance Study No.: 20780-0-447. Submitted to 3M Corporate Toxicology St. Paul, MN 55144-1000

Title: MUTAGENICITY TEST ON T-6295 IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY, 1996
TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS #2795-39-3

Remarks: T-6295, FC-95, purity not specified.

METHOD

Method/Guideline followed: Heddle, 1983

Test type: Micronucleus

GLP: Y

Year study performed: 1996

Species/Strain: Mouse; Crl:CD-1[®](ICR)BR

Sex: Males & Females

No. animals/sex/dose: 5/sex/dose

Vehicle (if used): Deionized water

Route of administration: Oral

Doses: 237.5 mg/kg, 450 mg/kg, 950 mg/kg

Frequency of treatment: Single dose

Statistical methods used: Analysis of variance; Dunnet's t-test

Remarks: There were no significant protocol deviations. (1) Animals were 9 weeks and 1 day old at start of dosing males; weight range for the males was 29.9 –37.0 g; for females it was 23.1-29.2 g; (2) the vehicle was deionized water; (3) the test lasted 72 hours; (4) the test material was administered as a single oral dose; (5) all treatment groups were sampled at 24, 48 and 72 hours; (6) the vehicle control was H₂O; the positive control was 80 mg/kg cyclophosphamide dissolved in water and administered by gavage. Controls were sampled at 24 hours only. Control groups consisted of 5 males and 5 females each. (7) No clinical examinations were made. (8) No necropsies or other gross examinations were made on these animals. (9) Micronuclei were evaluated in the bone marrow of treated animals. Frequency of PCEs vs. NCEs was determined by scoring the number of PCEs and NCEs in the optic fields while scoring the first 1000 erythrocytes. A positive was judged by an increase in micronucleated polychromatic erythrocytes over levels observed in the vehicle controls in either sex or at any harvest time. Bone marrow toxicity was judged by a significant reduction in PCE/NCE ratios in either sex at any harvest time. (10) The M.T.D. was chosen on the basis of 2 preliminary dose selection assays both of which showed significant toxicity at the highest dose tested.

RESULTS

Effect on mitotic index or PCE/NCE ratio by dose level and sex:

PCE:NCE Ratio

237.5 mg/kg

24 hours: males 0.57 ± 0.11 ; females 0.52 ± 0.10

48 hours: males 0.48 ± 0.04 ; females 0.80 ± 0.10

72 hours: males 0.39 ± 0.11 ; females 0.42 ± 0.14

450 mg/kg

24 hours: males 0.75 ± 0.11 ; females 0.59 ± 0.08

48 hours: males 0.71 ± 0.05 ; females 0.37 ± 0.07

72 hours: males 0.29 ± 0.06 ; females 0.40 ± 0.12

950 mg/kg

24 hours: males 0.56 ± 0.13 ; females 0.59 ± 0.08

48 hours: males 0.54 ± 0.08 ; females 0.44 ± 0.11

72 hours: males 0.17 ± 0.05 ; females 0.17 ± 0.05

Genotoxic effects (unconfirmed, dose-response, equivocal): Negative

Statistical results:

The PCE:NCE ratio was reduced in 237.5 mg/kg males at 48 and 72 hours; in 450 mg/kg males at 72 hours and in 450 mg/kg females at 48 hours and in 950 mg/kg males at 48 and 72 hours and in 950 mg/kg females at 72 hours. There was no statistically significant increase in the number of micronucleated PCEs over the controls in any treatment group. The positive control induced a significant increase in the number of mPCE in both males and females and reduced the PCE:NCE ratio in females only at 24 hours.

Remarks:

(1) Animals were examined approximately 1-2 hours before sampling for signs of toxicity and mortality. Animals in the 237.5 mg/kg group remained healthy throughout the treatment period. (2) Both males and females in the 950 mg/kg dose group began dying about 22 hours after treatment. Also at 22 hours 2 males in the 950 mg/kg dose group went into convulsions when their cage was opened but recovered in a few minutes. At about 46 hours after treatment 1 female from the 450 mg/kg dose group and more males and females from the 950 mg/kg dose group were found dead and at about 71 hours after treatment, one male from the 950 mg/kg dose group was found dead. All surviving animals appeared normal at that point. (3) No other clinical signs were noted or reported. (4) Body weight changes were not reported. (5) Food and water consumption were not reported. (6) There was no increase in the percent of micronucleated PCEs at any dose level tested or at any time period sampled.

CONCLUSIONS

The author concludes that PFOS is negative in the mouse bone marrow micronucleus assay. This is an accurate assessment.

REFERENCE

Murli, H. 1996. Mutagenicity Test on T-6295 in an *In Vivo* Mouse Micronucleus Assay. Corning Hazelton Inc. (CHV), Vienna, Virginia 22182. Final Report. CHV Study No.: 17403-0-455. Submitted to 3M St. Paul, Minnesota 55144-1000.

Title: *IN VITRO* MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2247 CoC AND T-2248 CoC, 1978

TEST SUBSTANCE

Identity: T-2247 CoC; L-4299, a 50% by weight solution of the diethanolammonium salt of perfluorooctanesulfonate in water T-2248 CoC; 22.5% of a reaction product of ethyl and methyl methacrylates and 22.5% of the pyridinium chloride salt of an: N-methylperfluorooctanesulfonamidoethanol-based glutaryl amide.

Remarks: T-2247 CoC, T-2248 CoC, purity not specified

METHOD

Method/Guideline followed: Ames et al., 1975; Zimmermann and Schwaier, 1967; Brusick and Mayer, 1973

Test type: Reverse Mutation; Recombination

Test system: *Salmonella typhimurium*; *Saccharomyces cerevisiae*

GLP: N

Year study performed: 1978

Species/Strain/cell-type/cell line: *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100; *Saccharomyces cerevisiae* D3

Metabolic activation: 0.5 ml of 10% S9 liver homogenate from Aroclor 1254 induced rats.

Concentrations tested: Plate incorporation assay: 10 µg/plate, 50 µg/plate, 100 µg/plate, 500 µg/plate, 1000 µg/plate, 5000 µg/plate

Dessicator method: 0.1 ml/dessicator, 0.5 ml/dessicator, 1.0 ml/dessicator, 5.0 ml/dessicator

Yeast recombination: 0.1%, 0.5%, 1.0%, 5.0%

Yeast repeat assay at 1.0%, 2.0%, 4.0%, 5.0%

Statistical methods used: None

Remarks: There were no significant protocol deviations. (1). The plate incorporation assay and the *S. cerevisiae* assay were performed with both chemicals and with one plate per test concentration; the dessicator assay was performed with T-2247 CoC using two plates per concentration but used only strains TA 98 and TA100 for the test. However, given the complexity of the dessicator assay and the limitations involved in setting it up, this is acceptable; (2) the positive controls were chosen according to the strain and activation conditions and included sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-anthramine for the plate incorporation assay; 1,1-dichloroethylene for the dessicator assay with T-2247 CoC and 1,2,3,4-diepoxybutane for the *S. cerevisiae* assay. The negative control group for all assays was water. (3) The plate incorporation assay with both agents and the yeast assay with T-2248 were repeated; the dessicator assay was run only once. (4) For the dessicator assay, plates were prepared as for the standard assay but no test chemical was added to the agar. The strains tested were *S. typhimurium* TA98 and TA 100. The test was performed both with and without metabolic activation. Plates without lids were placed side by side in

a perforated shelf in a 9-liter desiccator. A known volume of T-2247 was added to a glass Petri dish that was placed in the center of and attached to the bottom of the shelf. In decreasing order, 5.0 ml, 1.0 ml, 0.5 ml and 0.1 ml of test chemical were added to the desiccator. The negative control chemical was water; the positive control chemical was 1,1-dichloroethylene. Both were treated in the same manner as T-2247. The desiccator was sealed and placed on a magnetic stirrer plate in a room maintained at 37° C. A magnetic stirrer with vanes was placed in the base of each desiccator to ensure adequate dispersion of the chemical. Plates were incubated for 8 hours, removed from the desiccators, their lids replaced and they were incubated at 37° C for an additional 42 hours before revertants were counted.

RESULTS

Overall results: positive, negative, ambiguous: All tests were negative.

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative both with and without activation.

Cytotoxic concentration: T-2247 was not cytotoxic.

In the plate incorporation assay, T-2248 was toxic to strain TA1538 at 1000 µg/plate and to all other strains at 5000 µg/plate when tested without activation. It was toxic at 1000 µg/plate to strain TA1537 and at 5000 µg/plate for all other strains when tested with metabolic activation.

T-2248 was slightly toxic to *S. cerevisiae* D3 at 5% concentration without metabolic activation.

Statistical results: No statistical results were determined.

Remarks: In the first assay with T-2248 and *S. cerevisiae* D3 without metabolic activation there seemed to be some slight indication of mutagenicity at the highest concentration tested, 5%. The assay was repeated at 1%, 2%, 4%, and 5% concentrations with and without activation. There was no indication of a mutagenic dose response and the testing laboratory concluded that T-2248 did not cause recombination in *S. cerevisiae* D3. There were no test-specific confounding factors in any aspect of the test.

CONCLUSIONS

The testing laboratory concluded that T-2247 and T-2248 were nonmutagenic for *S. typhimurium* TA1535, TA100, TA1537, TA1538, and TA98 when tested in a plate incorporation assay with and without metabolic activation; that T2247 did not induce mutation in *S. typhimurium* TA98 and TA100 when tested in a dessicator assay for volatile chemical and that neither chemical induced recombination in *S. cerevisiae* D3.

These conclusions are accurate.

REFERENCE

Simmon, V.F. 1978. *IN VITRO* MICROBIOLOGICAL MUTAGENICITY ASSAYS OF 3M COMPANY COMPOUNDS T-2247 CoC AND T-2248 CoC. SRI International, Final Report. Prepared for 3M Company, St. Paul, Minnesota 55101.

REPEAT DOSE STUDIES

Title: First ninety-day rhesus monkey toxicity study, 1979

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95, purity not specified.

METHOD

Method/guideline followed: None

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1978

Species/strain: Rhesus monkey

Sex: Males and females

Number of animals per dose group: 2/sex/group

Route of administration: Gavage

Doses tested and frequency: 0, 10, 30, 100, 300 mg/kg/day

Post-observation period: None

Statistical methods used: None

Remarks: Distilled water was used for the vehicle control. The males weighed 3.05-3.80 kg at study initiation and the females weighed 2.75-4.10 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study. The study was terminated after 20 days due to the death of the monkeys. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

RESULTS

NOAEL (dose and effect): None

LOAEL (dose and effect): None

Toxic response/effects by dose level: All of the monkeys in the treated groups died.

Statistical results: None

Remarks: The monkeys in the 300 mg/kg/day group died between days 2-4, the monkeys in the 100 mg/kg/day group died between days 3-5, the monkeys in the 30 mg/kg/day group died between days 7-10, and the monkeys in the 10 mg/kg/day group died between days 11-20 of treatment. The monkeys from all the groups showed similar signs of toxicity including decreased activity, emesis with some diarrhea, body stiffening, general body trembling, twitching, weakness, convulsions and prostration. At necropsy, several of the monkeys in the 100 and 300 mg/kg/day groups had a yellowish-brown discoloration of the liver; histologic examination showed no microscopic lesions. Congestion, hemorrhage and lipid depletion of the adrenal cortex was noted in all treated groups. No other lesions were noted.

CONCLUSIONS

Remarks: Authors conclusions stated above in results. Reviewer agrees.

REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1979. Ninety-day subacute rhesus monkey toxicity study. Study No. 137-087, International Research and Development Corporation, Mattawan, MI.

Title: Second ninety-day rhesus monkey toxicity study, 1978

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95, purity not specified.

METHOD

Method/guideline followed: None

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1978

Species/strain: Rhesus monkey

Sex: Males and females

Number of animals per dose group: 2/sex/group

Route of administration: Gavage

Doses tested and frequency: 0, 0.5, 1.5, 4.5 mg/kg/day

Post-observation period: None

Statistical methods used: Body wts, hematological, biochemical and urinalysis and organ wts were compared by analysis of variance (one-way classification), Bartlett's test and the appropriate t-test using Dunnett's multiple comparison tables to judge significance of differences.

Remarks: Distilled water was used for the vehicle control. The males weighed 2.55-3.55 kg at study initiation and the females weighed 2.7-3.75 kg. The monkeys were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

RESULTS

NOAEL (dose and effect): None

LOAEL (dose and effect): 0.5 mg/kg/day: Soft stools, diarrhea, anorexia, emesis, occasional decreases in activity; slight reduction in serum alkaline phosphatase.

Toxic response/effects by dose level: 4.5 mg/kg/day - 4/4 monkeys died between weeks 5-7, clinical signs (anorexia, emesis, black stool, dehydration), significant reduction in serum cholesterol, marked diffuse lipid depletion in the adrenals, moderate diffuse atrophy of pancreatic acinar cells, moderate diffuse atrophy of serous alveolar cells.

1.5 mg/kg/day – clinical signs (soft stools, diarrhea), reduced body weight, reduced serum alkaline phosphatase activity and serum potassium (females), reduced serum cholesterol (1/2 females), reduced inorganic phosphate (1/2 females).

0.5 mg/kg/day – clinical signs, (soft stools, diarrhea), soft stools, diarrhea, anorexia, emesis, occasional decreases in activity; slight reduction in serum alkaline phosphatase.

Statistical results: The statistical results are presented, but should be viewed with caution due to the small number of animals.

4.5 mg/kg/day - significant reduction in serum cholesterol

1.5 mg/kg/day – significant reduction in serum alkaline phosphatase activity and serum potassium (females)

Remarks: All monkeys in the 4.5 mg/kg/day group died or were sacrificed *in extremis* between week 5 and 7 of the study. Beginning on the first or second day of the study, these monkeys exhibited signs of gastrointestinal tract toxicity including anorexia, emesis, black stool and dehydration. All of the monkeys had decreased activity and just prior to death showed marked to severe rigidity, convulsions, generalized body trembling and prostration. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.7 kg at week 5. After 30 days of treatment, there was a significant reduction in serum cholesterol and a 50% reduction in serum alkaline phosphatase activity. At necropsy, mean organ weights were comparable among the control and treated monkeys. Histologic examination showed several treatment related lesions. All the male and females had marked diffuse lipid depletion in the adrenals. One male and two females had moderate diffuse atrophy of the pancreatic exocrine cells with decreased cell size and loss of zymogen granules. Two males and one female had moderate diffuse atrophy of the serous alveolar cells characterized by decreased cell size and loss of cytoplasmic granules.

All monkeys in the 1.5 mg/kg/day group survived until the end of the study. During the first week of the study, the monkeys had decreased activity. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included black stool, diarrhea, mucous in the stool and bloody stool; at the end of the study, anorexia, dehydration or general body trembling were noted. Although statistical significance was not achieved, the mean body weight of the males dropped from 3.15 kg at the beginning of the study to 2.93 kg at the end of the study, and the mean body weight of the females dropped from 3.22 kg to 2.75 kg. One of the females had very low serum cholesterol and another had a reduction in inorganic phosphate. Necropsy revealed no treatment related lesions.

All monkeys in the 0.5 mg/kg/day group survived until the end of the study. Signs of gastrointestinal tract toxicity were noted occasionally during the study and included diarrhea, soft stools, anorexia and emesis. Occasionally, decreased activity was noted in three of the monkeys. Necropsy revealed no treatment related lesions.

CONCLUSIONS

Remarks: Authors conclusions stated above in results. Reviewer agrees.

REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1978. Ninety-day subacute rhesus monkey toxicity study. Study No. 137-092, International Research and Development Corporation, Mattawan, MI.

Title: Ninety day study in rats, 1978

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95, purity not specified.

METHOD

Method/guideline followed: None

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1978

Species/strain: CD rat

Sex: Males and females

Number of animals per dose group: 5/sex/group

Route of administration: Diet

Doses tested and frequency: 0, 30, 100, 300, 1000, 3000 ppm
Equivalent to 0, 2, 6, 18, 60, 200 mg/kg/day

Post-observation period: None

Statistical methods used: Body wts, hematological, biochemical and urinalysis and organ wts were compared by analysis of variance (one-way classification), Bartlett's test and the appropriate t-test using Dunnett's multiple comparison tables to judge significance of differences.

Remarks: The males weighed 196-232 g and the females weighed 165-206 g at study initiation. The animals were observed daily for general clinical signs and body weights were recorded weekly. Hematological and clinical chemistry analyses and urinalysis were conducted at the beginning of the study and after 30 and 90 days of treatment. At necropsy the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed. The thyroid/parathyroid were weighed after fixation. Tissues were preserved in buffered neutral 10% formalin; the eyes were preserved in Russell's fixative. The following organs from control and all treated groups were examined microscopically: adrenals, aorta, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, bone/bone marrow (rib junction), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder and vagina.

RESULTS

NOAEL (dose and effect): None

LOAEL (dose and effect): 30 ppm (2 mg/kg/day) based on a significant increase in relative and absolute liver weights.

Toxic response/effects by dose level: 3000 ppm – 10/10 rats died between days 7-8. 1000 ppm – 10/10 rats died between days 8-14. 300 ppm – 5/5 male rats died between days 13-25; 5/5 female rats died between days 18-28. At 300, 1000 and 3000 ppm – histologic lesions in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. 100 ppm – 2/5 males and 2/5 females died during week 5 and a third male died during week 11, mean body weights were reduced by 16.7% (males) and 16.3% (females) at study termination, food consumption significantly reduced, significant reduction in hematocrit (males), erythrocyte (males), hemoglobin (males & females), leukocyte (males), and reticulocyte (females) counts, significant increase in absolute (females) and relative (males & females) liver weight and relative kidney weight. At 100, 300, 1000 and 3000 ppm – slight to marked focal necrosis of hepatocytes.

30 ppm – Significant reduction in food consumption (males), significant increase in absolute and relative liver weight (females).

At all dose levels – very slight to slight cytoplasmic hypertrophy of hepatocytes in the centrilobular and midzonal regions, especially in males.

Statistical results: 100 ppm – significant reduction in food consumption

Remarks: All of the rats in the 300, 1000 and 3000 ppm groups died. Death occurred between days 13-25 and days 18-28 for the males and females, respectively, in the 300 ppm group. At 1000 ppm, death occurred between days 8-14, and at 3000 ppm, the rats died between days 7-8 of treatment. The rats in all groups showed signs of toxicity including emaciation, convulsions following handling, hunched back, red material around the eyes, yellow material around the anogenital region, increased sensitivity to external stimuli, reduced activity and moist red material around the mouth or nose.

Three males and two females in the 100 ppm group died prior to scheduled sacrifice. Two of the males and the two females died during week 5 and the third male died during week 11 of the study. At study termination, mean body weights were reduced by 16.7% and 16.3% in the male and female groups, respectively. Average food consumption during the entire study period (g/rat/day) was significantly reduced for males and females at 100 ppm. After 30 days of treatment, hematologic values were comparable among the control and 100 ppm groups. Clinical chemistry analyses at one month showed a significant increase in mean glucose in males, blood urea nitrogen values in males and females, and creatinine phosphokinase and alkaline phosphatase values for females. After 90 days of treatment at 100 ppm, the two surviving males had significantly reduced erythrocyte, hemoglobin, hematocrit and leukocyte counts; the three surviving females had significantly reduced hemoglobin and reticulocyte counts, as well as slightly lower erythrocyte, hematocrit and leukocyte counts. Two of the surviving females showed slight to moderate increases in plasma glutamic oxalacetic and pyruvic transaminase activities. Urinalysis results were comparable among treated and control groups at 30 and 90 days. Relative liver weight was significantly increased in the males and absolute and relative liver weights were significantly increased in the females.. Relative kidney weights were significantly increased in both sexes.

All rats in the 30 ppm group survived until the end of the study. At study termination, mean body weights were reduced by 8.7 and 8% in the males and females, respectively. Average food consumption during the

entire study period (g/rat/day) was significantly reduced for the males at 30 ppm. Hematologic values were comparable among the control and 30 ppm group at 30 and 90 days. One female showed a slightly elevated glucose level and one male showed a slightly increased alkaline phosphatase level at 30 days. At 90 days, one male showed moderate increases in glucose, blood urea nitrogen and γ -glutamyl transpeptidase activity. The females had significant increases in absolute and relative liver weights. The males had significant decreases in absolute and relative adrenal weights, absolute thyroid/parathyroid weight and absolute pituitary weight. The biological significance of the changes in male organ weights is unclear since similar changes were not noted in higher dose groups.

At necropsy, treatment related gross lesions were present in all treated groups and included varying degrees of discoloration and/or enlargement of the liver and discoloration of the glandular mucosa of the stomach. Histologic examination also showed lesions in all treated groups. Centrilobular to midzonal cytoplasmic hypertrophy of hepatocytes and focal necrosis was observed in the liver; the incidence and relative severity were greater in the males. In addition, especially among rats in the 300, 1000 and 3000 ppm groups, treatment related histologic lesions were noted in the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin. In the thymus, this consisted of depletion in the number and size of the lymphoid follicles and in the bone marrow hypocellularity was noted. The spleen was slightly atrophied with a corresponding decrease in the size and number of lymphoid follicles and cells and a similar depletion was noted in the mesenteric lymph nodes. Mucosal hyperkeratosis and/or acanthosis was observed in the forestomach and mucosal hemorrhages were noted in the glandular portion of the stomach. Decrease atrophy in the height and thickness of the villi were noted in the small intestine. Atrophy of the skeletal muscle was noted, as well as epidermal hyperkeratosis and/or acanthosis was noted in the skin.

CONCLUSIONS

Remarks: Authors conclusions stated above in results. Reviewer agrees.

REFERENCE

Goldenthal, E.I., D.C. Jessup, R.G. Geil and J.S. Mehring. 1978. Ninety-day subacute rat toxicity study. Study No. 137-085, International Research and Development Corporation, Mattawan, MI.

Title: 4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys. Unaudited Draft. 1998.

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No.: 2795-39-3

Remarks: T-6295, purity not specified.

METHOD

Method/guideline followed: Range finding – Unaudited Draft report, sections missing

Study duration: 28 days

GLP (Y/N): Y, report contains GLP statement, but as submitted, unaudited draft report with sections missing

Year study performed: 1998

Species/Strain: Monkey/Cynomolgus

Sex: both

Number of animals per dose: 2 per sex, 0 mg/kg/day; 3 per sex, 0.02 mg/kg/day;
1 per sex, 2.0 mg/kg/day

Route of administration: Capsule in stomach

Doses tested and frequency: 0 mg/kg/day; 0.02 mg/kg/day; 2.0 mg/kg/day

Post-observation period: none

Statistical methods used: none

Remarks: Monkeys were observed at least daily for general clinical signs and body weights were recorded twice weekly. Hematological and clinical chemistry analyses were conducted on samples collected before the beginning of the study at day -7 (baseline values) and day 29. Additional blood samples for clinical chemistry were collected on study days 2, 7, and 14. Blood samples for serum PFOS concentrations were taken on days -7, 2, 3, 7, 14, and 29. In addition, samples from day -7 and day 29 were analyzed for levels of estradiol, estrone, estriol, thyroid stimulating hormone, triiodothyronine, and thyroxine. The study animals were terminated as scheduled at 30 days. At necropsy a sample of liver was collected from each animal for palmitoyl CoA oxidase activity analyses. Samples of liver, testes, and pancreas were collected for proliferation cell nuclear antigen evaluation. A sample of liver was also collected from each animal for PFOS concentration analysis. The following organs from control and all treated groups were examined microscopically: adrenals, eye, kidney, liver, lung, spleen, pancreas, femoral bone marrow, testes, and thymus.

RESULTS

NOAEL (dose and effect): None determined.

LOAEL (dose and effect): None determined

Toxic response/effects by dose level: None determined

Statistical results: None, high-dose too few animals (1 male, 1 female)

Summary Hormone Analyses Data in Males on Females on Days -7 and 29

Dose Level	Sex	Day	Estradiol pg/mL	Estrone pg/mL	Estriol pg/mL	TSH ØU/mL	Triiodothyronine ng/dL	Thyroxin Øg/dL
0	Males	-7	32.03±4.002	28.23±14.701	0.00±0.000	2.82±0.686	139.74±29.232	5.43±2.001
0	Males	29	32.09±0.205	21.93±4.30	0.00±0.000	2.15±2.008	186.35±6.935	4.32±1.407
0.02	Males	-7	28.61±4.874	17.32±1.373	0.00±0.000	2.11±0.799	122.49±48.610	4.28±0.335
0.02	Males	29	34.25±7.998	14.72±4.767	0.00±0.000	3.10±0.584	188.34±26.229	4.19±0.612
2.0	Males	-7	28.71	13.98	0.00	1.71	97.61	3.86
2.0	Males	29	18.16	20.12	0.00	0.95	90.73	3.18
0	Females	-7	45.28±0.212	28.77±5.162	0.00±0.000	4.37±4.398	132.65±56.434	4.88±0.948
0	Females	29	53.47±20.082	25.97±2.128	0.00±0.000	2.95±0.530	162.70±29.366	4.26±0.438
0.02	Females	-7	48.12±21.124	30.40±10.852	0.00±0.000	2.51±1.506	131.09±56.434	4.76±1.057
0.02	Females	29	58.72±27.628	31.61±0.921	0.00±0.000	2.76±1.764	180.58±15.203	4.05±1.108
2.0	Females	-7	37.37	29.50	0.00	3.92	122.38	3.60
2.0	Females	29	19.82	25.85	0.00		90.17	3.38

CONCLUSIONS

Estradiol, estrone, thyroid stimulating hormone (TSH), thyroxin, and triiodothyronine levels were lower in the high dose animals at the end of the study. Since the numbers of tested animals are small (one male and one female in the high-dose group) and baseline levels are variable, it is not clear if these hormone level changes are treatment-related.

Remarks: None

REFERENCE

Thomford, P.J. 1998. 4-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys. Unaudited Draft. Study No.T-6295.6, for 3M, St. Paul, MN, by Covance Laboratories Inc., Madison, WI.

Title: 26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys, 2002

TEST SUBSTANCE

Identity: Potassium perfluorooctylsulfonate, CAS # 2795-39-3

Remarks: FC-95, T-6295, purity not specified.

METHOD

Method/guideline followed: No guideline followed.

Study duration: 78 weeks, 26-week treatment period, followed by 52 weeks recovery.

GLP (Y/N): Y

Year study performed: 2000

Species/strain: Cynomolgus monkeys

Sex: Males and females

Number of animals per dose group: 6 animals/sex/dose group for groups 1, 3, and 4;
4 animals/sex for group 2.

Route of administration: Oral capsule

Doses tested and frequency: 0 mg/kg/day, 0.03 mg/kg/day, 0.15 mg/kg/day, or 0.75 mg/kg/day

Post-observation period: 52 weeks

Statistical methods used: Levene's test for variance homogeneity: ANOVA, Dunnett's t-test ANCOVA, covariate-adjusted means, 5% two-tailed probability level.

Remarks: Animals were observed twice daily for mortality and moribundity and were examined at least once daily for abnormalities and signs of toxicity; food consumption was assessed qualitatively. Ophthalmic examinations were done before initiation of treatment and during weeks 26 and 52. Body weight data were recorded weekly before the start of treatment, on Days -1 and 1 and weekly thereafter. Blood and urine samples were collected for clinical hematology, clinical chemistry, and urinalysis before the start of treatment and at specified intervals during treatment and recovery. Blood samples were also taken for hormone determinations. Samples of serum were collected at various time points during the study and sent to 3M for analysis of PFOS levels. The following organs were weighed at scheduled and unscheduled sacrifices; paired organs were weighed separately: adrenal (2), brain, epididymis (2), kidney (2), liver, ovary (2), pancreas, testis (2), and thyroid (2) with parathyroid. The following tissues were collected for histopathology: adrenals (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eyes (2), femur with bone marrow, gallbladder, heart, ileum, jejunum, kidneys (2), lesions, liver, lung, mammary gland, mesenteric lymph node, ovary (2), pancreas, pituitary, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle (2), skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis (2), thymus, thyroid (2) with parathyroid, trachea, urinary bladder, uterus, and vagina. Liver specimens from the 0.15 and 0.75

mg/kg/day recovery animals were collected via biopsy and analyzed for PFOS levels.

Serum and liver specimens collected from test animals were sent to the 3M Laboratory and analyzed for the presence of PFOS. Serum was harvested from blood that was centrifuged within one hour of collection. Liver specimens were flash frozen in liquid nitrogen. Both liver and serum samples were stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Samples were shipped frozen and on dry ice from Covance Laboratories to 3M periodically from August 1998 through March 2000 which covered the in-life phase of the study. Once received at 3M specimens were stored in freezer at either -55°C ± 10-20°C or -20°C ± 10°C.

During the first 26 weeks of the study a total of 550 serum specimens and 30 liver specimens were collected. Of the serum specimens, 151 were from Group 1, 99 from Group 2, 152 from Group 3 and 148 from Group 4. Eight liver samples were collected from Group 1, 8 from Group 2, 12 from Group 3 and 14 from Group 4. In the recovery Groups, 72 serum and 4 liver samples were collected from Group 1; 72 serum and 4 liver samples from Group 3 and 80 serum and 4 liver samples from Group 4.

Liver and serum samples were extracted using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to extraction. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external standard calibration.

Liver samples were homogenized in water. An aliquot of each liver homogenate and all serum samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the samples and the ion pairs were partitioned into MtBE. The extracts were evaporated until dry on a nitrogen evaporator and then were reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter.

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of the fluorochemical of interest using HPLC/ES/MS/MS. Molecular ion 499, the primary ion for PFOS (C₈F₁₇SO₃⁻) analysis, was fragmented to produce ion 99 (FSO₃⁻). Ion 99 was monitored for quantitative analysis.

RESULTS

NOAEL (dose and effect): 0.15 mg/kg/day.

LOAEL (dose and effect): 0.75 mg/kg/day. Death, liver effects, effect on cholesterol

Toxic response/effects by dose level: Death at 0.75 mg/kg/day; increased absolute liver weight, liver to body weight percentages, liver to brain weight ratios in females at 0.75 mg/kg/day; absolute and relative liver weight; Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and lower high density lipoprotein cholesterol, liver organ weights.

Statistical results: The difference in weight at the end of treatment between the control and the 0.75 mg/kg/day female treatment groups was statistically significant; the effect on total cholesterol and high density lipoprotein cholesterol in the 0.75 mg/kg/dose group was statistically significant; in males in the 0.75 mg/kg/day dose group the liver organ weights and the organ-to body weight percentages were statistically significant and in females the liver weights, the organ-to-body weight percentage and the organ-brain weight ratio were all significant.

Remarks:

Males weighed 3.3-3.4 kg and females weighed 2.8-2.9 kg at the beginning of the study. At the end of 26 weeks of treatment, males weighed 3.7, 3.8, 3.5, and 3.3 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. Females weighed 3.1, 3.1, 3.1 and 2.8 kg for the 0, 0.03, 0.15 and 0.75 mg/kg/day treatment groups respectively. The difference between the control and the 0.75 mg/kg/day female treatment groups was statistically significant. At the end of the recovery period, differences in weight between the control and treated animals were no longer obvious.

Two males from the 0.75 mg/kg/day group did not survive to the scheduled sacrifice. One animal died after dosing on Day 155 (Week 23). Clinical signs noted in this animal included: constricted pupils, pale gums, few, mucoid, liquid and black-colored feces, low food consumption, hypoactivity, labored respiration, dehydration, and recumbent position. In addition, the animal was cold to the touch. An enlarged liver was detected by palpation. Cause of death was determined to be pulmonary necrosis with severe acute inflammation. On day 179, the second male was sacrificed in a moribund condition. Clinical signs noted included low food consumption, excessive salivation, labored respiration, hypoactivity and ataxia. Cause of death was not determined.

Males and females in the 0.75 mg/kg/day dose-group had lower total cholesterol and males and females in the 0.15 and 0.75 mg/kg/day groups had lower high density lipoprotein cholesterol during treatment. However, only the effect in the 0.75 mg/kg group was statistically significant. The effect on total cholesterol worsened with time. By day 182, mean total cholesterol for males and females in the high dose group were 68% and 49% lower, respectively, than levels in the control animals. The effect on high density lipoprotein was greater than that seen with cholesterol. On day 182, the mean high density lipoprotein levels were 79% and 62% lower in males and females, respectively, from the high dose group than they were in male and female control animals. Males in the high dose group also had lower total bilirubin concentrations and higher serum bile acid concentrations than males in either the control or other treatment groups. The effect on total cholesterol was reversed within 5 weeks of recovery and the effect on high density lipoprotein cholesterol was reversed within 9 weeks of recovery.

Estradiol values were lower in males given 0.75 mg/kg/day on days 62, 91, and 182 by because of variation only the day 182 value was significant. Estrone values were generally higher in the treated females on days 37 62 and 91 by again because of variation in the data none of these values were significantly different. Triiodothyronine values were notably lower on days 91 and 182 in males and females given 0.15 and 0.75 mg/kg/day. There were other instances in which hormone values in treated groups were different from those of controls but these differences were not consistent over time or between sexes, were not clearly dose-related and did not appear to be related to the administration of the test material. Apparent differences in the sexual maturity of both males and females used in the study complicates the interpretation of the hormone data.

At terminal sacrifice, females in the 0.75 mg/kg/day dose-group had increased absolute liver weight, liver-to-body weight percentages, and liver-to-brain weight ratios. In males, liver-to body weight percentages were increased in the high-dose group compared to the controls. "Mottled" livers were observed in two high-dose males and in one high-dose female. Of the two males not surviving until the scheduled terminal sacrifice, one had a "mottled" and large liver. Three of 4 high-dose males (including those that did not survive to scheduled sacrifice) had centrilobular or diffuse hepatocellular hypertrophy that was also observed in all high-dose females. Centrilobular or diffuse hepatocellular vacuolation occurred in 2 of 4 females and 2 of 4 males in the high-dose group.

No PFOS related lesions were observed at recovery sacrifice indicating that the effects seen at terminal sacrifice may be reversible.

Although low levels of PFOS were often detected in the sera and liver of the control animals, these levels were significantly lower than those found in the low dose test animals. PFOS levels in the sera of test animals increased with dose during treatment from 21.0 ± 1.57 and 20.4 ± 2.71 $\mu\text{g/ml}$ in the Group 4 males and females respectively at the end of Week 1 to 194 ± 8.94 and 160 ± 23.1 $\mu\text{g/ml}$ in males and females respectively in Group 4 at the end of Week 27. During recovery, PFOS levels in serum samples decreased over time until they reached 41.1 ± 25.9 $\mu\text{g/ml}$ in males and 41.4 ± 1.15 $\mu\text{g/ml}$ in females from Group 4 at 79 weeks post-treatment. Control values were < LOQ (the limit of quantitation) at Week 4 in both males and females and 0.0215 ± 0.00296 and 0.0243 ± 0.00355 $\mu\text{g/ml}$ in males and females respectively at the end of Week 79. The serum values for selected weeks of treatment and recovery are shown in the table below. There were no significant differences between PFOS levels in the sera of treated males and females.

**Average PFOS Concentrations ($\mu\text{g/ml}$) in Serum of Monkeys for
Selected Weeks During Treatment and Recovery**

	Group 1 0.0 mg/kg/day		Group 2 0.03 mg/kg/day		Group 3 0.15 mg/kg/day		Group 4 0.75 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
Week 1	<LOQ	<LOQ	0.869 ± 0.147	0.947 ± 0.110	4.60 ± 0.782	3.71 ± 0.455	21.0 ± 1.57	20.4 ± 2.71
Week 4	<LOQ	<LOQ	3.20 ± 0.577	3.40 ± 0.291	17.8 ± 1.68	16.5 ± 1.87	95.3 ± 70.4	92.7 ± 39.6
Week 16	0.0407 ± 0.0110	0.0432 ± 0.0081	11.2 ± 2.44	10.5 ± 1.90	56.2 ± 5.84	42.1 ± 4.04	189 ± 15.9	162 ± 19.3
Week 27	0.0529 ± 0.0145	0.0416 ± 0.0148	15.9 ± 5.54	11.1 ± 1.52	68.1 ± 5.75	58.5 ± 4.67	194 ± 8.93	160 ± 23.9
Week 35	0.0459 ± 0.00303	0.0723 ± 0.00352	Not Determined	Not Determined	84.5 ± 12.0	74.7 ± 9.53	181 ± 19.5	171 ± 10.1
Week 47	0.0355 ± 0.00221	0.0459 ± 0.00323	Not Determined	Not Determined	48.3 ± 3.69	42.6 ± 6.70	124 ± 25.9	98.3 ± 8.32
Week 57	0.0327 ± 0.00526	0.0445 ± 0.00385	Not Determined	Not Determined	30.2 ± 2.36	32.3 ± 1.34	78.0 ± 16.3	106 ± 3.84
Week 69	0.0406 ± 0.00313	0.0400 ± 0.00301	Not Determined	Not Determined	26.4 ± 2.59	34.5 ± 3.46	84.0 ± 52.4	75.0 ± 5.25
Week 79	0.0215 ± 0.00296	0.0243 ± 0.00355	Not Determined	Not Determined	19.1 ± 0.805	21.4 ± 2.01	41.1 ± 25.9	41.4 ± 1.15

LOQ = Lowest Observable Concentration

Liver values behaved in a manner similar to serum values and increased over time. At Week 27 mean

PFOS values on an RSD basis were 22.2 ± 0.0269 in Group 1 males and 16.8 ± 0.0178 in females in Group 1; 27.0 ± 4.66 and 9.73 ± 2.15 in males and females in Group 2; 33.1 ± 19.5 in males and 21.4 ± 14.9 in females in Group 3, and 6.03 ± 23.9 in males and 5.00 ± 13.6 in females in Group 4. At Week 79 values in the liver were 71.0 ± 33.4 in males and 21.4 ± 10.8 in females in Group 4. At Week 80, values were 14.9 ± 1.38 in Group 3 males and 23.5 ± 4.98 in Group 3 females.

CONCLUSIONS

PFOS is toxic to cynomolgous monkeys at 0.75 mg/kg/day causing death, alterations in total cholesterol, and effecting liver weight and causing hepatocellular hypertrophy and vacuolation in both treated males and females. However, the effects on cholesterol and the liver appear to be reversible after a 52 week recovery period.

REFERENCE

- Thomford, PJ. (2000). 26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T6295) in Cynomolgus Monkeys. Unaudited Draft Final Report Prepared for 3M, St Paul, Minnesota by Covance Laboratories, Inc., Madison Wisconsin 53704-2595. April 12, 2000. 502 pp.
- Seacat, AM. Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples. 3M Medical Department Study: T-6295.7; Covance In-Life Study:#6329-223. Analytical Study; FACT TOX-030; 3M Laboratory Request No. U2279.

DEVELOPMENTAL TOXICITY STUDIES

Title: Oral Teratology Study of FC-95 in Rats - Experiment No. 0680TR0008

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

Remarks: FC-95, Lot 640.

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): Actual guideline followed was not specified but appears to be similar in design to OECD 414.

GLP (Y/N): The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study was conducted according to the 1978 Good Laboratory Practice regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

Year study performed: 1980

Species/Strain: Sprague-Dawley rats

Number of animals per dose: 22

Route of administration: Gavage

Dosing regimen (list all with units): Four groups of 22 time-mated Sprague-Dawley rats were administered Potassium Perfluorooctylsulfonate in corn oil by gavage on gestation days 6-15. Doses were adjusted according to the most recent recorded body weight.

Doses: 0, 1, 5, and 10 mg/kg/day

Statistical methods used: The animals will be assigned cages according to a computer-generated random numbers table. The statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities.

Remarks – Detail and discuss any significant protocol parameters and deviations:

Potassium Perfluorooctylsulfonate was administered in corn oil by gavage to four groups of 22 time-mated Sprague-Dawley rats weighing 175-261g, at doses of 0, 1, 5, and 10 mg/kg/day PFOS on days 6-15 of gestation (Gortner, 1980). Purina Laboratory Chow and water were available ad libitum. The animals were dosed according to a constant dose volume of 5 ml/kg of body weight and observed daily from day 3 through day 20 of gestation for abnormal clinical signs.

Body weights were recorded on days 3, 6, 9, 12, 15, and 20 of gestation and the rats. All animals were sacrificed on day 20 by cervical dislocation and the ovaries, uteri and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning by the Wilson technique. The remaining fetuses were subjected to a

skeletal examination using alizarin red.

RESULTS

NOAEL (dose and effect) – maternal and developmental: A NOAEL of 5 mg/kg/day for maternal toxicity was indicated. No signs of maternal toxicity were reported at doses of 5 mg/kg/day and below. A NOAEL for developmental toxicity could not be established; signs of developmental toxicity were evident at all doses.

LOAEL (dose and effect) – maternal and developmental: A LOAEL of 10 mg/kg/day for maternal toxicity was indicated based on significant reductions in mean body weights during gestation day 12-20 at the high-dose group of 10 mg/kg/day. A LOAEL of 1 mg/kg/day for developmental toxicity was indicated based on abnormalities of the lens of the eye.

Toxic response/effects by dose level - maternal: Significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day.

Toxic response/effects by dose level - developmental: Unusually high incidences of developmental variations and abnormalities of the lens of the eye were observed in all dose groups.

Statistical results: At 10 mg/kg/day, mean maternal body weights were statistically significantly lower than controls (Dunnett's test $p < 0.05$). Mean litter data and pup weights were not significantly different from controls (Dunnett's t test $p < 0.05$). Number of fetuses with gross findings were not significantly different from controls (Chi-square $p < 0.05$).

Number and percent of fetuses with skeleton findings were not significantly different from controls (Chi-square $p < 0.05$).

Number and percent of fetuses with internal findings - developmental lens abnormalities with secondary lens aberrations were significantly higher than controls (Chi-square $p < 0.05$).

Remarks – Additional information to adequately assess the data: Signs of maternal toxicity consisted of significant reductions in mean body weights during GD 12-20 at the high-dose group of 10 mg/kg/day. No other signs of maternal toxicity were reported. Developmental toxicity evident at doses of 10 mg/kg/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and the mean numbers of viable male, female, and total fetuses, but the differences were not statistically significant. In addition, unusually high incidences of unossified, asymmetrical, bipartite, and missing sternebrae were observed in all dose groups; however, these skeletal variations were also observed in control fetuses at the same rate and therefore were not considered to be treatment-related. The most notable sign of developmental toxicity observed in all dose groups consisted of abnormalities of the lens of the eye, which was not seen in controls. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group. All eye abnormalities appeared to be localized to the area of the embryonal lens nucleus, although a variety of morphological appearances were present within that location. According to the authors, this abnormality appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. An amendment to the results and discussion section concludes that the gross finding of a lens cleft was an artifact created by freehand sectioning and the range of gross lens observations and the differences among the dose group incidences were due to the manner and frequency in which the lens cleft artifact was created by free-hand sectioning and the limitations inherent in visualizing the embryonal nucleus. Additionally, a subsequent study (Wetzel, 1893) of similar design was not able to repeat this finding.

CONCLUSIONS

Given the explanation that the eye abnormalities reported at doses as low as 1 mg/kg/day in this study may have been due to an artifact of sectioning and that these findings could not be repeated in a second study of similar design, it is entirely plausible that the lens defect observed in this study is not treatment-related.

REFERENCE

Gortner, E.G. 1980. Safety Evaluation Laboratory and Riker Laboratories, Inc. Experiment Number: 0680TR0008, December, 1980. "Oral Teratology Study of FC-95 in Rats".

Title: Rat Teratology Study T-3351 Final Report – Project No. 154-160

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3

Remarks: T-3351, Lot No. 80275

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): Actual guideline followed was not specified but appears to be similar in design to OECD 414.

GLP (Y/N): Quality Assurance inspections of the study and review of the final report were conducted according to the standard operating procedures of the Office of Quality Assurance and according to the general requirements of the Good Laboratory Practice regulations that were issued on December 22, 1978, by the Food and Drug Administration for compliance on and after June 20, 1979.

Year study performed: 1983

Species/Strain: Sprague-Dawley rats

Number of animals per dose: 25

Route of administration: Gavage

Dosing regimen (list all with units): Four groups of 25 pregnant Sprague-Dawley rats were administered Potassium Perfluorooctylsulfonate in corn oil by gavage on gestation days 6-15. Doses were adjusted according to the most recent recorded body weight.

Doses: 0, 1, 5, and 10 mg/kg/day

Statistical methods used: Statistical methods used for analysis of the data : Dunnett's t test for control vs. compound-treated group mean comparisons. If the variances were proved to be homogeneous, the data were analysed by one-way classification analysis of variance (ANOVA). Mean fetal body weights per litter were statistically analysed as follows: Bartlett's test for homogeneity of variances was performed by one-way classification of covariance (ANCOVA).

If ANCOVA was significant, control vs. treatment group comparisons were analysed using the Games and Howell modification of the Tukey-Kramer honestly significant difference test. Tests for homogeneity of variances, ANOVA, and ANCOVA were evaluated at the 5% one-tailed probability level. Control vs. compound-treated group mean comparisons of the above data were evaluated at the 5% two-tailed probability level. Percent fetal viability, percent fetal loss (dead and resorbing fetuses), percent early, late, and total resorptions, and the number of dead fetuses were analysed by nonparametric one-way ANOVA and the Terpstra-Jonckheere test for trend. The litter was used as the experimental unit. Teratology data were analysed using the Cochran-Armitage test for linear trend in proportions. If a significant trend was noted, the results of Fisher's "exact" test were evaluated at the one-tailed, 5% level. If a significant trend was not observed, or if there was a significant trend with severe departure from it, the results of Fisher's "exact" test were evaluated at the two-tailed, 5% level.

Remarks – Detail and discuss any significant protocol parameters and deviations:

Potassium perfluorooctylsulfonate was administered in corn oil by gavage to four groups of 25 pregnant Sprague-Dawley rats at doses of 0, 1, 5, and 10 mg/kg/day PFOS on gestation days (GD) 6-15 (Wetzel, 1983). Sexually mature Sprague-Dawley rats, one per sex per cage, were paired until confirmation of mating or until two weeks had elapsed. Mating was confirmed by daily vaginal examinations for the presence and viability of sperm or the presence of a copulatory plug. The day of confirmation of mating was designated as day 0 of gestation. Purina Rodent Laboratory Chow 5001 and tap water were available ad libitum. A dose volume of 3 ml/kg of body weight was administered and doses were adjusted according to the most recently recorded body weight measurements. Dams were observed twice daily for signs of mortality and moribundity and once daily for clinical signs of toxicity. Individual body weights and food consumption were recorded on GD 6, 8, 12, 16, and 20. Animals were sacrificed on GD 20 by CO₂ asphyxiation and the fetuses were delivered by cesarean section on GD 20. A gross necropsy was performed on all dams. The uterus from each female was excised, weighed and examined for the number and placement of implantation sites, number and of live and dead fetuses, number of early and late resorptions, and any abnormalities and then weighed again after the contents were removed. The ovaries were examined for the number of corpora lutea. Each female was examined by gross necropsy. Each fetus was sexed, weighed, and examined externally. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by the Wilson technique, with particular attention to the eyes, palate, and brain. The remaining fetuses were subjected to a skeletal examination that included evaluation of the skull, long bones, vertebral column, rib cage, extremities, and pectoral and pelvic girdles using alizarin red; bone alignment and degree of ossification were also evaluated.

RESULTS

NOAEL (dose and effect) – maternal and developmental: The NOAEL for maternal toxicity is 1 mg/kg/day. The NOAEL for developmental toxicity is 1 mg/kg/day.

LOAEL (dose and effect) – maternal and developmental: The LOAEL for maternal toxicity is 5 mg/kg/day, based on clinical signs of toxicity, decreases in body weight and food consumption, decreases in uterine weights, and an increased incidence in gastrointestinal lesions.

The LOAEL for developmental toxicity is 5 mg/kg/day, based on decreased fetal body weight and increases in external and visceral anomalies and variations.

Toxic response/effects by dose level - maternal: Clinical signs of toxicity, decreases in body weights and food consumption at 5 and 10 mg/kg/day; decreases in uterine weights, increased incidence in gastrointestinal lesions, and two deaths at 10 mg/kg/day.

Toxic response/effects by dose level - developmental: Decreased fetal weight at 5 and 10 mg/kg/day; external and visceral anomalies and skeletal variations at 10 mg/kg/day.

Statistical results: Statistically significant differences between controls and treated were noted for the following maternal endpoints: mean body weight gain, mean total food consumption, and mean gravid uterine weight. Nonparametric analysis of the mean incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss did not indicate statistical significance; however, there was a significant linear trend towards an increased incidence in these data with respect to control. The primary trend component was contributed by the high-dose group. Statistically significant treatment-related increases in the incidences of visceral anomalies and skeletal variants were also observed.

Remarks – Additional information to adequately assess the data: Evidence of maternal toxicity, that was observed at the 5 and 10 mg/kg/day dose groups both during and following treatment and considered to be treatment-related, consisted of hunched posture, anorexia, bloody vaginal discharge, uterine stains, alopecia, rough haircoat, and bloody crust. Significant decreases in mean body weight gains during GD 6-8, 6-16, and 0-20 were also observed at the 5 and 10 mg/kg/day dose groups. These reductions were considered to be treatment-related since mean body weight gains were greater than controls during the post-exposure period (GD 16-20). Significant decreases in mean total food consumption were observed on GD 17-20 in the 10 mg/kg/day dose group, and on GD 7-16 and 0-20 in both the 5 and 10 mg/kg/day dose groups. The mean gravid uterine weight in the 10 mg/kg/day dose group was significantly lower when compared with controls. The mean terminal body weights minus the gravid uterine weights were lower in all treated groups, with significant decreases at 5 and 10 mg/kg/day. High-dose animals also exhibited an increased incidence in gastrointestinal lesions. No significant differences were observed in pregnancy rates, number of corpora lutea, and number and placement of implantation sites among treated and control groups. Two dams in the 10 mg/kg/day dose group were found dead on GD 17.

Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although, none of these effects were statistically significantly different from controls. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg/day dose groups. The percent of male fetuses was 52%, 54%, and 60% for 1, 5, and 10 mg/kg/day, respectively, compared to 44% in controls. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups but not in the mid-dose group. Statistically significant increases in the incidences in the number of litters containing fetuses with visceral anomalies, delayed ossification, and skeletal variations were observed in the high dose group of 10 mg/kg/day. These included external and visceral anomalies of the cleft palate, subcutaneous edema, and cryptorchism as well as delays in skeletal ossification of the skull, pectoral girdle, rib cage, vertebral column, pelvic girdle and limbs. Skeletal variations in the ribs and sternbrae were also observed at this dose level.

CONCLUSIONS

The developmental eye abnormalities that were seen in Gortner (1980) were not observed in the present developmental toxicity study even though the study design and doses were the same. Findings of abnormalities in eye development were initially thought to be treatment-related but then later suggested as being artifacts of sectioning.

REFERENCE

Wetzel, L.T. 1983. Hazelton Laboratories America, Inc. Project Number: 154-160, December 19, 1983. "Rat Teratology Study, T-3351, Final Report".

Title: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF PFOS IN RABBITS – 3M T-6295.10, ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.10, 1999.

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate (PFOS), CAS No. 2795-39-3

Remarks: PFOS – Lot 217, 98.4% pure (SMD Analytical Request 53030) Analytical Documentation filed along with final report. Note: Same lot as used in two-year rat PFOS carcinogenicity study (T-6295, Covance 6329-183).

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): The requirements of the International Conference on Harmonization (ICH) Harmonized Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal products, stages C and D of the reproductive process in a non-rodent species were used as the basis for study design (U.S. Food and Drug Administration, 1994. Federal Register, September 22, 1994, Vol. 59, No. 183).

GLP (Y/N): The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (MHW) and the European Economic Community (EEC). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study.

Year study performed: 1999

Species/Strain: New Zealand White rabbits

Number of animals per dose: 22 (additional total of 19 rabbits, 3-5 per dose, were included in a satellite study to determine the concentrations of PFOS in maternal liver and serum.)

Route of administration: Gavage

Dosing regimen (list all with units): Four groups of 22 pregnant New Zealand White rabbits were administered Potassium Perfluorooctylsulfonate (PFOS) in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 ml/kg was administered, adjusted daily on the basis of individual body weights.

Doses: 0, 0.1, 1.0, 2.5, and 3.75 mg/kg/day (In the satellite study the number of does was 3, 5, 3, 3 and 5 in the control, 0.1, 1.0, 2.5 and 3.75 mg/kg/day groups, respectively).

Statistical methods used:

The animals will be assigned to individual housing on the basis of computer-generated random units. The litter was the unit of measurement. Clinical observation and other proportion data were analysed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (e.g., maternal body weights, body weight changes, feed consumption values and litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data and fetal ossification site data) were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance. If the Analysis of Variance was significant, Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test was used. In cases, in which Kruskal-Wallis Test was statistically significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. Count data obtained

at Caesarean-sectioning were evaluated using the procedures described for the Kruskal-Wallis Test.

Remarks – Detail and discuss any significant protocol parameters and deviations: Timed-pregnant New Zealand White rabbits (obtained from Covance Research Products, Inc.), 22 per group, were given doses of 0, 0.1, 1.0, 2.5 or 3.75 mg/kg/day PFOS in 0.5% Tween-80 by gavage on gestation days 7-20. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weights. The does were observed twice daily for viability, and clinical observations were recorded 1 hour prior to and after dosing during the treatment period and once daily during the post-treatment period (i.e. gestation days 20-29). Maternal body weights were recorded on gestation days 0 and 6-29; food consumption was recorded daily throughout the study. On gestation day 29, the does were euthanized; a gross necropsy of the thoracic, abdominal and pelvic viscera was conducted and the number of corpora lutea in each ovary was recorded. The uteri were examined for number and distribution of implantations, live and dead fetuses, and early and late absorptions. The fetuses were weighed, sexed and examined for external abnormalities. All fetuses were examined for visceral and skeletal abnormalities and the brain of one-half of the fetuses were free-hand cross-sectioned and examined in situ.

In the satellite study, the does were euthanized on gestation day 21, blood samples were collected, and the liver was weighed and sectioned. The fetuses were removed and examined for external abnormalities. Fetuses and placentae were pooled per litter. All samples were sent to the Sponsor (3M) for analysis. At this time, only the liver and serum analyses have been Reported (3M Environmental Laboratory, 2001).

RESULTS

NOAEL (dose and effect) – maternal and developmental: The NOAEL for maternal toxicity is 0.1 mg/kg/day. The NOAEL for developmental toxicity is 1.0 mg/kg/day.

LOAEL (dose and effect) – maternal and developmental: The LOAEL for maternal toxicity is 1.0 mg/kg/day, based on abortions, incidences of scant feces, and decreases in body weight gains and food consumption. The LOAEL for developmental toxicity is 2.5 mg/kg/day, based on reductions in body weight and increased incidences in fetal alterations.

Toxic response/effects by dose level - maternal: Maternal toxicity was evident at dose levels of 1.0 mg/kg/day and above and consisted of the following: abortions at 2.5 mg/kg/day and above occurring on GD 22-28; increased incidence of scant feces at 1.0 mg/kg/day and above; reductions in mean body weight and body weight gain at doses of 1.0 mg/kg/day and above; reductions in absolute and relative food consumption at 2.5 mg/kg/day and above.

Toxic response/effects by dose level – developmental: Developmental toxicity was evident at doses of 2.5 mg/kg/day and above and consisted of the following: reductions in mean fetal body weight at 2.5 mg/kg/day and above; delayed ossification at 2.5 mg/kg/day and above.

Statistical results:

Maternal data: Statistically significant increases in abortions were observed at 3.75 mg/kg/day. Incidences of scant feces at 3.75 mg/kg/day reached statistical significance ($p \leq 0.01$). Maternal body weight gains were reduced or body weight losses occurred in the 1.0, 2.5, and 3.75 mg/kg/day dosage groups at most measured intervals during dosing; these reductions were significant ($p \leq 0.05$ or 0.01) in the 3.75 mg/kg/day dose group on GDs 10-13, 13-16, and 16-19. Dosage-dependent, significant body weight reductions or body weight losses ($p \leq 0.05$ or 0.01) occurred in the 1.0, 2.5, and 3.75 mg/kg/day dosage groups for the entire dosage period (calculated as GD 7-21). Dosage-dependent reductions in body weight gains occurred in the 2.5 and 3.75 mg/kg/day dosage groups for the entire period of gestation (GD 0-29).

and for the gestation period after the initiation of dosing (GD 7-29; significant at $p \leq 0.01$ in the 2.5 mg/kg/day dosage group). Average body weights were significantly reduced ($p \leq 0.05$ or 0.01) on GD 17-24 in the 3.75 mg/kg/day dosage group. Absolute and relative food consumption values were significantly reduced ($p \leq 0.05$ or 0.01) in the 2.5 and 3.75 mg/kg/day dosage groups for the entire dosage period (GD 7-21), and the entire period after the initiation of dosage (GD 7-29).

Fetal data: Fetal body weights (total, male and female) were significantly reduced ($p \leq 0.05$ and $p \leq 0.01$, respectively) in the 2.75 and 3.75 dosage groups. Significant delays ($p \leq 0.05$ and 0.01) in litter and fetal averages for ossification were seen at both 2.5 and 3.75 mg/kg/day dosage groups.

Remarks:

Maternal toxicity was evident at doses of 1.0 mg/kg/day and above. One doe in the 2.5 mg/kg/day group and nine does in the 3.75 mg/kg/day aborted. All abortions occurred on gestation days 22-28 and were considered treatment-related by the study authors. There was a significant increase in the incidence of scant feces in the 3.75 mg/kg/day group. Scant feces were also noted in one and three does in the 1.0 and 2.5 mg/kg/day groups, respectively. Mean maternal body weight gains were significantly reduced in the 3.75 mg/kg/day group on gestation days 10-13, 13-16, 16-19 and 21-24. Mean body weight gains were also calculated for the treatment period (days 7-21), post-treatment period (days 21-29) and duration of the study (days 7-29). There was a significant reduction in mean maternal body weight gain during the treatment period in the 1.0, 2.5 and 3.75 mg/kg/day groups. Mean body weight gain for the entire study period was also significantly reduced in the 2.5 mg/kg/day group. Mean food consumption (g/kg/day) was significantly reduced in the 2.5 mg/kg/day group on gestation days 16-19, 19-21 and 21-24, as well as for the entire study period (days 7-29). Mean food consumption was significantly reduced in the 3.75 mg/kg/day group on gestation days 13-16, 16-19, 19-21 and 21-24, as well as the entire treatment period (GD 7-21) and the entire period after the initiation of dosage (GD 7-29). Pregnancy occurred in 20 (90%), 19 (86.4%), 19 (86.4%), 17 (77.3%), and 21 (95.4%) rabbits in each dosage group. Cesarean-sectioning observations on GD 29 were based on 20, 18, 19, 16, and 12 pregnant rabbits in each of the five respective dosage groups.

In the satellite study of does euthanized on gestation day 21, the liver and serum analyses were reported by 3M Environmental Laboratory (2001). All serum and liver samples (including those from untreated controls) had detectable levels of PFOS; the values are presented below. Data from analysis of fetal and placenta tissues were not presented in the report.

Average Concentration of PFOS in Rabbit Liver and Serum by Dose Group:

Dose group (mg/kg/day)	PFOS conc. liver (ug/g)	PFOS conc. serum (ug/ml)
0.0	0.239	0.0690
0.1	13.1	2.73
1.0	133	23.8
2.5	317	45.8
3.75	416	88.9

Qualitatively, increasing concentrations of PFOS were found in samples of liver and serum as doses of PFOS increased. The levels of PFOS are much higher in the liver than in the serum.

These values should be viewed with caution. It was stated that because radio-labeled reference material was not available, "it is not possible to verify true recovery of endogenous analyte from tissues." Matrix spike recovery indicates the accuracy of quantitation to be $\pm 30\%$. It is also noted that liver concentrations may be biased high. The only conclusion presented in the laboratory report is that "PFOS

was observed in the liver and serum of all rabbits dosed with the test article.”

Developmental toxicity was evident at doses of 2.5 mg/kg/day and above. The number of corpora lutea, resorptions, live/dead fetuses, litter size and sex ratio were comparable among treated and control groups. Mean fetal body weight (male, female and sexes combined) was significantly reduced in the 2.5 and 3.75 mg/kg/day groups. There was also a significant reduction in the ossification of the sternum (litter averages) in the 2.5 and 3.75 mg/kg/day groups, and a significant reduction in the ossification of the hyoid (litter averages), metacarpals (litter averages) and pubis (litter and fetal averages) in the 3.75 mg/kg/day group. Other fetal gross external, soft tissue and skeletal alterations (malformations and variations) were considered unrelated to treatment because the incidences were not dosage-dependent and/or were within historical control range.

CONCLUSIONS

Conclusions are summarized above and this reviewer agrees.

REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G. 1999. Argus Research Laboratories, Inc. Protocol Number: 418-012, January 1999. “Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits”.

3M Environmental Laboratory. 2001. Analytical Laboratory Report, FACT TOX-099, February 9, 2001. “Determination of the Concentration of Potassium Perfluorooctanesulfonate (PFOS) in Rabbit Liver and Serum Samples.”

REPRODUCTIVE TOXICITY STUDIES

Title: COMBINED ORAL (GAVAGE) FERTILITY, DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFOS IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER: 6295.9, 1999.

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3.

Remarks: The test article, FC-95 (lot 217), was received on May 20, 1998, and stored at room temperature. Prepared suspensions were stored at room temperature overnight. Information regarding the purity, identity, strength and composition of the test article is on file with the Sponsor.

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): This study was designed to evaluate ICH Harmonized Tripartite Guideline stages A-F. A modification of the requirements of the U.S. Food and Drug Administration (FDA) were used as a basis for the study design.

Type of study (one-generation, two-generation, etc.): Two-generation reproductive toxicity

GLP (Y/N): The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. Food and Drug Administration (FDA), the Japanese Ministry of Health and Welfare (MHW) and the European Economic Community (EEC). There were no significant deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study have been documented.

Year study performed: 1999

Species/Strain: Sprague Dawley rats

Sex (males/females/both): Both

Number of animals per dose: 35

Route of administration: Gavage

Dosing regimen (list all with units): Five groups of 35 rats per sex per dose group were administered PFOS by gavage for six weeks prior to and during mating. Treatment in male rats continued until one day before sacrifice (approximately 22 days total); female rats were treated throughout gestation, parturition, and lactation.

Doses: 0, 0.1, 0.4, 1.6, and 3.2 mg/kg/day

Premating exposure period for males/females (P and F1, if appropriate): Six weeks for P; Nine and a half weeks for F1.

Statistical methods used: Proportion data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (body weights, body weight changes, and feed consumption) were analyzed using Bartlett's Test of Homogeneity of Variance and Analysis of Variance (ANOVA). If the

ANOVA was significant ($p \leq 0.05$), Dunnett's Test was used to identify the statistical significance of the individual groups. If the ANOVA was not appropriate, the Kruskal-Wallis Test was used. In cases where the Kruskal-Wallis Test was statistically significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups.

If there were greater than 75% ties, Fisher's Exact Test was used. Fisher's Exact Test was also used to evaluate necropsy data for the pups which were stillborn or found dead. Data obtained at Cesarean-sectioning, natural delivery, preweaning reflex/physical developmental data and postweaning behavioral data involving discrete data (number of corpora lutea, number of pups per litter, trials to a criterion) were evaluated by the Kruskal-Wallis Test.

Remarks – Detail and discuss any significant protocol parameters and deviations:

F0 Generation:

Parental animals (F0) were observed twice daily for clinical signs. Body weights and food consumption values were recorded weekly during the treatment period in male rats; and weekly during mating and then daily during gestation, and on lactation days 1, 4, 7, 10, 14, and at sacrifice in female rats. Each dosage group consisted of two sets of female rats. One set consisted of the first ten female rats with confirmation of mating; this group was dosed until gestation day (GD) 10 and delivered via Cesarean-sectioning. The remaining females comprised the second set which delivered naturally. During the 21-day lactation period, the dams were evaluated for clinical signs during parturition and length of gestation, and then each litter was evaluated at least twice daily for size and pup viability at birth. Pup observations during the 21-day lactation period included physical signs, body weights, nursing behavior, surface righting reflex, pinna unfolding, eye opening, acoustic startle response and air righting reflex. Pupil constriction was evaluated only on lactation day 21. On lactation day 4, litters were randomly culled to four male and four female pups. The remaining pups were sacrificed and necropsied. The F0 male rats were sacrificed and necropsied after the end of dosing at the time of parturition (lactation day 1). The testes, epididymides, prostate, and seminal vesicles were weighed. Evaluations of sperm number, motility, and morphology were not included in the protocol. The F0 generation females that delivered by Cesarean-sectioning were sacrificed on GD 10 and necropsied. Pregnancy status was confirmed, the ovaries were examined for the number and distribution of corpora lutea, implantation sites were determined, and embryos were examined for viability. The F0 generation females that delivered naturally were sacrificed on lactation day (LD) 21 and necropsied. Ovaries were examined as above and the number and distribution of implantation sites was recorded. The liver from each parental rat was removed, weighed and analyzed. Blood samples were collected from 5 male rats that had mated and from 5 female rats on LD 21 for pharmacokinetic analysis; livers from the pups from the litters of these five dams were also collected for analysis.

F1 Generation:

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosages groups, only the 0.1 and 0.4 mg/kg/day dosage groups were carried into the second generation. Twenty-five F1 generation rats per sex per dose group were administered PFOS by gavage at doses of 0, 0.1, and 0.4 mg/kg/day beginning on LD 22 and continuing through the day before sacrifice. At 24 days of age, one rat per sex per litter in each dosage group was tested in a passive avoidance paradigm. On LD 28, females were evaluated for the age of vaginal patency and on LD 34, male rats were evaluated for the age of preputial separation. One rat per sex per litter were evaluated in a water-filled M-maze on LD 70. Assignment to cohabitation within each dosage group began on LD 90. Females with evidence of mating were considered to be at GD 0 and assigned to individual housing for the remainder of the dosing period. The F1 generation male rats were sacrificed after mating, necropsied and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver naturally. Dams that delivered litters were

sacrificed and necropsied on LD 21. All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups.

RESULTS

NOAEL (dose and effect) – for F0, F1, and F2 (as appropriate): The NOAEL for the F0 generation male and female parental animals = 0.1 mg/kg/day, the lowest dose tested. The NOAEL for the F1 generation male parental animals could not be determined since treatment related signs of toxicity were observed at 0.1 and 0.4 mg/kg/day; the NOAEL for the F1 generation female parental animals = 0.1 mg/kg/day. The NOAEL for the F1 generation offspring = 0.4 mg/kg/day. The NOAEL for the F2 generation offspring = 0.1 mg/kg/day.

LOAEL (dose and effect) – for F0, F1, and F2 (as appropriate): The LOAEL for the F0 generation male and female parental animals = 0.4 mg/kg/day, based on reductions in body weight gain and food consumption. The LOAEL for the F1 generation male animals = 0.1 mg/kg/day based on significant reductions in absolute food consumption; the NOAEL for the F1 generation female animals = 0.4 mg/kg/day based on reductions in body weight and food consumption. The LOAEL for the F1 generation offspring = 1.6 mg/kg/day, based on significant reductions in the number of implantation sites, litter size, pup viability, growth and survival. The LOAEL for the F2 generation offspring = 0.4 mg/kg/day, based on significant reductions in pup growth.

Toxic response/effects by dose level – parental/F1:

Toxic effects in F0 generation animals: reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dosage groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4. and 1.6 mg/kg/day dosage groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Signs of reproductive toxicity in the F0 generation males were seen at the highest dose group of 3.2 mg/kg/day and included significant reductions in the absolute weights of the seminal vesicles (with fluid) and the prostate. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dosage groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Toxic effects in F1 generation animals: F1 males; the only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels. F1 females; observations at the 0.4 mg/kg/day dosage group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation.

Toxic response/effects by dose level – offspring (F1/F2):

Toxic effects in the F1 generation pups consisted of reduced pup viability at the two highest dose groups (1.6 and 3.2 mg/kg/day). The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. Statistically significant increases were observed in the number of dams with stillborn pups at the 3.2 mg/kg/day dose group. Viability and lactation indices were significantly reduced in these same dosage groups (viability index = 0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day; lactation index = 94.6% at 1.6 mg/kg/day). Statistically significant reductions in pup body weights were also observed at the two highest dosage groups. Gestation

length was significantly reduced at 3.2 mg/kg/day. Significant reduction in the number of implantation sites followed by a concomitant reduction in litter size was observed at 3.2 mg/kg/day. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead.

Toxic effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7-14.

Statistical results:

F0 generation male animals: Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in body weight gains at 0.4 mg/kg/day and higher. Absolute and relative food consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups. A significant increase ($p \leq 0.01$) in the number of male rats in the 3.2 mg/kg/day dosage group with brown liver. The gross lesions of the liver were considered to be treatment related because the incidences were dosage-dependent. Significant reductions ($p \leq 0.05$ and $p \leq 0.01$) in terminal body weights were observed in the 1.6 and 3.2 mg/kg/day dosage groups. Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in the absolute weights of the seminal vesicles with fluid and the prostate were observed in the 3.2 mg/kg/day dosage group.

F0 generation female animals: Significant increases ($p \leq 0.05$ or $p \leq 0.01$) in localized alopecia were observed in the 0.4, 1.6, and 3.2 mg/kg/day dosages groups. Significant reductions in body weight and body weight gains and food consumption ($p \leq 0.05$ or $p \leq 0.01$) were observed in the 1.6 and 3.2 mg/kg/day dosage during pre-mating and gestation and then in 0.4 mg/kg/day dosage group and above during lactation. Significant reductions ($p \leq 0.01$) in gestation length, implantation sites, and litter size were observed at 3.2 mg/kg/day.

F1 generation offspring: Pup viability was significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups. Significant increases ($p \leq 0.05$ or $p \leq 0.01$) were observed in the number of dams with stillborn pups, while significant reductions ($p \leq 0.05$ or $p \leq 0.01$) were observed in the viability index, lactation index, and averages for surviving pups at 3.2 mg/kg/day. A dosage-dependent pattern of reduced pup body weight was evident in each dosage group, with statistical significance ($p \leq 0.01$) in the 1.6 and 3.2 mg/kg/day dosage groups.

F1 generation adult animals: Males - Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in absolute food consumption at 0.1 and 0.4 mg/kg/day; females - significant ($p \leq 0.05$) body weight loss on lactation days 1-4 at 0.4 mg/kg/day; and significant reductions ($p \leq 0.05$) in food consumption at 0.4 mg/kg/day on days 1-8 postweaning.

F2 generation offspring: Pup body weights tended to be reduced in the 0.1 mg/kg/day dosage group on lactation day 4 and 7, but were comparable to controls by lactation day 14. Pup body weights in the 0.4 mg/kg/day dosage group tended to be reduced, though not significantly, on lactation days 4-21, with significant reductions ($p \leq 0.05$ and $p \leq 0.01$, respectively) on lactation days 7 and 14, as compared to controls.

Remarks: In the F0 generation male rats, there were no treatment-related clinical signs of toxicity, no mortality, and no effects on mating or on any of the fertility parameters evaluated in any dose group tested. Reported effects included reductions in both body weight gains and in absolute and relative food consumption at the 1.6 and 3.2 mg/kg/day dosage groups during the pre-mating period. Following mating, food consumption was significantly reduced in the 0.4 and 1.6 mg/kg/day dosage groups. Terminal body weights were also significantly reduced in the 1.6 and 3.2 mg/kg/day dose groups. Body weights, body weight gains, absolute and relative food consumption were unaffected by treatment at the 0.1 mg/kg/day dosage group. Signs of reproductive toxicity in the F0 generation males were seen at the highest dose group of 3.2 mg/kg/day and included significant reductions in the absolute weights of the seminal vesicles

(with fluid) and the prostate. A significant increase in the number of males with brown liver at 3.2 mg/kg/day dose group was also reported.

In the F0 generation female rats, no deaths were reported at any dose level. In dams sacrificed on GD 10 for Caesarean-sectioning, there did not appear to be any effects on estrous cycling, mating and fertility parameters, the numbers of corpora lutea and implantations, or in the number of viable or non-viable embryos. The only findings reported in the F0 dams occurred in the 0.4, 1.6, and 3.2 mg/kg/day dosage groups and included localized alopecia during pre-mating, gestation, and lactation; and reductions in body weight and body weight gain and food consumption values observed during the pre-mating period and continuing throughout gestation and lactation.

Reversible delays in reflex and physical development were observed in the F1 generation offspring. The ability to surface right was significantly delayed in the 1.6 and 3.2 mg/kg/day dosage groups on LDs 3-10 (delays in the 3.2 mg/kg/day dose group were observed on LD 1, after which there were no surviving pups remaining for further observation). By the end of the observation period, however, all surviving pups in the 1.6 mg/kg/day dosage group had the ability to surface right. There were no delays observed in the ability to surface right in dose groups ≤ 0.4 mg/kg/day. Similar responses were seen for pinna unfolding and eye opening. Although there were transient delays seen with these signs of physical development across all dose groups, by the end of the observation period responses in pups were similar to controls. The time of development of the acoustic startle reflex and the ability to air right were both significantly reduced in the 1.6 mg/kg/day dosage group. No effects on these reflexes were observed in the low dose group of 0.1 mg/kg/day and only a transient delay (on LD 16 only) in the ability to air right was seen in the 0.4 mg/kg/day group. At the end of lactation (LD 21), all live pups in all dose groups (0, 0.1, 0.4, and 1.6 mg/kg/day) had pupil constriction response.

The most significant finding reported in the offspring was that of reduced pup viability at the two highest dose groups. The reductions in pup viability began to appear on LD 4 postculling in the 1.6 mg/kg/day dose group, with over 26% of the pups found dead between LD 2-4. In the 3.2 mg/kg/day dose group 45% of the pups were found dead on LD1; no pups survived beyond LD 1. As a result, the viability index was greatly reduced in these dosage groups (0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day). The lactation index was also significantly reduced (94.6%) in the 1.6 dosage group. In addition, gestation length was significantly reduced in the high-dose group and there also was a significant reduction in the number of implantation sites followed by a concomitant reduction in litter size. Statistically significant reductions in pup body weights were also observed at the two highest dosage groups. Other adverse signs in the 3.2 mg/kg/day dose level associated with reductions in pup viability and maternal care included litters with pups that were not nursing or who had no evidence of milk in the stomach, as well as maternal cannibalization of pups that were stillborn or found dead. The percentage of male pups was comparable across all dosage groups.

Since F1 generation pup viability was significantly reduced in the 1.6 and 3.2 mg/kg/day dosages groups, only the 0.1 and 0.4 mg/kg/day dosage groups were carried into the second generation.

Clinical observations in the F1 generation male rats appeared unremarkable. No treatment-related deaths were reported and no statistically significant differences were reported for any of the following parameters: body weights/body weight gains, average day of preputial separation; values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating or fertility parameters; necroscopic examinations; absolute or relative weights for the right or left testis, seminal vesicles, right epididymis, or prostate; and terminal body weights. The only reported effects were significant reductions in absolute food consumption on postweaning days 1-8 occurring at the 0.1 and 0.4 mg/kg/day dose levels.

Clinical observations for the F1 generation females were likewise unremarkable. Observations at the 0.4 mg/kg/day dosage group included, reductions in body weights on day 1 postweaning, significant losses in body weight on LDs 1-4, and significant reductions in food consumption on days 1-8 postweaning and during lactation. There were no statistically significant differences reported for any of the following parameters: values for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance or watermaze performance paradigm; mating and fertility parameters; gestation index; pregnancy rates; and necroscopic examinations.

Evidence of treatment-related effects in the F2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 mg/kg/day on LD 4 and 7. Body weights were comparable to control levels by LD 14. At 0.4 mg/kg/day, statistically significant reductions in mean pup body weights were observed on LDs 7-14. Mean body weights on LD21 continued to remain lower than controls, although the difference was not statistically significant (46.5 g in 0.4 mg/kg/day dose group vs. 50 g in controls). Clinical and necroscopic observations of the F2 generation pups were unremarkable. No other toxicologically significant effects were reported.

Liver and sera samples collected from the initial population of dosed animals (F0) and their offspring (F1) were analyzed for the presence of PFOS. The F0 results for the F0 animals were:

Dose group (mg/kg/day)	Average PFOS conc. in serum (ug/ml)	Average PFOS conc. in liver (ug/g)
0.0:	female 0.0307 male 0.0244	female 0.171 male 0.665
0.1:	female 5.28 male 10.5	female 14.8 male 84.9
0.4:	female 18.9 male 45.4	female 58.0 male 176
1.6:	female 82 male 152	female 184 male 323
3.2:	female NR* male 273	female NR* male 1360

*samples not received

Qualitatively, the F0 results indicate all rats (including controls) had detectable levels of PFOS in serum and livers. PFOS concentration increased with dose. PFOS concentrations were higher in the liver than in the serum, and males had greatly increased PFOS concentrations in serum and liver when compared with females of the same dose group. Pooled liver samples from the F1 animals sacrificed shortly after birth had lower PFOS concentrations than adults of the F0 generation of the same dose group. The average PFOS concentrations in pooled liver samples from F1 animals shortly after birth were 0.0511, 6.19, 57.6, and 70.4 ug/g in the 0.0, 0.1, 0.4, and 1.6 mg/kg/day dose groups, respectively. These quantitative values for the PFOS concentration in the liver and serum should be viewed with caution. The accuracy of quantitation is $\pm 30\%$, the purity of the analytical reference substance is unknown, and there were several uncorrected dilution errors.

CONCLUSIONS

Conclusions stated above and this reviewer agrees.

REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G. 1999b. Argus Research Laboratories, Inc. Protocol Number: 418-008, Sponsor Study Number: 6295.9, June 10, 1999. Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats.

3M Environmental Laboratory. 1999b. Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2795-39-3) in the Serum and Liver of Sprague-Dawley Rats Exposed to PFOS via Gavage. As amended April 19, 2000.

CROSS-FOSTERING/DEVELOPMENTAL TOXICITY STUDY

Title: ORAL (GAVAGE) CROSS-FOSTERING STUDY OF PFOS IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER 418-014, 3M T-6295.13, JULY 1999.

TEST SUBSTANCE

Identity: Potassium Perfluorooctylsulfonate, CAS No. 2795-39-3.

Remarks: The test article, FC-95 (Lot 217) was received on October 21, 1998, and stored at room temperature. Prepared suspensions were stored at room temperature. Information regarding the identity, strength, composition, and purity of the test article is on file with the sponsor.

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): For the cross-fostering study: The requirements of the U.S. Food and Drug Administration (FDA) were used as the basis of the study design, i.e., the International Conference on Harmonization: Guideline on detection of toxicity to reproduction for medicinal products. Federal Register, September, 22, 1994, Vol. 59. No. 183. For the PFOS bioanalysis: US FDA GLP Final Rule 21 CFR 58, with exceptions: Two separate study directors were assigned to the in-life phase and the analytical phase of this study. The ABS final report does not have a Statement of Compliance. The QAU statement in the ABS final report indicates compliance with EPA 40 CFR Part 792, rather than FDA 21 CFR Part 58. Dose confirmation analyses were not conducted according to the GLP regulations; analytical method was not fully validated. Not all raw data were verified by the group leader or designee.

GLP (Y/N): The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. FDA, the Japanese Ministry of Health and Welfare, and the European Economic Community. There were no deviations from the GLP regulations that affected the quality or integrity of the study. Quality assurance Unit findings derived from the inspections during the conduct of this study are documented.

Year study performed: 1999

Species/Strain: Sprague-Dawley rats

Number of animals per dose: 25

Route of administration: Gavage

Dosing regimen (list all with units): Two groups of 25 female rats were administered PFOS by gavage beginning 42 days prior to mating to untreated (breeder) males, and continuing throughout gestation and into day 21 of lactation.

Doses: 0, 1.6 mg/kg/day

Statistical methods used: Averages and percentages were calculated. Litter values were used where appropriate.

Remarks – Detail and discuss any significant protocol parameters and deviations: Two groups of 25

female Sprague-Dawley rats were administered 0 and 1.6 mg/kg/day PFOS in 0.5% Tween-80 by gavage, beginning 42 days prior to mating to untreated (breeder) males, and continuing throughout gestation and into day 21 of lactation. A dose volume of 5 mL/kg was administered, adjusted daily on the basis of individual body weight. Parental females were observed twice daily for viability and clinical observations were recorded 1 hour prior to and after dosing during the treatment period. Maternal body weights were recorded once during the acclimation period and then daily during the treatment period and at sacrifice; food consumption was also recorded once during the acclimation period and then daily during gestation and on days 1, 4, 7, 10, and 14 of lactation. During parturition, females were continually evaluated for clinical signs and also for duration of gestation, length of parturition, litter sizes, and pup viability at birth. Maternal behavior was recorded daily throughout lactation. All maternal rats were sacrificed by carbon dioxide asphyxiation on day 22 of lactation and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed; any gross lesions were preserved for future analysis. In addition, the number and distribution of implantation sites were recorded. Rats that did not deliver a litter were not included in the cross-fostering procedure and were sacrificed on lactation day 25, examined for gross lesions, and the uteri examined to confirm the presence/absence of implantation sites. Dams with no surviving pups were sacrificed after the last pup was found dead, missing, or presumed cannibalized.

Following completion of parturition, litters were immediately removed from their respective dams and placed with either a control- or PFOS-treated dam for rearing. This cross-fostering procedure resulted in four groups of 12-13 dams or pups as follows: A) control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; B) control dams with litters from control dams, i.e., negative control; C) PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure; and D) PFOS-treated dams with litters from control dams, i.e., post-natal exposure only.

On day 1 of lactation (birth), each pup was individually weighed and each litter was evaluated twice daily during lactation for viability. Pups were observed once daily for clinical signs and gross external physical anomalies. Pup body weights were recorded on days 1, 4, 7, 14, and 21 of lactation, and then at sacrifice. On day 4 of lactation, each cross-fostered litter was culled to 5 males and 5 females. On day 21 of lactation, all pups were sacrificed via decapitation and examined for gross lesions. Pups found dead or sacrificed because of moribundity were examined for gross lesions and for the cause of death or the moribund condition. The lungs, liver, and any gross lesions were collected from selected pups at various timepoints (for the F0 generation, blood, milk, and liver samples were collected on LD 14; milk was collected 2-6 hours post-dosing; for the F1 generation offspring, blood was collected on LD 14) and preserved for possible future analysis. Liver samples were evaluated via electron microscopy. In addition, samples of blood, milk (including the milk-secreting glands), and liver were collected from selected maternal rats and pups (blood and liver samples were pooled per litter) at various timepoints for analysis of PFOS concentration. Only the analysis of the sera samples are available at this time.

RESULTS

Toxic response/effects by dose level - maternal: Mean maternal body weight and body weight gains at 1.6 mg/kg/day were reduced compared to controls during premating and continuing throughout gestation. Mean maternal body weight changes in the treated group were comparable to those seen in control animals during lactation. During the premating period, and on into gestation and lactation, food consumption was reduced in treated animals as compared to controls. Reductions in gestation length, the average number of implantation sites, delivered sizes, and live litter size were observed in treated animals.

Toxic response/effects by dose level – developmental: Pup mortality was observed in two of the cross-fostered groups. On lactation days 2-4, approximately 19% of the pups in group C were either found dead or presumed cannibalized. Pup mortality was also observed in group A at a rate of 9%. In addition, the

number of live pups on day 4 of lactation, numbers of surviving pups per litter, and live litter sizes were also reduced in these two groups. Pup mortality in groups B and D during lactation days 2-4 were at 1.6% and 1.1%, respectively. Reductions in pup body weights were observed in groups A and C on day 1 of lactation. Pup body weights in group D were comparable to controls during that same period. From lactation day 4-21, pup body weights in groups A, C, and D were reduced when compared to group B (negative control), with the reductions greatest in group C. Two litters in group A and one litter on group C did not nurse. Milk analysis of the stomachs of pups found no milk in the stomachs of 57%, 100%, and 87% of the pups found dead and necropsied in groups A, C, and D, respectively.

Electron microscopic examination of the liver revealed an increase in the number of peroxisomes in pups from dams treated with 1.6 mg/kg/day PFOS.

Statistical results: The method of statistical analysis consisted of calculation of averages (mean \pm S.D.) and percentages. Statistical significance was not assessed. The data were presented according to day of gestation/lactation and compared by dose and by cross-fostered group. Litter values were used where appropriate. The data are too cumbersome to cite here.

Remarks – Additional information to adequately assess the data: All maternal rats survived to schedule sacrifice. Signs of clinical toxicity observed in the dams during the study period (e.g., chromorhinorrhea, scaly tail, abrasion on the head, neck, tail and/or forelimb, missing, broken and/or misaligned incisors, and localized alopecia, among others) were not considered to be treatment-related since they also occurred in the control animals. All pregnant animals delivered live offspring.

Following cross-fostering on LD 1, live litter sizes were comparable between treated and control. Sex ratios and the lactation index were comparable among all groups. Signs of clinical toxicity were observed in pups, but were not considered to be treatment-related since they also occurred in group B (negative control) at the same rate. No significant differences were observed between group B and the other groups following examination of pup lungs.

PFOS concentrations in the serum of untreated dams ranged from below the limit of detection (0.05 ug/ml) to 5.34 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with untreated dams, were below the limit of detection. Serum PFOS concentrations in the pups from treated dams, fostered with untreated dams, ranged from 47.6 ug/ml to 59.2 ug/ml. PFOS concentrations in the serum of treated dams ranged from 59.2 ug/ml to 157 ug/ml. Serum PFOS concentrations in the pups from untreated dams, fostered with treated dams, ranged from below the limit of detection to 35.7 ug/ml. Serum PFOS concentrations in the pups from treated dams, fostered with treated dams, ranged from 79.5 ug/ml to 96.9 ug/ml. These data indicate that exposure to PFOS can occur both *in utero* and via milk from treated dams.

CONCLUSIONS

Pups from control dams that were cross-fostered with PFOS-treated dams (post-natal exposure only) had the same low mortality rate (1.1%) as pups from control dams cross-fostered with control dams (1.6%; negative control). Mortality rates in the remaining two groups, however (i.e. control dams with litters from PFOS-treated dams, i.e., *in utero* exposure only; and PFOS-treated dams with litters from PFOS-treated dams, i.e., both *in utero* and post-natal exposure), had higher mortality rates at 9.6 % and 19.2%, respectively. Under the conditions of the study, this limited data appear to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure may also contribute to reduced pup survival. In contrast, exposure during lactation alone, through milk from exposed dams, does not appear to have any adverse affect on pup viability. Additionally, analysis of PFOS concentration showed that PFOS was observed in the sera of F0 female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera

samples taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed via lactation, but not exposed *in utero*.

These are the conclusions of the study authors and this reviewer agrees.

REFERENCE

Christian, M.S., Hoberman, A.M., and York, R.G.1999c. Testing Facility: Argus Research Laboratories, Inc., Protocol Number 418-014, Sponsor: 3M Corporate Toxicology, Study Number T-6295.13, July 23, 1999. Oral (Gavage) Cross-Fostering Study of PFOS in Rats.

3M Environmental Laboratory. 1999. Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) (CAS Number: 2759-39-3) in the Serum of Sprague-Dawley Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage, Laboratory Report No. U2779, Requestor Project No. 3M Tox 6295.13. Study initiation date: June 10, 1999. Completion at signing (2/11/00). Sample analysis completion June 28, 1999. Study Identification Number: FACT Tox-108

REPEAT DOSE DATA

Title: 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats

TEST SUBSTANCE

Identity: Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295).

Remarks: The test substance, Lot No. 217, is a light colored, free flowing powder, and is 86.9% pure. The impurities of the test substance were not indicated in the main body of the study report.

METHOD

Method/guideline followed: Guideline number not stated

Study duration: Two years

GLP (Y/N): Yes

Year study performed: 1998-2000

Species/strain: Sprague-Dawley rat [CrI:COBS^R CD(SD)BR]

Sex: Male and female

Number of animals per dose group: The control and high-dose groups contained 70 rats/sex and the low-, mid-, and mid-high dose groups contained 60 rats/sex. A high-dose recovery group contained 40 rats/sex.

Route of administration: Diet

Doses tested and frequency: Control (Group 1): 0 ppm ; low-dose (Group 2): 0.5 ppm ; mid dose (Group 3): 2 ppm; mid-high dose (Group 4): 5 ppm; high-dose (Group 5) : 20 ppm.

Post-treatment observation period: A high-dose recovery group (Group 6) was observed for 52 weeks after 52 weeks of treatment.

Statistical methods used: Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $P < 0.05$, transformations were used to stabilize the variance. One-way analysis of variance [ANOVA] was used to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, palmitoyl CoA oxidase activities, and organ weight data. ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparisons between treated and control groups. Group comparisons were evaluated at the 5.0%, two-tailed probability level.

Remarks: The animals were observed twice daily (a. m. and p.m.) for mortality and moribundity; findings were recorded as they were observed. At least once prior to treatment and weekly thereafter, each animal was removed from its cage and examined; abnormal findings or an indication of normal was recorded. Body weight data were collected weekly through Week 17, once every 4 weeks thereafter, and at Week

105. Food consumption data were collected weekly for the first 16 weeks and once every 4 weeks thereafter. During Weeks 4, 14, 27, and 53, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry tests from 10 animals/sex in Groups 1 through 5. Blood was collected for cholesterol and triglyceride determinations from all animals prior to the terminal sacrifice during Week 105 (Week 103 for females in Group 3) and the recovery sacrifice (Group 6) during Week 106. Blood films were also prepared for animals at the terminal and recovery sacrifices. Five animals/sex in Groups 1 through 5 were sacrificed during Week 4; livers were collected and weighed. [The liver samples were collected for PFOS analysis, mitochondrial activity, hepatocellular proliferation rate measurements by proliferation cell nuclear antigen (PCNA), and determination of palmitoyl-CoA oxidase activity. In addition, liver samples were collected for PFOS analysis (Weeks 14, 53, 103, 105, and 106), hepatocellular proliferation rate measurement [by PCNA at Week 14 and by bromodeoxyuridine (BrdU) immunohistochemistry at Week 53] and palmitoyl-CoA oxidase determination (Week 4 and 14).]

RESULTS

Survival rates:

There was a significant increased trend in survival that occurred in the males that was due to significant increases in survival in mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control group. None of the other treated groups in the males revealed any significant differences in survival. No significant trend was noted in survival in females. There was a significant decrease in survival in the mid-dose (2.0 ppm) group and not in the mid-high (5.0 ppm) and high-dose (20.0 ppm) groups as compared to that of the control.

Neoplastic effects:

The results of the study show that PFOS is carcinogenic, inducing tumors of the liver, and of the thyroid and mammary glands.

Tumors	Tumor incidence (%)					
	Male					
Liver Hepatocellular adenoma*	0	0.5 ppm	2 ppm	5 ppm	20 ppm	20 ppm recovery#
	0 (0/60)	6.0 (3/50)	6.0 (3/50)	2.0 (1/50)	11.7** (7/60)	
Thyroid						
Follicular cell adenoma	5.0 (3/60)	10.2 (5/49)	8.0 (4/50)	8.2 (4/49)	6.8 (4/59)	23.1** (9/39)
Follicular cell carcinoma	5.0 (3/60)	2.0 (1/49)	2.0 (1/50)	4.1 (2/49)	1.7 (1/59)	2.6 (1/39)
Combined	10.0 (6/60)	12.2 (26/49)	10.0 (5/50)	10.2 (5/49)	8.5 (5/59)	25.6 (10/39)
	Female					
Liver						
Hepatocellular adenoma*	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	8.3** (5/60)	
Hepatocellular carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	0 (1/50)	1.7 (1/60)	
Combined*	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	10.0** (6/60)	
Thyroid						
Follicular cell adenoma	0 (0/60)	0 (0/50)	0 (0/49)	4.0 (2/50)	1.7 (1/60)	
Follicular cell carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	2.0 (1/50)	0 (0/60)	
Combined	0 (0/60)	0 (0/50)	0 (0/49)	6.0** (3/50)	1.7 (1/60)	
Mammary						
Fibroadenoma/ adenoma	38.3 (23/60)	60.0** (30/50)	45.8 (22/48)	52.04 (26/50)	25 (15/60)	
Carcinoma	18.3 (11/60)	24.0 (12/50)	31.2 (15/48)	22.0 (11/50)	23.3 (14/60)	
Combined	48.3 (29/60)	72.0** (36/50)	64.6** (31/48)	58.0 (29/50)	40.0 (24/60)	

*Significant positive trend ($P < 0.03$).

** Significantly increased over the control ($P < 0.05$).

Recovery group; after 52 weeks of treatment.

Remarks:

At terminal of the studies, mean body weights of the surviving males and females were not significantly different from the controls. Food consumption for males and females was similar in all treated groups compared to animals given the control material except for the high-dose females which had statistically significantly lower food consumption during weeks 2 through 44. Except for the mid-dose (2.0 ppm) female group, there were no significant decreases in survival in any other male and female treatment groups as compared to that of the control. Therefore, it appears that higher dose levels could have been tolerated by the high-dose groups.

Nonneoplastic effects:	NOEL (dose and effect):	0.5 ppm (Male)
		2 ppm (Female)
	LOEL (dose and effect):	2 ppm (Male)
		5 ppm (Female)

Hepatotoxicity, characterized by significant increases in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, centrilobular hepatocytic pigment, or centrilobular hepatocytic

vacuolation was noted in male and/or female rats given 5 or 20 ppm. A significant increase in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Significant increases in the incidence of cystic hepatocellular degeneration was found in all the male treated groups (0.5, 2, 5, or 20 ppm); however, this liver lesion is believed to be due to old age of the animals and is not considered to be treatment-related. Therefore, based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is considered to be 2 ppm in male rats and 5 ppm in female rats.

Results of Statistical Analyses of Nonneoplastic Lesions in Male Rats.

Group	1 Control	2 Low	3 Mid	4 Mid- High	5 High	6 High Recovery	Groups 5 vs. 6
Liver – Vacuolation, Hepatocellular Midzonal/Centrilobular							
	0/65	0/55	6/55	10/55	19/55	9/40	0.0060+**
p-values	.0000+**	NA	.1690	.0024+**	.0001+**	.4152+	
Liver – Hypertrophy, Hepatocellular Centrilobular							
	0/65	2/55	4/55	22/55	42/565	3/40	.0000-**
p-values	.0000+**	.2080+	.0415+*	.0000+**	.0000+**	.0527+	
Liver – Granular Cytoplasm, Eosinophilic, Centrilobular							
	0/65	0/55	0/55	0/55	14/65	0/40	.0007-**
p-values	.0000+**	NA	NA	BA	.0139+*	NA	
Liver – Pigment Hepatocellular, Centrilobular							
	0/65	0/55	0/55	0/55	6/65	0/40	.0513
-values	.006+**	NA	NA	NA	.0139+*	NA	
Liver – Necrosis, Individual Hepatocyte							
	5/65	4/55	6/55	5/55	14/65	4/40	.1024
p-values	.0106+*	NA	NA	NA	.0224+*	.0699+	
Liver – Degeneration, Cystic							
	5/65	15/55	19/55	17/55	22/65	15/40	NA
p-values	.0007+**	.0041+**	.0003+**	.0011+**	.002+**	.0002+**	
							.4306+

Results of Statistical Analyses of Nonneoplastic Lesions in the Female Rats.

Group	1 Control	2 Low	3 Mid	4 Mid-High	5 High	6 High Recovery	Groups 5 vs. 6
Liver - Infiltrate, Lymphohistiocytic	42/65	42/55	38/55	41/55	56/65	32/40	.2852
p-values	.0080 +**	NA	.3738+	NA	.0038 +**	.0709+	
Liver – Hypertrophy, Hepatocellular, Centrilobular	2/65	1/55	4/55	16/55	52/65	2/40	.0000 -**
p-values	.0000 +**	NA	.2641+	.0001 +**	.0000 +**	NA	
Liver - Granular Cytoplasm, Eosinophilic, Centrilobular	0/65	0/55	0/55	7/55	36/65	1/40	.0000 -**
p-values	.0000 +**	NA	NA	.0034 +**	.0000 +**	.3810+	
Liver - Pigment, Hepatocellular, Centrilobular	0/65	0/55	0/55	1/55	36/65	0/40	.0000 -**
p-values	.0000 +**	NA	NA	NA	.0000 +**	NA	
Liver - Necrosis, Individual Hepatocyte	7/65	6/55	6/55	6/55	15/65	3/40	.0329 -*
p-values	.0359 +*	NA	NA	NA	.0500 +*	.4254-	
Liver – Degeneration, Cystic	0/65	1/55	1/55	2/55	4/65	1/40	.3660
p-values	.0187 +*	NA	NA	.2080+	.0596+	.3810+	
Liver - Hypertrophy, Hepatocellular, Periportal	12/65	10/55	9/55	4/55	3/65	7/40	.0344 +*
p-values	.0026 -**	.5796-	.4778-	.0614-	.0127 -*	NA	
Liver - Infiltrate, Macrophage, Pigmented	2/65	3/55	5/55	6/55	23/65	7/40	.0383 -*
p-values	.0000 +**	NA	.1567+	.0889	.0000 +**	.0147 +*	

List of statistically different non-neoplastic effects (increased compared with controls, $p < 0.05$):

Males (0.5ppm):

Liver - Degeneration, Cystic

Males (2 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Males (5 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Vacuolation, Hepatocellular Midzonal / Centrilobular

Males (20 ppm):

Liver - Degeneration, Cystic

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Vacuolation, Hepatocellular Midzonal / Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Pigment Hepatocellular, Centrilobular

Liver - Necrosis, Individual Hepatocyte

Females (0.5 ppm):

None

Females (2 ppm):

None

Females (5 ppm):

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Infiltrate, Macrophage, Pigmented

Females (20 ppm):

Liver - Hypertrophy, Hepatocellular Centrilobular

Liver - Granular Cytoplasm, Eosinophilic, Centrilobular

Liver - Infiltrate, Macrophage, Pigmented

Liver - Pigment Hepatocellular, Centrilobular

Liver - Necrosis, Individual Hepatocyte

Liver - Infiltrate, Lymphohistiocytic

Liver - Hypertrophy, Hepatocellular, Periportal

Remarks:

The cystic degeneration of the liver is believed to be due to old age of the animals and is not considered to be treatment-related. However, A significant increase in hepatocellular centrilobular hypertrophy was also observed in mid-dose (2 ppm) male rats. Therefore, the NOAEL for the male rat is considered to be 0.5 ppm. The author concluded that the NOAEL for both male and female rats are 2 ppm (which we do not agree).

There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) at weeks 4 and 14, or by bromodeoxyuridine (BrdU) at week 53.

Serum and Liver level of PFOS

Under the conditions of the studies, PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals.

Summary of PFOS Concentration-Serum (ug/mL)

Timepoint	Sex	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Group 6
							High Recovery
		Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD
Week 0	Male	<LOQ ^b (n=5)	0.907 \pm 0.0619 (n=5)	4.33 \pm 1.16 (n=5)	7.57 \pm 2.17 (n=5)	41.8 \pm 7.92 (n=5)	
	Female	0.0259 \pm 0.00663 (n = 5)	1.61 \pm 0.207 (n = 5)	6.62 \pm 0.499 (n = 5)	12.6 \pm 1.73 (n = 5)	54.0 \pm 7.34 (n = 5)	
Week 14 ^a	Male	<LOQ ^c (n = 5)	4.04 \pm 0.801 (n = 5)	17.1 \pm 1.22 (n = 5)	43.9 \pm 4.90 (n = 5)	148 \pm 13.8 (n = 5)	
	Female	2.67 \pm 4.58 (n = 5)	6.96 \pm 0.993 (n = 4 ^d)	27.3 \pm 2.34 (n = 5)	64.4 \pm 5.48 (n = 5)	223 \pm 22.4 (n = 5)	
Week 53	Male	0.0249 \pm 0.0182 (n = 5)				146 \pm 33.5 (n = 4)	
	Female	0.395 \pm 0.777 (n = 5)				220 \pm 44.0 (n = 5)	
Day 719	Male						
	Female			20.2 \pm 13.3 (n = 9)			
Week 105	Male	0.0118 \pm 0.0104 (n = 11)	1.31 \pm 1.30 (n = 10)	7.60 \pm 8.60 (n=17)	22.5 \pm 23.5 (n = 25)	69.3 \pm 57.9 (n = 22)	
	Female	0.0836 \pm 0.134 (n = 24)	4.35 \pm 2.78 (n = 15)		75.0 \pm 45.7 (n = 15)	233 \pm 124 (n = 25)	
Week 106	Male						2.42 \pm 5.09 (n = 10)
	Female						9.51 \pm 8.70 (n = 17)

a Not corrected for purity of the standard material.

b LOQ-Limit of Quantitation = 0.00910 pg/mL

c LOQ-Limit of Quantitation = 0.0457 pg/mL

d C92987F sample spilled during extraction, no sample remaining for analysis.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated (that the sera data are accurate to \pm 30%; liver data are accurate to \pm 50%.

Summary of PFOS Concentration-Liver (ug/g)

Timepoint	Sex	Group 1 Control Average \pm SD	Group 2 Low Average \pm SD	Group 3 Mid Average \pm SD	Group 4 Mid-High Average \pm SD	Group 5 High Average \pm SD	Group 6 High Recovery Average \pm SD
Week 0	Male	0.104 \pm 0.0673 (n = 5)	11.0 \pm 2.31 (n = 5)	31.3 \pm 5.84 (n = 5)	47.6 \pm 12.5 (n = 5)	282 \pm 45.3 (n = 5)	
	Female	0.107 \pm 0.0486 (n = 5)	8.71 \pm 0.552 (n = 5)	25.0 \pm 6.11 (n = 5)	83.0 \pm 14.1 (n = 5)	373 \pm 44.1 (n = 5)	
Week 10	Male	0.459 \pm 0.0573 (n = 5)	23.8 \pm 3.45 (n = 5)	74.0 \pm 6.16 (n = 5)	358 \pm 28.8 (n = 5)	568 \pm 107 (n = 5)	
	Female	12.0 \pm 22.4 (n = 5)	19.2 \pm 3.77 (n = 5)	69.2 \pm 3.46 (n = 5)	370 \pm 22.3 (n = 5)	635 \pm 49.0 (n = 5)	
Week 53	Male	0.635 \pm 1.04 (n = 10)				435 \pm 96.9 (n = 9)	
	Female	0.923 \pm 1.77 (n = 10)				560 \pm 180 (n = 10)	
Day 719	Male						
	Female			55.1 \pm 31.5 (n = 9)			
Week 105	Male	0.114 \pm 0.148 (n = 11)	7.83 \pm 7.34 (n = 10)	26.4 \pm 20.4 (n = 17)	70.5 \pm 63.1 (n = 25)	189 \pm 141 (n = 22)	
	Female	0.185 \pm 0.184 (n = 24)	12.9 \pm 6.81 (n = 15)		131 \pm 61.4 (n = 15)	381 \pm 176 (n = 25)	
Week 106	Male						3.12 \pm 5.97 (n = 10)
	Female						12.9 \pm 10.4 (n = 17)

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

CONCLUSIONS

The study results are summarized as follows:

1. Treatment-related changes were found more commonly in males than in females of each of the treatment groups, which were supported by earlier pharmacokinetic studies demonstrating a higher retention of the compound by males than females.
2. The test material was considered to be carcinogenic in the rat, inducing tumors of the liver and the thyroid gland in the males and tumors of the liver and of the thyroid and mammary gland in females.
3. Based on the pathological findings in the liver, the no-observed-adverse-effect level (NOAEL) for PFOS is considered to be 0.5 ppm in male rats and 2 ppm in female rats; the low observed-adverse-effect level (LOAEL) is considered to be 2 ppm in male rats and 5 ppm in female rats.

4. There was no effect on hepatic palmitoyl-CoA oxidase activity. There were also no statistically significant increases in cell proliferation as measured by proliferative cell nuclear antigen (PCNA) or by bromodeoxyuridine (BrdU).
5. PFOS was observed in the serum and liver of rats dosed with perfluorooctane sulfonic acid potassium salt (PFOS T-6295). Trace levels of PFOS were often detected in the serum and liver of the control animals.

REFERENCE

3M, (2002). 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats. Final Report, 3M T-6295 (Covance study no.: 6329-183), Volumes I-IX , 4068 pages, January 2, 2002. 3M, St. Paul, Minnesota.

Seacar, A.M., Thomford, P.J., and Butenhoff, J.L. Terminal observations in Sprague-Dawley rats after lifetime dietary exposure to potassium perfluorooctanesulfonate. Toxicol. Sci./Toxicologist, 66 (1-S):185, 2002.

EPIDEMIOLOGIC DATA

Title: Identification of Fluorochemicals in Sera of American Red Cross Adult Blood Donors

TEST SUBSTANCE

Identity: PFOS and 6 other fluorochemicals

Remarks: The results reported are preliminary. The final report is expected November 2001.

METHOD

Study design: Cross-sectional

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the levels of PFOS in the serum of American Red Cross blood banks in 6 regions of the U.S.

Study period: 2000

Setting: N/A

Total population: Serum pooled from 6 ARC blood banks in various geographic regions in the US: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD.

Subject selection criteria: Unknown

Total # of subjects in study: 652 donors, age 20-69 years

Comparison population: N/A

Participation rate: N/A

Subject description: No information was provided on the individuals from whom the sera samples were taken.

Health effects studied: PFOS levels in blood

Data collection methods: Blood sera samples were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS).

Details on data collection: No information was provided as to how the blood was drawn, stored, etc.

Exposure period: Unknown--PFOS serum levels used as surrogate for exposure.

Description/delineation of exposure groups/categories: N/A

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

Other methodological information: N/A

RESULTS

Describe results: The mean serum PFOS level was 43.7 ppb. The range was 4.27 to 1656 ppb. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOS levels in the general population. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small. Blood donors cannot be considered representative of the general population of the US.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: Mean PFOS levels reported in this study are similar to those reported earlier in pooled blood samples, although they are on the higher end of the range.

CONCLUSIONS

N/A

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of American Red Cross adult blood donors. Interim report. June 25, 2001.

EPIDEMIOLOGIC DATA

Title: An Epidemiologic Analysis of Episodes of Care of 3M Decatur Chemical and Film Plant Employees, 1993-1998

TEST SUBSTANCE

Identity: POSF-based chemicals used at the Decatur plant

Remarks: Episodes of care analyses are not often used in occupational epidemiologic studies.

METHOD

Study design: Episode of care comparison

Manufacturing/Processing/Use: The 3M Decatur, Alabama plant began production in 1961. It is made up of the film plant and the chemical plant. The 3 major product groups in the chemical plant are protective chemicals, performance chemicals, and fluoroelastomers. Perfluorooctanesulfonyl fluoride (POSF) is the major sulfonate fluorochemical manufactured at Decatur and is used as the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers.

Hypothesis tested: To use episodes of care methodology as a screen for morbidity outcomes associated with long-term, high exposure to POSF-based production at the 3M facility in Decatur, Alabama.

Study period: Episodes of care experience of 652 chemical employees and 659 film plant employees were analyzed for workers at the plant who were employed for at least 1 year between January 1, 1993 and December 31, 1998.

Setting: 3M plant in Decatur, Alabama.

Total population: 1311 workers were eligible for the cohort (at least 1 year of employment at the plant). The total worker population was not reported.

Subject selection criteria: All workers employed at the Decatur plant for at least 1 year between Jan. 1, 1993 and Dec. 31, 1998. Episodes of care were limited to their Decatur time of employment for employees hired, terminated, or died during the study period. However, records of employees on Medicare, long-term disability or who chose HMO coverage were not in the database and would not be included in the episodes of care for that employee.

Comparison population: Chemical and film plant employees were analyzed separately and then compared to each other. Employee comparison groups were defined according to their potential workplace exposure to POSF fluorochemical production. Group A: all chemical plant employees and all film plant employees eligible for the cohort. Group B: all chemical plant employees who worked solely in the chemical plant and all film plant employees who worked exclusively in the film plant. Group C: all chemical plant employees with high fluorochemical exposures compared to their job counterparts in the film plant. Group D: all plant workers with high fluorochemical exposure for at least 10 years prior to the study onset compared to their job counterparts in the film plant.

Participation rate: 97% of Decatur employees were eligible for participation in the study.

Subject description: 82% of the employees in the cohort were male (530 in the chemical plant and 558 in the film plant). The mean age was 45.1 in the chemical plant and 48.6 in the film plant. Sixty percent of the chemical plant employees had worked only in the chemical plant and a similar percentage of film plant workers had worked exclusively in the film plant. Seventy-six percent of the chemical plant workers had high exposure jobs.

Health effects studied: Morbidity. Based on animal data and epidemiologic studies on PFOA and PFOS, certain episodes of care were considered *a priori*. They included: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, gastrointestinal disorders of the liver and biliary tract, and reproductive disorders.

Data collection methods: The Clinical Care Groups episode of care software developed by Ingenix, Inc. was used to provide a comprehensive grouping of all visits (inpatient and outpatient), procedures, ancillary services, and prescription drugs used in the diagnosis, treatment and management of more than 400 diseases or conditions. The software code constructs an episode of care around the index-eligible record by searching backward and forward in time for the health claims records that are related to the disease or condition on the index record. The index record consists of either procedure codes indicative of a face-to-face encounter or a pharmacy record for a delineating drug.

Exposure period: The episodes of care that were included in the study were those experienced between Jan. 1, 1993 to Dec. 31, 1998.

Description/delineation of exposure groups/categories: Workers were placed into groups according to potential workplace exposures: workers who were employed solely in the chemical or film plants, those who had high exposure jobs, and those who worked at least 10 years in jobs with high potential for fluorochemical exposure.

Measured or estimated exposure: estimated based on job history information.

Exposure levels: Not measured. Employees were placed into exposure categories based on job description.

Statistical methods: A risk ratio episode of care (RREpC) provided the estimate of risk between the observed to expected episodes of care for chemical plant employees compared to the observed to expected episodes of care among film plant employees. The expected number of episodes of care for both the film and chemical plant employees was calculated from health claims data of the 3M manufacturing population in the U.S. Because the chemical and film plant cohorts had slightly different age and gender structures, an adjusted ratio was calculated and compared to the unadjusted risk ratio. In most cases, the risk ratios were comparable. Therefore, 95% confidence intervals were only calculated for the unadjusted risk ratios.

Other methodological information: It should be noted that from an epidemiologic perspective, an episode of care could represent any and all incident cases, prevalent cases, and/or misclassified cases (both false positive and false negative). In addition, types and counts of episodes of care may differ by the software used, and it is possible that 2 different diagnoses may be assigned to the same episode. Certain services, such as lab procedures and prescriptions may not be reported for the episode. Also, the endpoint of an episode may vary among software programs. The clinical flexibility of the algorithm may differ depending on the software program.

RESULTS

Describe results: The only increased risk of episodes for the conditions of a priori interest were for

neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding is important because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study. However, the update of the study did not confirm this finding.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

Study strengths and weaknesses: See “other methodological information” section for limitations of episodes of care software. The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. Episodes of care are reported, not disease incidence; therefore, this parameter cannot be interpreted in any other manner. The data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease. In addition, many of the risk ratios for episodes of care had very wide confidence intervals. The analysis was limited to 6 years. Also, the utilization of health care services may reflect local medical practice patterns. Individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications. Episodes of care may include the same individual several times. Not all employees were included in the database, such as those on long-term disability.

Research sponsors: 3M Company

Consistency of results: No other morbidity studies have been conducted on fluorochemicals.

CONCLUSIONS

This study should only be used for hypothesis generation regarding workers employed at the Decatur plant who are employed in jobs with high exposure to POSF-based fluorochemicals.

REFERENCE

Olsen, GW, Burlew, MM, Hocking, BB, Skratt, JC, Burris, JM, Mandel, JH. An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. Final Report. May 18, 2001.

EPIDEMIOLOGIC DATA

Title: Identification of Fluorochemicals in Sera of Children in the United States

TEST SUBSTANCE

Identity: PFOS and 6 other fluorochemicals

Remarks: The results reported are preliminary. The final report is expected November 2001.

METHOD

Study design: Cross-sectional.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the serum concentrations of selected fluorochemicals in a sample of children to provide a more specific understanding of the distribution of these compounds in children.

Study period: Child sera samples were collected from January 1994 to March 1995. The sera samples were analyzed in Spring 1999.

Setting: N/A

Total population: Not reported

Subject selection criteria: The sera samples were provided to 3M by the University of Minnesota Department of Pediatrics. They were obtained from a large clinical trial on Group A streptococcal infections in children. The children were residents of 23 states in the US. These children presented with signs and symptoms of acute-onset pharyngitis. All of the children had positive throat cultures at the initial visit.

Total # of subjects in study: n = 599 children, age 2-12 years

Comparison population: N/A

Participation rate: N/A

Subject description: No information was provided on the children from whom the sera samples were taken.

Health effects studied: PFOS serum levels in blood, as well as 6 other fluorochemicals.

Data collection methods: Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). The samples were collected from equal numbers of male and female children residing in 23 states.

Details on data collection: No information was provided as to how the blood was drawn, stored, etc.

Exposure period: N/A

Description/delineation of exposure groups/categories: Blood sera samples were collected from children 2 – 12 years old.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

Other methodological information: N/A

RESULTS

Describe results: The mean PFOS serum level was 43.5 ppb. The range was 6.7 - 515 ppb. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOS levels in U.S. children. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: To date, no other data have been collected on PFOS serum levels in children.

CONCLUSIONS

N/A

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of children in the United States. Interim Report. June 25, 2001.

EPIDEMIOLOGIC DATA

Title: Mortality Study of Workers Employed at the 3M Decatur Facility

TEST SUBSTANCE

Identity: Several perfluorooctanesulfonyl fluoride-based fluorochemicals, including PFOS

Remarks: This study is an update of the study published by Mandel and Johnson, 1995.

METHOD

Study design: Retrospective cohort mortality study.

Manufacturing/Processing/Use: The 3M Decatur, Alabama plant began production in 1961. It is made up of the film plant and the chemical plant. The 3 major product groups in the chemical plant are protective chemicals, performance chemicals, and fluoroelastomers. Perfluorooctanesulfonyl fluoride (POSF) is the major sulfonate fluorochemical manufactured at Decatur and is used as the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers.

Hypothesis tested: To determine whether occupational exposure to fluorochemicals is related to mortality of employees of the 3M facility in Decatur, Alabama

Study period: The study population worked at the plant for at least 1 year since it began production in 1961. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

Setting: 3M plant in Decatur, Alabama.

Total population: 3512 workers were identified. Of these workers, 2083 worked at the plant for at least one year. Eighty-four percent of the cohort was male.

Subject selection criteria: All workers employed at the Decatur plant for at least 1 year. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

Comparison population: In SMR analyses, Minnesota population death rates for whites were used. Mortality reference rates from 7 regional counties were also used to rule out large variations based on regional mortality reporting differences.

Participation rate: Death certificates were obtained for 96% (n = 139) of the cohort who were deceased.

Subject description: 84% of the employees in the cohort were male. The mean age at follow-up was 51.1 years, and the mean number of years worked at the plant was 14.9. The number of person-years at follow-up was 50970. There were 145 deaths identified in the cohort. The high exposure group was slightly younger than the other 2 groups but worked 3 years longer at the plant than the other 2 exposure groups.

Health effects studied: Mortality

Data collection methods: A review of employee work history records of any employee with at least 1 year employment were abstracted to record the workers' name, SSN, 3M identification number, date of

birth, and dates of work history. This cohort was linked to records from the original cohort to update the employment information and verify other data. The National Death Index was searched for all of the workers. Discrepancies with the original cohort were resolved and deaths before 1979 were verified in the Social Security Death Index. A licensed nosologist coded the death certificates to ICD 8.

Exposure period: The potential exposure period was from the plant opening in 1961 to Dec. 31, 1997.

Description/delineation of exposure groups/categories: Workers were placed into 3 exposure groups based on job history information: high exposure, low exposure, and no exposure. There were 145 deaths in the cohort: 65 in the high exposure group, 27 in the low exposure group and 53 in the non-exposed group.

Measured or estimated exposure: estimated based on job history information.

Exposure levels: Not measured. Employees were placed into 3 exposure categories based on job description: low exposure (n = 289), high exposure (n = 782), non-exposed (n = 812).

Statistical methods: Standardized Mortality Ratios (SMRs) and 95% confidence intervals were derived using the PC Life Table Analysis System software developed by NIOSH. This program computes age, gender, and race-specific SMRs using standard life table methods. The expected number of deaths is estimated by multiplying the age, gender, race, and calendar period tabulated person-years of follow up to the corresponding cause-specific mortality reference rates. Mortality rates for white Minnesotans were used as reference data.

Other methodological information:

RESULTS

Describe results: 145 deaths were identified in the cohort: 65 of these deaths were in the high exposure group. When the entire cohort was analyzed, SMRs were not elevated for most of the cancer types and for non-malignant causes. SMRs that were above 1 (cancer of the esophagus, liver, breast, urinary organs, bladder, and skin) were also elevated when the cohort was limited to any employee ever employed in a high exposure job (except breast cancer). Only 2 or 3 deaths were reported for each of these cause-specific categories and were not statistically significant, except for bladder cancer.

Workers who were employed in high exposure jobs were greater than 12 times more likely to die of bladder cancer than the general population of Alabama (SMR = 12.77, 95% CI = 2.63 - 37.35). This effect remained when the data were analyzed using county death rates. Three male employees in the cohort died of bladder cancer, and all of the deaths occurred in employees who had worked in high exposure jobs for at least 5 years (SMR = 24.49, 3 observed deaths, 0.12 expected). All of them had worked at the Decatur plant for more than 20 years. In the previous cohort mortality study, 1 bladder cancer death was reported.

Two deaths were reported for liver cancer. One was in the low exposure group and one in the high exposure group. The SMR for workers who were employed in either high or low exposure jobs was 3.08 (95% CI = 0.37 – 11.10). Five cases of cirrhosis of the liver were reported in this cohort, 2 in the high exposure group, 1 in the low exposure group, and 2 in the non-exposed. The observed did not exceed the expected mortality experience in any of these groups.

Study strengths and weaknesses: A larger cohort was followed in this update of the original study, thus reporting twice as many deaths; however, the cohort is fairly young and the number of deaths is still small. There were small numbers of deaths in many of the categories for males and especially for females in all categories, thus limiting the power of the study. Death certificates were located for 96% of the cohort, but

the 6 not obtained could greatly impact the results of the analyses since the number of deaths in most of the sub-cohorts was very small. Biological measurements of fluorochemicals were not available; therefore, exposure categories based on job descriptions were used as a surrogate for exposure. The categories are more specific in this update of the study; however, there is still a potential for misclassification of exposure. Based on the results of the biomonitoring conducted on employees in a random sample of Decatur employees in 1998, the geometric mean of PFOS in film plant employees was 0.136 ppm. However, in this study, film plant employees are considered non-exposed. In addition to fluorochemicals, workers were exposed to other chemicals in the workplace.

Research sponsors: University of Minnesota

Consistency of results: In the first study, there was an excess of bladder cancer; however, this was based on only 1 death. Three additional deaths have now been reported.

CONCLUSIONS

Workers employed at the Decatur plant who are employed in jobs with high exposure to POSF-based fluorochemicals are at increased risk of death from bladder cancer.

REFERENCE

Alexander, B.H. April 26, 2001. Mortality study of workers employed at the 3M Decatur facility. Final Report. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota.

OTHER

This study is the second update of the mortality study.

EPIDEMIOLOGIC DATA

Title: Identification of Fluorochemicals in Human Sera. I. American Red Cross Adult Blood Donors

TEST SUBSTANCE

Identity: PFOS and 6 other fluorochemicals

Remarks: This is a final report. The preliminary report was dated June 25, 2001.

METHOD

Study design: Cross-sectional

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the levels of PFOS in the serum of American Red Cross blood banks in 6 regions of the U.S.

Study period: 2000

Setting: N/A

Total population: 6 ARC blood banks in various geographic regions in the US provided 645 serum samples from adult donors. The 6 regions included: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD.

Subject selection criteria: Unknown

Total # of subjects in study: 645 donors, age 20-69 years

Comparison population: N/A

Participation rate: N/A

Subject description: The only demographic factors known were age, gender, and location.

Health effects studied: Levels of 7 fluorochemicals in human blood serum, including PFOS. The other chemicals were: PFOA, PFOSAA, M570, M556, PFOSA, PFHS.

Data collection methods: Each blood bank was requested to provide approximately 10 samples per 10-year age intervals (20-29, 30-39, etc.) for each sex.

Details on data collection: Blood sera samples were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). 24 samples were split and analyzed to provide an estimate of the reliability of the analyses conducted. The analytical lab was blind to the identity of these split samples. These analyses were performed concurrently with all other analyses of the study to minimize experimental error.

Exposure period: Unknown--PFOS serum levels used as surrogate for exposure.

Description/delineation of exposure groups/categories: 332 male donors, 313 female donors. There were 10 or more subjects for both males and females in each age category (20-29, 30-39, etc.) except the 60-69 age group where there were fewer.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: A reliability assessment was done. Geometric means, 95% confidence intervals of the geometric means, range, interquartile range, raw cumulative 90th percentile, and frequency distributions, were calculated. Central tendency and distribution of the data by age, gender, and location were presented. Bootstrap analysis was done to calculate mean serum PFOS values for each 6 locations adjusted for 10-year age intervals, gender, and their interaction terms. Multivariable regression analyses. A reliability assessment was also done for the chemical analyses.

Other methodological information: N/A

RESULTS

Describe results: The results of the reliability analysis indicate that there was a strong correlation between the split samples ($r = .9$) for PFOS (as well as PFOA and PFHS).

PFOS is the only chemical that met the criteria for a log-normal distribution based on the Shapiro-Wilk test.

The geometric mean serum PFOS level for all locations and gender was 34.9 ppb (95%CI, 33.3-36.5 ppb). The range was < LLOQ (4.3 ppb) to 1656 ppb. Males had significantly higher ($p < .05$) geometric mean PFOS levels than females. The geometric mean for all males was 37.8 ppb (95% CI, 35.5-40.3) and was 31.3 ppb for all females (95% CI, 30.0 – 34.3). Age was not an important predictor of adult serum fluorochemical concentrations.

When stratified by geographic location, the highest geometric mean for PFOS was in the samples from Charlotte, NC (51.5 ppb, range: 19.3 – 166.0) and the lowest from Boston (28.0 ppb, range: 4.3 – 87.2). The other PFOS geometric means and ranges by location were: Los Angeles, 40.4 ppb (6.6 – 205.0); Minneapolis/St. Paul, 33.1 ppb (7.7 – 207.0); Portland, 27.0 ppb (6.0 – 1656); Hagerstown, 35.3 ppb (7.6 – 226.0). The cumulative 90% were highest for Charlotte (105.3), Minneapolis/St. Paul (71.7), Los Angeles (70.1), and Hagerstown (69.8). Portland and Boston were much lower (49.4 and 48.7, respectively).

The results from a bootstrap analysis, done to calculate mean serum PFOS values for each 6 locations adjusted for 10-year age intervals, gender, and their interaction terms, resulted in similar means for Boston (29.0 ppb), Los Angeles (35.0 ppb), Minneapolis/St. Paul (34.8 ppb), and Hagerstown (34.9 ppb). However the mean for Charlotte was much lower (39.0 ppb) and the mean for Portland was slightly higher (32.8 ppb).

Bootstrap analyses for PFOS calculated a mean of the 95% tolerance limit of 88.5 ppb with an upper 95% confidence limit of 100.0 ppb. The mean of the 99% tolerance limit was 157.3 ppb with an upper 95% confidence limit of 207.0 ppb. At the lowest tolerance limit analyzed (90%), the mean for PFOS was 70.7 ppb with an upper 95% confidence limit of 74.3 ppb.

The highest serum PFOS measurement in this sample was 1656 ppb from a male blood donor, 67 years old

from Portland. The next highest donor level was 329 ppb from a male donor, 62 years old also from Portland. The next 8 highest serum PFOS values (range 139 – 226 ppb) were measured in 4 females and 4 males representing Charlotte (n=4), Hagerstown (n=2), Los Angeles (n=1) and Minneapolis/St. Paul (n=1).

PFOS and PFOA were strongly correlated ($r = .63$) PFOS had a lower correlation with PFOSAA ($r = .42$) and M570 ($r = .20$). Both PFOSAA and M570, adjusted for age, gender, and their interaction, were significant predictors of PFOS in a multivariable model. PFOSAA was the stronger of the 2 independent variables. Age and gender were not significant predictors in models that examined the significant association between PFOS and PFOA.

Study strengths and weaknesses: Blood donors cannot be considered representative of the general population of the US.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: Mean PFOS levels reported in this study are similar to those reported in other pooled blood samples.

CONCLUSIONS

N/A

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in human sera. I. American Red Cross adult blood donors. Final report. February 25, 2002.

EPIDEMIOLOGIC DATA

Title: Identification of Fluorochemicals in Human Sera. II. Elderly Participants of the Adult Changes in Thought Study, Seattle, Washington

TEST SUBSTANCE

Identity: PFOS and 6 other fluorochemicals

Remarks: The results reported are final.

METHOD

Study design: Cross-sectional.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the serum concentrations of selected fluorochemicals in a sample of elderly persons to provide a more specific understanding of the distribution of these compounds in this age group.

Study period: 9/29/2000

Setting: N/A

Total population: 238 serum samples from elderly adult donors from the Adult Changes in Thought study.

Subject selection criteria: Donors were 65-96 years old. Subjects were identified during an enrollment phase of this community-based prospective cohort study of dementia and normal aging conducted collaboratively between the U. of Washington and Group Health Cooperative (HMO). Eligible individuals were those with no known history of neuropsychiatric disease or dementia.

Total # of subjects in study: 238

Comparison population: N/A

Participation rate: N/A

Subject description: 238 adults--118 males, 120 females. The mean age was 76 years. Female subjects had resided in the Seattle area for 53.3 years, males 50.2 years.

Health effects studied: PFOS serum levels in blood, as well as 6 other fluorochemicals.

Data collection methods: Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS).

Exposure period: N/A

Description/delineation of exposure groups/categories: 65-75 years (n= 121), 75+ to 85 years (n = 93), 85+ to 96 years (n = 24).

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms. A reliability assessment was also done.

Other methodological information: N/A

RESULTS

Describe results:

The geometric mean of PFOS for all samples was 31.0 ppb (95% CI, 28.8-33.4). The range was 3.4 – 175 ppb. There was no significant ($p < .05$) difference in geometric means for males and females. In simple linear regression analyses, age was negatively ($p < .05$) associated with PFOS in men but not in women. The mean of the 95% tolerance limit for PFOS was 84.1 ppb with an upper 95% confidence limit of 104.0 ppb.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOS levels in elderly. Very little descriptive information about the subjects is available. The subjects only characterize PFOS levels in the Seattle region. To date, they are the only data available characterizing serum PFOS levels in the elderly.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: To date, no other data have been collected on PFOS serum levels in elderly.

CONCLUSIONS

PFOS levels in these Seattle residents are similar to those found in adults and children in the U.S.

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. February 25, 2002. Identification of fluorochemicals in human sera. II. Elderly participants of the Adult Changes in Thought study, Seattle, Washington. Final Report. 3M Company, Medical Department.

EPIDEMIOLOGIC DATA

Title: Identification of Fluorochemicals in Human Sera. III. Pediatric Participants in a Group A Streptococci Clinical Trial Investigation

TEST SUBSTANCE

Identity: PFOS and 6 other fluorochemicals

Remarks: The results reported are final.

METHOD

Study design: Cross-sectional.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the serum concentrations of selected fluorochemicals in a sample of children to provide a more specific understanding of the distribution of these compounds in children.

Study period: Child sera samples were collected from January 1994 to March 1995. The sera samples were analyzed in Spring 1999.

Setting: N/A

Total population: Not reported

Subject selection criteria: The sera samples were provided to 3M by the University of Minnesota Department of Pediatrics. They were obtained from a large clinical trial on Group A streptococcal infections in children. The children were residents of 23 states in the US. These children presented with signs and symptoms of acute-onset pharyngitis. All of the children had positive throat cultures at the initial visit.

Total # of subjects in study: n = 599 children, age 2-12 years

Comparison population: N/A

Participation rate: N/A

Subject description: 299 male children, 300 female children from 23 states and the District of Columbia.

Health effects studied: PFOS serum levels in blood, as well as 6 other fluorochemicals.

Data collection methods: Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). Sera were frozen at -20 degrees C prior to the request for analysis.

Exposure period: N/A

Description/delineation of exposure groups/categories: Blood sera samples were collected from children 2 – 12 years old.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms. A reliability assessment was also done.

Other methodological information: N/A

RESULTS

Describe results:

The geometric mean of PFOS for all of the participants was 37.5 ppb (95% CI, 33.3-36.5). The range was 6.7 to 515.0 ppb. Male children had significantly ($p < .01$) higher geometric mean PFOS levels than females: 40.1 ppb and 35.2 ppb, respectively. In bootstrap analyses, the mean of the 95% tolerance limit for PFOS was 88.5 ppb with an upper 95% confidence limit of 97.0 ppb. When stratified by age, the geometric mean tended to rise for each age group from age 2 (28.6 ppb) through age 9 (42.8 ppb) where it was highest, and then started to decrease gradually to 32.8 ppb at 12 years. In simple linear regression analyses, age was not significantly ($p < .05$) associated with PFOS. Although the data were not provided, a graphical presentation of log PFOS levels for each state by gender were similar across the states, however, it is difficult to interpret these data given the limited sample size for each gender/location subgroup.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOS levels in U.S. children. Very little descriptive information about the subjects is available. To date, they are the only data available characterizing serum PFOS levels in children.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: To date, no other data have been collected on PFOS serum levels in children.

CONCLUSIONS

N/A

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of children in the United States. Interim Report. June 25, 2001.

EPIDEMIOLOGIC DATA

Title: A Cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology, and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program

TEST SUBSTANCE

Identity: PFOS, PFOA

Remarks:

METHOD

Study design: cross-sectional

Manufacturing/Processing/Use: Facilities in Decatur, Alabama and Antwerp, Belgium which manufacture perfluorooctanesulfonyl fluoride products. These fluorochemicals can metabolize in the body to PFOS.

Hypothesis tested: To provide an aggregate analysis of the hematology, clinical chemistries, and hormonal parameters of volunteer employees in relation to serum PFOS and PFOA levels as measured in the medical surveillance examinations of Antwerp and Decatur employees in 2000.

Study period: March 1, 2000. End date was not reported.

Setting: Occupational. 3M plants located in Antwerp, Belgium and Decatur, Alabama.

Total population: 340 Antwerp employees and 500 Decatur employees working in the chemical plant area were eligible for inclusion in the surveillance.

Subject selection criteria: Voluntary participation in medical surveillance program in Y2000.

Total # of subjects in study: 255 Antwerp employees (206 male and 49 female) and 263 Decatur employees (215 male and 48 female).

Comparison population: N/A

Participation rate: 75% of employees at the Antwerp plant and 50% of the employees at the Decatur plant who were eligible participated. 73% of the participating Antwerp male employees and 75% of the Decatur employees were engaged in production activities. Only 12% of the participating Antwerp female employees were engaged in production activities compared to 63% of the Decatur female employees.

Subject description: Male Antwerp employees had lower PFOS and PFOA levels, were significantly younger than Decatur male employees, had lower BMIs, worked fewer years, had higher self-reported daily consumption of alcohol, had lower mean alkaline phosphatase, GGT, AST, ALT and triglyceride values and higher total bilirubin and HDL values. Comparable results were observed for Antwerp female employees vs. Decatur females.

Health effects studied: To determine if there were differences in the following parameters based on

PFOS/PFOA levels: hematology (hematocrit, hemoglobin, RBCs, WBCs, platelet count), clinical chemistries (alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, blood urea nitrogen, creatinine, glucose, cholesterol, low density lipoproteins, high density lipoproteins, and triglycerides), and thyroid hormones (thyroid stimulating hormone, serum thyroxine, free thyroxine, serum triiodothyronine, thyroid hormone binding ratio, and free thyroxine).

Data collection methods: Medical questionnaire, work history questionnaire, blood sera samples, measurements of height, weight, and blood pressure, urinalysis (Decatur only), and standard clinical chemistry and hematology tests, thyroid hormone measurement, and pulmonary function tests. Values used for reference ranges were not provided.

Details on data collection: The site-specific work history questionnaire was administered to all participants. The data were self-reported. Questionnaire content, design, administration, etc. were not provided in this report. Data on blood collection (amount, etc.) not provided. Urinalysis was only assessed for Decatur employees via standard urine microstick analysis which tested for urine glucose, albumin, and RBCs.

TSH, free T4 and T3 were determined by immunochemiluminometric assay. T4 and THBR were determined by a cloned enzyme donor immunoassay. FTI was calculated by multiplying T4 and THBR.

Sera samples were extracted using an ion-pairing extraction procedure. In addition to PFOA and PFOS, the extracts were also analyzed for PFHS, PFOSAA, PFOSA, and M556 (perfluorooctanesulfonamidoacetate) using high-pressure liquid chromatography electrospray tandem mass spectrometry and evaluated versus an extracted curve from a human serum matrix. All serum values for PFOS and PFOA were above the LLOQ.

Exposure period: Unknown. PFOS/PFOA serum levels indicate exposure.

Description/delineation of exposure groups/categories: Workers were stratified by plant location as well as by serum PFOS distribution, production status (production vs. non-production workers), and gender.

Mean serum PFOS levels for all employees participating in this study at Antwerp (n = 206) and Decatur (n = 215) were 0.96 and 1.40 ppm, respectively. Levels among production employees were higher. At Antwerp, the mean PFOS level of male production employees was 1.16 ppm and 1.63 ppm at Decatur.

Mean PFOA levels for all employees were 1.03 and 1.90 ppm at Antwerp and Decatur, respectively. Levels among production employees were higher. At Antwerp, the mean PFOA level of male production employees was 1.28 ppm and 2.34 ppm at Decatur.

Measured or estimated exposure: Serum PFOS and PFOA levels were used to estimate exposure.

Statistical methods: Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance, and multivariable regression were used to evaluate associations between PFOS and PFOA and each hematological and clinical chemistry test and thyroid hormone assay. For stratified analyses, employees were divided into quartiles of their serum PFOS distribution. Potential confounding factors considered in the analyses included: age, BMI, alcohol consumption, cigarette use, years worked at either plant, and type of job.

Multivariable regression models were fitted with PFOS/PFOA analyzed as continuous variables. Natural

log transformations of the dependent variables were performed, when necessary, to normalize variables and to enhance model fit. SAS was used to analyze the data.

Other methodological information:

RESULTS

Describe results:

Antwerp and Decatur employees were different in several ways (see “Subject description” above). Therefore, univariate analyses were initially stratified by location and then those analyses were stratified by gender and production status. They were placed into quartiles depending on production status. Therefore, the PFOS values in the quartiles are different for male production, non-production, and female employees.

Antwerp

The mean PFOS level for all employees at this plant was 0.96 ppm (range 0.04 - 6.24 ppm). When stratified by production status, the mean was 1.16 ppm for production employees and 0.42 ppm for non-production employees. The mean for female employees was 0.13 ppm.

Male production employees were placed into the following quartiles based on PFOS levels: Q1 (mean, 0.29 ppm; range, 0.04 – 0.41 ppm), Q2 (mean, 0.58 ppm; range 0.41 – 0.78 ppm), Q3 (mean 1.18 ppm; range 0.79 – 1.66 ppm), Q4 (mean, 2.61 ppm; range, 1.67 – 6.24 ppm).

In male production employees (n = 150), the highest quartile mean serum PFOS level was 2.61 ppm (range 1.76 – 6.24 ppm) and the lowest was 0.29 ppm (range 0.04 – 0.41 ppm). Production employees in the highest quartile were significantly ($p < .05$) older and worked more years at Antwerp than employees in the lowest quartile. The only difference in clinical chemistries for production workers was in BUN. When compared by quartile of serum PFOS distribution, no significant ($p < .05$) differences among male production employees were observed for thyroid (TSH, T4, free T4, T3, THBR, FTI) or for hematology (HCT, HGB, RBC, WBC, platelets). The same held true for non-production employees (n = 56).

For all female employees, BUN was significantly different (higher) between 1st quartile and 3rd and 4th quartile. Thyroid and hematology results were not significantly different between any of the quartiles for females.

Decatur

The mean PFOS level for all employees at this plant was 1.40 ppm (range 0.11 – 10.06 ppm). When stratified by production status, the mean was 1.63 ppm for production employees and 0.73 ppm for non-production employees. The mean for female employees was 0.93 ppm. 75% of male employees worked in production jobs (n = 161) and 63% of female employees worked in production jobs.

Male production employees were placed into the following quartiles based on PFOS levels: Q1 (mean, 0.55 ppm; range, 0.11 – 0.75 ppm), Q2 (mean, 1.01 ppm; range 0.76 – 1.30 ppm), Q3 (mean 1.74 ppm; range 1.32 – 2.29 ppm), Q4 (mean, 3.22 ppm; range, 2.31 – 10.06 ppm).

When male production workers were placed into quartiles, the only significant ($p < .05$) difference between the quartiles was in ALT (highest quartile different from all 3 others).

No significant differences between quartiles were observed for thyroid (TSH, T4, free T4, T3, THBR, FTI), hematology (HCT, HGB, RBC, WBC, platelets), or urinalysis (albumin, blood, sugar).

For female employees (both production and non-production employees), there were no significant differences among quartiles for demographics, thyroid, hematology, or urinalysis except for mean platelet count where the third quartile was significantly lower than the 1st quartile, but the 4th was not.

When results were analyzed by number of employees who had values above the reference range for hepatic clinical chemistry tests and liver enzyme and bilirubin tests, there was a higher percentage of male Decatur production workers in the highest PFOS quartile for ALT, GGT, and total liver panel than the other quartiles. Most notable were the results for ALT where 8% of employees in the lowest exposure group (Q1) and 28% in the highest exposure group (Q4) had values above the reference range, while the percentages for total liver panel (which includes alkaline phosphatase, AST, ALT, GGT, and total and direct bilirubin) were 18% and 35%, respectively. This trend was not evident in Decatur non-production employees (although the n was only 54), in Decatur females, or in any of the Antwerp employees. However, each sub-population had a different serum PFOS quartile distribution. Therefore, they cannot be directly compared.

Analyses combining employees from both plants

When clinical chemistry results of all male employees from both plants were combined (both production and non-production) (n = 421) and placed into quartiles (n = 105 per quartile), mean values for triglycerides, alkaline phosphatase, total bilirubin, and ALT were significantly ($p < .05$) higher in the 4th quartile (mean PFOS level 2.69, range 1.69 – 10.06 ppm) than in the first (mean PFOS level 0.27 ppm, range 0.04 – 0.42 ppm). It should be noted that the number of Antwerp production employees were evenly distributed among the quartiles while this was not the case for Decatur employees. The highest number of Decatur employees was in the 4th quartile. In addition, overall the employees in the 1st quartile were slightly younger, had a lower BMI, and worked fewer years than employees in the other quartiles.

Thyroid results for this same group indicated that T3 was significantly higher ($p < .05$) and THBR was significantly lower ($p < .05$) in Q4 than Q1. In female employees combined for both plants (n = 97), alkaline phosphatase and GGT were significantly higher ($p < .05$) and total bilirubin significantly lower in Q4 than in Q1. Most of the Decatur female employees worked in production jobs while most of the Antwerp females worked in non-production jobs. Therefore, Q4 was 92% female production workers and all of them worked at the Decatur plant.

The combined plant data were analyzed for employees who had values above the reference range for alkaline phosphatase, AST, ALT, GGT, and total liver panel. For male employees for all of these measures, the levels increased from Q1 to Q4. In Q1, 4% of the employees had values above the reference range for ALT and 6% for GGT, while 12% was reported for Q4 for both of these tests. For total liver panel, 14% of the employees had values above the reference range in Q1 as compared to 23% in Q4. The numbers of female employees with values above the reference range was very small (n = 8).

The above data were not adjusted for potential confounders; therefore, multivariable regression analyses were conducted. There was a positive significant ($p = .04$) association between PFOS and cholesterol and also a positive significant ($p = .05$) association between PFOA and cholesterol. When both PFOS and PFOA were included in the model, neither were statistically significant at $p = .05$. PFOS was not significant with HDL although PFOA was negatively associated with HDL ($p = .04$). Triglycerides were positively associated with PFOS ($p = .01$) and PFOA ($p = .002$). When both were left in the model, PFOA remained significant ($p = .02$). Total organic fluorine (TOF) was highly significant for triglycerides ($p = .0009$). No significant associations were observed with PFOS, PFOA, or TOF in relation to alkaline phosphatase, GGT, AST or total bilirubin. A significant ($p = .02$) positive association was observed for TOF and ALT. A positive significant ($p = .04$) association between T3 and PFOS was observed. Plant location was highly significant ($p < .0001$) in the model. BMI, cigarettes/day, alcohol/day were also significant. [In the

univariate analyses, Antwerp employees had higher mean T3 levels than Decatur employees overall. However, for each plant (individually) T3 values increased by quartile as PFOS serum levels increased, although the differences were not statistically significant.] THBR, as well as the other thyroid hormones, were not significant in the regression analyses. Most of the thyroid hormones, including TSH, T4, THBR, and FTI, were not significantly associated with PFOS, PFOA, or TOF. However, PFOS, PFOA and TOF were positively associated ($p = .04$, $.01$, and $.004$, respectively) with T3.

Study strengths and weaknesses: Cross-sectional design, voluntary participation, the Decatur and Antwerp populations were significantly different in certain demographic and clinical chemistry results as well as in PFOS serum levels, PFOS serum levels are declining thereby making it harder to detect an effect if one is present, plant populations cannot be compared because quartiles are different for each subgroup, only one measurement at a certain point in time was collected for each test, other perfluorinated chemicals, such as PFOA, are present in the plants.

Research sponsors: 3M

Consistency of results: These results are somewhat consistent with those of the 1995 and 1997 cross-sectional medical surveillance data, in that all of them conclude that there were no significant abnormalities in hematological and clinical chemistry parameters of the Antwerp or Decatur workers. No decline in cholesterol levels was observed as PFOS serum levels increased. The hormone data collected in 1995 was different from that collected in 2000 and therefore cannot be compared. A longitudinal analysis of these data did not reveal any significant changes in hepatic or lipid clinical chemistry values; however, there were many limitations to the study.

CONCLUSIONS

The authors concluded that these data continue to suggest that Antwerp and Decatur fluorochemical production and non-production employees do not have significant changes in serum cholesterol, lipoproteins, or hepatic enzymes that are consistent with toxicological findings in laboratory animals.

REFERENCE

Olsen GW, Burlew MM, Burris JM, Mandel JH. October 11, 2001. A cross-sectional analysis of serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to clinical chemistry, thyroid hormone, hematology and urinalysis results from male and female employee participants of the 2000 Antwerp and Decatur fluorochemical medical surveillance program. Final report. 3M Medical Department.

EPIDEMIOLOGIC DATA

Title: A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997, and 2000 Fluorochemical Medical Surveillance Program

TEST SUBSTANCE

Identity: PFOS, PFOA

Remarks:

METHOD

Study design: longitudinal

Manufacturing/Processing/Use: 3M Decatur, Alabama plant and Antwerp, Belgium plant.

Hypothesis tested: To determine whether occupational exposure to fluorochemicals over time is related to changes in clinical chemistry and lipid results in employees of 2 3M facilities.

Study period: There were 3 time periods during which medical surveillance took place at the plants—1994/95, 1997, and 2000.

Setting: 3M plants in Decatur, Alabama and Antwerp, Belgium.

Total population: 175 male employees participated in 2000 and at least one of the other sampling periods.

Subject selection criteria: Employees participated voluntarily.

Comparison population: n/a

Participation rate: 106/175 (61%) participated in 1994/95, 110/175 (63%) participated in 1997, and 175 participated in 2000. 24% participated in all 3 sampling periods (n = 41, Antwerp 20, Decatur 20), 37% in 1994/95 and 2000 (n = 65, Antwerp 45, Decatur 20), and 39% (n = 69, Antwerp 34, Decatur 35) in 1997 and 2000.

Subject description: Male employees volunteered to participate in biomonitoring offered at the plants. In general, Antwerp male employees were significantly younger, had lower BMIs and a higher daily consumption of alcohol reported than Decatur male employees. Antwerp male employees also had lower mean alkaline phosphatase and triglyceride values and higher total bilirubin and HDL values than the Decatur male employees.

Health effects studied: To determine whether workers' lipid and hepatic clinical chemistry results are affected by PFOS and PFOA levels.

Data collection methods: Clinical chemistries and hematology collected—cholesterol (mg/dl), high density lipoproteins (HDL, mg/dl), triglycerides (mg/dl), alkaline phosphatase (IU/L), gamma glutamyl transferase (GGT, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), total and direct bilirubin (mg/dl). Demographic data collected via questionnaire.

Details on data collection: Details on data collection methods including questionnaire content, design, administration, etc. and blood collection methods were not provided.

PFOS and PFOA methods of analysis differed slightly each year. In 1994/95, the method used tetrabutylammonium to ion-pair with PFOS and PFOA in the serum. The ion-pairs were then extracted with ethyl acetate and the abstraction product was then analyzed using high-performance liquid chromatography-thermospray mass spectrometry. In 1997, the serum samples were analyzed by liquid chromatography/mass spectrometry, using selected ion monitoring in the negative-ion mode. In 2000, sera samples were extracted using an ion-pairing extraction procedure. High-performance liquid chromatography/electrospray tandem mass spectrometry was used. The samples were evaluated versus an extracted curve from a human serum matrix.

Exposure period: Unknown. PFOS, PFOA levels measured in blood serum.

Description/delineation of exposure groups/categories: The groups of employees were broken into subpopulations A, B, and C. A was comprised of the employees who participated in all 3 years of surveillance, B contained those employees who participated in 1994/95 and 2000, and C contained those who participated in 1997 and 2000.

Measured or estimated exposure: PFOS and PFOA levels were measured in workers' blood serum. No ambient exposure data are available.

Exposure levels:

	Mean PFOS levels	
	Antwerp	Decatur
1994/95	1.87 ppm	2.62 ppm
1997	1.42 ppm	1.85 ppm
2000	1.16 ppm	1.67 ppm

	Mean PFOA levels	
	Antwerp	Decatur
1994/95	1.08 ppm	1.90 ppm
1997	1.54 ppm	1.41 ppm
2000	1.43 ppm	1.83 ppm

Statistical methods: repeated measures incorporating the random subject effect fitted to a mixed model using SAS. Restricted maximum likelihood estimates of variance parameters were computed. Adjusted regression models were built by introducing all covariates and testing the covariance structure. Covariates included in the model were age, BMI, number of alcoholic drinks per day, and cigarettes smoked per day.

Other methodological information: A total of 175 male employees (100 Antwerp and 75 Decatur) who participated in the 2000 surveillance year also participated in at least one previous fluorochemical medical surveillance exam since 1994/95. Therefore, this provided an opportunity to undertake a longitudinal assessment.

RESULTS

PFOS results

When mean serum PFOS levels were compared by surveillance year, PFOS levels have been decreasing in the participants in medical surveillance in both plants. When the data were analyzed by the 3 subcohorts (those who participated in 2 or more medical exams between 1995 and 2000), Antwerp and Decatur employees in each of the 3 subcohorts had lower mean serum PFOS levels in 2000 than at their year of entry.

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was no association between PFOS and serum cholesterol or triglycerides in male participants over time. There were also no significant associations between PFOS and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

PFOA results

When mean serum PFOA levels were compared by surveillance year, PFOA levels in the employees participating in medical surveillance at the Antwerp plant increased between 1994/95 and 1997 and then decreased slightly between 1997 and 2000. At the Decatur plant, PFOA serum levels decreased between 1994/95 and 1997 and then increased between 1997 and 2000. When the data were analyzed by plant and the 3 subcohorts (those who participated in 2 or more medical exams between 1995 and 2000), there were no consistent changes across subcohorts at the Antwerp plant. However, among the 3 Decatur subcohorts, mean PFOA levels tended to increase.

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between PFOA and serum cholesterol ($p = .0008$) and triglycerides ($p = .0002$) over time. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees ($p = .005$) and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years ($p = .001$). However, the association between PFOA and triglycerides was also statistically significant ($p = .02$) for subgroup B (employees who participated in biomonitoring in 1994/95 and 2000). There was not a significant association between PFOA and triglycerides among Decatur workers.

There were no significant associations between PFOA and changes over time in HDL, alkaline phosphatase, GGT, AST, ALT, total bilirubin, and direct bilirubin.

Total Organic Fluorine (TOF) results

When analyzed using mixed model multivariable regression and combining Antwerp and Decatur employees, there was a statistically significant positive association between TOF and serum cholesterol ($p = .007$) and triglycerides ($p = .008$) over time. However, the interaction term with time (years) was not significant. This association was more consistent for Antwerp employees than Decatur employees.

Study strengths and weaknesses: Study limitations include the following:

1. A very small number of employees participated in all 3 study periods (only 24%, $n = 41$)
2. different labs were used each year for analysis and different analytical techniques for PFOS
3. could not analyze female employees due to small numbers
4. PFOS levels in employees are decreasing over time and are below those levels causing effects in laboratory animals
5. serum PFOS levels were approximately 0.5 ppm lower in Antwerp employees than Decatur
6. more Antwerp employees than Decatur participating in this study (57% vs. 43%)
7. PFOA levels fluctuating (direction depending on the plant)
8. there are several consistent differences between the Antwerp and Decatur male populations (eg.,

statistically significant differences in BMI, age, consumption of alcohol, and differences in chemistry profiles)

9. low levels of PFOS and PFOA measured in each program year among these employees as compared with those that cause effects in laboratory animals
10. blood sampling was conducted only once per sampling period at a certain point in time.

Research sponsors: 3M

Consistency of results: This is the first longitudinal analysis of the surveillance data and will probably be the last since PFOS is being phased out.

The positive association between PFOA and serum cholesterol and triglycerides is not consistent with the hypolipidemia effect observed in rodents (and not observed in primates). In addition, this effect has not been observed at 3M's Cottage Grove facility where PFOA serum levels in workers are much higher than at the Decatur or Antwerp plant.

CONCLUSIONS

A longitudinal analysis over a six-year period of 175 Antwerp and Decatur male employees did not show significant changes, consistent with toxicological data, of lipid or hepatic clinical chemistry values associated with PFOS. A positive statistically significant association was observed between PFOA and cholesterol and triglycerides. When analyzed by plant and also by subcohort, these associations were limited to the Antwerp employees and, in particular, the 21 Antwerp employees who participated in all 3 surveillance years.

REFERENCE

Olsen, G.W., Burlew, M.M, Burris, J.M., Mandel, J.H. A Longitudinal Analysis of Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA) Levels in Relation to Lipid and Hepatic Clinical Chemistry Test Results from Male Employee Participants of the 1994/95, 1997, and 2000 Fluorochemical Medical Surveillance Program. 3M Final Report. October 11, 2001.

COMMISSION REGULATION (EC) No 552/2009**of 22 June 2009****amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XVII****(Text with EEA relevance)**

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC ⁽¹⁾, and in particular Article 131 thereof,

Whereas:

- (1) Council Directive 76/769/EEC of 27 July 1976 on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations ⁽²⁾ established in its Annex I restrictions for certain dangerous substances and preparations. Regulation (EC) No 1907/2006 repeals and replaces Directive 76/769/EEC with effect from 1 June 2009. Annex XVII to that Regulation replaces Annex I to Directive 76/769/EEC.
- (2) Article 67 of Regulation (EC) No 1907/2006 provides that substances, mixtures or articles may not be manufactured, placed on the market or used unless they comply with the conditions of any restrictions laid down in their regard in Annex XVII.
- (3) Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006 amending for the 30th time Council Directive 76/769/EEC (perfluorooctane sulfonates (PFOS)) ⁽³⁾ and Commission Directive 2006/139/EC of 20 December 2006 amending Council Directive 76/769/EEC as regards restrictions on the marketing and use of arsenic compounds for the purpose of adapting its Annex I to technical progress ⁽⁴⁾ amending Annex I to Directive 76/769/EEC were adopted shortly before Regulation (EC) No 1907/2006 was adopted, in December 2006, but the restrictions concerned have not yet been included in

Annex XVII to that Regulation. Therefore Annex XVII should be amended to include the restrictions corresponding to Directives 2006/122/EC and 2006/139/EC as otherwise the restrictions concerned will be repealed on 1 June 2009.

- (4) Pursuant to Article 137(3) of Regulation (EC) No 1907/2006, any amendment to the restrictions adopted under Directive 76/769/EEC from 1 June 2007 is to be incorporated in Annex XVII to that Regulation with effect from 1 June 2009.
- (5) Directive 2007/51/EC of the European Parliament and of the Council amending Council Directive 76/769/EEC relating to restrictions on the marketing of certain measuring devices containing mercury ⁽⁵⁾ was adopted on 25 September 2007. Decision 1348/2008/EC of the European Parliament and Council amending Council Directive 76/769/EEC as regards restrictions on the marketing and use of 2(2-methoxyethoxy)ethanol, 2-(2-butoxyethoxy)ethanol, methylenediphenyl diisocyanate, cyclohexane and ammonium nitrate ⁽⁶⁾ was adopted on 16 December 2008. The restrictions concerned have not yet been included in Annex XVII to that Regulation. Annex XVII should be amended to incorporate the restrictions on certain measuring devices containing mercury adopted under Directive 2007/51/EC and the restrictions on 2(2-methoxyethoxy)ethanol, 2-(2-butoxyethoxy)ethanol, methylene-diphenyl diisocyanate, cyclohexane and ammonium nitrate adopted under Decision 1348/2008/EC.
- (6) Account should be taken of the relevant provisions of Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directive 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 ⁽⁷⁾.
- (7) Since the provisions of Title VIII of Regulation (EC) No 1907/2006 and in particular Annex XVII are to be directly applicable from 1 June 2009, the restrictions should be clearly drafted in order to allow operators and enforcement authorities to apply them correctly. Therefore the drafting of the restrictions should be reviewed. The terminology for the different entries should be harmonised and made more coherent with the definitions contained in Regulation (EC) No 1907/2006.

⁽¹⁾ OJ L 396, 30.12.2006, p. 1.

⁽²⁾ OJ L 262, 27.9.1976, p. 201.

⁽³⁾ OJ L 372, 27.12.2006, p. 32.

⁽⁴⁾ OJ L 384, 29.12.2006, p. 94.

⁽⁵⁾ OJ L 257, 3.10.2007, p. 13.

⁽⁶⁾ OJ L 348, 24.12.2008, p. 108.

⁽⁷⁾ OJ L 353, 31.12.2008, p. 1.

- (8) Council Directive 96/59/EC of 16 September 1996 on the disposal of polychlorinated biphenyls and polychlorinated terphenyls (PCB/PCT) ⁽¹⁾ requires that equipment containing PCBs and PCTs is decontaminated and disposed of as soon as possible and sets the conditions for the decontamination of equipment containing those substances. Therefore the entry in Annex XVII to Regulation (EC) 1907/2006 concerning PCTs should not include provision concerning equipment containing PCTs as that is fully regulated under Directive 96/59/EC.
- (9) The existing restrictions for the substances 2-naphthylamine, benzidine, 4-nitrobiphenyl, 4-aminobiphenyl are ambiguous as it is unclear whether the prohibition concerns only the supply to the general public or also the supply to professional users. These should be clarified. Since Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents ⁽²⁾ prohibits the production, manufacture and use at work of those substances, the restrictions in Annex XVII to Regulation (EC) No 1907/2006 relating to those substances should be consistent with Directive 98/24/EC.
- (10) The substances carbon tetrachloride and 1,1,1-trichloroethane are severely restricted under Regulation (EC) No 2037/2000 of the European Parliament and of the Council of 29 June 2000 on substances that deplete the ozone layer ⁽³⁾. Regulation (EC) No 2037/2000 imposes for carbon tetrachloride a prohibition with exceptions, and for 1,1,1-trichloroethane a complete prohibition. Restrictions on carbon tetrachloride and 1,1,1-trichloroethane in Annex XVII to Regulation (EC) 1907/2006 are therefore superfluous and should be deleted.
- (11) Since mercury in batteries is regulated under Directive 2006/66/EC of the European Parliament and of the Council of 6 September 2006 on batteries and accumulators and waste batteries and accumulators ⁽⁴⁾, the provisions on mercury in batteries currently contained in Annex XVII to Regulation (EC) No 1907/2006 are superfluous and should therefore be deleted.
- (12) Pursuant to Article 2(2) of Regulation (EC) No 1907/2006, waste is considered not to be a substance, a mixture, or an article within the meaning of Article 3 of Regulation (EC) No 1907/2006. Therefore since waste is not covered by the restrictions under that Regulation, provisions in Annex XVII thereto excluding waste are redundant and should be deleted.
- (13) Certain restrictions in Annex XVII to Regulation (EC) 1907/2006 should be modified in order to take into account the definitions of 'use' and 'placing on the market' contained in Article 3 of that Regulation.
- (14) The entry in Annex I to Directive 76/769/EEC concerning asbestos fibres included an exemption for diaphragms containing chrysotile. It should be specified that this exemption will be reviewed following receipt of reports that Member States who are making use of this exemption will have to submit. Moreover in the light of the definition of 'placing on the market' in Regulation (EC) No 1907/2006, Member States should be able to allow the placing on the market of some articles containing such fibres where the articles were already installed or in service before 1 January 2005, under specific conditions ensuring a high level of protection of human health
- (15) It should be made clear that, for substances which have been incorporated in Annex XVII to Regulation (EC) No 1907/2006 as a consequence of restrictions adopted in the framework of Directive 76/769/EEC (Entries 1 to 58), the restrictions do not apply to storage, keeping, treatment, filling into containers, or transfer from one container to another of the substances for export, unless the manufacture of the substances is prohibited.
- (16) Unlike Directive 76/769/EEC, Regulation (EC) No 1907/2006 defines the term 'article'. In order to cover the same items as provided for in the original restriction on Cadmium, the term 'mixtures' should be added in some of the provisions.
- (17) It should be made clear that the restrictions incorporated in Annex XVII to Regulation (EC) No 1907/2006 on the marketing of certain measuring devices containing mercury do not apply to devices already in use in the Community at the time of entry into force of the restriction.
- (18) In the entries in Annex XVII to Regulation (EC) 1907/2006 for the substances diphenylether, pentabromo derivatives and diphenylether, octabromo derivatives, it should be provided that the restrictions do not apply to articles already in use at the date from which the restriction was to apply as those substances were incorporated in articles which have a long lifecycle and are sold on the second hand market, such as aeroplanes and vehicles. Moreover, since the use of the substances in electrical and electronic equipments is regulated under Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain hazardous substances in electrical and electronic equipment ⁽⁵⁾ that equipment should not be subject to the restrictions concerned.

⁽¹⁾ OJ L 243, 24.9.1996, p. 31.

⁽²⁾ OJ L 131, 5.5.1998, p. 11.

⁽³⁾ OJ L 244, 29.9.2000, p. 1.

⁽⁴⁾ OJ L 266, 26.9.2006, p. 1.

⁽⁵⁾ OJ L 37, 13.2.2003, p. 19.

- (19) It should be made clear in the restriction on nonylphenol and nonylphenol ethoxylate that the validity of existing national authorisations for pesticides and biocidal products containing nonylphenol ethoxylate as co-formulant should not be affected, as provided for in Article 1(2) of Directive 2003/53/EC of the European Parliament and of the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement) ⁽¹⁾.
- (20) It should be made clear that the restriction incorporated in Annex XVII to Regulation (EC) No 1907/2006 concerning perfluorooctane sulfonates does not apply to products which were already in use in the Community when the restriction entered into force.

- (21) The measures provided for in this Regulation are in accordance with the opinion of the Committee established under Article 133 of Regulation (EC) No 1907/2006,

HAS ADOPTED THIS REGULATION:

Article 1

Annex XVII to Regulation (EC) No 1907/2006 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the day following its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 22 June 2009.

For the Commission

Günter VERHEUGEN

Vice-President

⁽¹⁾ OJ L 178, 17.7.2003, p. 24.

ANNEX

Annex XVII to Regulation (EC) No 1907/2006 is amended as follows:

1. the title is replaced by the following:

'Restrictions on the manufacture, placing on the market and use of certain dangerous substances, mixtures and articles';

2. the table setting out the designation of the substances, groups of substances and mixtures and the conditions of restriction is replaced by the following:

'For substances which have been incorporated in this Annex as a consequence of restrictions adopted in the framework of Directive 76/769/EEC (Entries 1 to 58), the restrictions shall not apply to storage, keeping, treatment, filling into containers, or transfer from one container to another of these substances for export, unless the manufacture of the substances is prohibited.

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
1. Polychlorinated terphenyls (PCTs)	Shall not be placed on the market, or used: <ul style="list-style-type: none"> — as substances, — in mixtures, including waste oils, or in equipment, in concentrations greater than 50 mg/kg (0,005 % by weight).
2. Chloroethene (vinyl chloride) CAS No 75-01-4 EC No 200-831-0	Shall not be used as propellant in aerosols for any use. Aerosols dispensers containing the substance as propellant shall not be placed on the market.
3. Liquid substances or mixtures, which are regarded as dangerous according to the definitions in Council Directive 67/548/EEC and Directive 1999/45/EC.	<ol style="list-style-type: none"> 1. Shall not be used in: <ul style="list-style-type: none"> — ornamental articles, intended to produce light or colour effects by means of different phases, for example in ornamental lamps and ashtrays, — tricks and jokes, — games for one or more participants, or any article intended to be used as such, even with ornamental aspects. 2. Articles not complying with paragraph 1 shall not be placed on the market. 3. Shall not be placed on the market if they contain a colouring agent unless required for fiscal reasons or perfume or both and if they: <ul style="list-style-type: none"> — present an aspiration hazard and are labelled with R65 or H304, and, — can be used as fuel in decorative lamps, and, — are packaged in containers of a capacity of 15 litres or less. 4. Without prejudice to the implementation of other Community provisions relating to the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of substances and mixtures covered by paragraph 3, where intended for use in lamps, is marked visibly, legibly and indelibly as follows: <p>"Keep lamps filled with this liquid out of the reach of children".</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>4. Tris (2,3 dibromopropyl) phosphate CAS No 126-72-7</p>	<p>1. Shall not be used in textile articles, such as garments, undergarments and linen, intended to come into contact with the skin.</p> <p>2. Articles not complying with paragraph 1 shall not be placed on the market.</p>
<p>5. Benzene CAS No 71-43-2 EC No 200-753-7</p>	<p>1. Shall not be used in toys or parts of toys where the concentration of benzene in the free state is greater than 5 mg/kg (0,0005 %) of the weight of the toy or part of toy.</p> <p>2. Toys and parts of toys not complying with paragraph 1 shall not be placed on the market.</p> <p>3. Shall not be placed on the market, or used,</p> <ul style="list-style-type: none"> — as a substance, — as a constituent of other substances, or in mixtures, in concentrations equal to, or greater than 0,1 % by weight. <p>4. However, paragraph 3 shall not apply to:</p> <ul style="list-style-type: none"> (a) motor fuels which are covered by Directive 98/70/EC; (b) substances and mixtures for use in industrial processes not allowing for the emission of benzene in quantities in excess of those laid down in existing legislation.
<p>6. Asbestos fibres</p> <p>(a) Crocidolite CAS No 12001-28-4</p> <p>(b) Amosite CAS No 12172-73-5</p> <p>(c) Anthophyllite CAS No 77536-67-5</p> <p>(d) Actinolite CAS No 77536-66-4</p> <p>(e) Tremolite CAS No 77536-68-6</p> <p>(f) Chrysotile CAS No 12001-29-5 CAS No 132207-32-0</p>	<p>1. The manufacture, placing on the market and use of these fibres and of articles containing these fibres added intentionally is prohibited.</p> <p>However, Member States may exempt the placing on the market and use of diaphragms containing chrysotile (point (f)) for existing electrolysis installations until they reach the end of their service life, or until suitable asbestos-free substitutes become available, whichever is the sooner.</p> <p>By 1 June 2011 Member States making use of this exemption shall provide a report to the Commission on the availability of asbestos free substitutes for electrolysis installations and the efforts undertaken to develop such alternatives, on the protection of the health of workers in the installations, on the source and quantities of chrysotile, on the source and quantities of diaphragms containing chrysotile, and the envisaged date of the end of the exemption. The Commission shall make this information publicly available.</p> <p>Following receipt of those reports, the Commission shall request the Agency to prepare a dossier in accordance with Article 69 with a view to prohibit the placing on the market and use of diaphragms containing chrysotile.</p> <p>2. The use of articles containing asbestos fibres referred to in paragraph 1 which were already installed and/or in service before 1 January 2005 shall continue to be permitted until they are disposed of or reach the end of their service life. However, Member States may, for reasons of protection of human health, restrict, prohibit or make subject to specific conditions, the use of such articles before they are disposed of or reach the end of their service life.</p> <p>Member States may allow placing on the market of articles in their entirety containing asbestos fibres referred to in paragraph 1 which were already installed and/or in service before 1 January 2005, under specific conditions ensuring a high level of protection of human health. Member States shall communicate these national measures to the Commission by 1 June 2011. The Commission shall make this information publicly available.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	3. Without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances and mixtures, the placing on the market and use of articles containing these fibres, as permitted according to the preceding derogations, shall be permitted only if suppliers ensure before the placing on the market that articles bear a label in accordance with Appendix 7 to this Annex.
7. Tris(aziridinyl)phosphin oxide CAS No 545-55-1 EC No 208-892-5	1. Shall not be used in textile articles, such as garments, undergarments and linen, intended to come into contact with the skin. 2. Articles not complying with paragraph 1 shall not be placed on the market.
8. Polybromobiphenyls; Polybrominatedbiphenyls (PBB) CAS No 59536-65-1	1. Shall not be used in textile articles, such as garments, undergarments and linen, intended to come into contact with the skin. 2. Articles not complying with paragraph 1 shall not be placed on the market.
9. (a) Soap bark powder (<i>Quillaja saponaria</i>) and its derivatives containing saponines CAS No 68990-67-0 EC 273-620-4 (b) Powder of the roots of <i>Helleborus viridis</i> and <i>Helleborus niger</i> (c) Powder of the roots of <i>Veratrum album</i> and <i>Veratrum nigrum</i> (d) Benzidine and/or its derivatives CAS No 92-87-5 EC No 202-199-1 (e) o-Nitrobenzaldehyde CAS No 552-89-6 EC No 209-025-3 (f) Wood powder	1. Shall not be used, in jokes and hoaxes or in mixtures or articles intended to be used as such, for instance as a constituent of sneezing powder and stink bombs. 2. Jokes and hoaxes, or mixtures or articles intended to be used as such, not complying with paragraph 1 shall not be placed on the market. 3. However, paragraphs 1 and 2 shall not apply to stink bombs containing not more than 1,5 ml of liquid.
10. (a) Ammonium sulphide CAS No 12135-76-1 EC No 235-223-4 (b) Ammonium hydrogen sulphide CAS No 12124-99-1 EC No 235-184-3 (c) Ammonium polysulphide CAS No 9080-17-5 EC No 232-989-1	1. Shall not be used, in jokes and hoaxes or in mixtures or articles intended to be used as such, for instance as a constituent of sneezing powder and stink bombs. 2. Jokes and hoaxes, or mixtures or articles intended to be used as such, not complying with paragraph 1 shall not be placed on the market. 3. However, paragraphs 1 and 2 shall not apply to stink bombs containing not more than 1,5 ml of liquid.

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>11. Volatile esters of bromoacetic acids:</p> <p>(a) Methyl bromoacetate CAS No 96-32-2 EC No 202-499-2</p> <p>(b) Ethyl bromoacetate CAS No 105-36-2 EC No 203-290-9</p> <p>(c) Propyl bromoacetate CAS No 35223-80-4</p> <p>(d) Butyl bromoacetate CAS No 18991-98-5 EC No 242-729-9</p>	<p>1. Shall not be used, in jokes and hoaxes or in mixtures or articles intended to be used as such, for instance as a constituent of sneezing powder and stink bombs.</p> <p>2. Jokes and hoaxes, or mixtures or articles intended to be used as such, not complying with paragraph 1 shall not be placed on the market.</p> <p>3. However, paragraphs 1 and 2 shall not apply to stink bombs containing not more than 1,5 ml of liquid.</p>
<p>12. 2-Naphthylamine CAS No 91-59-8 EC No 202-080-4 and its salts</p> <p>13. Benzidine CAS No 92-87-5 EC No 202-199-1 and its salts</p> <p>14. 4-Nitrobiphenyl CAS No 92-93-3 Einecs EC No 202-204-7</p> <p>15. 4-Aminobiphenyl xenylamine CAS No 92-67-1 Einecs EC No 202-177-1 and its salts</p>	<p>The following shall apply to entries 12 to 15:</p> <p>Shall not be placed on the market, or used, as substances or in mixtures in concentrations greater than 0,1 % by weight.</p>
<p>16. Lead carbonates:</p> <p>(a) Neutral anhydrous carbonate (PbCO₃) CAS No 598-63-0 EC No 209-943-4</p> <p>(b) Trilead-bis(carbonate)-dihydroxide 2Pb CO₃-Pb(OH)₂ CAS No 1319-46-6 EC No 215-290-6</p>	<p>Shall not be placed on the market, or used, as substances or in mixtures, where the substance or mixture is intended for use as paint.</p> <p>However, Member States may, in accordance with the provisions of International Labour Organisation (ILO) Convention 13 on the use of white lead and sulphates of lead in paint, permit the use on their territory of the substance or mixture for the restoration and maintenance of works of art and historic buildings and their interiors.</p>
<p>17. Lead sulphates:</p> <p>(a) PbSO₄ CAS No 7446-14-2 EC No 231-198-9</p> <p>(b) Pb_x SO₄ CAS No 15739-80-7 EC No 239-831-0</p>	<p>Shall not be placed on the market, or used, as substances or in mixtures, where the substance or mixture is intended for use as paint.</p> <p>However, Member States may, in accordance with the provisions of ILO Convention 13 on the use of white lead and sulphates of lead in paint, permit the use on their territory of the substance or mixture for the restoration and maintenance of works of art and historic buildings and their interiors.</p>
<p>18. Mercury compounds</p>	<p>Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is intended for use:</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>(a) to prevent the fouling by micro-organisms, plants or animals of:</p> <ul style="list-style-type: none"> — the hulls of boats, — cages, floats, nets and any other appliances or equipment used for fish or shellfish farming, — any totally or partly submerged appliances or equipment; <p>(b) in the preservation of wood;</p> <p>(c) in the impregnation of heavy-duty industrial textiles and yarn intended for their manufacture;</p> <p>(d) in the treatment of industrial waters, irrespective of their use.</p>
<p>18a. Mercury</p> <p>CAS No 7439-97-6</p> <p>EC No 231-106-7</p>	<p>1. Shall not be placed on the market:</p> <p>(a) in fever thermometers;</p> <p>(b) in other measuring devices intended for sale to the general public (such as manometers, barometers, sphygmomanometers, thermometers other than fever thermometers).</p> <p>2. The restriction in paragraph 1 shall not apply to measuring devices that were in use in the Community before 3 April 2009. However Member States may restrict or prohibit the placing on the market of such measuring devices.</p> <p>3. The restriction in paragraph 1(b) shall not apply to:</p> <p>(a) measuring devices more than 50 years old on 3 October 2007;</p> <p>(b) barometers (except barometers within point (a)) until 3 October 2009.</p> <p>4. By 3 October 2009 the Commission shall carry out a review of the availability of reliable safer alternatives that are technically and economically feasible for mercury containing sphygmomanometers and other measuring devices in healthcare and in other professional and industrial uses. On the basis of this review or as soon as new information on reliable safer alternatives for sphygmomanometers and other measuring devices containing mercury becomes available, the Commission shall, if appropriate, present a legislative proposal to extend the restrictions in paragraph 1 to sphygmomanometers and other measuring devices in healthcare and in other professional and industrial uses, so that mercury in measuring devices is phased out whenever technically and economically feasible.</p>
<p>19. Arsenic compounds</p>	<p>1. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is intended for use to prevent the fouling by micro-organisms, plants or animals of:</p> <ul style="list-style-type: none"> — the hulls of boats, — cages, floats, nets and any other appliances or equipment used for fish or shellfish farming, — any totally or partly submerged appliances or equipment. <p>2. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is intended for use in the treatment of industrial waters, irrespective of their use.</p> <p>3. Shall not be used in the preservation of wood. Furthermore, wood so treated shall not be placed on the market.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>4. By way of derogation from paragraph 3:</p> <p>(a) Relating to the substances and mixtures for the preservation of wood: these may only be used in industrial installations using vacuum or pressure to impregnate wood if they are solutions of inorganic compounds of the copper, chromium, arsenic (CCA) type C and if they are authorised in accordance with Article 5(1) of Directive 98/8/EC. Wood so treated shall not be placed on the market before fixation of the preservative is completed.</p> <p>(b) Wood treated with CCA solution in accordance with point (a) may be placed on the market for professional and industrial use provided that the structural integrity of the wood is required for human or livestock safety and skin contact by the general public during its service life is unlikely:</p> <ul style="list-style-type: none"> — as structural timber in public and agricultural buildings, office buildings, and industrial premises, — in bridges and bridgework, — as constructional timber in freshwater areas and brackish waters, for example jetties and bridges, — as noise barriers, — in avalanche control, — in highway safety fencing and barriers, — as debarked round conifer livestock fence posts, — in earth retaining structures, — as electric power transmission and telecommunications poles, — as underground railway sleepers. <p>(c) Without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that all treated wood placed on the market is individually labelled "For professional and industrial installation and use only, contains arsenic". In addition, all wood placed on the market in packs shall also bear a label stating "Wear gloves when handling this wood. Wear a dust mask and eye protection when cutting or otherwise crafting this wood. Waste from this wood shall be treated as hazardous by an authorised undertaking".</p> <p>(d) Treated wood referred to under point (a) shall not be used:</p> <ul style="list-style-type: none"> — in residential or domestic constructions, whatever the purpose, — in any application where there is a risk of repeated skin contact, — in marine waters, — for agricultural purposes other than for livestock fence posts and structural uses in accordance with point (b), — in any application where the treated wood may come into contact with intermediate or finished products intended for human and/or animal consumption. <p>5. Wood treated with arsenic compounds that was in use in the Community before 30 September 2007, or that was placed on the market in accordance with paragraph 4 may remain in place and continue to be used until it reaches the end of its service life.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>6. Wood treated with CCA type C that was in use in the Community before 30 September 2007, or that was placed on the market in accordance with paragraph 4:</p> <ul style="list-style-type: none"> — may be used or reused subject to the conditions pertaining to its use listed under points 4(b), (c) and (d), — may be placed on the market subject to the conditions pertaining to its use listed under points 4(b), (c) and (d). <p>7. Member States may allow wood treated with other types of CCA solutions that was in use in the Community before 30 September 2007:</p> <ul style="list-style-type: none"> — to be used or reused subject to the conditions pertaining to its use listed under points 4 (b), (c) and (d), — to be placed on the market subject to the conditions pertaining to its use listed under points 4(b), (c) and (d).
<p>20. Organostannic compounds</p>	<p>1. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is acting as biocide in free association paint.</p> <p>2. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture acts as biocide to prevent the fouling by micro-organisms, plants or animals of:</p> <ul style="list-style-type: none"> (a) all craft irrespective of their length intended for use in marine, coastal, estuarine and inland waterways and lakes; (b) cages, floats, nets and any other appliances or equipment used for fish or shellfish farming; (c) any totally or partly submerged appliance or equipment. <p>3. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is intended for use in the treatment of industrial waters.</p>
<p>21. Di-μ-oxo-di-n-butylstanniohydroxyborane/Dibutyltin hydrogen borate C₈H₁₉BO₃Sn (DBB)</p> <p>CAS No 75113-37-0 EC No 401-040-5</p>	<p>Shall not be placed on the market, or used, as a substance, or in mixtures in a concentration equal to, or greater than 0,1 % by weight.</p> <p>However, the first paragraph shall not apply to this substance (DBB) or mixtures containing it if these are intended solely for conversion into articles, among which this substance will no longer feature in a concentration equal to or greater than 0,1 %.</p>
<p>22. Pentachlorophenol</p> <p>CAS No 87-86-5 EC No 201-778-6 and its salts and esters</p>	<p>Shall not be placed on the market, or used,</p> <ul style="list-style-type: none"> — as a substance, — as a constituent in other substances, or in mixtures, in a concentration equal to or greater than 0,1 % by weight.
<p>23. Cadmium</p> <p>CAS No 7440-43-9 EC No 231-152-8 and its compounds</p>	<p>For the purpose of this entry, the codes and chapters indicated in square brackets are the codes and chapters of the tariff and statistical nomenclature of Common Customs Tariff as established by Council Regulation (EEC) No 2658/87 (*).</p> <p>1. Shall not be used to give colour to articles manufactured from the following substances and mixtures:</p> <ul style="list-style-type: none"> (a) — polyvinyl chloride (PVC) [3904 10] [3904 21] [3904 22] — polyurethane (PUR) [3909 50]

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<ul style="list-style-type: none"> — low-density polyethylene (ld PE), with the exception of low-density polyethylene used for the production of coloured masterbatch [3901 10] — cellulose acetate (CA) [3912 11] [3912 12] — cellulose acetate butyrate (CAB) [3912 11] [3912 12] — epoxy resins [3907 30] — melamine — formaldehyde (MF) resins [3909 20] — urea — formaldehyde (UF) resins [3909 10] — unsaturated polyesters (UP) [3907 91] — polyethylene terephthalate (PET) [3907 60] — polybutylene terephthalate (PBT) — transparent/general-purpose polystyrene [3903 11] [3903 19] — acrylonitrile methylmethacrylate (AMMA) — cross-linked polyethylene (VPE) — high-impact polystyrene — polypropylene (PP) [3902 10] <p>(b) paints [3208] [3209]</p> <p>However, if the paints have a high zinc content, their residual concentration of cadmium shall be as low as possible and shall in any event be less than 0,1 % by weight.</p> <p>In any case, whatever their use or intended final purpose, articles or components of articles manufactured from the substances and mixtures listed above coloured with cadmium shall not be placed on the market if their cadmium content (expressed as Cd metal) is greater than 0,01 % by weight of the plastic material.</p> <p>2. However, paragraph 1 shall not apply to articles to be coloured for safety reasons.</p> <p>3. Shall not be used to stabilise the following mixtures or articles manufactured from polymers or copolymers of vinyl chloride:</p> <ul style="list-style-type: none"> — packaging materials (bags, containers, bottles, lids) [3923 29 10], — office or school supplies [3926 10], — fittings for furniture, coachwork or the like [3926 30], — articles of apparel and clothing accessories (including gloves) [3926 20], — floor and wall coverings [3918 10], — impregnated, coated, covered or laminated textile fabrics [5903 10], — imitation leather [4202], — gramophone records, — tubes and pipes and their fittings [3917 23], — swing doors, — vehicles for road transport (interior, exterior, underbody), — coating of steel sheet used in construction or in industry, — insulation for electrical wiring.

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>In any case, whatever their use or intended final purpose, the placing on the market of the above mixtures, articles or components of articles manufactured from polymers or copolymers of vinyl chloride, stabilised by substances containing cadmium is prohibited, if their cadmium content (expressed as Cd metal) exceeds 0,01 % by weight of the polymer.</p> <p>4. However, paragraph 3 shall not apply to mixtures and articles using cadmium-based stabilisers for safety reasons.</p> <p>5. For the purpose of this entry, "cadmium plating" means any deposit or coating of metallic cadmium on a metallic surface.</p> <p>Shall not be used for cadmium plating metallic articles or components of the articles used in the following sectors/applications:</p> <p>(a) equipment and machinery for:</p> <ul style="list-style-type: none"> — food production [8210] [8417 20] [8419 81] [8421 11] [8421 22] [8422] [8435] [8437] [8438] [8476 11] — agriculture [8419 31] [8424 81] [8432] [8433] [8434] [8436] — cooling and freezing [8418] — printing and book-binding [8440] [8442] [8443] <p>(b) equipment and machinery for the production of:</p> <ul style="list-style-type: none"> — household goods [7321] [8421 12] [8450] [8509] [8516] — furniture [8465] [8466] [9401] [9402] [9403] [9404] — sanitary ware [7324] — central heating and air conditioning plant [7322] [8403] [8404] [8415] <p>In any case, whatever their use or intended final purpose, the placing on the market of cadmium-plated articles or components of such articles used in the sectors/applications listed in points (a) and (b) above and of articles manufactured in the sectors listed in point (b) above is prohibited.</p> <p>6. The provisions referred to in paragraph 5 shall also be applicable to cadmium-plated articles or components of such articles when used in the sectors/applications listed in points (a) and (b) below and to articles manufactured in the sectors listed in (b) below:</p> <p>(a) equipment and machinery for the production of:</p> <ul style="list-style-type: none"> — paper and board [8419 32] [8439] [8441] textiles and clothing [8444] [8445] [8447] [8448] [8449] [8451] [8452] <p>(b) equipment and machinery for the production of:</p> <ul style="list-style-type: none"> — industrial handling equipment and machinery [8425] [8426] [8427] [8428] [8429] [8430] [8431] — road and agricultural vehicles [chapter 87] — rolling stock [chapter 86] — vessels [chapter 89] <p>7. However, the restrictions in paragraphs 5 and 6 shall not apply to:</p> <ul style="list-style-type: none"> — articles and components of the articles used in the aeronautical, aerospace, mining, offshore and nuclear sectors whose applications require high safety standards and in safety devices in road and agricultural vehicles, rolling stock and vessels, — electrical contacts in any sector of use, where that is necessary to ensure the reliability required of the apparatus on which they are installed. <p>(*) OJ L 256, 7.9.1987, p. 42.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>24. Monomethyl — tetrachlorodiphenyl methane</p> <p>Trade name: Ugilec 141</p> <p>CAS No 76253-60-6</p>	<p>1. Shall not be placed on the market, or used, as a substance or in mixtures.</p> <p>Articles containing the substance shall not be placed on the market.</p> <p>2. By way of derogation, paragraph 1 shall not apply:</p> <p>(a) in the case of plant and machinery already in service on 18 June 1994, until such plant and machinery is disposed of;</p> <p>(b) in the case of the maintenance of plant and machinery already in service within a Member State on 18 June 1994.</p> <p>For the purposes of point (a) Member States may, on grounds of human health protection and environmental protection, prohibit within their territory the use of such plant or machinery before it is disposed of.</p>
<p>25. Monomethyl-dichloro-diphenyl methane</p> <p>Trade name: Ugilec 121</p> <p>Ugilec 21</p>	<p>Shall not be placed on the market, or used, as a substance or in mixtures.</p> <p>Articles containing the substance shall not be placed on the market.</p>
<p>26. Monomethyl-dibromo-diphenyl methane bromobenzylbromotoluene, mixture of isomers</p> <p>Trade name: DBBT</p> <p>CAS No 99688-47-8</p>	<p>Shall not be placed on the market, or used, as a substance or in mixtures.</p> <p>Articles containing the substance shall not be placed on the market.</p>
<p>27. Nickel</p> <p>CAS No 7440-02-0</p> <p>EC No 231-111-4 and its compounds</p>	<p>1. Shall not be used:</p> <p>(a) in any post assemblies which are inserted into pierced ears and other pierced parts of the human body unless the rate of nickel release from such post assemblies is less than 0,2 µg/cm²/week (migration limit);</p> <p>(b) in articles intended to come into direct and prolonged contact with the skin such as:</p> <ul style="list-style-type: none"> — earrings, — necklaces, bracelets and chains, anklets, finger rings, — wrist-watch cases, watch straps and tighteners, — rivet buttons, tighteners, rivets, zippers and metal marks, when these are used in garments, <p>if the rate of nickel release from the parts of these articles coming into direct and prolonged contact with the skin is greater than 0,5 µg/cm²/week.</p> <p>(c) in articles referred to in point (b) where these have a non-nickel coating unless such coating is sufficient to ensure that the rate of nickel release from those parts of such articles coming into direct and prolonged contact with the skin will not exceed 0,5 µg/cm²/week for a period of at least two years of normal use of the article.</p> <p>2. Articles which are the subject of paragraph 1 shall not be placed on the market unless they conform to the requirements set out in that paragraph.</p> <p>3. The standards adopted by the European Committee for Standardisation (CEN) shall be used as the test methods for demonstrating the conformity of articles to paragraphs 1 and 2.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>28. Substances which appear in Part 3 of Annex VI to Regulation (EC) No 1272/2008 classified as carcinogen category 1A or 1B (Table 3.1) or carcinogen category 1 or 2 (Table 3.2) and listed as follows:</p> <ul style="list-style-type: none"> — Carcinogen category 1A (Table 3.1)/carcinogen category 1 (Table 3.2) listed in Appendix 1 — Carcinogen category 1B (Table 3.1)/carcinogen category 2 (Table 3.2) listed in Appendix 2 <p>29. Substances which appear in Part 3 of Annex VI to Regulation (EC) No 1272/2008 classified as germ cell mutagen category 1A or 1B (Table 3.1) or mutagen category 1 or 2 (Table 3.2) and listed as follows:</p> <ul style="list-style-type: none"> — Mutagen category 1A (Table 3.1)/mutagen category 1 (Table 3.2) listed in Appendix 3 — Mutagen category 1B (Table 3.1)/mutagen category 2 (Table 3.2) listed in Appendix 4 <p>30. Substances which appear in Part 3 of Annex VI to Regulation (EC) No 1272/2008 classified as toxic to reproduction category 1A or 1B (Table 3.1) or toxic to reproduction category 1 or 2 (Table 3.2) and listed as follows:</p> <ul style="list-style-type: none"> — Reproductive toxicant category 1A adverse effects on sexual function and fertility or on development (Table 3.1) or reproductive toxicant category 1 with R60 (May impair fertility) or R61 (May cause harm to the unborn child) (Table 3.2) listed in Appendix 5 — Reproductive toxicant category 1B adverse effects on sexual function and fertility or on development (Table 3.1) or reproductive toxicant category 2 with R60 (May impair fertility) or R61 (May cause harm to the unborn child) (Table 3.2) listed in Appendix 6 	<p>Without prejudice to the other parts of this Annex the following shall apply to entries 28 to 30:</p> <p>1. Shall not be placed on the market, or used,</p> <ul style="list-style-type: none"> — as substances, — as constituents of other substances, or, — in mixtures, <p>for supply to the general public when the individual concentration in the substance or mixture is equal to or greater than:</p> <ul style="list-style-type: none"> — either the relevant specific concentration limit specified in Part 3 of Annex VI to Regulation (EC) No 1272/2008, or, — the relevant concentration specified in Directive 1999/45/EC. <p>Without prejudice to the implementation of other Community provisions relating to the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures is marked visibly, legibly and indelibly as follows:</p> <p>“Restricted to professional users”.</p> <p>2. By way of derogation, paragraph 1 shall not apply to:</p> <ul style="list-style-type: none"> (a) medicinal or veterinary products as defined by Directive 2001/82/EC and Directive 2001/83/EC; (b) cosmetic products as defined by Directive 76/768/EEC; (c) the following fuels and oil products: <ul style="list-style-type: none"> — motor fuels which are covered by Directive 98/70/EC, — mineral oil products intended for use as fuel in mobile or fixed combustion plants, — fuels sold in closed systems (e.g. liquid gas bottles); (d) artists' paints covered by Directive 1999/45/EC.

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>31. (a) Creosote; wash oil CAS No 8001-58-9 EC No 232-287-5</p> <p>(b) Creosote oil; wash oil CAS No 61789-28-4 EC No 263-047-8</p> <p>(c) Distillates (coal tar), naphthalene oils; naphthalene oil CAS No 84650-04-4 EC No 283-484-8</p> <p>(d) Creosote oil, acenaphthene fraction; wash oil CAS No 90640-84-9 EC No 283-484-8 EC No 292-605-3</p> <p>(e) Distillates (coal tar), upper; heavy anthracene oil CAS No 65996-91-0 EC No 266-026-1</p> <p>(f) Anthracene oil CAS No 90640-80-5 EC No 292-602-7</p> <p>(g) Tar acids, coal, crude; crude phenols CAS No 65996-85-2 EC No 266-019-3</p> <p>(h) Creosote, wood CAS No 8021-39-4 EC No 232-419-1</p> <p>(i) Low temperature tar oil, alkaline; extract residues (coal), low temperature coal tar alkaline CAS No 122384-78-5 EC No 310-191-5</p>	<p>1. Shall not be placed on the market, or used, as substances or in mixtures where the substance or mixture is intended for the treatment of wood. Furthermore, wood so treated shall not be placed on the market.</p> <p>2. By way of derogation from paragraph 1:</p> <p>(a) The substances and mixtures may be used for wood treatment in industrial installations or by professionals covered by Community legislation on the protection of workers for in situ retreatment only if they contain:</p> <p>(i) benzo[a]pyrene at a concentration of less than 50 mg/kg (0,005 % by weight), and</p> <p>(ii) water extractable phenols at a concentration of less than 3 % by weight.</p> <p>Such substances and mixtures for use in wood treatment in industrial installations or by professionals:</p> <p>— may be placed on the market only in packaging of a capacity equal to or greater than 20 litres,</p> <p>— shall not be sold to consumers.</p> <p>Without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures is visibly, legibly and indelibly marked as follows:</p> <p>“For use in industrial installations or professional treatment only”.</p> <p>(b) Wood treated in industrial installations or by professionals according to subparagraph (a) which is placed on the market for the first time or retreated in situ may be used for professional and industrial use only, for example on railways, in electric power transmission and telecommunications, for fencing, for agricultural purposes (for example stakes for tree support) and in harbours and waterways.</p> <p>(c) The prohibition in paragraph 1 on the placing on the market shall not apply to wood which has been treated with substances listed in entry 31 (a) to (i) before 31 December 2002 and is placed on the second-hand market for re-use.</p> <p>3. Treated wood referred to under paragraph 2(b) and (c) shall not be used:</p> <p>— inside buildings, whatever their purpose,</p> <p>— in toys,</p> <p>— in playgrounds,</p> <p>— in parks, gardens, and outdoor recreational and leisure facilities where there is a risk of frequent skin contact,</p> <p>— in the manufacture of garden furniture such as picnic tables,</p> <p>— for the manufacture and use and any re-treatment of:</p> <p>— containers intended for growing purposes,</p> <p>— packaging that may come into contact with raw materials, intermediate or finished products destined for human and/or animal consumption,</p> <p>— other materials which may contaminate the articles mentioned above.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
32. Chloroform CAS No 67-66-3 EC No 200-663-8	Without prejudice to the other parts of this Annex, the following shall apply to entries 32 to 38. 1. Shall not be placed on the market, or used,
34. 1,1,2-Trichloroethane CAS No 79-00-5 EC No 201-166-9	— as substances, — as constituents of other substances, or in mixtures in concentrations equal to or greater than 0,1 % by weight,
35. 1,1,2,2-Tetrachloroethane CAS No 79-34-5 EC No 201-197-8	where the substance or mixture is intended for supply to the general public and/or is intended for diffusive applications such as in surface cleaning and cleaning of fabrics.
36. 1,1,1,2-Tetrachloroethane CAS No 630-20-6	2. Without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of such substances and mixtures containing them in concentrations equal to or greater than 0,1 % by weight is visibly, legibly and indelibly marked as follows:
37. Pentachloroethane CAS No 76-01-7 EC No 200-925-1	“For use in industrial installations only”.
38. 1,1-Dichloroethene CAS No 75-35-4 EC No 200-864-0	By way of derogation this provision shall not apply to: (a) medicinal or veterinary products as defined by Directive 2001/82/EC and Directive 2001/83/EC; (b) cosmetic products as defined by Directive 76/768/EEC.
40. Substances meeting the criteria of flammability in Directive 67/548/EEC and classified as flammable, highly flammable or extremely flammable regardless of whether they appear in Part 3 of Annex VI to Regulation (EC) No 1272/2008 or not.	1. Shall not be used, as substance or as mixtures in aerosol dispensers where these aerosol dispensers are intended for supply to the general public for entertainment and decorative purposes such as the following: — metallic glitter intended mainly for decoration, — artificial snow and frost, — “whoopee” cushions, — silly string aerosols, — imitation excrement, — horns for parties, — decorative flakes and foams, — artificial cobwebs, — stink bombs. 2. Without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances, suppliers shall ensure before the placing on the market that the packaging of aerosol dispensers referred to above is marked visibly, legibly and indelibly with: “For professional users only”. 3. By way of derogation, paragraphs 1 and 2 shall not apply to the aerosol dispensers referred to Article 8 (1a) of Council Directive 75/324/EEC (**). 4. The aerosol dispensers referred to in paragraphs 1 and 2 shall not be placed on the market unless they conform to the requirements indicated.
41. Hexachloroethane CAS No 67-72-1 EC No 200-666-4	Shall not be placed on the market, or used, as substance or in mixtures, where the substance or mixture is intended for the manufacturing or processing of non-ferrous metals.

(**) OJ L 147, 9.6.1975, p. 40.

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>42. Alkanes, C₁₀-C₁₃, chloro (short-chain chlorinated paraffins) (SCCPs)</p> <p>EC No 287-476-5 CAS No 85535-84-8</p>	<p>Shall not be placed on the market, or used as substances, or as constituents of other substances or in mixtures in concentrations greater than 1 % by weight, where the substance or mixture is intended for:</p> <ul style="list-style-type: none"> — metalworking, — fat liquoring of leather.
<p>43. Azocolourants and Azodyes</p>	<p>1. Azodyes which, by reductive cleavage of one or more azo groups, may release one or more of the aromatic amines listed in Appendix 8, in detectable concentrations, i.e. above 30 mg/kg (0,003 % by weight) in the articles or in the dyed parts thereof, according to the testing methods listed in Appendix 10, shall not be used, in textile and leather articles which may come into direct and prolonged contact with the human skin or oral cavity, such as:</p> <ul style="list-style-type: none"> — clothing, bedding, towels, hairpieces, wigs, hats, nappies and other sanitary items, sleeping bags, — footwear, gloves, wristwatch straps, handbags, purses/wallets, briefcases, chair covers, purses worn round the neck, — textile or leather toys and toys which include textile or leather garments, — yarn and fabrics intended for use by the final consumer. <p>2. Furthermore, the textile and leather articles referred to in paragraph 1 shall not be placed on the market unless they conform to the requirements set out in that paragraph.</p> <p>3. Azodyes, which are contained in Appendix 9, "List of azodyes" shall not be placed on the market, or used, as substances, or in mixtures in concentrations greater than 0,1 % by weight, where the substance or the mixture is intended for colouring textile and leather articles.</p>
<p>44. Diphenylether, pentabromo derivative</p> <p>C₁₂H₅Br₅O</p>	<p>1. Shall not be placed on the market, or used,</p> <ul style="list-style-type: none"> — as a substance, — in mixtures in concentrations greater than 0,1 % by weight. <p>2. Articles shall not be placed on the market if they, or flame-retarded parts thereof, contain this substance in concentrations greater than 0,1 % by weight.</p> <p>3. By way of derogation, paragraph 2 shall not apply</p> <ul style="list-style-type: none"> — to articles that were in use in the Community before 15 August 2004, — to electrical and electronic equipment within the scope of Directive 2002/95/EC of the European Parliament and Council (**). <p>(**) OJ L 37, 13.2.2003, p. 19.</p>
<p>45. Diphenylether, octabromo derivative</p> <p>C₁₂H₂Br₈O</p>	<p>1. Shall not be placed on the market, or used:</p> <ul style="list-style-type: none"> — as a substance, — as a constituent of other substances, or in mixtures, in concentrations greater than 0,1 % by weight. <p>2. Articles shall not be placed on the market if they, or flame-retardant parts thereof, contain this substance in concentrations greater than 0,1 % by weight.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>3. By way of derogation, paragraph 2 shall not apply:</p> <ul style="list-style-type: none"> — to articles that were in use in the Community before 15 August 2004, — to electrical and electronic equipment within the scope of Directive 2002/95/EC.
<p>46. (a) Nonylphenol $C_6H_4(OH)C_9H_{19}$ CAS 25154-52-3 EC 246-672-0</p> <p>(b) Nonylphenol ethoxylates $(C_2H_4O)_nC_{15}H_{24}O$</p>	<p>Shall not be placed on the market, or used, as substances or in mixtures in concentrations equal to or greater than 0,1 % by weight for the following purposes:</p> <ul style="list-style-type: none"> (1) industrial and institutional cleaning except: <ul style="list-style-type: none"> — controlled closed dry cleaning systems where the washing liquid is recycled or incinerated, — cleaning systems with special treatment where the washing liquid is recycled or incinerated. (2) domestic cleaning; (3) textiles and leather processing except: <ul style="list-style-type: none"> — processing with no release into waste water, — systems with special treatment where the process water is pre-treated to remove the organic fraction completely prior to biological waste water treatment (degreasing of sheepskin); (4) emulsifier in agricultural teat dips; (5) metal working except: <ul style="list-style-type: none"> uses in controlled closed systems where the washing liquid is recycled or incinerated; (6) manufacturing of pulp and paper; (7) cosmetic products; (8) other personal care products except: <ul style="list-style-type: none"> spermicides; (9) co-formulants in pesticides and biocides. However national authorisations for pesticides or biocidal products containing nonylphenol ethoxylates as co-formulant, granted before 17 July 2003, shall not be affected by this restriction until their date of expiry.
<p>47. Chromium VI compounds</p>	<p>1. Cement and cement-containing mixtures shall not be placed on the market, or used, if they contain, when hydrated, more than 2 mg/kg (0,0002 %) soluble chromium VI of the total dry weight of the cement.</p> <p>2. If reducing agents are used, then without prejudice to the application of other Community provisions on the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that the packaging of cement or cement-containing mixtures is visibly, legibly and indelibly marked with information on the packing date, as well as on the storage conditions and the storage period appropriate to maintaining the activity of the reducing agent and to keeping the content of soluble chromium VI below the limit indicated in paragraph 1.</p> <p>3. By way of derogation, paragraphs 1 and 2 shall not apply to the placing on the market for, and use in, controlled closed and totally automated processes in which cement and cement-containing mixtures are handled solely by machines and in which there is no possibility of contact with the skin.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
48. Toluene CAS No 108-88-3 EC No 203-625-9	Shall not be placed on the market, or used, as a substance or in mixtures in a concentration equal to or greater than 0,1 % by weight where the substance or mixture is used in adhesives or spray paints intended for supply to the general public.
49. Trichlorobenzene CAS No 120-82-1 EC No 204-428-0	Shall not be placed on the market, or used, as a substance or in mixtures in a concentration equal to or greater than 0,1 % by weight for any use except: <ul style="list-style-type: none"> — as an intermediate of synthesis, or, — as a process solvent in closed chemical applications for chlorination reactions, or, — in the manufacture of 1,3,5-triamino — 2,4,6-trinitrobenzene (TATB).
50. Polycyclic-aromatic hydrocarbons (PAH)(a) Benzo[a]pyrene (BaP) CAS No 50-32-8 (b) Benzo[e]pyrene (BeP) CAS No 192-97-2 (c) Benzo[a]anthracene (BaA) CAS No 56-55-3 (d) Chrysen (CHR) CAS No 218-01-9 (e) Benzo[b]fluoranthene (BbFA) CAS No 205-99-2 (f) Benzo[j]fluoranthene (BjFA) CAS No 205-82-3 (g) Benzo[k]fluoranthene (BkFA) CAS No 207-08-9 (h) Dibenzo[a,h]anthracene (DBAhA) CAS No 53-70-3	<p>1. From 1 January 2010, extender oils shall not be placed on the market, or used for the production of tyres or parts of tyres if they contain:</p> <ul style="list-style-type: none"> — more than 1 mg/kg (0,0001 % by weight) BaP, or, — more than 10 mg/kg (0,001 % by weight) of the sum of all listed PAHs. <p>These limits shall be regarded as kept, if the polycyclic aromatics (PCA) extract is less than 3 % by weight as measured by the Institute of Petroleum standard IP346: 1998 (Determination of PCA in unused lubricating base oils and asphaltene free petroleum fractions — Dimethyl sulphoxide extraction refractive index method), provided that compliance with the limit values of BaP and of the listed PAHs, as well as the correlation of the measured values with the PCA extract, is controlled by the manufacturer or importer every six months or after each major operational change, whichever is earlier.</p> <p>2. Furthermore, tyres and treads for retreading manufactured after 1 January 2010 shall not be placed on the market if they contain extender oils exceeding the limits indicated in paragraph 1.</p> <p>These limits shall be regarded as kept, if the vulcanised rubber compounds do not exceed the limit of 0,35 % Bay protons as measured and calculated by ISO 21461 (Rubber vulcanised — Determination of aromaticity of oil in vulcanised rubber compounds).</p> <p>3. By way of derogation, paragraph 2 shall not apply to retreaded tyres if their tread does not contain extender oils exceeding the limits referred to in paragraph 1.</p> <p>4. For the purpose of this entry “tyres” shall mean tyres for vehicles covered by:</p> <ul style="list-style-type: none"> — Directive 2007/46/EC of the European Parliament and of the Council of 5 September 2007 establishing a framework for the approval of motor vehicles and their trailers (****), — Directive 2003/37/EC of the European Parliament and of the Council of 26 May 2003 on type-approval of agricultural or forestry tractors, their trailers and interchangeable towed machinery, together with their systems, components and separate technical units (*****), and — Directive 2002/24/EC of the European Parliament and of the Council of 18 March 2002 relating to the type-approval of two or three-wheel motor vehicles and repealing Council Directive 92/61/EEC (*****). <p>(****) OJ L 263, 9.10.2007, p. 1. (*****) OJ L 171, 9.7.2003, p. 1. (*****) OJ L 124, 9.5.2002, p. 1.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
<p>51. The following phthalates (or other CAS and EC numbers covering the substance):</p> <p>(a) Bis (2-ethylhexyl) phthalate (DEHP) CAS No 117-81-7 EC No 204-211-0</p> <p>(b) Dibutyl phthalate (DBP) CAS No 84-74-2 EC No 201-557-4</p> <p>(c) Benzyl butyl phthalate (BBP) CAS No 85-68-7 EC No 201-622-7</p>	<p>1. Shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles.</p> <p>2. Toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market.</p> <p>3. The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this entry in the light of new scientific information on such substances and their substitutes, and if justified, these measures shall be modified accordingly.</p> <p>4. For the purpose of this entry "childcare article" shall mean any product intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children.</p>
<p>52. The following phthalates (or other CAS- and EC numbers covering the substance):</p> <p>(a) Di-"isononyl" phthalate (DINP) CAS No 28553-12-0 and 68515-48-0 EC No 249-079-5 and 271-090-9</p> <p>(b) Di-"isodecyl" phthalate (DIDP) CAS No 26761-40-0 and 68515-49-1 EC No 247-977-1 and 271-091-4</p> <p>(c) Di-n-octyl phthalate (DNOP) CAS No 117-84-0 EC No 204-214-7</p>	<p>1. Shall not be used as substances or in mixtures, in concentrations greater than 0,1 % by weight of the plasticised material, in toys and childcare articles which can be placed in the mouth by children.</p> <p>2. Such toys and childcare articles containing these phthalates in a concentration greater than 0,1 % by weight of the plasticised material shall not be placed on the market.</p> <p>3. The Commission shall re-evaluate, by 16 January 2010, the measures provided for in relation to this entry in the light of new scientific information on such substances and their substitutes, and if justified, these measures shall be modified accordingly.</p> <p>4. For the purpose of this entry "childcare article" shall mean any product intended to facilitate sleep, relaxation, hygiene, the feeding of children or sucking on the part of children.</p>
<p>53. Perfluorooctane sulfonates (PFOS) C8F17SO2X (X = OH, Metal salt (O-M+), halide, amide, and other derivatives including polymers)</p>	<p>1. Shall not be placed on the market, or used, as substances or in mixtures in concentrations equal to or greater than 50 mg/kg (0,005 % by weight).</p> <p>2. Shall not be placed on the market in semi-finished products or articles, or parts thereof, if the concentration of PFOS is equal to or greater than 0,1 % by weight calculated with reference to the mass of structurally or microstructurally distinct parts that contain PFOS or, for textiles or other coated materials, if the amount of PFOS is equal to or greater than 1 µg/m² of the coated material.</p> <p>3. By way of derogation, paragraphs 1 and 2 shall not apply to the following items, nor to substances and mixtures needed to produce them:</p> <p>(a) photoresists or anti-reflective coatings for photolithography processes;</p> <p>(b) photographic coatings applied to films, papers, or printing plates;</p> <p>(c) mist suppressants for non-decorative hard chromium (VI) plating and wetting agents for use in controlled electroplating systems where the amount of PFOS released into the environment is minimised, by fully applying relevant best available techniques developed within the framework of Directive 2008/1/EC (*****);</p> <p>(d) hydraulic fluids for aviation.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>4. By way of derogation from paragraph 1, fire-fighting foams that were placed on the market before 27 December 2006 may be used until 27 June 2011.</p> <p>5. By way of derogation, paragraph 2 shall not apply to articles that were in use in the Community before 27 June 2008.</p> <p>6. Paragraphs 1 and 2 shall apply without prejudice to Regulation (EC) No 648/2004 of the European Parliament and of the Council (*****).</p> <p>7. As soon as new information on details of uses and safer alternative substances or technologies for the uses becomes available, the Commission shall review each of the derogations in paragraph 3(a) to (d) so that:</p> <p>(a) the uses of PFOS will be phased out as soon as the use of safer alternatives is technically and economically feasible;</p> <p>(b) a derogation can only be continued for essential uses for which safer alternatives do not exist and where the efforts undertaken to find safer alternatives have been reported on;</p> <p>(c) releases of PFOS into the environment have been minimised, by applying best available techniques.</p> <p>8. The Commission shall keep under review the ongoing risk assessment activities and the availability of safer alternative substances or technologies related to the uses of perfluorooctanoic acid (PFOA) and related substances and propose all necessary measures to reduce identified risks, including restrictions on marketing and use, in particular when safer alternative substances or technologies, that are technically and economically feasible, are available.</p> <p>(*****) OJ L 24, 29.1.2008, p. 8. (*****) OJ L 104, 8.4.2004, p. 1.</p>
<p>54. 2-(2-methoxyethoxy)ethanol (DEGME)</p> <p>CAS No 111-77-3 EC No 203-906-6</p>	<p>Shall not be placed on the market after 27 June 2010, for supply to the general public, as a constituent of paints, paint strippers, cleaning agents, self-shining emulsions or floor sealants in concentrations equal to or greater than 0,1 % by weight.</p>
<p>55. 2-(2-butoxyethoxy)ethanol (DEGBE)</p> <p>CAS No 112-34-5 EC No 203-961-6</p>	<p>1. Shall not be placed on the market for the first time after 27 June 2010, for supply to the general public, as a constituent of spray paints or spray cleaners in aerosol dispensers in concentrations equal to or greater than 3 % by weight.</p> <p>2. Spray paints and spray cleaners in aerosol dispensers containing DEGBE and not conforming to paragraph 1 shall not be placed on the market for supply to the general public after 27 December 2010.</p> <p>3. Without prejudice to other Community legislation concerning the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that paints other than spray paints containing DEGBE in concentrations equal to or greater than 3 % by weight of that are placed on the market for supply to the general public are visibly, legibly and indelibly marked by 27 December 2010 as follows:</p> <p>“Do not use in paint spraying equipment”.</p>
<p>56. Methylenediphenyl diisocyanate (MDI)</p> <p>CAS No 26447-40-5 EC No 247-714-0</p>	<p>1. Shall not be placed on the market after 27 December 2010, as a constituent of mixtures in concentrations equal to or greater than 0,1 % by weight of MDI for supply to the general public, unless suppliers shall ensure before the placing on the market that the packaging:</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>(a) contains protective gloves which comply with the requirements of Council Directive 89/686/EEC (*****);</p> <p>(b) is marked visibly, legibly and indelibly as follows, and without prejudice to other Community legislation concerning the classification, packaging and labelling of substances and mixtures:</p> <p>— Persons already sensitised to diisocyanates may develop allergic reactions when using this product.</p> <p>— Persons suffering from asthma, eczema or skin problems should avoid contact, including dermal contact, with this product.</p> <p>— This product should not be used under conditions of poor ventilation unless a protective mask with an appropriate gas filter (i.e. type A1 according to standard EN 14387) is used.”</p> <p>2. By way of derogation, paragraph 1(a) shall not apply to hot melt adhesives.</p> <p>(*****) OJ L 399, 30.12.1989, p. 18.</p>
<p>57. Cyclohexane</p> <p>CAS No 110-82-7</p> <p>EC No 203-806-2</p>	<p>1. Shall not be placed on the market for the first time after 27 June 2010, for supply to the general public, as a constituent of neoprene-based contact adhesives in concentrations equal to or greater than 0,1 % by weight in package sizes greater than 350 g.</p> <p>2. Neoprene-based contact adhesives containing cyclohexane and not conforming to paragraph 1 shall not be placed on the market for supply to the general public after 27 December 2010.</p> <p>3. Without prejudice to other Community legislation concerning the classification, packaging and labelling of substances and mixtures, suppliers shall ensure before the placing on the market that neoprene-based contact adhesives containing cyclohexane in concentrations equal to or greater than 0,1 % by weight that are placed on the market for supply to the general public after 27 December 2010 are visibly, legibly and indelibly marked as follows:</p> <p>— This product is not to be used under conditions of poor ventilation.</p> <p>— This product is not to be used for carpet laying.”.</p>
<p>58. Ammonium nitrate (AN)</p> <p>CAS No 6484-52-2</p> <p>EC No 229-347-8</p>	<p>1. Shall not be placed on the market for the first time after 27 June 2010 as a substance, or in mixtures that contain more than 28 % by weight of nitrogen in relation to ammonium nitrate, for use as a solid fertiliser, straight or compound, unless the fertiliser complies with the technical provisions for ammonium nitrate fertilisers of high nitrogen content set out in Annex III to Regulation (EC) No 2003/2003 of the European Parliament and of the Council (*****).</p> <p>2. Shall not be placed on the market after 27 June 2010 as a substance, or in mixtures that contain 16 % or more by weight of nitrogen in relation to ammonium nitrate except for supply to:</p> <p>(a) downstream users and distributors, including natural or legal persons licensed or authorised in accordance with Council Directive 93/15/EEC (*****);</p> <p>(b) farmers for use in agricultural activities, either full time or part time and not necessarily related to the size of the land area.</p>

Column 1 Designation of the substance, of the group of substances or of the mixture	Column 2 Conditions of restriction
	<p>For the purposes of this subparagraph:</p> <p>(i) “farmer” shall mean a natural or legal person, or a group of natural or legal persons, whatever legal status is granted to the group and its members by national law, whose holding is situated within Community territory, as referred to in Article 299 of the Treaty, and who exercises an agricultural activity;</p> <p>(ii) “agricultural activity” shall mean the production, rearing or growing of agricultural products including harvesting, milking, breeding animals and keeping animals for farming purposes, or maintaining the land in good agricultural and environmental condition as established under Article 5 of Council Regulation (EC) No 1782/2003 (*****);</p> <p>(c) natural or legal persons engaged in professional activities such as horticulture, plant growing in greenhouses, maintenance of parks, gardens or sport pitches, forestry or other similar activities.</p> <p>3. However, for the restrictions in paragraph 2, Member States may until 1 July 2014, for socioeconomic reasons, apply a limit of up to 20 % by weight of nitrogen in relation to ammonium nitrate for substances and mixtures placed on the market within their territories. They shall inform the Commission and other Member States thereof.</p> <p>(*****) OJ L 304, 21.11.2003, p. 1. (*****) OJ L 121, 15.5.1993, p. 20. (*****) OJ L 270, 21.10.2003, p. 1.’</p>

3. in the Appendices 1 to 6, the foreword is replaced by the following:

FOREWORD

Explanations of column headings

Substances:

The name corresponds to the International Chemical Identification used for the substance in Part 3 of Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Whenever possible, substances are designated by their IUPAC names. Substances listed in EINECS (European Inventory of Existing Commercial Chemical Substances), ELINCS (European List of Notified Substances) or the list of “No-longer-polymers” are designated using the names in these lists. Other names, such as usual or common names, are included in some cases. Whenever possible, plant protection products and biocides are designated by their ISO names.

Entries for groups of substances:

A number of group entries are included in Part 3 of Annex VI to Regulation (EC) No 1272/2008. In these cases, the classification requirements will apply to all substances covered by the description.

In some cases, there are classification requirements for specific substances that would be covered by the group entry. In such cases a specific entry is included in Part 3 of Annex VI to Regulation (EC) No 1272/2008 for the substance and the group entry will be annotated with the phrase “except those specified elsewhere in Annex VI to Regulation (EC) No 1272/2008”.

In some cases, individual substances may be covered by more than one group entry. In these cases, the classification of the substance reflects the classification for each of the two group entries. In cases where different classifications for the same hazard are given, the most severe classification will be applied.

Index number:

The Index number is the identification code given to the substance in Part 3 of Annex VI to Regulation (EC) No 1272/2008. Substances are listed in the Appendix according to this index number.

EC numbers:

The EC number, i.e. EINECS, ELINCS or NLP, is the official number of the substance within the European Union. The EINECS number can be obtained from the European Inventory of Existing Commercial Chemical Substances (EINECS). The ELINCS number can be obtained from the European List of Notified Substances. The NLP number can be obtained from the list of "No-longer-polymers". These lists are published by the Office for Official Publications of the European Communities.

The EC number is a seven-digit system of the type XXX-XXX-X which starts at 200-001-8 (EINECS), at 400-010-9 (ELINCS) and at 500-001-0 (NLP). This number is indicated in the column entitled "EC No".

CAS number:

Chemical Abstracts Service (CAS) numbers have been defined for substances to help in their identification.

Notes:

The full text of the notes can be found in Part 1 of Annex VI to Regulation (EC) No 1272/2008.

The notes to be taken into account for the purposes of this Regulation are the following:

Note A:

Without prejudice to Article 17(2) of Regulation (EC) No 1272/2008, the name of the substance must appear on the label in the form of one of the designations given in Part 3 of Annex VI to that Regulation.

In that Part, use is sometimes made of a general description such as "... compounds" or "... salts". In this case, the supplier who places such a substance on the market is required to state on the label the correct name, due account being taken of Section 1.1.1.4 of Annex VI to Regulation (EC) No 1272/2008.

Note C:

Some organic substances may be marketed either in a specific isomeric form or as a mixture of several isomers.

Note D:

Certain substances which are susceptible to spontaneous polymerisation or decomposition are generally placed on the market in a stabilised form. It is in this form that they are listed in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

However, such substances are sometimes placed on the market in a non-stabilised form. In this case, supplier who places such a substance on the market must state on the label the name of the substance followed by the words "non-stabilised".

Note J:

The classification as a carcinogen or mutagen need not apply if it can be shown that the substance contains less than 0,1 % w/w benzene (EC No 200-753-7).

Note K:

The classification as a carcinogen or mutagen need not apply if it can be shown that the substance contains less than 0,1 % w/w 1,3-butadiene (EC No 203-450-8).

Note L:

The classification as a carcinogen need not apply if it can be shown that the substance contains less than 3 % DMSO extract as measured by IP 346.

Note M:

The classification as a carcinogen need not apply if it can be shown that the substance contains less than 0,005 % w/w benzo[a]-pyrene (EC No 200-028-5).

Note N:

The classification as a carcinogen need not apply if the full refining history is known and it can be shown that the substance from which it is produced is not a carcinogen.

Note P:

The classification as a carcinogen or mutagen need not apply if it can be shown that the substance contains less than 0,1 % w/w benzene (EC No 200-753-7).

Note R:

The classification as a carcinogen need not apply to fibres with a length weighted geometric mean diameter, less two standard errors, greater than 6µm.;

4. in Appendices 1, 2, 3, 5 and 6, in the entries, in the column headed 'notes', references to notes E, H and S are deleted;
5. in Appendix 1, the heading is replaced by 'Entry 28 — Carcinogens: category 1A (Table 3.1)/category 1 (Table 3.2)';
6. Appendix 2 shall be amended as follows:
 - (a) the heading is replaced by 'Entry 28 — Carcinogens: category 1B (Table 3.1)/category 2 (Table 3.2)';
 - (b) in the entries index Nos 024-017-00-8, 611-024-00-1, 611-029-00-9, 611-030-00-4 and 650-017-00-8, the words 'Annex I to Directive 67/548/EEC' shall be replaced by 'Annex VI to Regulation (EC) No 1272/2008';
 - (c) the entries with index numbers 649-062-00-6, 649-063-00-1, 649-064-00-7, 649-065-00-2, 649-066-00-8, 649-067-00-3, 649-068-00-9, 649-069-00-4, 649-070-00-X, 649-071-00-5, 649-072-00-0, 649-073-00-6, 649-074-00-1, 649-075-00-7, 649-076-00-2, 649-077-00-8, 649-078-00-3, 649-079-00-9, 649-080-00-4, 649-081-00-X, 649-082-00-5, 649-083-00-0, 649-084-00-6, 649-085-00-1, 649-086-00-7, 649-087-00-2, 649-089-00-3, 649-090-00-9, 649-091-00-4, 649-092-00-X, 649-093-00-5, 649-094-00-0, 649-095-00-6, 649-096-00-1, 649-097-00-7, 649-098-00-2, 649-099-00-8, 649-100-00-1, 649-101-00-7, 649-102-00-2, 649-103-00-8, 649-104-00-3, 649-105-00-9, 649-106-00-4, 649-107-00-X, 649-108-00-5, 649-109-00-0, 649-110-00-6, 649-111-00-1, 649-112-00-7, 649-113-00-2, 649-114-00-8, 649-115-00-3, 649-116-00-9, 649-117-00-4, 649-119-00-5, 649-120-00-0, 649-121-00-6, 649-122-00-1, 649-123-00-7, 649-124-00-2, 649-125-00-8, 649-126-00-3, 649-127-00-9, 649-128-00-4, 649-129-00-X, 649-130-00-5, 649-131-00-0, 649-132-00-6, 649-133-00-1, 649-134-00-7, 649-135-00-2, 649-136-00-8, 649-137-00-3, 649-138-00-9, 649-139-00-4, 649-140-00-X, 649-141-00-5, 649-142-00-0, 649-143-00-6, 649-144-00-1, 649-145-00-7, 649-146-00-2, 649-147-00-8, 649-148-00-3, 649-149-00-9, 649-150-00-4, 649-151-0-X, 649-152-00-5, 649-153-00-0, 649-154-00-6, 649-155-00-1, 649-156-00-7, 649-157-00-2, 649-158-00-8, 649-159-00-3, 649-160-00-9, 649-161-00-4, 649-162-00-X, 649-163-00-5, 649-164-00-0, 649-165-00-6, 649-166-00-1, 649-167-00-7, 649-168-00-2, 649-169-00-8, 649-170-00-3, 649-171-00-9, 649-172-00-4, 649-173-00-X, 649-174-00-5, 649-177-00-1, 649-178-00-7, 649-179-00-2, 649-180-00-8, 649-181-00-3, 649-182-00-9, 649-183-00-4, 649-184-00-X, 649-185-00-5, 649-186-00-0, 649-187-00-6, 649-188-00-1, 649-189-00-7, 649-190-00-2, 649-191-00-8, 649-193-00-9, 649-194-00-4, 649-195-00-X, 649-196-00-5, 649-197-00-0, 649-198-00-6, 649-199-00-1, 649-200-00-5, 649-201-00-0, 649-202-00-6, 649-203-00-1, 649-204-00-7, 649-205-00-2, 649-206-00-8, 649-207-00-3, 649-208-00-9, 649-209-00-4 and 649-210-00-X are deleted;
7. in Appendix 3, the heading is replaced by 'Entry 29 — Mutagens: category 1A (Table 3.1)/category 1 (Table 3.2)';
8. in Appendix 4, the heading is replaced by 'Entry 29 — Mutagens: category 1B (Table 3.1)/category 2 (Table 3.2)';
9. in Appendix 5, the heading is replaced by 'Entry 30 — Toxic to reproduction: category 1A (Table 3.1)/category 1 (Table 3.2)';
10. in Appendix 6, the heading is replaced by 'Entry 30 — Toxic to reproduction: category 1B (Table 3.1)/category 2 (Table 3.2)';
11. in Appendix 8, the heading is replaced by 'Entry 43 — Azocolourants — List of aromatic amines';
12. In Appendix 9, the heading is replaced by 'Entry 43 — Azocolourants — List of azodyes';
13. Appendix 10 shall be amended as follows:
 - (a) the heading is replaced by 'Entry 43 — Azocolourants — List of testing methods';
 - (b) in the footnote, the addresses of CEN and Cenelec are replaced by the following:

'CEN: Avenue Marnix 17, 1000 Brussels, Belgium, tel. +32 2550 08 11, fax +32 2550 08 19 (<http://www.cen.eu/cenorm/homepage.htm>)

Cenelec: Avenue Marnix 17, B-1000 Brussels, tel. +32 25196871, fax +32 2519 69 19 (<http://www.cenelec.eu/Cenelec/Homepage.htm>)'



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON HEALTH AND ENVIRONMENTAL RISKS

SCHER

Opinion on

**“RPA’s report “Perfluorooctane Sulphonates
Risk reduction strategy and analysis of advantages and
drawbacks”**

(Final report - August 2004)

Adopted by the SCHER
during the 4th plenary of 18 March 2005

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1. BACKGROUND

Perfluorooctane sulfonate is a fully fluorinated moiety which is found in a large family of PFOS related substances. Most members of this group are polymers of high molecular weights in which PFOS constitutes only a small fraction of the total polymer molecule and of the final product.

On 16 May 2000, the major global producer of PFOS (3M) announced a voluntary phase out from 2001 onwards. An OECD hazard assessment was produced according to which the production of PFOS has now ceased, apart from a small-scale uses.

A study has been undertaken by RPA in association with the British authorities. The report recommends a risk reduction strategy to be taken forward.

2. TERMS OF REFERENCE

The SCHER is requested to examine the following issues:

1. To assess the overall scientific quality of the RPA report, and in considering this, to comment on the methodology, findings, conclusions and recommendation of the report.
2. To evaluate the contribution of the ongoing uses to the overall risks for the environment and to human health.
 - a. Does the SCHER think it likely that all PFOS derivatives would fulfil the criteria for classification as PBT and whether they would qualify for the classification under POP criteria?
 - b. How rapidly and to what level does the SCHER think that the concentrations of PFOS found in the environment will reduce as a consequence of the recent phase out of numerous uses of PFOS?
 - c. Does the SCHER expect that the concentrations of PFOS in the environment will reduce to the extent that they will reach a level at which there is no risk?
 - d. Does the SCHER think that the current emissions into the environment from ongoing uses have a significant influence on the rate of reduction of the concentrations of PFOS found in the environment?

3. OPINION

3.1. Overall considerations

The proposed Risk Reduction Strategy by Risk & Policy Analysts Limited (RPA, 2004) applies to PFOS, its salts and related substances including polymers, which contain one or more perfluorooctylsulphonate groups (i.e. $C_8F_{17}SO_2$) and may potentially degrade or metabolise to PFOS under certain conditions. This strategy thus does not include perfluoroalkylsulphonates with other chain lengths than eight carbons, or perfluorocarboxylates. Several of these substances have similar properties as PFOS, and may be used as substitutes.

PFOS is not on an EU priority list and there is no EU Risk Assessment Report available. It was undergoing a national environmental risk assessment by the UK in 2004 (RER, 2004). Further to preliminary indications of persistence and toxicity, the Organisation for Economic Co-operation and Development (OECD) performed in 2002 an assessment of the hazards associated with PFOS. With regard to the environmental fate, the OECD concluded that PFOS is persistent and bioaccumulative; the OECD reports toxicity studies on several aquatic and terrestrial species. PFOS has been detected in tissues of a number of species of wildlife (including marine mammals), in surface water and sediment, in wastewater treatment plant effluent, sewage sludge and in landfill leachate.

The RPA report is based on the UK Risk Evaluation Report (RER, 2004) which has mainly taken the information provided in the OECD (2002) report on PFOS. In this RER, risk characterisation is performed according to the procedures suggested in the TGD (2003) and using the EUSESv2 model. The SCHER is of the opinion that considering the particular properties of PFOS and related substances this approach is not suitable for these particular compounds. The physico-chemical properties including surface-tension activity produce an unusual environmental behaviour. Therefore the conventional partitioning approaches cannot be applied and consequently the PEC calculations cannot be supported by the SCHER. It is noted that the PECs are not sufficiently validated (including assessment of uncertainties) against the available monitoring data. The SCHER is particularly concerned on the way bioaccumulation data is used in the assessment of secondary poisoning. The SCHER is of the opinion that the procedure used in the RER is not suitable for PFOS and related substances. The reasons for this are: (1) the bioaccumulation potential of PFOS is not related to the typical mechanisms associated with accumulation in lipid-rich tissues observed for other organic chemicals, (2) in fact, the bioaccumulation is associated with a rapid assimilation, low elimination rate and protein binding, (3) the toxico-kinetic information indicates multi-compartmental kinetics and therefore the bioaccumulation/bioconcentration potential is related to the exposure level and cannot be reduced to a single BAF.

Based on the concerns expressed above, the SCHER cannot support the conclusions of the environmental risk characterisation for the present and possible future uses of PFOS (and related substance) proposed in the RER. However, concentrations in some aquatic and terrestrial organisms have been reported in several areas, indicating a potential concern for secondary poisoning even at regional level. Due to the contribution of historical emissions, the relevance of the proposed PEC/PNEC ratios for assessing the potential risk of current and future uses cannot be established.

The SCHER has also reservations regarding the conclusions reached by RPA on the risks of PFOS and related substances to human health. RPA used the 3M Risk Assessment Report (2003) as basis, where “adequate margins of exposure” were claimed for the general population, and medical surveillance data were used to demonstrate the absence of adverse effects in workers. As PFOS has been detected in the serum of occupational and general populations with elimination half-lives in the order of years, and because the lowest serum effect levels found in animal long-term repeated-dose studies were in the same range as the serum levels of some workers, a scientific human health risk assessment should be performed, taking into account the substantial differences in elimination kinetics between animal species and man.

Use of PFOS and PFOS related substances in consumer applications such as carpets, leather/apparel, textiles/upholstery, paper and packaging, coatings, industrial and

household cleaning products, pesticides and insecticides has been largely abandoned following the announcement of the main global producer (3M) to voluntarily phase out manufacture and use of PFOS consumer applications. Continued use in some of these applications is, however, reported from single European countries. According to RPA, there is no evidence of use in Europe in medicinal products or medical devices, flame retardants, mining/oil surfactants and adhesives. These uses were therefore not considered any further in the RPA risk reduction strategy.

Ongoing industrial/professional usage of PFOS and PFOS-related substances has been confirmed for five sectors in the EU (current demand):

- Metal (chromium) plating (8,600 - 10,000 kg/year)
- Fire fighting foams (estimated quantity held in current stock: 122 tonnes)
- Photographic industry (approximately 850 kg PFOS-related substances/year)
- Semiconductor industry (436 kg/year)
- Aviation industry (hydraulic fluids; approximately 730 kg/year).

3.2. Question 1

The SCHER is asked to assess the overall scientific quality of the RPA report and in considering this, to comment on the methodology, findings, conclusions and recommendation of the report.

In response to this question, the SCHER will focus its comments on the scientific basis used for the management strategy, but will not comment on the risk reduction strategy itself:

As pointed out earlier in this document, some fundamental assumptions made in the RPA report lack substantiation, and the SCHER cannot therefore support the estimations presented in the environmental risk characterisation for present and possible future uses of PFOS (and related substance) proposed in the RER, and taken over into the RPA report. However, the SCHER agrees, that measured concentrations in some aquatic and terrestrial organisms indicate a potential concern for secondary poisoning, even at the regional level.

The SCHER has reservations also with regard to RPA's conclusion on the risks to human health, which were uncritically taken from the 3M Risk Assessment Report (2003). In particular, the summary statement that "...the observed levels of PFOS in human serum demonstrate adequate margins of exposure and should not be associated with increased health risk" cannot be supported by the SCHER.

The SCHER acknowledges that no consistent associations between exposure to PFOS and haematological, hormonal, or biochemical parameters were found in surveys of occupationally exposed workers with serum levels of up to 10 µg/ml, and that no conclusions with regard to human carcinogenicity can be drawn from the available epidemiology data, including a retrospective cohort study with a reported increased risk of bladder cancer in workers who were highly exposed to fluorochemicals, but also other chemicals, primarily in non-production jobs, including maintenance and incinerator and

wastewater treatment plant operations (SMR 12.77; 95% CI 2.63-37.3; Alexander et al., 2003).

The toxicity profile of PFOS is, however, fairly consistent across different animal species, and includes liver toxicity, hypolipidemia, liver tumours, and toxicity to the developing organism. As PFOS has not shown a genotoxic activity in a wide variety of tests, it is justified to assume a non-genotoxic mechanism for the development of liver, thyroid and mammary gland tumours. The key health hazards identified in animal studies were therefore repeated dose and developmental toxicity, with the lowest effect level found in a chronic dietary toxicity study on rats at 2 ppm (corresponding to ~17 µg/ml serum; mild hepatocellular centrilobular hypertrophy in males). The NOAEL in this study was at 0.5 ppm (4 µg PFOS/ml serum). Dose-dependent mortality was seen in rodent offspring after exposure of dams to PFOS in postnatal, reproductive and developmental toxicity studies. Exposure to 3 mg/kg bw/day (rat, corresponding to 72 µg/ml serum) and 10 mg/kg bw/day (mouse) resulted in the death of 50% of the offspring within four days, possibly related to an interference of the chemical with lung maturation. In this study, the benchmark dose that predicts a 5% increase in pup mortality over background was estimated at 0.58 mg/kg bw/day. In a rat 2-generation reproductive study the NOAEL and LOAEL for second generation offspring (F2 pups) were 0.1 mg/kg bw/day and 0.4 mg/kg bw/day based on reductions in pup weight.

Substantial differences exist in the elimination kinetics between animal species and man. These differences have to be taken into account in order to judge whether or not "...the observed levels of PFOS in human serum demonstrate adequate margins of exposure". No such information is presented in the RPA report. Whilst simply comparing serum levels in the general population (0.030 – 0.040 µg/ml, geometric mean) with the no effect level in the chronic rodent studies (4 µg/ml serum) indeed reveals Margins of Exposure ≥ 100 , indicating a low risk for the general population, serum levels of workers (up to 13 µg/ml) are already in the range of those levels at which toxicity has been observed in animal studies. The SCHER is therefore of the opinion that a full human health risk assessment should be performed.

Potential risk reduction measures that were examined by RPA were those of the TGD document. These measures were assessed against the following decision criteria (in accordance with the TGD): a) effectiveness, b) practicality, c) economic impact, and d) monitorability. Where controls on the marketing and use of PFOS and PFOS containing substances were proposed, an analysis of the advantages and drawbacks of the substance itself and its substitutes was undertaken by RPA. The recommended risk reduction measures included: manufacturing process changes, environmental emission control, exposure control, provision of information and guidance, waste disposal, marketing and use restrictions, and measures for "previous uses" to prevent re-introduction. Additionally, classification and labelling were proposed (R51/R53; R48).

3.2.1. *Findings and recommendations for on-going uses:*

- Metal (chromium) plating

PFOS is used as mist suppressant in hard, decorative and plastic chromium plating. According to the RPA report, there are no means of reducing emissions from its use in these applications to near zero level. Alternative processes/operations and/or emission controls for hard and plastic chromium platers include additional extraction ventilation and tank enclosure; and for decorative chromium platers alternatively also a move away

from Cr(VI) processes to Cr(III) processes. A voluntary agreement with the plating industry was considered unlikely to succeed given that previous initiatives to promote a shift to Cr(III) technology have had little effect. Therefore, RPA proposed marketing and use restrictions through national regulations in accordance with EU Directive 76/769/EEC.

The SCHER notes that the risk reduction strategy assumes 100% release into the environment, without taking into consideration recycling measures and waste disposal by incineration. For Europe, Industry has calculated a total emission of only 517 kg based on data from a German plant and extrapolated to the European situation.

- Fire fighting foams

PFOS based substances are no longer used in the manufacture of fire fighting foams. The RPA report notes that 95% of the substitute foams that are currently available in the UK are based on perfluorocarboxylates and telomer sulphonates with unknown long-term effects on the environment. Further toxicity data has also to be generated on the fluorine-free alternative foams.

Given these uncertainties, the immediate destruction and replacement of PFOS based foams is not recommended in the RPA report. Instead, a five year delay in destruction of the remaining foams is suggested to allow for provision of better data on the impact of the substitutes. The ongoing use of remaining stocks should be subject to a number of conditions, such as that they are not used at incidents where firewater containment is not possible.

RPA proposes marketing and use restrictions through national regulations in accordance with EU Directive 76/769/EEC.

The SCHER notes, that releases into the environment were estimated to be 570 kg PFOS-acid/year (based on a use rate of 15%/year and a PFOS-concentration of 1% in the foams). This is probably overstating the actual releases, because a use rate of only 0.5% for PFOS-based foams is reported elsewhere (UK Environment Agency, 2004: Risk Evaluation Report, p. 21).

- Photographic industry

PFOS itself is not used in the photographic industry. The use of PFOS related substances in the photographic industry has been reduced by more than 80% through replacement by telomer products. It is noted, that telomers are currently under review in various countries. If restrictions will be placed on telomers, it may become more difficult to further reduce or eliminate PFOS in this sector. Furthermore, for a few applications no alternatives are currently available, including electrostatic charge control, which may affect workers safety. Current releases into the environment were estimated to be about 6.75 kg PFOS-related substances/year from film development, and 1.02 kg PFOS-acid/year into waste water and 0.051 kg PFOS-acid/year into air from the manufacturing of films.

RPA proposes marketing and use restrictions through national regulations in accordance with EU Directive 76/769/EEC with a conditional derogation period of 5 years for the critical photographic applications where no replacement is currently available. Conditions for permitted use should include that PFOS related substances are only used

in closed systems, and high temperature incineration of all PFOS containing waste. Any prolongation of the 5-year-derogation period would need detailed justification (e.g. providing evidence of research progress). Efforts to secure an industry voluntary agreement aimed at ensuring effective emissions control and high temperature incineration of wastes containing PFOS related substances are also suggested by RPA.

- Semiconductor industry

Substitutes (of unknown identity) are available only for developer applications. There are currently no alternatives available for anti-reflective coatings. For photoresists alternative processes are in the early stages of development.

RPA proposes marketing and use restrictions through national regulations in accordance with EU Directive 76/769/EEC with a conditional derogation period of 5 years for the critical applications where no replacement is currently available. Conditions for permitted use should include that PFOS related substances are only used in closed systems, and high temperature incineration of all PFOS containing waste. Any prolongation of the 5-yr-derogation period would need detailed justification (e.g. providing evidence of research progress). Efforts to secure an industry voluntary agreement aimed at ensuring effective emissions control and high temperature incineration of wastes containing PFOS related substances are also suggested by RPA.

The SCHER notes that waste incineration with high destruction and removal efficiency was not taken into account when estimating current releases. If complete elimination of the use in developers is assumed (as indicated by industry), about 43 kg PFOS-related substance/year are estimated to be released into the environment (use in developers: 195 kg/year; incineration of 83% of the remaining 241 kg/year with 99.99% destruction and removal efficiency).

- Aviation industry

According to the RPA report, there are no current alternatives to the PFOS related substances in hydraulic fluids for aircraft systems. In recognition of the long time frames involved in replacing this use this application should be derogated from marketing and use restrictions with no set deadline for phase-out but with continuing review. PFOS related substances should be subject to conditions of permitted use, involving provisions for the collection and disposal of aviation hydraulic fluids via high temperature incineration. Efforts to secure an industry voluntary agreement are suggested.

Releases into waste water were estimated to be 3.94 kg PFOS-acid/year, and to soil 9.2 kg PFOS-acid/year.

3.2.2. *Former uses*

The risk reduction strategy proposes marketing and use restrictions through national regulations and EU Directive 76/769/EEC for all former uses in order to prevent the re-introduction and re-use of PFOS related substances in these applications. The SCHER supports this recommendation, but notes that high uncertainty exists on the actual versus “historical” uses. Certain EU member countries have indeed confirmed that single applications that were classified “historical” in the UK are still on-going in their country.

In conclusion, in order to answer to Question 1, it is opinion of the SCHER that:

- the RPA report suffers from a lack of scientific detail to substantiate the recommendations, and the information presented does not appear to be a sufficient basis for decision-making. The RPA report is based on the UK Risk Evaluation Report, which used the methodology of the TGD (2003). The SCHER is of the opinion that considering the particular properties of PFOS and related substances, this approach is not suitable for a science-based risk characterisation and consequently the PEC calculations cannot be supported by the SCHER, nor can the SCHER support the conclusions for the present and possible future uses of PFOS (and related substance). Concentrations in some aquatic and terrestrial organisms in several areas indicate a potential concern for secondary poisoning even at regional level.
- the SCHER has reservations also with regard to RPA's conclusion on the risks to human health. The data as presented in the RPA report is not sufficient to adequately judge the risk of PFOS and PFOS related substances to human health.

3.3. Question 2

The SCHER was asked "to evaluate the contribution of the ongoing uses to the overall risks for the environment and to human health"

- a. Does the SCHER think it likely that all PFOS derivatives would fulfil the criteria for classification as PBT and whether they would qualify for the classification under POP criteria*
- b. How rapidly and to what level does the SCHER think that the concentrations of PFOS found in the environment will reduce as a consequence of the recent phase out of numerous uses of PFOS?*
- c. Does the SCHER expect that the concentrations of PFOS in the environment will reduce to the extent that they will reach a level at which there is no risk?*
- d. Does the SCHER think that the current emissions into the environment from ongoing uses have a significant influence on the rate of reduction of the concentrations of PFOS found in the environment?*

Given that risks are not properly characterized (Question1) the SCHER cannot address this question relating to the contribution to risk. The following therefore relates only to the contribution to exposure reduction.

In order to respond to these questions, the SCHER compared exposures from former and ongoing uses:

Environmental and human exposure assessment before phase out of major applications, including the contribution of other sources like formation of PFOS from degradation of PFOS related compounds

Before their phase out PFOS and PFOS-related substances were mainly used in the textile and leather industry, in household cleaning products, in pesticides and insecticides, in paper, packaging, and coatings. According to the RPA report, in the UK alone more than 100 tonnes of PFOS were used in these applications in 2000 (>25 tonnes for paper and packaging protecting, >25 tonnes for carpet protection, >10 tonnes for

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coatings (additives), >10 tonnes for apparel and leather protection, >10 tonnes for fabric and upholstery protection, and >1 tonne for fire fighting foams. Additives for household products, chemical intermediates, electroplating/etching bath surfactants and others accounted for less than 1 tonne each). For Europe, the ECB noted 10 PFOS-related raw chemicals in trade (imported and/or manufactured) since 1996 or 2000, each in volumes less than 1000 tonne/year (OECD, 2004). According to the 3M Company, U.S. production and import of these chemicals in 2000 was estimated at 2,950 metric tons (OECD, 2004). The global consumption in 2000 was estimated based on data from the 3M Company to be \approx 4,500 metric tons (OECD, 2002).

The OECD survey (2004) also gives the following estimates for the use in the EU, largely describing the situation before the PFOS products were removed from the market:

Table 1: EU uses of PFOS related substances in 2000 (OECD, 2004)

Use	Substance Type	EU quantity (tonnes/year)	Note
Chromium plating	acid	10	Estimate from Germany
Photolithography	substance	0.47	ESIA/SEMI estimate
Photography	acid	0.85	EPCI estimate
	polymer	0.75	EPCI estimate, use of film
Aviation	acid	0.73	One third of world use estimate
Fire fighting foams	acid	0.57	From UK estimate of foam use
Fabric treatment	polymer	240	From UK estimate of 48 tons, assuming UK is 20% of EU
Paper treatment	substance	160	From UK estimate of 32 tons, assuming UK is 20% of EU
Coatings	substance	90	From UK estimate of 18 tons, assuming UK is 20% of EU
Total		\approx 500 tonnes/year	

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For major former uses emission estimates have been made by RPA on basis of EU emission scenarios and information from industry (relating to the year 2000). These are summarised in the following:

Table 2: EU emissions estimates for the year 2000

	Compartment	EU emissions per year
<u>PFOS-related substances</u>		
Fabrics treatment	Waste water	30 kg
Fabrics service life	Water	1,018 kg
	Soil	599 kg
Paper treatment	Waste water	7,200 kg
Coating	Waste water	405 kg
<u>PFOS-polymers</u>		
Fabrics treatment	Waste water	3,024 kg
Fabrics service life	Water	101,800 kg
	Soil	59,900 kg
Total		174 tonnes / year

PFOS was found in surface water and sediment, downstream of production sites, waste water treatment plant effluents, sewage sludge, landfill leachate, and in wildlife species all over the world, including very remote areas in the Arctic. At present, it is unclear which exposure pathways are responsible for the small quantities of PFOS (and several other perfluorinated compounds) now found in the serum of the general population around the world. Surveys of American adults, children, and elderly subjects showed geometric mean serum concentrations in the range of 30-40 ng/ml with no correlation with age, and liver concentrations of PFOS from < 4.5 to 57 ng/g in human organ donors from the general population (Olsen, 2003a). In other studies (e.g. Kärman et al., 2004; Kannan et al., 2004) serum concentrations of <1 to 82 ng/ml were found, with values greater than 30 ng/ml in samples from the United States and Poland, the highest of the countries surveyed. PFOS concentrations in samples from Japan, Korea, Malaysia, Belgium, and Brazil averaged between 10 and 25 ng/ml, and samples from Italy and Colombia were between 4 and 10 ng/ml. The lowest concentration was found in samples from India (on average less than 3 ng/ml).

Mean serum PFOS levels of 1-2 ppm (range 0.1 – 13 µg/ml) were reported in 3M fluorochemical production workers in 1999; later, geometric means in random sample of

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126 3M chemical plant employees were reported as 0.941 µg/ml (95% confidence interval: 0.787-1.126; Olsen et al.,1999; 2003b).

Evaluation of present and possible future exposure (after phasing out of previous uses)

3M's voluntary phase-out of PFOS production has led to a significant reduction in the use of PFOS and PFOS-related substances in the EU, as PFOS has been substituted by other perfluoroalkylates or other chemicals in most of the former major uses. PFOS and PFOS-related chemicals are however still manufactured by a few countries, including Germany (20 – 60 tonnes in 2003), Italy (< 22 tonnes in 2003) and Japan (< 1000 tonnes), mostly for industrial uses. The total volume is not known (OECD, 2004).

Products containing PFOS and related chemicals are also still imported and/or manufactured in the EU. The total volume of PFOS and related chemicals reported in these products is imprecise but is likely to be at least 30 tonnes/year worldwide (OECD, 2004). As PFOS and PFOS-related chemicals may not always be listed on MSDS for products/mixtures, importers may not be aware of the presence of PFOS and related chemicals within products/mixtures and so these products/mixtures may not have been always be reported.

There is no precise quantitative assessment of the emissions from on-going uses into the different environmental departments available; nevertheless the following emissions can be estimated:

Table 3: Emission estimates for on-going uses (2004)

	Compartment	Emissions
<u>PFOS</u>		
Metal plating	Waste water	9,000 kg ¹
Fire-fighting foams	Surface water	257 kg ²
	Soil	257 kg ²
Photographic industry	Air	0.051 kg
	Waste water	1.02 kg
Aviation	Waste water	3.94 kg
	Soil	9.2 kg
<u>PFOS related substances</u>		
Photographic industry	Waste water	6.75 kg
Semiconductor industry	Waste water	43 kg ³
Total		9578 kg (without metal plating: 576

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	Compartment	Emissions
		kg)

¹ This figure assumes that all used material is released into the environment; according to industry calculations the emissions are only 517 kg, based on German data extrapolated for Europe

² Based on a use rate of 15%/year and a PFOS-concentration of 1% in the foams; with a more realistic use rate of 0.05%/year, only 17 kg instead of 514 kg would be released from fire-fighting foams

³ Based on new information from industry; the figure given in the RPA report is 226 kg

Emissions from ongoing uses in the photographic, semiconductor and aviation industry amount to 64 kg, i.e. less than 0.3% of the emissions caused by the former uses. A possibly relevant environmental exposure is to be expected by the on-going use in metal plating, potentially accounting for >5% of the previous major uses, if re-cycling of the material is not taken into account (industry calculations indicate a total of only 517 kg for the EU). If allowed to re-occur, emissions from the previous uses would be orders of magnitude higher than the emissions from on-going uses. The RPA report, therefore, rightly points to the fact that any risk reduction measures that are implemented in relation to known current uses should be accompanied by measures to prevent re-occurrence of previous uses, should alternative sources of PFOS become available again.

According to the recent OECD survey, PFOS chemicals are now present in products in concentrations ranging between 0.001% and 50%. For uses in electronic etching or metal plating, PFOS or related chemicals were noted as present at 5% to 25% (OECD, 2004). A typical use concentration in hydraulic fluids for the aviation industry is 0.05%.

Only in recent years the analytical techniques have become sufficiently sophisticated to detect and to reliably determine PFOS concentrations in environmental samples and biological materials. Some efforts have been undertaken to re-construct the PFOS concentrations in environmental and tissue samples over the course of time from archived samples.

- a. *Does the SCHER think it likely that all PFOS derivatives would fulfil the criteria for classification as PBT and whether they would qualify for the classification under POP criteria*

As previously expressed by the CSTEE, there are several scientific concerns with regard to the criteria applied for the classification of substances as PBT. The SCHER considers that the criteria are particularly problematic for substances with properties such as PFOS, particularly related to bioaccumulation and toxicity. The bioaccumulation potential of PFOS should not be based on the BCF but on scientifically sound information on the toxicokinetics of this chemical. Considering the oral assimilation in fish and mammals and the low elimination rate, the SCHER concludes that PFOS has similar environmental concerns for bioaccumulation than those associated to vB substances. The toxicity associated to the oral exposure route is confirmed as well as a high persistency. Therefore a scientifically based assessment of Persistence-Bioaccumulation and Toxicity should indicate that PFOS fulfils the criteria for vP, vB and T.

PFOS

To be considered as a POP under the Stockholm Convention a substance has to be persistent, bioaccumulative, have a potential for long range environmental transport and have the potential to give adverse effects. PFOS fulfils the criteria for all these properties given in Annex D of the Convention.

- b. How rapidly and to what level does the SCHER think that the concentrations of PFOS found in the environment will reduce as a consequence of the recent phase out of numerous uses of PFOS?*

At present, there is insufficient data available to respond to this question. On-going and future monitoring of PFOS-concentrations in the environment, biota and humans will be necessary to elucidate the kinetics of distribution, accumulation, and elimination.

PFOS concentration may initially increase even if all productions ceases due to degradation of precursors already in the environment.

- c. Does the SCHER expect that the concentrations of PFOS in the environment will reduce to the extent that they will reach a level at which there is no risk?*

If re-occurrence of former major uses is not allowed, the concentrations of PFOS in the environment may eventually diminish.

- d. Does the SCHER think that the current emissions into the environment from ongoing uses have a significant influence on the rate of reduction of the concentrations of PFOS found in the environment?*

Current emissions from ongoing uses will most likely influence the rate of reduction of the PFOS concentrations in the environment only on a local level, and will insignificantly affect the overall concentration found in the environment. Local contributions from metal platters, airports, and from the use of PFOS-containing fire fighting foam may still be significant.

In conclusion, in order to answer to Question 2, it is opinion of the SCHER that:

- The contribution of the confirmed on-going industrial/professional uses to the overall risks for the environment and for the general public are probably negligible with regard to the sectors photographic industry, semiconductor industry, and aviation industry. Emissions from the plating industry must however be restricted. With regard to human health, occupational exposure analyses should be performed, and a scientific risk assessment be performed. Regarding environmental risks, a long-term assessment of the risk associated to secondary poisoning considering the specificities of these chemicals and covering historical and on-going uses is required.

4. CONCLUSIONS

Because PFOS and PFOS related substances pose potential risk to human health and the environment, the SCHER agrees that risk reduction measures might be necessary. These measures should be substantiated by scientific risk assessment, taking into account the exceptional physico-chemical and toxicological properties of PFOS and PFOS-related substances, and the considerable reduction in production

volume after the phase-out of PFOS by the major producer the SCHER agrees that the re-occurrence of former uses (e.g. textile industry) must not be allowed, and that significant new uses must not be introduced in the future. The SCHER also agrees with the proposed restrictions for the plating industry, if there are no other measures available that could be applied to reduce emissions during metal plating to a significantly lower level. On-going critical uses in the aviation industry, the semiconductor industry, and the photographic industry do, however, not appear to pose a relevant risk to the environment or human health, if releases into the environment and workplace exposure are minimised. With regard to fire-fighting foams, the SCHER agrees that health and environmental risks of the proposed substitutes must be assessed before a final decision is taken.

5. LIST OF ABBREVIATIONS

BAF	Bio-accumulation factor
BCF	Bio-concentration factor
ECB	European Chemical Bureau
EUSES	European Union System for the Evaluation of Substances
LOAEL	Lowest Observed Adverse Effect Level
MSDS	Material Safety Data Sheets
NOAEL	No Observed Adverse Effect Level
OECD	Organization for Economic Cooperation and Development
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PFOS	Perfluorooctane sulfonate
POP	Persistent Organic Pollutant
RER	Risk Evaluation Report
RPA	Risk & Policy Analysts Limited
SMR	Standardized Mortality Rate
TGD	Technical Guidance Document

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ENVIRONMENTAL RISK EVALUATION REPORT: PERFLUOROOCTANESULPHONATE (PFOS)

D Brooke, A Footitt, T A Nwaogu

Research Contractor:

Building Research Establishment Ltd
Risk and Policy Analysts Ltd

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It is not the intention of this report to imply that any particular substance should or should not be used for any particular purpose. It is the aim of this report to evaluate concerns in respect of a wide range of different substances. This report should only be used as a tool to assist in identifying priorities for environmental risk assessment. It should not be used in isolation to assess whether any particular substance should be used for any particular purpose.

The Agency has used all reasonable skill and expertise to ensure that the findings of this report are factually correct. The Agency accepts no liability for:

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Dissemination Status

Internal: Release to Regions
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Statement of Use

This report summarises the environmental hazards and risks of PFOS based on its recent and current use pattern in the EU. The information will be used by the Chemicals Policy function of the Agency and the Department of Environment, Food & Rural Affairs to inform decisions on the need for risk management.

Keywords

PFOS, perfluorooctane sulphonate, perfluoroalkylated, hazard, risk, PBT

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Foreword

Perfluorooctane sulphonate (PFOS) is a fully fluorinated anion, the related compounds of which are members of the large family of perfluoroalkylated substances (PFAS). The term PFOS-related substances is used in this document to represent any substance containing the PFOS moiety ($\text{C}_8\text{F}_{17}\text{SO}_2$) with the potential to degrade to PFOS in the environment.

In 2000, 3M (a major global producer of PFOS based in the United States) announced that the company would phase out the production of PFOS voluntarily from 2001 onwards. Production by 3M has now ceased. At a meeting of the OECD Task Force on Existing Chemicals following this announcement, several OECD countries agreed to work together informally to collect information on the effects of PFOS on the environment and on human health to allow hazard assessment to be produced. This hazard assessment concluded that the presence and persistence of PFOS in the environment, as well as its toxicity and bioaccumulation potential, indicate a cause for concern for the environment and for human health.

In response to these findings, the Environment Agency has commissioned this study to review the risks arising from current uses of PFOS-related substances. This work is being undertaken in tandem with a study commissioned by the Department for Environment, Food and Rural Affairs (Defra) to prepare a Risk Reduction Strategy for PFOS-related substances, including an analysis of the advantages and drawbacks of potential risk reduction options. This Risk Reduction Strategy will follow the provision of the EU Existing Substances Regulation according to which, where controls on the marketing and use of the substances in question are proposed, an analysis of the advantages and drawbacks of the substance should be undertaken.

A PBT assessment has been carried out as part of this risk evaluation. Although this PBT assessment concludes that PFOS is a PBT, calculations to allow a PEC:PNEC comparison have also been conducted to help establish priorities for the risk reduction strategy.

This risk evaluation report (RER) has been prepared in accordance with the principles of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of “existing” substances (the “Existing Substances Regulation” or “ESR”) and the methods laid down in Commission Regulation (EC) 1488/94¹ which is supported by a technical guidance document (TGD)². The layout follows the format of an ESR RAR with a few small modifications, so that a reader familiar with such assessments can quickly find the information in which they are interested. It has been produced using publicly available data gathered and assessed by the contractor for the Environment Agency. Additional information has been submitted by various organisations and companies as part of the information gathering and consultation for the preparation of the Risk Reduction Strategy. Comments received during a consultation on a draft version of this report in June 2004 have also been taken into account.

Peer review process: The Environment Agency wishes to ensure that the data used in this report are as complete and accurate as possible. Original reports have been obtained and assessed for reliability where possible (it is clearly indicated where this is not the case), except where this has already been done for OECD SIDS purposes. In addition, results from the robust summaries in the 3M assessment (3M, 2003) have been included on the basis of the

¹ O.J. No. L 161, 29/06/1994 p. 0003 – 0011

² Technical Guidance Document, (First edition), Part I-V, ISBN 92-827-801[1234] and Second Edition, Parts I-IV, EUR 20418 EN/1-4

validity markings given in them. The report is currently under discussion by stakeholders with the aim of reaching consensus. However, it has not been possible to obtain as much industry-specific information as initially hoped. The information contained in this report does not, therefore, necessarily provide a sufficient basis for decision-making regarding the hazards, exposures or the risks associated with the substance.

In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised contact the Environment Agency beforehand.

The Environment Agency would like to thank those who contributed information to the Risk Reduction Strategy and hence to this risk evaluation.

Date of Last Literature Search:

February 2004

Executive Summary

PFOS is the perfluorooctane sulphonate anion and is not a substance as such. It is or was commercially available in the form of salts, derivatives (PFOS-substances) and polymers (PFOS-polymers). The PFOS moiety is very stable, and has been found in biota in large parts of the world. This evaluation has been produced to support the development of a risk reduction strategy for PFOS, and has been carried out using the methods of the EU Technical Guidance Document.

The term PFOS-related substance is used to refer to any or all of the substances which contain the PFOS moiety (defined as the $C_8F_{17}SO_2$ group) and may break down in the environment to give PFOS. PFOS, the substances and the polymers are made from a common source material. The major producer of the substances, 3M, has voluntarily ceased production. Hence the use in some areas has reduced significantly or even stopped, although the potential market for use remains since there are other known suppliers.

The major uses for the PFOS-related substances were in providing grease, oil and water resistance to materials such as textiles, carpets, paper and in general coatings. The substances used in these areas were largely PFOS-polymers for fabrics and PFOS-substances for paper treatment and coatings. Other smaller volume uses, which are continuing for the present, are in chromium plating, photolithography, photography and in hydraulic fluids for aviation. One further use in fire-fighting foams is discussed; the inclusion of PFOS-related substance in new foams has ceased, but stockpiles of foams containing PFOS still exist and may be used.

In order to simplify the calculations and evaluation, the use areas have been treated as if they use one or more of: PFOS itself; an example PFOS substance; or a hypothetical polymer. Estimates of emissions from each use area have been made, including for those uses which are considered to have reduced or ceased (as it is possible that other suppliers could provide products for use in these areas). The estimates are made on the basis of a mixture of information from industry, emission scenario documents and the default values from the Technical Guidance Document. The emission estimates are for emissions of PFOS, PFOS-substance or PFOS-polymer as appropriate. The calculations of the environmental distribution and concentrations have been made using the EUSES 2 program, considering various combinations of the use patterns and different rates at which the substances and polymers may break down to PFOS in the environment.

PFOS is not degradable, either abiotically or through aerobic or anaerobic biodegradation. It has low volatility. It accumulates in fish, with a measured bioconcentration factor of 2796. Measured sorption coefficients show moderate sorption in soils, less in sediment.

The ecotoxicity data for PFOS have been reviewed. Although limited, the data do not show any obvious differences in toxicity between different salts. PFOS is moderately toxic to aquatic organisms, with acute toxicity values ($L(E)C_{50}$) in the range 1 - 10 mg/l. Coupled with the lack of degradability, this indicates that it would be classified as dangerous for the environment, with the risk phrases R51 (toxic to aquatic organisms) and R53 (may cause long term adverse effects on the aquatic environment). There are several long term test results with aquatic organisms available, the lowest no effect concentration being 0.25 mg/l for Mysid shrimp. The PNECs for freshwater and marine water are derived from this value as 25 μ g/l and 2.5 μ g/l respectively.

Mammalian toxicity data have not been reviewed for this evaluation; instead the evaluation carried out for the OECD hazard assessment has been used. The key study reviewed for the OECD report was a two-year feeding study with rats which had a no observed adverse effect level of 0.5 ppm for liver effects in male rats. This has been used to give a PNEC for secondary poisoning of 0.0167 mg/kg wwt. An alternative PNEC of 0.067 mg/kg has also been considered.

A PBT assessment shows that PFOS meets the P (and likely the vP), B and T criteria. Strictly this means that a conventional risk evaluation through a PEC:PNEC comparison is not required. However, it was considered useful to carry out such a comparison in order to assist in the setting of priorities for the risk reduction strategy. The risk evaluation shows possible risks for secondary poisoning for all use areas in all of the scenarios used to examine the effects of different rates of break down and different combinations of releases. Risks are also indicated for secondary poisoning through exposure to the calculated regional background concentrations, for the freshwater and marine food chains. The same is true when using the alternative PNEC value. As both the PNECs are based on long term studies, possible refinement of the evaluation seems most likely through revision of the emission estimates. For freshwater, the regional emissions would need to be reduced to less than one twelfth of the estimated values in order to remove the risk at the regional level (assuming a similar distribution of emissions), or to less than one third if the alternative PNEC is used. There are also indications of possible effects from the releases of fire fighting foams to water. Again the PNEC is unlikely to be revised upwards; here, the release estimates are necessarily arbitrary to some degree, as they relate to the use in fighting fire.

There are a number of areas of uncertainty in the evaluation, in particular in relation to the degradation of PFOS-substances to PFOS in the environment (both the rate and the yield). A number of different assumptions about this have been included in the evaluation. Although some of the alternative assumptions result in a reduction in the PEC/PNEC ratios, in most cases the risks for secondary poisoning remain. A comparison of the PNEC(s) with concentrations measured in aquatic biota suggests that levels of concern may be present in some areas.

N.B. No assessment of risk to humans has been carried out.

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1

GENERAL SUBSTANCE INFORMATION

1.1

IDENTIFICATION OF THE SUBSTANCE

The subject of this risk evaluation is not a discrete substance and does not have a CAS number. It is the perfluorooctane sulphonate anion, known as PFOS. The parent sulphonic acid and some of its commercially important salts are:

Perfluorooctane sulphonic acid (CAS No. 1763-23-1)

Potassium salt (CAS No. 2795-39-3)

Diethanolamine salt (CAS No. 70225-39-5)

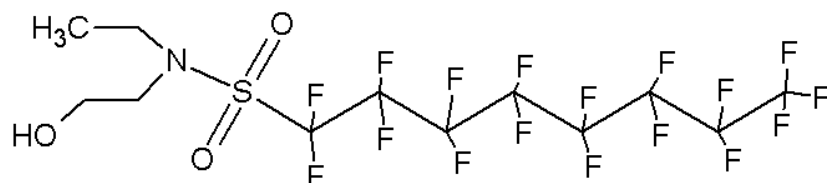
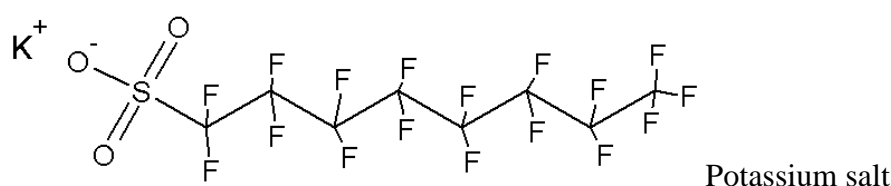
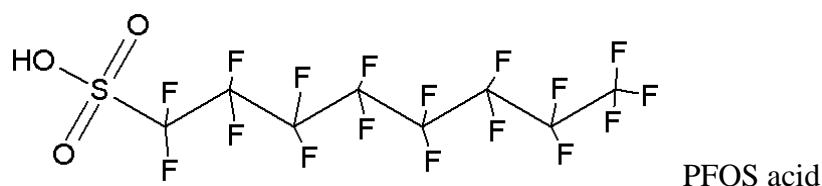
Ammonium salt (CAS No. 29081-56-9)

Lithium salt (CAS No. 29457-72-5)

The following sections deal with the properties of this group of substances, generally determined for the potassium salt. The risk evaluation also considers possible releases of PFOS from other products, either directly or from the breakdown of other substances. Section 2 discusses the production of these substances and the properties necessary for assessing their environmental behaviour are discussed in Section 3. Some issues relating to the purity and composition are considered in the current section.

For the purpose of this evaluation, all substances (simple or polymeric) which contain the PFOS moiety ($C_8F_{17}SO_2^-$) are termed PFOS-related substances.

Structure



Example amido derivative, N-EtFOSE (n-ethylperfluorooctanesulphonamidoethanol)

It is expected that the anion will be the form present in the environment, probably associated with metal cations (3M, 2003).

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Composition

The production processes for PFOS-related substances are described in Section 2. Although the starting material is n-octane sulphonyl fluoride, this will contain some non-linear C₈ compounds. The fluorination process is expected to lead to some fragmentation of the chain. Thus the product of the fluorination step will contain linear and non-linear chains, mostly C₈ but with other chain lengths present. RIKZ (2002) quote 3M as reporting a final product (as PFOSF, see Section 2) of approximately 70% n-PFOSF and 30% branched impurities including odd and even chain lengths. An alternative description of the content is 90% of C₈ molecules, of which 25% are branched, with 5-10% C₆ compounds and the remainder C₇ (2-5%) and C₅ compounds. A similar distribution is assumed to apply to all products based on the ECF process (see Section 2.1.1), whether produced by 3M or by other companies. No specific information on other companies' products has been identified.

These figures relate to the major precursor of PFOS-related substances, and are assumed to apply to the products made from this. For the purpose of this evaluation, the presence of differing chain lengths is ignored. The quantities of PFOS-related substances produced and used are assumed to relate to the C₈ moiety, recognising that this may lead to some over-estimation of releases.

Other issues relating to the composition of substances and polymers made from the precursor are considered in Section 2, in particular in relation to the presence of residual monomers or unreacted substances in products.

1.2.2 Additives

No information on possible additives has been located.

1.3 PHYSICO-CHEMICAL PROPERTIES

This section discusses the properties relevant to the PFOS anion, mainly in the form of the potassium salt. Data are taken largely from the OECD hazard assessment (OECD, 2002) and the 3M assessment (3M, 2003). The original sources of these data have not been reviewed; in many cases they are 3M reports, which are summarised in the robust summaries in the 3M (2003) document.

1.3.1 Physical state (at ntp)

The potassium salt of PFOS is a white powder at normal temperature and pressure.

1.3.2 Melting point

No indications of melting were seen with the potassium salt at up to 400°C, which was the maximum temperature specified for the instrument used. A value of >400°C will be used in this evaluation.

1.3.3 Boiling point

In view of the high melting point, no attempts have been made to measure the boiling point.

1.3.4 Relative density

The relative densities (specific gravities) of the salts are given in the OECD assessment as: potassium ~0.6; lithium ~1.1; ammonium ~1.1; diethanolamine ~1.1.

1.3.5 Vapour pressure

A vapour pressure of 3.31×10^{-4} Pa has been measured for the potassium salt, using the spinning rotor method (OECD 104). A note in the 3M assessment comments that this result is thought to be due to volatile impurities in the substance. This is supported to some extent by a calculated vapour pressure of 1.9×10^{-9} Pa using the modified Grain method in the MPBPVPPWIN (v 1.41) program, with a melting point of 400°C selected. Calculated values for other substances range from 3.1×10^{-11} Pa for the diethanolamine salt to 0.85 Pa for the acid. The measured value will be used in this evaluation, recognising its limitations.

1.3.6 Water solubility

The OECD assessment quotes a value of 570 mg/l in pure water from 3M (1999) reports. The more recent 3M assessment (2003) has the results of two determinations. Both involved the equilibration of an excess of the substance with pure water at 30°C, followed by equilibration at a lower temperature (either 20°C or 24-25°C). Solutions in the 24-25°C experiment were centrifuged before sampling and analysis, this process was not noted for the 20°C experiment. The results were 519 mg/l at 20±0.5°C, and 680 mg/l at 24-25°C. The 3M assessment takes a mean value of 600 mg/l. Both studies are given a validity rating of 1 and so are considered fully valid. For this evaluation the value at 20°C, 519 mg/l, will be used as closer to environmental conditions.

Solubility has also been determined in salt waters (3M, 2003), and is reduced from that in pure water. In natural seawater a solubility of 12.4 mg/l at 22-23°C was measured. A value of 20.0 mg/l was obtained for a sodium chloride solution at 3.5% salinity, the same as the natural seawater.

1.3.7 n-Octanol-water partition coefficient

The surface active properties of the substance make a direct determination of the octanol-water partition coefficient impossible. In a preliminary study reported by 3M an inseparable emulsion was formed. 3M determined the solubility of PFOS in octanol as 56 mg/l, and calculated the log Kow from the ratio of solubilities, giving a value of -1.08. The study summary notes that this is not a real value.

Using the KOWWIN program, a value of 4.13 is calculated for log Kow for the potassium salt, and a value of 6.28 for the acid. The reliability of this program for substances of this type is unknown.

The octanol-water partition coefficient is often used to estimate other properties such as bioconcentration factors and sorption coefficients. The lack of a reliable measured value for this substance means this cannot be done here. However, a number of the required properties have been measured directly, and although there are limitations to these measurements they will be used in the evaluation. Where values for properties have not been measured, a surrogate value for the log Kow will be estimated from suitable measured data. These values will be discussed in Section 3.

1.3.8 Henry's Law constant

3M report a study designed to measure air-water partitioning directly. Although a non-standard method, it was considered suitable for substances with low air-water partition coefficients (K_{aw}). The conclusion from the study was that PFOS was essentially non-volatile, significantly less so than water, which has a K_{aw} of 2×10^{-5} (Henry's law constant of $0.044 \text{ Pa m}^3/\text{mole}$).

From the solubility and vapour pressure values above, the Henry's Law constant can be calculated as the ratio, at $3.19 \times 10^{-4} \text{ Pa m}^3/\text{mole}$ ($K_{aw} = 1.35 \times 10^{-7}$). This value will be used in the evaluation as an indication of the properties of the substance.

1.3.9 pKa

No direct measurement of the pKa of the acid has been located. A calculated value of -3.27 has been provided in comments from Finland, obtained from the ACD/I-Lab Web Service. 3M (personal communication) confirm that the substance is considered to be a strong acid. The value indicates that PFOS will be present in the environment completely in the ionised form, and that all determination of properties here which involve the substance in solution relate to the anionic form.

1.3.10 Summary of physico-chemical properties

A summary of the physico-chemical data used for the risk assessment is given in Table 1.1.

Table 1.1 Physico-chemical properties

Property	Value and comment
Physical state at ntp	Solid
Molecular weight	500 for acid; 538 for potassium salt
Vapour Pressure	$3.31 \times 10^{-4} \text{ Pa}$
Water solubility	519 mg/l
n-Octanol-water partition coefficient (K_{ow})	Not possible to measure
Henry's Law constant	$3.19 \times 10^{-4} \text{ Pa m}^3 \text{ mole}^{-1}$ – from vapour pressure/solubility ratio
Acid dissociation constant (pKa)	-3.27 (calculated)

From the very low pKa value, all properties involving solution relate to the ionised form.

2

GENERAL INFORMATION ON EXPOSURE

Information in this Section and Section 3 has been taken from a number of sources. Where these are published sources, this has been indicated. In some cases the information was obtained as part of the consultations carried out for the production of the Risk Reduction Strategy report, which included consultation on aspects required for this risk evaluation. Reference to “the consultation” in this report is intended to cover these cases. More detail can be found in the Risk Reduction Strategy report in some cases.

2.1 PRODUCTION

It is understood that production of significant quantities of PFOS-related substances has never taken place in the UK. All such substances have been imported. Therefore this life cycle step is not considered further in this evaluation in terms of quantities produced or emissions. However, a description of the production processes is needed to explain the source of the different types of substances considered in this evaluation, and to contribute to the basis on which they are later grouped.

2.1.1 Production process

Information in this section is taken from the 3M assessment (3M, 2003) and the OECD hazard assessment (OECD, 2002). PFOS-related substances are manufactured by a process known as Simons Electro-Chemical Fluorination (ECF). In this process, organic feedstocks are dispersed in liquid anhydrous hydrogen fluoride, and an electric current is passed through the solution, leading to the replacement of all of the hydrogen atoms in the molecule with fluorine atoms.

The starting feedstock for this process is 1-octanesulphonyl fluoride, and the initial product is perfluorooctanesulphonyl fluoride (PFOSF). This product is sold commercially to some extent, but is mainly used as an intermediate in the production of other substances. The simplest of these is PFOS itself, produced by hydrolysis of PFOSF. The various salts are then produced from this.

The majority of PFOSF is reacted first with either methylamine or ethylamine to give either N-methyl- or N-ethyl perfluorooctane sulphonamide. These intermediates can be used to make various amides, oxazolidinones, silanes, carboxylates and alkoxylates which are available commercially.

The sulphonamide derivatives can be reacted with ethyl carbonate to form either N-methyl- or N-ethylperfluorooctanesulphonamidoethanol (N-MeFOSE and N-EtFOSE). These then form the basis of adipates, phosphate esters, fatty acid esters, urethanes, copolymers and acrylates as commercialised products. The majority of the PFOSF-related products made by 3M were from this group of products.

It should be noted that the secondary reactions producing the various products are single or sequential batch reactions, and do not necessarily lead to pure products. There may be varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials or intermediate products) carried forward into the final product. According to 3M these residues are present at around 1% or less in the final commercial products. Where information has been provided on fluorochemical residuals for this evaluation, it has been assumed that it relates to these unreacted materials. Possible releases of these residuals are

considered along with releases of the products themselves in Section 3, with an assumed content of 1% if no more specific information is available.

Figure 2.1 shows the flow of PFOS-related substances from initial production to final products.

2.2 GROUPING OF PFOS-RELATED SUBSTANCES

As part of the work in developing the Risk Reduction Strategy, a list of substances containing the PFOS moiety was produced (Appendix 1). These substances are considered to have the potential to lead to releases of PFOS to the environment. These include salts of PFOS, simple derivatives and polymeric materials. In attempting to assess the emissions of PFOS-related substances to the environment, it is neither practical nor realistic to attempt to assess the release and fate of each individual substance. Rather, these substances have been grouped in a way that takes account of the apparent relative ease with which PFOS could be produced. This has been done on a fairly subjective basis, as there is little information on the breakdown of these substances in the environment. In grouping these substances, the general type and description of the PFOS-related substances used in each sector have been used to select the relevant group for the substances. A three way division has been employed on the above basis.

The first group includes substances which are effectively PFOS itself, in the form of salts of perfluorooctane sulphonic acid - salts with potassium, lithium, sodium, ammonium (including quaternary ammonium) and diethanolamine. The use of products containing these substances can lead to the direct emission of PFOS to the environment. The properties of PFOS salts have been used as far as possible in estimating emissions and behaviour in the environment. Measured property values have been used as far as possible, rather than the usual estimates from QSAR approaches - in particular, measurements of sorption coefficients and bioaccumulation. For the purpose of estimating releases and environmental behaviour, these substances will be called **PFOS-salts** (salts rather than acids as the species will be fully ionised in water in the environment).

In the second group are individual substances which are made from perfluorooctane sulphonyl fluoride (PFOSF) in a parallel route to the production of PFOS. These are the FOSA and FOSE-type substances, together with their relatively simple derivatives. These are considered to be potential sources of PFOS in the environment through degradation. There is some evidence for this with the substance N-EtFOSE, but little or none for any other substance. The 3M report (3M, 2003) comments that no production of PFOS was seen through hydrolysis or aqueous photolysis of a number of compounds; formation of PFOS was only seen through biodegradation, largely in studies on N-EtFOSE. Thus assumptions about the extent to which this happens, and the rate, have been made in the model calculations in Section 3. It is not possible to treat each substance of this group individually, so a generic set of properties has been used to estimate emissions and behaviour. There is also little information on the properties of these substances. Most of the information available relates to N-EtFOSE, and this will be used as the basis for these calculations. For the purpose of estimating releases, these substances will be called **PFOS-substances**.

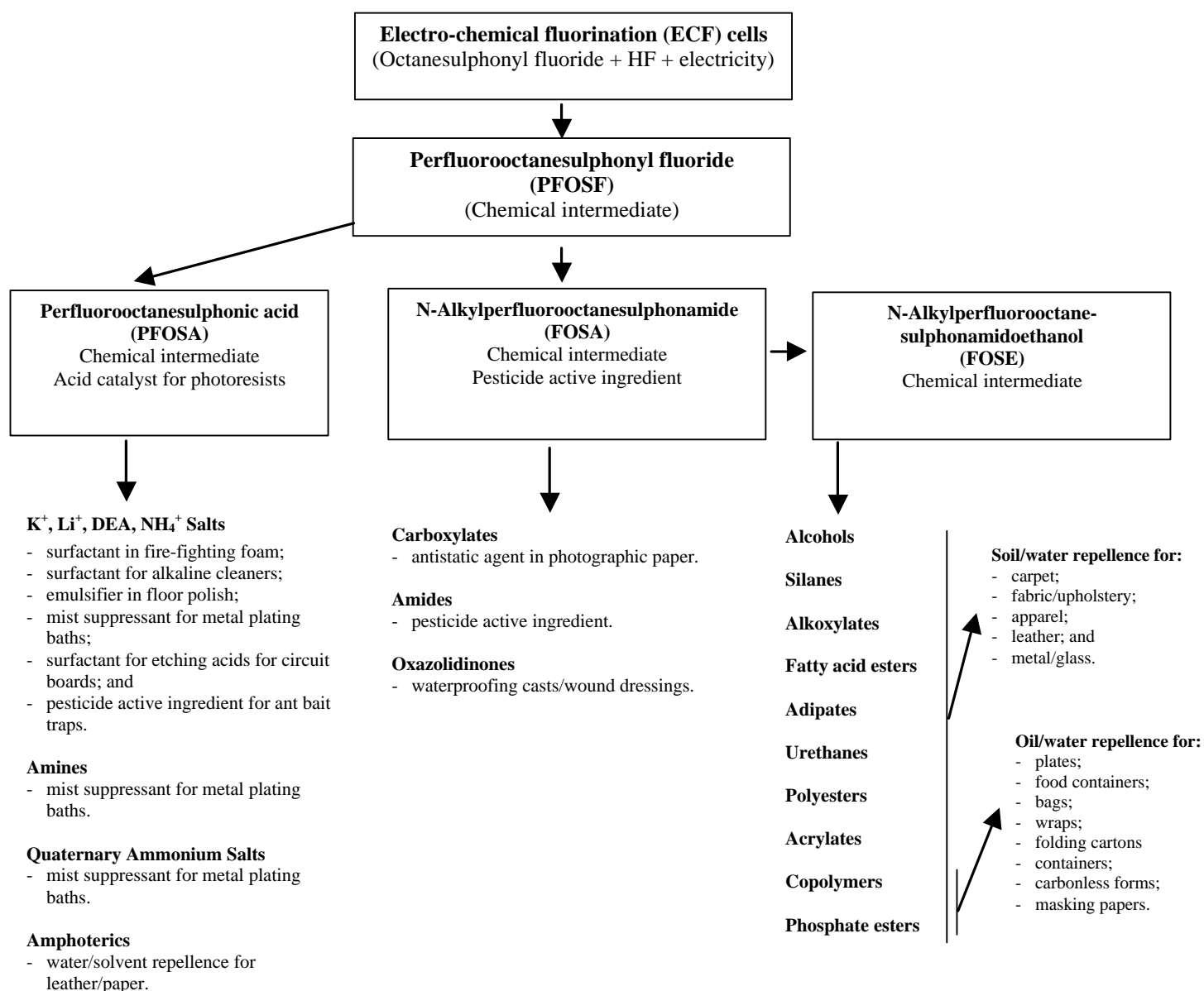


Figure 2.1 Major Product Categories and Applications for Perfluorooctylsulphonates (OECD, 2002)

The third group are polymeric materials, higher molecular weight polymers derived largely from the FOSE-type substances. These have also been suggested as potential sources of PFOS in the environment. RIKZ (2002) assumed that all of the PFOS contained in these substances was released. 3M in contrast considered that the polymers were non-degradable. No specific evidence on this has been located. The properties of these polymers may vary over a considerable range, and generic values will be needed. It has been assumed that they have low vapour pressures, low solubilities and a higher affinity for solid phases in the environment. Assumptions have been made about the extent to which they will break down to PFOS in the environment in the model calculations in Section 3. These substances may contain residual PFOS-substances, and releases of these will also be considered. This group of substances will be called **PFOS-polymers**.

It has been assumed throughout that only substances and polymers derived originally from PFOSF have the potential to degrade to PFOS in the environment. Hence telomer-derived substances and materials are not included, nor are perfluorooctanoic acid (PFOA) related substances. It should however be noted that in some areas, it is not always possible to tell which specific type of material is being used. Similarly, the distinctions between the three groups (PFOS-salts, PFOS-substances and PFOS-polymers) are not always clear, and hence in some cases materials may be incorrectly allocated. In some cases the allocation has been simplified by assuming that all of the material used in a particular area is of one group, where the reality may be that substances from different groups are used.

2.3 USES

2.3.1 General information on uses

The use pattern for PFOS-related substances in the UK and the EU has changed significantly since 3M announced their intention to cease the manufacture of a range of these substances. Information collected during the consultation indicates that their use in a number of the major areas has effectively ceased, as users have moved to different types of substance to provide a similar function.

The discussion of uses will initially address those uses expected to continue after the cessation of manufacture by 3M, either because there are no alternatives available at present³, or where there are stocks of PFOS-related substances to use up. These include:

- metal plating;
- semi-conductors;
- photographic;
- aviation; and
- fire fighting foams stock.

Uses which have taken place in the UK in the past but which are not considered to be relevant at present will also be considered, especially where products treated with PFOS-related substances may be in use from some time. These include:

³ Although 3M have ceased production of these substances, there are other potential suppliers for continuing uses. Consultation identified a number of possible suppliers to the UK, for more details see the Risk Reduction Strategy. The EA review of perfluoroalkylated substances (EA, 2001) also has some information on potential other producers of PFOS-related substances, in the confidential project record.

- carpets;
- leather/apparel;
- textiles/upholstery;
- paper and packaging; and
- coatings and coating additives.

Other uses which are not considered relevant to the UK or the EU will be mentioned.

In order to provide a common baseline, usage data from 2000 have been used in the evaluation wherever possible. These are largely from information supplied by 3M on the amounts of PFOS-related substances imported into the UK.

2.3.2 Metal (chromium) plating

PFOS-related substances are used to lower the surface tension of metal plating solutions to prevent the formation of mists containing potentially harmful components from the baths. As such they are used in chromium plating, in anodising and in acid pickling. The substances used are of the PFOS-salt type. Four companies supplying mist suppressants containing PFOS-related substances in the UK and the EU indicated the use of the tetraethylammonium salt of PFOS for this purpose.

The amount used in the UK has been estimated as <0.5 tonnes per year from information collected during the consultation. This level of use is considered to have been stable for the last few years. The Risk Reduction Strategy estimated that there were around 300 chromium platers in the UK. A company in Germany estimated that the EU market was 8.6 - 10 tonnes per year for PFOS-related substances, and this level of use will be used in the estimations.

2.3.3 Photolithography (semi-conductors)

PFOS-related substances are used in a number of functions within the semi-conductor industry. These relate to photolithography, which is the process by which the circuits are produced on the semi-conductor wafers. This process uses photoresists, materials whose composition is altered on exposure to light, making them either easier or more difficult to remove and so allowing structures to be built up in the wafer. The PFOS-related substances can form part of the photoresist itself, acting as a photoacid generator in the chemical amplification of the effect of exposure. They can also be used to add a thin coating to the resist to reduce reflections, either to the top (top anti-reflective coatings, TARC) or bottom (bottom anti-reflective coatings, BARC). PFOS-related substances may also be used as surfactants in developers, or in ancillary products such as edge bead removers (EBRs). ESIA (European Semi-Conductor Industry Association) and SEMI (Semiconductors Equipment & Materials International) have provided information on the use of PFOS-related substances in the EU in 2002 and this is presented in Table 2.1. The total use is 470 kg per year.

Table 2.1 Typical Concentrations and EU Consumption of PFOS Related Substances in Preparations Used in Semiconductor Applications

Application	PFOS Concentration	EU Consumption (kg/y)
Photoresists	0.02 - 0.1%	46
EBR	Not available	86
TARCs	ca. 0.1%	136
BARCs		8
Developers (surfactant)	ca. 0.01%	195

There is little information about the specific substances which are used in this area, and the companies involved are reluctant to identify them. For the purpose of this evaluation, it is assumed that they are all PFOS-substance type. This assumption will result in greater emissions of PFOS than if they were assumed to be polymers.

2.3.4 Photography

Information collected through the consultation indicates that PFOS-related substances are used in coatings applied to photographic films, papers and printing plates. The substances were also used in developing solutions in the past, but recent information from EPCI (European Photographic Chemicals Industry) states that they have been removed from such products. The main function of the substances is to act as anti-static agents. This is to prevent static discharge, both for the possible effects on workers handling the material, and from the possible exposure of photographic materials to the discharge. The substances also help in reducing friction, thereby improving transport in cameras, printers and projectors, and are essential to the laying down of multiple thin layers of photographic material on film, creating coatings of high complexity in a highly consistent and rapid manner. EPCI indicate that the main area of use for PFOS-related substances is in the medical area (x-rays), with 85% of EU use in this sector.

In the course of the consultation, six substances which are used in this area were identified. Of these, one is of the PFOS-salt group, one of the PFOS-substance group and the other four are PFOS-polymers. The OECD assessment indicates that the carboxylate derivatives of N-alkyl-perfluorooctane sulphonamide are used as anti-static agents. For these calculations the substances used in this area are assumed to be PFOS-salt for the production of film step, and PFOS-polymers for subsequent steps. The polymers are assumed to contain 1% of PFOS-substances as residuals. Information on the relative proportions of the various types would allow the estimates to be improved.

EPCI have provided data on the use of PFOS-related substances in the EU. The amount of PFOS-related substance used in the production of film in the EU is estimated to be 850 kg per year. In addition to this, finished articles containing PFOS-related substances imported into the EU add a further 150 kg per year. Exports of articles containing PFOS-related substances account for 250 kg per year. Hence the overall amount of PFOS-related substance in film used in the EU is 750 kg per year.

Information from EPCI indicates a concentration of PFOS-related substance in film of 0.1 - 0.8 $\mu\text{g}/\text{cm}^2$. According to the emission scenario document on the photographic industry in the Technical Guidance document (TGD), x-ray film has a double coating of photographic material. As this is the main use area of PFOS-related substances according to the information from EPCI, the high end of the composition range will be used in the calculations. Using this, the amount of film which would contain 750 kg is $9.4 \times 10^{11} \text{ cm}^2$. For comparison, the amount of x-ray film used in the EU according to the ESD is $7.9 \times 10^{11} \text{ cm}^2$, which is in good agreement.

2.3.5 Aviation

PFOS-related substances are used as a component of fire-resistant hydraulic fluids in aircraft. They act to inhibit erosion and damage to parts of the hydraulic systems and are present in the fluids at ppm levels. The specific substance used in these fluids is potassium perfluoroethylcyclohexyl sulphonate, rather than the perfluorooctane sulphonate which is

PFOS. Nevertheless they are included in this evaluation, as the perfluorooctane salt may be the replacement should the ethylcyclohexyl substance become unavailable. The ethylcyclohexyl compound has also been identified by other regulatory agencies in the US and Canada, and 3M have also included this in the products which they have discontinued.

The manufacture of these fluids takes place outside the EU, and so this step is not considered in the evaluation. The world-wide use of PFOS-related substances in this area is estimated to be 2.2 tonnes per year (information collected through the consultation). For the purpose of this evaluation, it is assumed that one third of this, or 0.73 tonnes, is used in the EU each year. The substance is of the PFOS-salt type.

2.3.6 Fire-fighting foams

Foams have been developed to assist in fighting fires involving flammable liquids, where the use of water is unable to extinguish the fire and may contribute to its spread. Fluorocarbon surfactants are a component of such foam concentrates, along with synthetic foaming agents, solvents and other substances. In use these foam concentrates are mixed with water and aspirated with air to produce the finished foam. In aqueous film-forming foams, the fluorinated surfactants move to the solution-air interface and produce a very thin film which spreads over the liquid fuel fire. They are also used in alcohol-resistant foams for fires involving more polar solvents, which tend to disintegrate conventional foams. Information on foam products provided during the consultation indicates that the PFOS-related substances used are of the PFOS-salt type.

From the consultation, the majority of fluorocarbon surfactants included in foams produced currently are derived from the alternative telomer based technology, and so will not give rise to PFOS (the substances involved in the telomer products are largely based on C₆ chains rather than C₈). However, PFOS-related substances have been used in such foams and so the production and use of these foams is included in this evaluation.

In terms of quantities, it has to be considered that the foam concentrates are stockpiled by potential users, to be used as and when needed. There are two types of holders of such stocks within the UK, the Fire Authorities (FA) and major installations (which hold stocks for their own use and as part of mutual agreements between FAs and industry for provision of fire cover at major accidents). Information on the current size of such stockpiles at each of these holders has been gathered for the Risk Reduction Strategy. The resulting figures are ~76,000 litres of PFOS-based foam concentrate held by Fire Authorities, and 2,367,000 litres by major installations (in the UK).

The stocks held by major installations are not considered in this evaluation. These will be used in the event of a major incident, and such use is not considered to be part of the 'normal' use of the substances. The frequency of use by Fire Authorities is expected to be much higher. The average use of fire fighting foams in general by Fire Authorities in the UK has been estimated as 15% per year.

The estimate of use of PFOS-based foams is therefore based on the amounts held by Fire Authorities. Assuming a density of 1, there are 76 tonnes of foam concentrate in the UK, which at a content of 1% of PFOS corresponds to 760 kg. Based on a use rate of 15%, the use of PFOS-related substances in foams is 114 kg per year. Taking the UK as 20% of the EU, the total use is 570 kg per year, with 57 kg in the region.

The production of foam concentrates can be considered to be a formulation process. There are a limited number of companies producing foams of this type - ten are reported for the European Economic Area, with none in the UK. Information on the quantity produced is only available for one company, this will be used to estimate local emissions. The use of PFOS-related substances at this site was 40 tonnes per year, which at a concentration of 1% in the foam concentrate made a total of 4,000 tonnes of concentrate. Note that these are historical data, as the company no longer uses PFOS-related substances in its foams.

2.3.7 Protective coatings for fabrics (carpets, textiles and leather)

PFOS-related substances have been used on a range of fabrics and other materials to provide soil, water and oil resistance. They have generally been applied as a coating to the surface, to create a protective barrier. The types of PFOS-related substances are the acrylate, adipate and urethane polymers produced from the intermediate substance N-ethylperfluorooctane sulphonamido ethanol. Therefore, for the purpose of this evaluation they are considered to be PFOS-polymers. As such, they contain residual levels of low molecular weight substances from the manufacturing processes. This residual material is assumed to be PFOS-substances, and is present at a level of 1% of the polymer (information provided for the consultation).

The treatment of fabrics with PFOS-related substances is no longer thought to take place in the UK. For the purpose of this evaluation, the possible emissions from the treatment process will be considered as an example calculation. Although no longer produced in the UK, treated fabrics are in use at present and will be so for some time, as at least some of the treated product (carpets, upholstery) have significant lifetimes. Therefore estimates are made of the possible releases of polymers and of PFOS-substances from such materials in use.

Information on the use of PFOS-related substances in this area was obtained through the consultation exercise. The approximate figure for use in the UK was 48 tonnes of PFOS-related substance (as polymer), with 23 tonnes on carpets, 15 tonnes on apparel and leather, and 10 tonnes on upholstery. These figure have been used to make estimates of the possible emissions from this area. Assuming that the UK accounts for 20% of EU use, the total for the EU would be 240 tonnes.

2.3.8 Paper treatment

PFOS-related substances have been used to treat a range of paper types and products. As for fabrics, the main function is to impart grease, oil and water resistance. Such products have been used in food contact applications. The major type of substance used appears to be phosphate derivatives of N-EtFOSE (3M, 1999), and they are therefore considered to be PFOS-substances for this evaluation. They are consider to be mainly applied during the paper making process, rather than being added to finished paper in subsequent operations. The approximate use of PFOS-related substances in this area in 2000 was 32 tonnes; assuming that the UK accounts for 20% of the use then the EU total would be 160 tonnes.

Releases from the paper making process are estimated in Section 3. For paper, there is the possibility of recycling. However, the nature of the use of these treated papers (food wrapping etc) suggests that they are more likely to be disposed of in household or municipal waste rather than entering the recycling streams. Therefore a paper recycling scenario is not considered.

2.3.9 Coatings

Perfluorinated substances (as a more general class than PFOS-related) have been indicated as being used in a wide range of areas in the coatings industries. Some of these areas overlap with areas covered above, such as textiles. No information on specific uses of PFOS-related substances has been found in this area, and it may be that such uses are no longer relevant for the UK. For the purpose of this evaluation, it is assumed that the use was in paints, and that PFOS-substances were used. The amount used in the UK in 2000 was 18 tonnes; assuming the UK to account for 20% of the EU gives an EU total of 90 tonnes. The content of PFOS-related substances in coatings is indicated as 0.1 - 1.0% from the consultation information; assuming an average of 0.55% means that 3,273 tonnes of coating were produced in the UK per year, and 16,346 tonnes in the EU.

2.3.10 Other uses

2.3.10.1 Industrial and household cleaning products

A wide range of applications of fluorosurfactants in the household and personal care and industrial cleaning areas has been identified in the consultation for the Risk Reduction Strategy. For more details, see the Risk Reduction Strategy report. As far as PFOS-related substances are concerned, specific mention is made of products marketed as alkaline cleaners, floor polishes, denture cleaners and shampoos. These products may have been used by consumers as well as in industrial contexts. PFOS-related substances have been found in floor polishes and waxes in a survey in Denmark (this might also be considered as a coating use). There is no information on the amounts used in these areas, and so it is not possible to make any emission estimates.

2.3.10.2 Pesticides

The use of PFOS-related substances in the manufacture of baits against ants and beetles has been described by 3M. The consultation exercise found one company in the EU producing such baits, using 0.5 tonnes of PFOS-related substances (PFOS-salt type) per year. No such use has been identified in the UK. There are no scenarios available for emissions from the use of such baits. If all of this quantity were released to the environment then it could make a significant contribution, but no information on the fate of component in such baits is available.

2.3.11 Summary

Table 2.2 summarises the quantities estimated as being used in the EU from the information in the preceding sections. Note that these figures relate largely to the situation before some of the PFOS products were removed from the market.

Table 2.2 Estimated EU use for each area

Use area	EU quantity (tonnes/year)	Substance type	Notes
Chromium plating	10	acid	Estimate from German company
Photolithography	0.47	substance	ESIA/SEMI estimate
Photography	0.85	acid	EPCI estimate, production of film
	0.75	polymers	EPCI estimate, use of film
Aviation	0.73	acid	One third of world use estimate
Fire fighting foams	0.57	acid	From UK estimate of foam use
Fabric treatment	240	polymer	From UK estimate of 48 tonnes, assuming UK is 20% of EU
Paper treatment	160	substance	From UK estimate of 32 tonnes, assuming UK is 20% of EU
Coatings	90	substance	From UK estimate of 18 tonnes, assuming UK is 20% of EU

2.4 TRENDS

The major world producer of PFOS-related substances was 3M. Quantitative data on production are only available for this company, but it is considered that the combined capacity of the other producers was very much less than that of 3M. The EA review of perfluoroalkylated substances (Environment Agency, 2001) indicated that 3M had the vast majority of the market for PFOS-based substances. In 2000, 3M produced around 3,665 tonnes of PFOSF, the precursor for PFOS-related substances. In 2003 this had been reduced to zero following 3M's decision to cease manufacturing.

As a result, the level of use in many areas has decreased significantly over the last two or three years, in some cases to zero. Users have moved to alternative fluorine-based products (telomer based) in some areas, and to other technologies in other areas. Details of these changes are included in the Risk Reduction Strategy report. The main areas where use is continuing at present are included in the first list in Section 2.3.1. It is expected that the use in fire-fighting foams will reduce further and eventually stop when existing stocks are used up or exceed their shelf life (unless they are disposed of without being used). The level of use in the other areas may depend on the outcome of the risk reduction strategy.

2.5 LEGISLATIVE CONTROLS

There is currently no legislation on the use of PFOS-reported substances in the EU directly related to their potential environmental and/or human health effects.

As noted above, the main producer of PFOS-related substances, 3M, voluntarily decided to phase out the production of these substances in 2000. A number of industry sectors have taken voluntary measures to reduce the potential emissions and risks from PFOS-related substances. More details of these are included in the Risk Reduction Strategy.

There have been a number of international initiatives. The US EPA introduced a significant new use rule (SNUR), requiring companies to inform them before manufacturing or importing any listed PFOS chemicals. There have been two such SNURs, listing 88 substances and allowing some derogations for essential uses (so that the rule does not apply to substances for these uses). Work is being carried out under the auspices of OSPAR to identify groups of PFOS substances which should be added to the OSAPR list of Chemicals for Priority Action.

3

ENVIRONMENTAL EXPOSURE

The evaluation of PFOS is complicated by the number of substances involved and the lack of information about the degree to which they act as sources of PFOS in the environment. As described in Section 2, the PFOS-related substances have been grouped into three types to simplify the evaluation. The approach taken here is to estimate the emissions of each of these three groups in the form that they used - acid, substance or polymer. Information on the fate and behaviour of PFOS is considered, along with any similar information for the substances or polymers. The evaluation considers the effect of different assumptions about the rate at which the substances and polymers break down in the environment on the predicted levels of PFOS. Various combinations of uses are also considered, to provide indications of possible levels from past uses and possible levels if certain uses were to continue.

3.1 ENVIRONMENTAL RELEASES

3.1.1 General introduction

Emissions have been estimated using a range of different sources of information. It is preferable to use information related to the specific substance and the specific area of use. In this evaluation there are specific estimates of PFOS-related substance emissions from photolithography, and some information on losses of polymeric materials from treated fabrics. For the other areas, use has been made of emission scenario documents (ESDs) where these are relevant. Documents used come from the Technical Guidance document, and also drafts from the OECD Task Force on Environmental Exposure Assessment. Where there is no suitable ESD, the default A and B tables from the Technical Guidance document have been used.

Releases have been estimated for local sources on a daily basis where appropriate. Larger scale emissions are presented on an annual basis. A large number of assumptions have had to be made in the course of this evaluation. In order to allow some comparison between the different use areas, the standard assumption of 10% activity in the region has been used as the basis for the regional emission estimates (with the exception of some formulation steps). This has been maintained even where there is specific information about the level of use in the UK.

3.1.2 Metal (chromium) plating

The substances used in this area are considered to be of the PFOS-salt type. Information from the consultation indicates that any formulation step for these products is likely to be simple dilution, and so only emissions from their use are considered here.

Emissions to water are estimated using the approach taken in the risk assessment for chromium (VI) compounds under the Existing Substances Regulation⁴. This approach was in turn based on an Emission Scenario Document on Metal Finishing (Environment Agency, 1997). It is assumed that a large scale processor treats 40 m² of metal per hour, over a 12 hour day for 240 days per year. Losses can occur from the treatment tank through solution remaining on the metal articles as they are removed from the tank – this is called drag-out. For a rack deposition system, the typical drag-out rate is 5 litres per 100 m² of surface treated. Hence for the site above, the drag-out rate would be 2 litres per hour. Information received

⁴ Draft risk assessment report available from the European Chemicals Bureau at <http://ecb.jrc.it/existing-chemicals/>, search on ESIS for substance name sodium dichromate

indicates that PFOS-salt substances are present in the treatment bath at ppm levels (personal comm.). Assuming a level of 10 ppm, then the removal rate for PFOS would be 20 mg per hour. Chromium plating takes place at ~40°C, and consequently there is some evaporation of water from the tank. This allows some of the drag out or rinse water to be returned to the treatment tank. The ESD suggests that 25% of the drag-out can be returned in this way, and so the amount lost is reduced by 25%, to 15 mg per hour. The drag out is removed from the metal by rinsing, so this substance is diluted in the rinse water, but the rate of loss is not affected by this. For a 12 hour day, the daily loss to water is therefore 180 mg/day. Over 240 days the annual loss is 43 g/year.

There may also be the possibility of emissions to air from this process. This should be low, as the function of the substance is to prevent mist formation during the plating process, and the substance has a low vapour pressure. An approach to estimating such emissions is to consider the maximum limit for chromium (VI) in air of 0.05 mg/m³, and to assume that all components of the treatment bath are present in any mist at their 'working' concentrations. From the ESD, the concentration of chromium (VI) in a hard hexavalent chromium bath is ~130 g/l. The volume containing 0.05 mg is therefore 3.8x10⁻⁷ litres. At a concentration of 10 ppm, this contains 3.8x10⁻⁶ mg of PFOS, hence the air concentration of PFOS is 3.8x10⁻⁶ mg/m³. No specific information on air flow rates in chromium plating works is available, but a rate of 7,200 m³/hour has been used for large lubricant blending sites (Environment Agency 1997a) and is used here as an illustration. For a 12 hour day, this gives a daily removal of air of 86,400 m³, and hence a release of PFOS of 0.33 mg/day. For a 240 day year, this is an annual emission of 79 mg.

From the calculations, the overall emission from the site is 43 g/year. This appears to be too low to account for the amount of PFOS used in this industry in the UK, which is estimated at 500 kg per year (Section 2.3.2). It would require over 10,000 sites of the size for which the calculation has been performed to account for this amount of PFOS, whereas in Section 2.6.6 there are estimated to be around 300 platers in the UK. The reasons for this difference are not clear. Consultation with the UK Health and Safety Executive (HSE) has indicated that the mist suppressant is added periodically to the tanks every two weeks, which suggests that the ability of the substance to provide the suppressant function is lost or reduced over this length of time. However, no specific information on amounts added is available. Losses of the substance are also possible when the contents of plating baths are disposed of, but information from the ESD and from industry is that such baths are maintained in use over long periods of time without disposal. Bath solutions can be cleaned up by filtration and ion exchange to remove particulates and other metals, but these methods are unlikely to remove PFOS. For this evaluation, the daily emissions estimated above will be used to estimate local concentrations. On the larger scale, it will be assumed that all of the substances sold for use in this area in a year are released to waste water during the course of a year. Emissions to air on this scale are neglected.

Total use in the EU was estimated in Section 2.3.2 to be 10 tonnes per year. Releases are assumed to be 10% (1 tonnes) to the region and 90% (9 tonnes) to the continent, to waste water. Table 3.1 below provides a summary of emissions from chromium plating.

Table 3.1 Summary of Emissions from Chromium Plating

Local (mg/day)	Regional (kg/year)	Continental (kg/year)
180 (waste water) 0.33 (air)	1000 (waste water)	9000 (waste water)

Note: all releases are of PFOS-salt.

3.1.3 Photolithography

For this sector, use has been made of a draft Emission Scenario Document on photoresist use in semiconductor manufacturing produced by the United States Environmental Protection Agency. This document has been reviewed by the OECD Task Force on Environmental Exposure Assessment, and is intended for publication in the OECD Environmental Health and Safety Publications series on Emission Scenario Documents.

The PFOS-related substances used in this industry are considered to be PFOS-substances (see Section 2.3.3).

The Emission Scenario Document (ESD) has information on the use of photoresists at sites in the USA, with up to 36 kg being used on site per day for larger sites, and for up to 360 days per year. These figures have been used here⁵. From information provided for the Risk Reduction Strategy, the content of PFOS-substances in the photoresists is up to 0.1%, hence the amount used per day would be up to 36 g. This assumes that all photoresists used at the site contain PFOS-substances; although this may not be the case, as there is no information relating to this the worst case assumption has thus been taken. This level of use over 360 days corresponds to an annual use of up to 13 kg. This would appear to fit reasonably with the total use of PFOS-substances in the EU, which is given as 471 kg/year in Table 2.2. However, this overall figures covers use in a range of areas and the specific use in photoresists only accounts for 46 kg/year. The estimated site use is still consistent with this, but may be a high estimate. For the calculation of releases on the regional and continental scales in this section, an overall EU use of 500 kg/year is applied.

The ESD estimates emissions from semiconductor manufacturing for a series of processes:

- packaging/container residuals: the loss from this step is estimated as 0.6%, with the losses going to landfill or to incineration. For a use of 36 g/day, the loss is 0.22 g/day. For a use of 500 kg in the EU the overall loss is 3 kg/year, with 0.3 kg to the region and 2.7 kg to the continent;
- equipment cleaning: losses from this step are estimated as 1% again to landfill and incineration. The estimated loss for the site is 0.358 g/day. The total EU loss is 4.98 kg/year, with 0.498 kg to the region and 4.482 kg to the continent;
- application excess: an amount of resist is applied to the semiconductor wafer which is then spun. The percentage of resist adhering to the wafer is considered to range from 1% to 7%; an average of 4% is used here, hence 96% of the application goes as waste. For the site, the estimated waste is 34 g, which goes to incineration. The total waste for the EU is estimated as 472 kg/year, with 47.2 kg to the region and 425 kg to the continent;
- loss in developer: the ESD assumes that 50% of the resist on the wafer is removed in the developer. The amount released at the site is 0.72 g/day, this is considered to go to waste water. The total loss for the EU is 9.94 kg/year, with 0.994 kg to the region and 8.946 kg to the continent; and

⁵ For comparative purposes, the ESD has information from Germany that suggests a use of 11.25 kg photoresist per day, somewhat lower than the value chosen.

- loss on etching and stripping: the ESD assumes that the rest of the resist is removed at this stage. As 50% was removed at the previous stage, the emissions at this step are the same as those for developing: 0.72 g/day to water at the local site, 9.94 kg/year for the EU, 0.994 kg/year for the region and 8.946 kg/year for the continent. These emissions are to waste water or to recycling, release to waste water has been assumed as a worst case.

Table 3.2 below provides a summary of emissions based on the ESD.

Table 3.2 Summary of emissions for photolithography based on ESD

Step	Local emission (g/day)	EU (kg/year)
Container residual (l/i)	0.22	3
Equipment clean (l/i)	0.358	4.98
Application excess (l/i)	34	472
Developer (ww)	0.72	9.94
Etching/stripping (ww)	0.72	9.94

Notes: l/i – landfill/incineration ww – waste water

The overall breakdown of the fate of the PFOS-substances according to the ESD is ~20 kg to waste water and 480 kg to incineration/landfill. This is based on the use of 500 kg per year. ESIA and SEMI estimated a mass balance for the EU based on a use level of 471 kg per year, and concluded a larger proportional release to water, 251 kg/year to waste water and 218 kg/year to incineration. Examining the mass balance, the differences arise largely through the use of PFOS-substances in areas other than as photoresists. For the anti-reflective coatings, the mass balance assumes 40% is present on the wafer when it goes to the developing step, whereas the ESD has only 4% of the photoresist material. On developing, all of the remaining anti-reflective coating is removed, thus entering the waste water stream rather than going to incineration as in the ESD. Part of the PFOS-substances are used in the developer in the EU, and this is considered to go directly into waste water according to the mass balance. The net effect is that a greater proportion of the PFOS-substances used go into waste water.

The mass balance is considered to be more relevant for the EU, and so the overall emissions from this will be used for the regional and continental emissions.

These give 25 kg/year to waste water on the regional scale, and 226 kg/year to waste water on the continental scale. For the local scale, the emissions estimated with the ESD will be used, but with the addition of a release for the use of PFOS-substances in the developer. It is assumed that the site uses developer and resist materials containing PFOS-substances in the same proportions. The site used for calculations uses 13 kg of PFOS-substance as resists etc, from a total of 276 kg for this type of use (or 4.7%). The amount of PFOS-substance in developers is therefore 4.7% of the total in developer (195 kg/year), or 9.2 kg/year. For 360 days of use, this is 25.6 g/day. Combined with the estimated releases from resist materials of 1.43 g/day, the local emission is 27 g/day. Table 3.14 below provides a summary of emissions from photolithographic processes.

Table 3.3 Summary of emissions from photolithography

Local (g/day)	Regional (kg/year)	Continental (kg/year)
27	25	226

Note: All emissions are to waste water and are of PFOS-substances.

In comments on the consultation for the risk reduction strategy, ESIA/SEMI indicated that the use of PFOS-related substances in developers is not a critical use (in contrast to the use in resists) and so could be replaced. The effect of phasing out the use of PFOS-related substances in this area is considered in Appendix 3.

3.1.4 Photography

From Section 2.3.4, both PFOS-salts and PFOS-polymers may be used in this area. For the purpose of these calculations, it will be assumed that they are PFOS-salt for the production of film step, and PFOS-polymers for subsequent steps. The polymers are assumed to contain 1% PFOS-substance. Information on the relative proportions of the various types would allow the estimates to be improved.

The use of PFOS-related chemicals for the production of film in the EU is estimated in Section 2.3.4 as 850 kg/year. The Technical Guidance Document for the Existing Substances Regulation in the EU has been used to estimate releases from the production of film, considered as a formulation step. The emission factors for a substance used in the production of films etc are 0.0001 to air (for vapour pressures below 1 Pa) and 0.002 to water (for any function other than control of crystal growth). The content of PFOS-related substances in film is $0.1 - 0.8 \mu\text{g}/\text{cm}^2$. Taking the highest value as representative of use in medical applications, which account for 85% of use in this area, and assuming the use of 850 kg per year, a total of $1.06 \times 10^{12} \text{ cm}^2$ of film containing PFOS-related substances could be produced.

The most common backing material for film is PET (polyethylene terephthalate), according to Kirk-Othmer (1994). The material has a density of $1.39 \text{ g}/\text{cm}^3$. The depth of film in Kirk-Othmer is suggested as $45 \mu\text{m}$, with $10 \mu\text{m}$ of base layer (backing) and $35 \mu\text{m}$ of coating. In the absence of other information, the density will be assumed to apply to the whole film. The mass per unit area is then given as $1.39 \times 45 \times 10^{-4}$ or $6.3 \times 10^{-3} \text{ g}/\text{cm}^2$. For a production of $1.06 \times 10^{12} \text{ cm}^2$ this gives 6,630 tonnes of film. This quantity of film can be used in the B tables of the TGD (Table B2.8) to estimate the amount produced at one site and the number of days of operation. This gives a fraction of 0.4 used at one site, applied to the total tonnage, and use over 300 days. The amount of PFOS-related substances used in the EU is 850 kg, so 40% is 340 kg, at 1.13 kg/day. From the emission factors above, the releases to air are 0.11 g/day and to waste water 2.27 g/day. Overall EU emissions are 0.085 kg/year to air and 1.7 kg/year to waste water. As the site accounts for 40% of emissions, these are used for the regional releases. So the regional releases are 0.034 (air) and 0.68 (waste water) kg per year, and the continental releases are 0.051 (air) and 1.02 (waste water) kg per year.

There is little information on whether any of the substances are released from the film when it is processed. EPCI commented that approximately 5-10% of one PFOS material may be released from film into film developer. None of the other PFOS materials used in imaging would be expected to be released on developing. The substances are intended to remain in the film, in order to perform their function. The Emission Scenario Document on the photographic industry in the TGD has a default release factor of 1 in the absence of information, i.e. all of a substance is removed on processing, but this is considered to be inappropriate for these substances. For the purpose of this evaluation, it will be assumed that any PFOS-substance present will be released on developing, and that polymeric material will remain in the film.

From the Emission Scenario Document, the amount of film (taken as x-ray film) processed in one day at a representative site is 110 m^2 . The concentration of PFOS-polymer in the film is $0.8 \mu\text{g}/\text{cm}^2$, or $8 \times 10^{-6} \text{ kg}/\text{m}^2$. Hence the amount of PFOS-polymer in the film processed in

one day is 0.88 g. The amount of PFOS-substance is 1% of this, or 8.8 mg, and this is assumed to be released to water. Across the EU, the 750 kg of PFOS-related substance in films used in this area would give rise to 7.5 kg of PFOS-substance released to waste water, with 0.75 kg to the region and 6.75 kg to the continent.

The polymers not released at this time will remain in the film or other material and may be kept for considerable periods of time. At the end of their use, X-ray film, movie film and commercial films are typically collected by brokers, and sold for secure disposal (movie film) or recycling of silver and/or PET polymer. These usually result in the incineration of residual materials.

Table 3.4 below provides a summary of emissions from the photographic industry.

Table 3.4 Summary of Emissions from Photographic Industry

Step	Local (g/day)	Regional (kg/year)	Continental (kg/year)
Film production (formulation)	0.11 (air) 2.27 (waste water)	0.034 (air) 0.68 (waste water)	0.051 (air) 1.02 (waste water)
Film development (processing)	0.0088 (waste water)	0.75 (waste water)	6.75 (waste water)

Note: formulation emissions are of PFOS-salt; processing emissions are of PFOS-substance.

3.1.5 Aviation

PFOS-related substances are used in hydraulic fluids in the aviation industry (Section 2.3.5). These are considered to be PFOS-salt for these calculations (see note on composition in Section 2.3.5). The manufacture of these fluids takes place outside the EU, so there is no need for a formulation step.

Information relating to emissions of hydraulic fluids is taken from an Emission Scenario Document on Lubricants and Lubricant Additives (Environment Agency, 1997a). Losses are most likely to occur on installation into equipment, during maintenance and on removal for disposal. Aviation systems are expected to be well sealed and so losses during use are expected to be low. It is assumed that all of the substances sold during a year go to replace the fluids lost during the same period, as the suggested replacement or reconditioning frequency for these types of fluids is one year. The suggested fate of the fluids is 2% loss to the environment (1.4% to soil, 0.6% to water) over the service life and 98% taken to chemical disposal from which no significant emissions are expected.

The world use of PFOS-related substances in this area is 2.2 tonnes, the EU is assumed to use one third of this, or 0.73 tonnes. The losses in the EU are therefore 4.4 kg/year to water and 10.2 kg/year to soil. In other work it has been assumed that 10% of the EU releases could relate to one large airport. Hence the local and regional annual emissions are 0.44 kg to water and 1.02 kg to soil. The local site emissions are assumed to take place over 300 days, hence the daily emissions are 1.5 g/day to waste water and 3.4 g/day to soil.

Table 3.5 below provides a summary of emissions from hydraulic fluids used in the aviation industry.

Table 3.5 Summary of aviation releases

Local (g/day)	Regional (kg/year)	Continental (kg/year)
1.5 (waste water) 3.4 (soil)	0.44 (waste water) 1.02 (soil)	3.94 (waste water) 9.2 (soil)

Note: all as PFOS-substances

3.1.6 Fire-fighting foams

3.1.6.1 Formulation

This calculation has been included to indicate possible releases from the formulation of fire-fighting foams containing PFOS-related substances. Such foams are not thought to be produced in the UK any longer.

Section 2.3.6 presented information relating to a producer of foams, which gave a use level of 40 tonnes of PFOS-related substances per year (the company no longer uses PFOS-based products). At a concentration of 1% in the foams this would make 4,000 tonnes of concentrate. This seems high in comparison to the UK stock figures below – these indicate a total stock of all foams (not just those with PFOS) of just under 4×10^6 litres, or 4,000 tonnes assuming a density of 1 g/cm³. Nevertheless this tonnage is used as an illustration.

There are no specific data regarding emissions from the production of the foams, so the default values from the Technical Guidance Document are used, treating the process as a formulation. The relevant emission factors are 0.001 to air and 0.02 to water, with production over 300 days per year. The resulting emissions are 0.13 kg/d to air and 2.7 kg/d to water. As noted in the next section, these are considered to be PFOS-salt. Larger scale emissions are not considered for this use pattern, as full release on use is included in the next section.

3.1.6.2 Use

The fraction of the stock of foams (all types) used by fire services in 2002 was 15%, although the fraction of PFOS-containing foams used was only 0.5%. For the purposes of these calculations it will be assumed that the higher rate of use is the normal rate, and that the remaining stock will be used at this rate⁶. The shelf life of the foams, based on information from the fire services through the consultation, is 10-20 years; a use rate of 15% would use up the existing stock within this time frame, whereas the reduced use rate would leave much of the stockpile to be disposed of. If the PFOS-containing foam is not used then the releases below will be over-estimates. The volume of PFOS-containing foam concentrates stockpiled in the UK is estimated as 76,000 litres (Section 2.3.6).

Confidential information on the quantities and compositions of formulations imported into the UK in the past has been provided. From this, it is reasonable to assume a concentration of 1% PFOS related substance in the foams as stored (i.e. corresponding to the volumes above). From the information provided the major part of the PFOS containing material was of the PFOS-salt type, and so this will be assumed for these calculations.

Assuming a density of 1 kg/l for the concentrates, the amount of foam is 76 tonnes, containing 760 kg of PFOS-salt. At a use rate of 15% per year, this is a use of 114 kg per year for the UK. It is assumed that all of this is released to the environment. As every fire will have different characteristics, there is probably no such scenario as a typical fire. As an illustrative calculation, it is assumed that 1% of this total is used at a fire⁷. Two scenarios for the release to the environment are considered, as possible extremes. In the first (use A), there

⁶ An alternative calculation assuming use of only 0.5% of PFOS-containing foams is included in Appendix 4 and is considered in the risk evaluation.

⁷ Foams may also be used in training exercises. As the assumption of 1% release is only for illustration, the scenario can be considered to cover use in large training exercises. As the water and foam from such exercises is likely to be collected, then use B may be more relevant to the training situation.

is no containment of the foam and water, and so 50% of the release (0.57 kg) goes to surface water without treatment and 50% (0.57 kg) to soil. In the second (use B), it is assumed that the foam and water are collected and passed to a waste water treatment plant, hence 1.14 kg to waste water treatment. The release is assumed to take place over one day.

The UK emissions are assumed to be 20% of those for the EU for these calculations, hence the EU release is 570 kg/year. This is assumed to be split evenly between surface water and soil. The regional emissions are 28.5 kg/year to surface water and to soil, and the continental emissions are 257 kg/year to surface water and to soil.

Table 3.6 below provides a summary of emissions from use of fire fighting foams from fire service use.

Table 3.6 Summary of Emissions from Use of Fire Fighting Foams

Local (kg/day)	Regional (kg/year)	Continental (kg/year)
Use A: 0.57 (drain) 0.57 (soil)	28.5 (surface water) 28.5 (soil)	257 (surface water) 257 (soil)
Use B: 1.14 (wwtp)		

Note: all emissions are of PFOS-salt

These calculations do not specifically address the possible releases from the use of foams on offshore installations, or in fire protection systems on ships. No specific information on these areas of use has been located for this evaluation, although Norway indicated that PFOS-containing foams were used in these areas. As the amounts used at a fire as estimated above are based on an arbitrary choice, it is suggested that in the first instance these may also be considered for the evaluation of offshore use. The calculations for the marine environment would be the most relevant (for Use B, the marine calculation will assume direct emission to the sea without passing through a wwtp) .

3.1.7 Fabric treatment

3.1.7.1 Treatment step

A number of different types of fabric have been treated with PFOS-related substances. For the estimation of releases from the treatment step, the treatment of textiles will be used as an example. Releases from the treatment of other materials may differ to some extent.

To estimate emissions from this treatment step, information from the risk assessment of decabromodiphenyl ether (EC 2002) and a draft Emission Scenario Document on textile processing produced by the Umweltbundesamt in Germany were used. This ESD has been reviewed by the OECD Task Force on Environmental Exposure Assessment, and is intended for publication in the OECD Environmental Health and Safety Publications series on Emission Scenario Documents. The substances used here are considered to be PFOS-polymer, with a residual 1% content of PFOS-substance.

The assessment of decabromodiphenyl ether considers losses from the backcoating of textiles, and this will be taken as being similar to the treatment of textiles with PFOS-related substances. The loss estimated was of 1 kg of formulation per batch used. This was lost to waste water from the setting up and washing down of the coating equipment. It was also assumed that a representative site would process five batches per day, hence the daily loss would be 5 kg of formulation. From data on products imported to the UK, the average content

of PFOS-related substances in formulations for apparel, carpets and fabrics was ~27%. Hence the daily emissions to waste water from the site would be 1.35 kg/day.

From Section 2.3.7, the amount of PFOS-related substances used in the apparel, carpet and fabric areas was 48 tonnes. At an average content of 27% this equates to ~180 tonnes of formulation. RIKZ (2002) indicated that a content of 2-3% by weight of perfluoro product was required in the fabric, which indicates that ~1900 tonnes of fabric could be treated.

From the ESD on textiles, the suggested realistic worst case amount of fabric treated at a site per day is 13 tonnes, with 225 days production giving 2,925 tonnes of fabric treated per year. As it is unlikely that all of a site's production each day will be of only one finish on one fabric type, a factor of 0.3 is used to adjust these figures. The result is 3.9 tonnes of fabric treated with a specific finish per day, and 878 tonnes per year. This yearly figure is just under half of the total estimated above for the whole of the UK, which may indicate that the substances are used over a smaller number of days, or that the amount used on a site is less than estimated.

Taking the 3.9 tonnes per day, at a content of 2.5% PFOS-related substance the amount of PFOS-related substance used per day would be 97.5 kg. The estimated release per day was 1.35 kg, giving an emission factor of 1.4%. Applying this to the UK use level of 48 tonnes gives an annual emission of 672 kg. Taking the UK as 20% of the EU, the total EU emissions would be 3,360 kg. The regional emission would be 336 kg/year, and the continental emission 3,024 kg/year. These values relate to the PFOS-polymer. Assuming a 1% content of PFOS-substance, the releases of PFOS-substance would be 3.4 kg/year (regional) and 30 kg/year (continental).

3.1.7.2 Service life releases

As noted earlier, the use of PFOS-related substances in treatment of fabrics has effectively ceased in the UK. However, there are materials currently in use in articles which contain PFOS-related substances from earlier treatments, and these may contribute to releases of PFOS during the course of the service life of the articles. The possible emissions from these articles are estimated in this section.

The substances used in treating fabrics were for the most part polymeric materials, i.e. PFOS-polymers. They also contained a small amount of residual PFOS-substances. For these calculations, it is assumed that the level of residual material is 1% (based on the information provided for the consultation exercise).

The relevant quantities of substances for these calculations are assumed to be those which were used annually on fabric treatment up until 2000. The use in the different areas was presented in Section 2.3.7, and is 23 tonnes in carpets, 15 tonnes in apparel/leather and 10 tonnes in fabrics (upholstery).

Carpets

Releases during the service life of carpets may arise from cleaning (vacuum or washing) or through wear. RIKZ (2002) quote 3M as providing a worst case estimate of 95% loss of PFOS from carpets over their working life, with 50% through abrasion from walking and vacuuming, and 45% through steam cleaning.

Walking and vacuuming losses may be considered to go to land or to water. The use of vacuum cleaners would probably be expected to lead to removal to solid waste and disposal with household refuse and consequently landfill or incineration. However there is no information to apportion losses to walking (wear) and to vacuuming, so it will be assumed that releases are to the environment as a worst case. In ESR assessments the loss of particulates as wear from plastics has been considered as 'waste remaining in the environment' and distributed as 75% to soil and 25% to water. Assuming a similar distribution for these releases gives 37.5% to soil and 12.5% to water. Releases from steam cleaning are assumed to go to water, hence 45% to water. The overall releases are therefore 57.5% to water and 37.5% to soil. The amount remaining on the carpet at the end of the lifetime is assumed to be disposed of with the carpet, to landfill or to incineration.

The loss factors are estimated over the whole working life. As such they can be applied to the annual use level (this assumes a constant level of use). For a tonnage of 23 tonnes, the UK emissions would therefore be 13.2 tonnes to water and 8.6 tonnes to soil. As before, it is assumed that the UK accounts for one fifth of the EU, so the overall totals are 66 tonnes to water and 43 tonnes to soil. The regional emissions are 6.6 tonnes (water) and 4.3 tonnes (soil), the continental emissions are 59.4 tonnes (water) and 38.7 tonnes (soil). The above are emissions of polymeric material, and would be expected to be mostly associated with particulate material worn from the fabric.

The polymers are considered to contain 1% of PFOS-substances, so the releases of these are: regional 66 kg/year (water), 43 kg/year (soil); continental 0.59 tonnes/year (water), 0.39 tonnes/year (soil).

Upholstery and Furnishing Fabrics

There is no specific information on losses of PFOS-related substances from these materials so the information on carpets will be used as the basis for the assumptions. These materials will be cleaned, although probably not frequently, and so the loss through washing is taken as the same as that for steam cleaning of carpets, i.e. 45% to water. The degree of wear is assumed to be less than that for carpets, and is taken as half of that figure, i.e. 25%. As for carpets this is split between water and soil in the ratio 25:75, although some would be to solid waste. The overall emission factors are therefore 51.25% to water and 18.75% to soil. The amount remaining on the fabrics at the end of the lifetime is assumed to be disposed of with the fabric to landfill or to incineration.

From a tonnage of 10 tonnes per year, the UK emissions would be 5.1 tonnes to water and 1.9 tonnes to soil. Assuming the UK is 20% of the EU, the total emissions would be 25.5 tonnes to water and 9.5 tonnes to soil. The regional emissions are 2.6 tonnes (water) and 0.95 tonnes (soil), the continental emissions are 23 tonnes (water) and 8.6 tonnes (soil). These are emissions of polymeric substances, probably in association with particulate material. As before, taking the content of PFOS-substances as 1% the emissions of these substances are: regional 26 kg/year (water), 9.5 kg/year (soil); continental 0.23 tonnes/year (water), 0.09 tonnes/year (soil).

Apparel and Leather

These two areas are treated together as use in treating clothing. There is no specific information about the loss of PFOS-related substances from clothes, so the information on carpets has again been used as the basis for the assumptions. Clothing will be washed more frequently than carpets or upholstery, although the purpose of the treatment is to reduce the

staining of fabrics. Against this, the lifetime of most clothing is much shorter than that of the two categories above. Washing losses are therefore taken as half of those above, i.e. 22.5%. Wear is expected to be less than for carpets, and is taken as the same as for fabrics above, i.e. 25%. This is again split as 75% to soil, 25% to water, or 18.75% to soil and 6.25% to water. The overall emission factors are therefore 28.75% to water and 18.75% to soil. From a tonnage of 15 tonnes per year, the UK emissions would be 4.3 tonnes to water and 2.8 tonnes to soil. Assuming the UK is 20% of the EU, the total emissions would be 21.5 tonnes to water and 14 tonnes to soil. The regional emissions are 2.15 tonnes (water) and 1.4 tonnes (soil), the continental emissions are 19.4 tonnes (water) and 12.6 tonnes (soil). These are emissions of polymeric substances, probably in association with particulate material. As before, taking the content of PFOS-substances as 1% the emissions of these substances are: regional 22 kg/year (water), 14 kg/year (soil); continental 0.19 tonnes/year (water), 0.13 tonnes/year (soil).

Tables 3.7 below provides a summary of emissions from treatment of fabrics (carpets, upholstery and leather).

Table 3.7 Summary of Emissions from Treatment of Fabrics

Area	PFOS-polymer		PFOS-substance	
	Regional (tonnes/year)	Continental (tonnes/year)	Regional (kg/year)	Continental (kg/year)
Carpets	6.6 (w)	59.4 (w)	66 (w)	594 (w)
	4.3 (s)	38.7 (s)	43 (s)	387 (s)
Upholstery & Furnishing Fabrics	2.6 (w)	23 (w)	26 (w)	230 (w)
	0.95 (s)	8.6 (s)	9.5 (s)	86 (s)
Apparel & Leather	2.15 (w)	19.4 (w)	22 (w)	194 (w)
	1.4 (s)	12.6 (s)	14 (s)	126 (s)
Total	11.35 (w)	101.8 (w)	114 (w)	1018 (w)
	6.65 (s)	59.9 (s)	66.5 (s)	599 (s)

3.1.8 Paper treatment

PFOS-related substances have been used to treat a range of paper types and products (grease proof paper, food cartons etc.). The major type of substance used appears to be phosphate derivatives of N-EtFOSE, and these will be considered here as PFOS-substances. They are considered to be applied mostly during the paper making process, rather than as a coating in subsequent operations.

A level of use of 1 – 1.5% by weight of paper is indicated (RIKZ, 2002). The approximate usage in the UK was 32 tonnes, so that 2,100-3,200 tonnes of paper could be treated. For the EU, assuming the UK accounted for 20% of use, the figures would be 160 tonnes of substances, and 10,700-16,000 tonnes of paper.

The Appendices in the Technical Guidance document have been used to estimate the emissions from paper. The substances are intended to remain in the paper, so the main category is 2, use resulting in inclusion into a matrix. The use category is 31 (impregnating agent). The resulting emission factors are zero to air and 0.05 to waste water.

The information in Section 2.3.8 suggests there were only a few users for this type of treatment. Large companies is chosen as the category for the B table, which results in a fraction of main source of 0.333 and 300 days production. The fraction of main source is applied to the UK tonnage in this case, as this gives a result in keeping with the information

available. This results in the use of 10.7 t of PFOS-substance at the site per year, or 35.7 kg/day. The estimated release to waste water is 1.8 kg/day.

From above, the total use in the EU was estimated as 160 tonnes per year. The release is therefore estimated as 8 tonnes per year, with 800 kg to the region and 7.2 tonnes for the continent. These are releases of PFOS-substance to waste water.

The possibility of emissions from paper in use could be considered. However, the lifetime of such papers is not expected to be very long. On disposal, such papers as food wrappings etc might be expected to be disposed of with household waste rather than entering the paper recycling streams. Hence most of the substances used are likely to be disposed of to landfill or incineration.

3.1.9 Coatings

A range of possible uses in coatings of various kinds is described in Section 2.3.9. In some cases there appears to be some overlap with areas which have already been addressed. In order to obtain some indication of the possible emissions from this area, it has been assumed that the default emission factors for paints, lacquers and coatings in the Technical Guidance Document can be applied.

A use of ~18 tonnes for the UK is assumed for these calculations. The content of PFOS-related substances in coatings is indicated to be 0.1-1.0%; taking an average of 0.55% gives a quantity of coating containing PFOS-related substances of 3,270 tonnes per year.

The substances are treated as PFOS-substances for simplicity, and are considered to be surface active agents, use category 50. Assuming the paints are water based, the emission factors are zero to air and 0.005 to waste water. Considering the UK as 20% of the EU, the amount of paints containing PFOS-substances in the EU would be 16,364 tonnes. From the B tables this indicates a fraction of main source of 0.1, or use of 1,636 tonnes per year at the representative site. This would be over 300 days, and equates to the use of 9 tonnes of PFOS-substance at the site. Using the factor of 0.005, the emission to waste water would be 45 kg/year, or 0.15 kg/day. The total emissions for the EU would be 450 kg/year, with 45 kg to the region and 405 kg to the continent. These are emissions of PFOS-substances to waste water.

There would also be the possibility of emissions of the PFOS-substances from the coatings during the course of their service life. No information on these possible releases is available, and so no estimates are possible at this time.

3.1.10 Summary

The releases estimated in Sections 3.1.2 to 3.1.9 are summarised in Table 3.8.

3.2 ENVIRONMENTAL FATE AND DISTRIBUTION

3.2.1 Atmospheric degradation

There are no experimental data. The AOP program (v1.91) estimates a rate constant for the reaction of PFOS (as the acid) with OH radicals of $1.4 \times 10^{-13} \text{ cm}^3 \text{ molec}^{-1} \text{ s}^{-1}$. With a

concentration of $5 \times 10^5 \text{ molec cm}^{-3}$, this gives a half life of 114 days. Combined with the low volatility, this indicates that degradation in the atmosphere is not likely to be significant.

The PFOS-substance N-EtFOSE is calculated to be more reactive, with a half life of 16 hours. This is almost entirely due to hydrogen abstraction from the amide substituent group, and may indicate that this part of the substance may break down to leave the PFOS backbone.

Table 3.8 Summary of Emissions from PFOS-salts, PFOS-substances and PFOS-polymers

Use area	Compartment	Local (per day)	Regional (per year)	Continental (per year)
<i>PFOS-salts</i>				
Chromium Plating	air	0.33 mg		
	waste water	180 mg	1000 kg	9000 kg
Photographic	air	0.11 g	0.034 kg	0.051 kg
	waste water	2.27 g	0.68 kg	1.02 kg
Aviation	waste water	1.5 g	0.44 kg	3.94 kg
	soil	3.4 g	1.02 kg	9.2 kg
Fire fighting foams - formulation	air	0.13 kg		
	waste water	1.07 kg		
Fire fighting foams – use (alternative local)	surface water	0.57 kg	28.5 kg	257 kg
	soil	0.57 kg	28.5 kg	257 kg
	waste water	1.14 kg		
<i>PFOS-substances</i>				
Photolithography	waste water	27 g	25 kg	226 kg
Photographic	waste water	8.8 mg	0.75 kg	6.75 kg
Fabrics – treatment	waste water	13.5 g	3.4 kg	30 kg
Fabrics – service life	water		114 kg	1018 kg
	soil		66.5 kg	599 kg
Paper treatment	waste water	1.8 kg	800 kg	7.2 tonnes
Coatings	waste water	0.15 kg	45 kg	405 kg
<i>PFOS-polymers</i>				
Fabrics – treatment	waste water	1.35 kg	336 kg	3024 kg
Fabrics – service life	water		11.35 tonnes	101.8 tonnes
	soil		6.65 tonnes	59.9 tonnes

Notes: Waste water - all releases treated in wwtp.
Surface water - release direct to surface water and not treated.
Water - releases split 80:20 wwtp:direct to surface water.

3.2.2 Aquatic degradation

3.2.2.1 Abiotic degradation

3.2.2.1.1 Hydrolysis

The hydrolysis of the potassium salt of PFOS has been studied over a wide range of pH values, from 1.5 to 11 (3M, 2003). Tests were carried out at 50°C to accelerate any reaction. No loss of PFOS was seen in any of the experiments. Based on the limit of quantification, and adjusting for the higher temperature of the tests, the half life at 25°C was estimated to be >41 years.

RIKZ (2002) quote 3M studies showing that some PFOS-related substances can undergo hydrolysis. The acrylate derivative of N-EtFOSE and N-MeFOSE have half lives of 35 and 99 days respectively at pH 7 and 25°C. The products of hydrolysis were not identified, but RIKZ suggested that they were likely to include acrylic acid and N-EtFOSE and N-MeFOSE.

Longer half life times of 6.3 years for N-MeFOSE and 7.3 years for N-EtFOSE are also quoted by RIKZ from 3M reports.

3.2.2.1.2 Photolysis

The possible photolysis in water of the potassium salt of PFOS has been studied in various test matrices, allowing for both direct and indirect photolysis (3M, 2003). No evidence of direct or indirect photolysis of PFOS was observed under any of the conditions tested. From the limit of quantitation of loss of PFOS, the minimum half life for photolysis in water was estimated to be >3.7 years.

RIKZ (2002) quote a 3M study showing no photolysis of N-EtFOSE.

3.2.2.2 Biodegradation

3.2.2.2.1 Aerobic

The OECD Hazard Assessment (OECD, 2002) and the 3M assessment (3M, 2003) concluded that PFOS was not biodegradable. Information on the main studies reviewed in these assessments is presented below.

A MITI test (Kurume Lab, 2002, quoted in OECD 2002) found zero removal as measured by oxygen demand, 3% by removal of parent compound and 6% by reduction in total organic carbon. These values indicated no significant degradation.

The OECD assessment includes a study summary for a 35-day biodegradation test. Activated sludge from a municipal waste water treatment plant was used, with 50 ml of settled sludge added per litre of mineral salts medium used. The concentration of PFOS (as the potassium salt) was 2.582 mg/l. The study found no evidence of degradation over the 35 day period. The recovery of PFOS from the sludge was good.

The summary also indicates that a number of other compounds were also quantified. None were detected. The majority of the substances listed are other PFOS-related substances, which may break down to give PFOS. It appears unlikely that any of these might be produced from the degradation of PFOS. These would seem to relate better to other work on the degradation of N-EtFOSE, which is considered below.

The 3M (2003) assessment includes a summary of an 18 day biodegradation test on PFOS (potassium salt). The experimental details are similar to those reported for the 35 day study above, except that the activated sludge was allowed to settle for five weeks rather than two days after collection, and the exposure concentration was 2.45 mg/l. No degradation of PFOS was measured in this study. As above, a group of other PFOS-related substances was also quantified, and none were detected. A note in the summary relates to a previous study to evaluate the biodegradation of N-EtFOSE over 35 days and to determine its metabolites. This seems a more relevant study for the determination of the other substances. There is no further information on this study in the documentation available, but Cahill and Mackay (2002) made use of this information in a recent study (see below).

Further experiments on the biodegradation of PFOS are included in study summaries in the 3M assessment (3M, 2003). An inoculum was produced from a mix of activated sludge from two waste water treatment plants, with material from a rotating biological contactor plant, sediment from below a water treatment plant outfall, and sandy soil. The test medium

included the inoculum, primary influent from one of the waste water treatment plants, and OECD 301A medium. The initial PFOS concentration was 20.8 mg/l. At weekly intervals, 70% of the bioreactor contents were removed and replaced with fresh medium including freshly collected waste water treatment plant effluent and inoculum, and additional PFOS. The final concentration of PFOS was 28.4 mg/l. There were no indications of biodegradation in this study. The PFOS was associated primarily with the biomass at the end of the study.

After 12 weeks of acclimation, activated sludge from the above study was added to OECD 301A medium, with PFOS at two concentrations, and placed in closed serum vials. The vials were sampled at intervals of up to 63 days. No clear evidence of the biodegradation of PFOS was seen in this study.

Other substances

RIKZ (2002) presented a degradation pathway for N-EtFOSE in waste water sludge, taken from two 3M reports. The ultimate products are shown as PFOS (anion) and perfluorooctanoic acid (PFOA). There is no indication of the rate of biodegradation, the overall yield of the two products or any intermediate product, or the relative amount of the two products formed. RIKZ (2002) considered that it was likely that N-MeFOSE would follow a similar degradation pathway. They quote a 3M report as stating that the likely endpoints of aerobic biodegradation of ECF-products are PFOS and PFOA. In the sequence of studies carried out by 3M (3M, 2001), the initial products of aerobic biodegradation of N-EtFOSE were identified, and then tested in further aerobic biodegradation tests. The process of identifying and testing products was continued with eventual tests on five intermediates products, PFOS and PFOA. Each of the intermediate products was biodegradable to some degree in the 18-day studies. PFOS and PFOA were not degradable and so represent the end of the chain. These were produced at intermediate stages as well as from degradation of the last intermediate product. The extent of degradation varied between the different substances tested.

Cahill and Mackay (2002) reported the development of a multi-species model, with PFOS-related substances being used in demonstrating the model. For the degradation of N-EtFOSE they used a rate constant of $0.014 - 0.0014 \text{ h}^{-1}$. The upper value was derived from a 3M study (3M (2000), assumed to be that referred to above), the lower rate was taken as 10-fold lower to reflect the lower densities of microbial communities in the environment compared to the test. This lower rate will be used in the modelling calculations later in this evaluation, along with other illustrative degradation rates, in the absence of more specific information. Cahill and Mackay (2002) also quote the same 3M report (3M, 2000) as reporting preliminary results showing that the microbial degradation of N-EtFOSE and M-EtFOSE predominantly resulted in PFOS formation (92%), with 8% of PFOA. In the executive summary for the completed studies (3M, 2001) there are no comments on the overall extent of production of PFOS from the biodegradation of N-EtFOSE. For the main calculations in this evaluation, complete conversion of PFOS-substances to PFOS through biodegradation in the environment will be assumed, recognising that this is a worst case assumption. Alternative assumptions will be considered in the later discussion on uncertainties in the evaluation (Section 5.6).

3.2.2.2.2 Anaerobic

A study on the degradation of PFOS (potassium salt) under anaerobic conditions is summarised in 3M (2003). The inoculum was derived from the anaerobic digester of a waste

water treatment plant, and exposures took place for 56 days in the dark. The concentration of PFOS in the test was 20.8 mg/l (nominal). No apparent degradation was seen in the study.

The 3M report (3M, 2003) also summarises a study using four pure microbial cultures to try to degrade PFOS (potassium salt). None of the species appeared able to degrade PFOS.

In contrast to these results, Schroder (2003) observed the disappearance of PFOS spiked into a laboratory-scale closed-loop bioreactor using columns filled with glass foam beads. PFOS was added initially at 5-10 mg/l; the inoculum was taken from the anaerobic sludge stabilisation tank of the Aachen-Soers STP, Germany. Anaerobic conditions were maintained and monitored. After two days the concentration of PFOS had fallen to below the detection limit. PFOA remained stable during this period, but following the reduction in the PFOS the level of PFOA also fell, to below the detection limit after 25 days. The fluoride ion concentration was monitored during the experiments and showed no increase, indicating that complete mineralization was not occurring. This may indicate some potential for degradation under certain conditions, however at present this will not be included in the evaluation due to the lack of information on the relevance to an environmental evaluation.

3.2.3 Degradation in soil

The degradation of PFOS, potassium salt, has been studied in a mixed soil and sediment culture (study summarised in 3M, 2003). Soils were obtained from three locations in the USA, sediments were taken from a brackish site below a waste water treatment plant outfall and from one other location. Soil and sediment samples were air dried, sieved and mixed in equal dry weight proportions. A nutrient mixture, containing sterile potting soil extract, trace mineral salts, yeast extract and water, was added to the soil/sediment mixture, to 75% of water holding capacity. Tests were conducted in the dark at 22°C for 20 weeks. No PFOS degradation was observed in the test system (by parent compound analysis).

3.2.4 Evaluation of environmental degradation data

None of the tests carried out show any indication of the biodegradation of PFOS in aquatic systems, under either aerobic or anaerobic conditions. There are also no indications of abiotic degradation in water. The rate estimated for reaction with hydroxyl radicals in air is slow. The one available study in soil also shows no degradation.

The limited information on other PFOS-related substances indicates that N-EtFOSE can be biologically degraded and that one of the products is PFOS. For the purpose of this evaluation, it will be assumed that PFOS is not degradable. For PFOS-substances, it will be assumed that they can be degraded to PFOS. This degradation will be assumed to take place in water, aerobic sediment and in soil. In the absence of more specific information it will be assumed as a worst case that this transformation is quantitative, i.e. one mole of PFOS-substance gives rise to one mole of PFOS. The effect of considering different rates for this process will be considered in the modelling section.

3.2.5 Environmental distribution

3.2.5.1 Adsorption

For most substances, adsorption is predicted on the basis of the log K_{ow} value. As a value for this property cannot be measured for PFOS, this is not possible. Measurements are available

on the sorption of PFOS to soils, sediment and sludge, and these will be used in this evaluation.

Sorption of the potassium salt of PFOS to three types of soil, a sediment and a sludge from a domestic waste water treatment plant has been measured using a method based on OECD 106 (3M, 2003). Adsorption occurred rapidly in all cases, and the concentrations remained fairly constant after 16 hours. Desorption was also investigated - again, the desorption which occurred took place rapidly, and after eight hours the concentration in water did not vary significantly. Values for the sorption and desorption coefficients were calculated. These are presented in Table 3.9

Table 3.9 Sorption and desorption coefficients for various matrices

Matrix type	Kd (L/kg)	Kdes (L/kg)	Mean
Clay soil	18.3	47.1	32.7
Clay loam soil	9.72	15.8	12.8
Sandy loam soil	35.3	34.9	35.1
River sediment	7.42	10	8.7
WWTP sludge			1028

Note: mean values are mean of sorption and desorption coefficients. For sludge, value is the mean of the Freundlich coefficients for sorption and desorption, as direct values are only reported as limit values.

Mean values from sorption and desorption have been used in this evaluation. The average value from the three soils, 26.9, has been used for soil. The value for sediment has been used for both sediment and suspended sediments. The single sludge value has been used to represent the different types of sludge in the EUSES calculations. The $K_{\text{comp-water}}$ values derived from these measurements using the TGD methods are: sediment, 5.11; suspended sediment, 3.08; soil, 40.6.

For comparison, a soil Kd value of 25, similar to that derived from the measurements, would be estimated from a log Kow value of 3.7 using the default QSAR equation from the Technical Guidance document. The corresponding estimated sediment and suspended sediment values would be 62.6 and 125 respectively, which are somewhat larger than the value from the measurements. For sludge, the estimated values would be 375 and 413, which are of a similar order but a little lower than that from the measurements.

It is recognised that these measured values are not necessarily representative of the environment. They also do not give any indication of the environmental factors which may affect the sorption of PFOS. As there is no reliable value for log Kow, no comments can be made on whether the sorption behaviour is different from that normally assumed, relating to organic carbon, or whether other processes are significant. However, they are considered to give at least an indication of the probable sorptive behaviour of PFOS.

3.2.5.2 Volatilisation

PFOS is not expected to volatilise significantly. It has a low vapour pressure and a solubility of ~500 mg/l. Attempts to measure the air-water partition coefficient using the potassium salt are reported in 3M (2003). The substance did not volatilise to any measurable extent. On the basis of the result, the value for the air-water partition coefficient was considered to be $<2 \times 10^{-6}$, and to be essentially zero. A value of 1.35×10^{-7} is used in this evaluation, derived from the estimated Henry's Law constant (Section 1.3.8). Note that some of the PFOS-substances have considerably higher vapour pressure and are more likely to be volatile to some extent. This may allow the wider transport of potential PFOS-precursors through the air than is possible for PFOS itself.

3.2.5.3 Precipitation

The low vapour pressure and volatility means that PFOS is not expected to be found in air to any great extent. Hence precipitation is not considered to be a significant factor, unless PFOS is formed in air through the breakdown of PFOS-related substances in air. There is no available information on this.

3.2.5.4 Distribution in waste water treatment plant

The distribution of PFOS in waste water treatment plants has been modelled with the SimpleTreat model included in the EUSES 2 program. There is no degradation in the plant. The results are:

To air	$4.5 \times 10^{-4}\%$
To water	72.1%
To sludge	27.9%

3.2.5.5 Distribution in environment

The distribution of PFOS in the environment has been estimated using the EUSES 2 program using the partitioning and degradation data presented in the preceding sections and the physico-chemical data from Section 1. The program was run with a fixed release to air, to water and to soil in turn to examine the fate of releases to different compartments. The results from these calculations are in Table 3.10. It should be noted that the properties of PFOS, in particular the partitioning behaviour, mean that this type of model may not be as appropriate as for other substances. The use of directly measured properties for partitioning should help to compensate for this to some extent. However the results here, and the predicted environmental concentrations in the following sections, should be considered to be indicative.

Table 3.10 Distribution of PFOS in the model environment (releases to individual compartments)

Compartment	Release to		
	Air	Water	Agricultural soil
Freshwater	0.38%	83.18%	0.26%
Seawater	0.04%	9.06%	0.03%
Air	<0.01%	<0.01%	<0.01%
Soil (combined)	99.55%	3.42%	99.7%
Freshwater sediment	0.02%	4.20%	0.01%
Marine sediment	<0.0%1	0.14%	<0.01%

The results show that releases to air are eventually found almost completely in the soil, through deposition, with a small amount in water. Releases to water remain for the most part in the water (either freshwater or seawater) with some found in sediment and some in soil. Releases to soil remain almost completely in soil.

The distribution of PFOS has also been obtained from the calculations for predicted environmental concentrations, i.e. the distributions from the EUSES model using the estimated emissions (see Section 3.3.1). The results for the seven scenarios are presented in Table 3.11. The emissions included in the different scenarios are described in Section 3.3.1.3.

Table 3.11 Distribution of PFOS in seven scenarios

Compartment	Scenario						
	1	2	3	4	5	6	7
Freshwater	2.08%	2.06%	1.07%	1.29%	0.51%	0.55%	1.97%
Seawater	0.23%	0.23%	0.12%	0.14%	0.06%	0.06%	0.22%
Air	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
Soil (combined)	97.59%	97.60%	98.75%	98.50%	99.40%	99.37%	97.71%
Freshwater sediment	0.10%	0.10%	0.05%	0.06%	0.03%	0.03%	0.10%
Marine sediment	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%

For all scenarios the majority of the PFOS is found in the soil (combined for the three types, but mostly in the agricultural soil, through sludge application). The remainder is mostly in the water compartment. These results reflect the fact that the estimated emissions of PFOS-salt are mainly to waste water, and hence result in emissions to surface water and soil (through sludge) after water treatment. Indirect emissions through the degradation of PFOS-substances or PFOS-polymers are significant only for soil and/or air, and so ultimately contribute mainly to the soil compartment.

The EUSES 2 model also includes three environments on global scales, for the arctic, temperate and tropical zones. For PFOS, the majority of the substance at steady state is found in the water compartments in these environments, i.e. it has been removed from the regional and continental environments. Together, the water compartments of the three global environments account for 98.3 % of the total burden at steady state.

3.2.6 Bioaccumulation and metabolism

3.2.6.1 Aquatic

There are a limited number of studies available on bioaccumulation of PFOS. A flow-through study on bluegill sunfish (*Lepomis macrochirus*) is cited in both 3M and the OECD assessment. The bioconcentration factors for edible tissues, non-edible tissues and whole fish were calculated from the rates of uptake and depuration because steady state had not been reached after 56 days of exposure.⁸ The values obtained were 1124 (edible), 4103 (non-edible) and 2796 (whole fish). The exposure concentration was 0.086 mg/l.

A flow-through study on carp (*Cyprinus carpio*) resulted in lower values of 720 at 20 µg/l exposure and 200-1500 at 2 µg/l exposure. Higher values of 6,300 - 125,000 have been reported (for bioaccumulation factors) for in situ measurements at the scene of a spill of fire fighting foam, but these were considered to be due to the uptake of derivatives which were then metabolised to PFOS, hence the values were over-estimated.

Martin et al (2003) measured BCF values in rainbow trout plasma and liver of 2900 and 3100 respectively following exposure in a flow-through system to a combination of perfluorinated acids (both carboxylates and sulphonates).

The value of 2796 will be used as the bioconcentration factor in fish in this evaluation. The Technical Guidance Document indicates a biomagnification factor of 2 for this BCF value,

⁸ The robust summary in the OECD hazard assessment (2002) has different values to those used in the main OECD text (which are those cited here). The 3M (2003) report explains that the original study used an inappropriate method to estimate the kinetic BCF values, and that those were revised in a later amended study report. This is assumed to explain the different values in the OECD robust summary, as the BCF values in the main report and the 3M report agree.

and this is also used in the calculation of levels in biota for the assessment of secondary poisoning.

Martin et al (2003a) studied the uptake of PFOS from food in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were fed food spiked with a mixture of perfluorinated substances - PFOS and its butyl and hexyl analogues, C₅-C₁₄ perfluoroalkanoic acids (excluding C₁₃). The concentration of PFOS in the food was 0.54 µg/g. The exposure period was 34 days, and was followed by a depuration period of 41 days. Fish were fed in such a way that the food was consumed rapidly after addition, to minimise the possible transfer of substances from food to water. Water samples were taken before and after feeding on day 30 to confirm that the water concentrations remained low. Fish were sampled at intervals during the uptake and depuration periods.

The measured concentrations in fish were corrected for the growth of the fish. The assimilation efficiency for PFOS calculated from the results was 120±7.9%, the depuration half life was 13±1.8 days, the bioaccumulation factor (BAF) was 0.32±0.05 and the time to steady state was estimated as 43 days. The results show that PFOS will not biomagnify from food in juvenile trout. The authors suggest caution in extrapolating these results to larger fish, e.g. mature trout, as the half lives of other substances have been shown to increase by up to a factor of 10 times in mature fish compared to juveniles. They also considered that these results did not mean that PFOS would not accumulate in higher organisms from food. Elimination through the gills is an important route for fish which is not available to birds for example, and elimination from the lungs is expected to be much lower in view of the low vapour pressure. In view of these comments the default BMF value from the TGD is used.

In the course of a study on the toxicity of PFOS to freshwater mussels (*Unio complamatus*) the concentration of PFOS in the mussel tissues was measured. The toxicity study was reviewed for the OECD assessment, the results are included in this evaluation. The measured concentrations in water and in mussel tissues are in Table 3.12

Table 3.12 Accumulation of PFOS in mussel tissue

Mean measured exposure concentration (mg/l)	% mortality at 96 hours	Mean measured tissue concentration (mg/g)	Calculated BCF
5.3	0	3.69	0.7
12	0	5.22	0.44
20	0	7.33	0.37
41	5	11.85	0.29
79	90	88.8	1.12

As no mortality was seen in the lower concentrations over the exposure period, the results at these concentrations can be considered suitable to determine bioconcentration factors. The average of the three results is 0.5.

3.2.6.2 Terrestrial

A 14-day toxicity study with earthworms has been reported (3M, 2003, see Section 4.2.1). The concentrations in worms were measured at the end of the exposures. The worms were allowed to clear soil from their guts before the concentrations were measured. These levels can be used together with the concentrations in the soil to calculate bioaccumulation factors. The data are in Table 3.13.

Table 3.13 Bioaccumulation in worms

Soil concentration (mg/kg ww)	Worm concentration (mg/kg ww)	BCF
77	195	2.5
141	203	1.4
289	252	0.87
488	1105	2.3

Effects were seen on the earthworms in the test at all concentrations above 77 mg/kg, so the factor used in this evaluation will be taken from the 77 mg/kg exposure.

The BCF for earthworms as used in the revised Technical Guidance is based on the pore water concentration. Using the $K_{\text{soil-water}}$ partition coefficient of 40.6, the pore water concentration at 77 mg/kg in soil is 3.22 mg/l. The BCF based on this concentration is 60.5 l/kg ww.

An alternative approach to a BCF value for worms could be to derive a surrogate log K_{ow} from the fish bioconcentration value. Using the default QSAR equation from the TGD, the fish BCF value of 2796 would be predicted from a log K_{ow} value of 4.88. Using this surrogate log K_{ow} value, a BCF for worms of 911 would be predicted. This is much higher than the value obtained from the measured levels. The measured value will be used in the evaluation.

There are limited data for uptake into plants. As part of a study on the toxicity of PFOS (potassium salt) to seven plant species (Section 5.4.2.1), concentrations of PFOS in plant tissues were measured. The concentrations in plant vegetative tissues were generally in the range of 1-2 times the concentration in soil. The values around twice the soil level were found at lower exposure concentrations, where the plants were less likely to be affected by the exposure. Concentrations in fruit were lower, less than 10% of the soil level. No specific data were located on transfer to meat or milk, processes which are considered in the estimation of exposure to humans through the environment. There are limited measurements of levels in food, which are discussed in Section 3.3.5. In the absence of specific data, uptake and accumulation factors for these are usually estimated from the log K_{ow} value. For the purpose of this evaluation, the surrogate log K_{ow} value estimated above from the fish data will be used. This is a log K_{ow} value of 4.88.

3.2.6.3 Absorption in mammals

PFOS appears to be well absorbed in mammals following ingestion. After a single oral dose in solution of 4.2 mg/kg bw to rats using labelled PFOS, at least 95% of the 14-C label was systematically absorbed after 24 hours (Johnson et al (1979a), in OECD 2002). Approximately 86% of this dose was found in the carcass at 24-48 hours. There was some excretion of total carbon-14 in urine, 1-2% per day. The half life for elimination from plasma was estimated to be 7.5 days. The same authors (Johnson et al (1979b), in OECD 2002) also gave the same dose intravenously. After 89 days, the mean urinary excretion was ~30%, and the mean faecal excretion was 12.6%.

3.3 ENVIRONMENTAL CONCENTRATIONS

3.3.1 Background to calculations

The calculation of predicted environmental concentrations for PFOS is complicated by a number of factors. One is that some of the uses have effectively ceased, while others are being reduced, so that the use pattern is changing. This is addressed in this evaluation by defining a number of scenarios to include or exclude various sources. The baseline situation is taken to be use as it was in the UK around 2000, so before the removal of products from the market.

A second factor is the possible contribution of PFOS-substances and PFOS-polymer materials to the levels of PFOS in the environment. The rate and extent to which these breakdown and produce PFOS is not known. Therefore a number of calculations based on various assumptions about this degradation have been included.

The EUSES 2.0 model has been used for the calculations in this evaluation. It should be noted that the regional concentrations calculated in the model relate to the steady-state situation. For a persistent substance such as PFOS, the steady state may take a considerable time to be reached, and so to some extent the calculated concentrations may be projections of the concentrations expected in the future, assuming that the emissions in the calculation remain constant.

3.3.1.1 Substance properties for modelling

3.3.1.1.1 PFOS-salt

The physico-chemical properties of the potassium salt, as described in Section 1, are used for this group. As noted in the relevant sections, it is not possible for this substance to predict properties from the log Kow value, and measured values have been used where possible. The full set of values as used in the model is presented in Table 3.14.

There are a number of other factors related to uptake in plants, crops and cattle which are used in the estimation of exposure of humans through the environment. These are usually estimated from the log Kow value. For the purpose of this evaluation, the surrogate log Kow value of 4.88 has been used (for explanation, see Section 3.2.6.2).

Table 3.14 Properties of PFOS-salt for modelling

Property	Value	Section
Molecular weight	538	Table 1.1
Vapour pressure	3.31×10^{-4} Pa	Table 1.1
Water solubility	519 mg/l	Table 1.1
Henry's law constant	3.19×10^{-4} Pa m ³ mole ⁻¹	Table 1.1
K _{sed-water}	5.16	Section 3.2.5.1
K _{susp-water}	3.08	Section 3.2.5.1
K _{soil-water}	40.6	Section 3.2.5.1
BCF fish	2,796	Section 3.2.6.1
BCF worm	60.5	Section 3.2.6.2
BMF ₁ , BMF ₂	2	Section 3.2.6.2
Biodegradation	Not biodegradable	Section 3.2.4
Photodegradation in air	114 d half life	Section 3.2.1
Other abiotic processes	EUSES defaults	

3.3.1.1.2 PFOS-substances

Although there are a large number of substances which are included in this group, specific property values are not available for the majority. Some data are available for the most widely used, and of these N-EtFOSE (CAS 1691-99-2) has the most extensive data set. This has been used as the model compound for this group. The properties of this group are shown in Table 3.15 below.

Table 3.15: Summary of property values for PFOS-substances (RIKZ, 2002)

Property	Value Used
Molecular weight	571.25
Melting point	57°C
Solubility	0.15 mg/l
Vapour pressure	0.5 Pa
Log Kow	4.4

A degradation half life in air of 16 hours was estimated in Section 3.2.1. For biodegradation, a rate constant of 0.0014 h^{-1} will be used (see Section 3.2.2.2.1). This rate will be used for water, soil and aerobic sediment in the model. This is assumed to be the rate of disappearance of the substance, and may therefore over-estimate the rate of formation of PFOS. An alternative half life of one year, to allow for the possible slower degradation of other substances, is also used in the same way. In all media the final product of degradation is assumed to be PFOS (see Section 3.3.1.2). Further consideration of the rate of degradation and the degree of production of PFOS is included in Appendices 3 and 5.

The fraction of N-EtFOSE adsorbed to particulates in air was measured as 65% in outdoor air samples (see Section 3.3.4.2). This value is used in the calculations rather than estimating this percentage from the log Kow value.

It is recognised that other substances considered in this group may have somewhat different properties. These calculations are intended to give a rough idea of possible behaviour. It should be noted that the possible accumulation and effects of the substances themselves are not considered in this evaluation.

3.3.1.1.3 PFOS-polymers

There are no data on the properties of the polymeric substances. Therefore properties have been chosen to represent the expected behaviour, i.e. low volatility, low solubility, and tendency to be associated with solid phases as shown in Table 3.16 below. The effect of higher values for the log Kow value is considered in Appendix 6.

Table 3.16 Summary of values for PFOS-polymers

Property	Value Used
Molecular weight	10000
Melting point	treated as a solid (100°C used)
Solubility	10^{-6} mg/l
Vapour pressure	10^{-6} Pa
Log Kow	6
Henry's Law constant	$10^{-4} \text{ Pa m}^3 \text{ mole}^{-1}$

There are no data on degradation rates for the polymers, or on the extent to which PFOS may be produced, so again values have been selected to represent possible outcomes. The half lives for polymers are expected to be longer than those for the substances, so a half life of

30 years has been used. This is an arbitrary value. Note that the residual fluorocarbons present in the polymers have been treated as PFOS-substances and so this rate does not apply to them.

3.3.1.2 Releases of PFOS to environment

The emission estimates from Section 3.1.10 are used. The PFOS-salt emissions are considered as emissions of PFOS itself, and are entered directly. For the PFOS-substances, the approach depends on the assumptions made about the breakdown of the substances to PFOS. The first assumption is that the PFOS-substances effectively break down immediately to PFOS on release (or are converted to PFOS before release). In this case the emissions of PFOS-substance are converted to PFOS-salt emissions. It is assumed that the degradation proceeds to PFOS with no by-products, so that the yield is 100%. The relative molecular weights for the chosen representative substances mean the yield is 0.94 kg for 1 kg of PFOS-substance. The resulting emissions are added to those of PFOS-salt direct.

Where the degradation of the PFOS-substances is assumed to take a longer time, the emissions of PFOS-substance are modelled using the properties for PFOS-substance above to allow for the effect of movement of air and water in the model. From the concentrations predicted and the appropriate degradation half lives the rate of degradation of PFOS-substance in each environmental compartment at steady state can be calculated. As above, degradation is assumed to proceed to PFOS with no by-products, so that 1 kg/day degradation of PFOS-substance is assumed to give 0.94 kg/day PFOS. Hence the rates of degradation of PFOS-substance are converted to rates of production of PFOS in each compartment, and these are added to the direct releases of PFOS-salt. The results presented in the tables are for the combined releases as appropriate to the particular scenario. This is done for both half lives chosen. The rates of production of PFOS from the breakdown of PFOS-substances for these scenarios are included in Appendix 2.

It is recognised that the assumption of 100% production of PFOS from the PFOS-substances is a worst case assumption (although Cahill and Mackay (2002) quote an estimated yield of 92% from N-EtFOSE). Other possible assumptions are considered in Appendix 3.

The approach for PFOS-polymer is similar to that for the substances. From the information on polymer composition provided for the Risk Reduction Strategy consultation, the PFOS moiety makes up on average about 30% of the polymer by weight, so a yield of 30% by weight has been used. The production rates of PFOS from polymer degradation are included in Appendix 2.

3.3.1.3 Scenarios

The baseline scenario is considered to be the situation in 2000. This includes all of the uses for which emission estimates have been made in Section 3.1, including the service life of treated fabrics. In order to consider the possible contributions of PFOS-substances and PFOS-polymers, calculations have been carried out with and without these included. Hence there are five scenarios using the baseline emissions:

- 1 - PFOS-salt releases only
- 2 - PFOS-salt plus PFOS-substances, assuming instant degradation to PFOS.
- 3 - PFOS-salt plus PFOS-substances, assuming a 20 day half life for degradation to PFOS.
- 4 - PFOS-salt plus PFOS-substances, assuming a 1 year half life for degradation to PFOS.

5 - PFOS-salt, PFOS-substances with 20 day half life, PFOS-polymer with 30 year half life degradation to PFOS.

There are no data for the breakdown of PFOS-polymers, and so the fifth scenario is considered to be much more speculative than the others. A 30 year half life is assumed, with complete release of the PFOS moiety from the polymer on this time scale. It should be noted that all of these scenarios reflect the situation in the past as far as emissions are concerned.

Two further scenarios have been used to try to consider the 'current' situation and a possible future. For the 'current' scenario (Scenario 6), continuing use in chromium plating, photolithography, photography and aviation has been assumed, with use of stocks of fire fighting foam and continuing release from fabrics in use. These last two uses will only continue for a number of years, whereas the calculations assume a continuous use to steady state, and so will overestimate.

The future scenario (Scenario 7) is that where the use of stocks of PFOS foams has been completed and the fabrics have reached the end of their service lives.

Local concentrations have been calculated for all releases of PFOS-salt substances where there is a local source. Local concentrations have also been calculated for releases of the PFOS-substances where instant conversion to PFOS has been assumed (in Scenario 2). For other scenarios, the breakdown of the PFOS-substances (or PFOS-polymer) occurs after dispersion in the environment and so local scenarios are not appropriate. Appendix 7 shows the contribution of each use area to the total regional emissions for each scenario.

3.3.2 Aquatic compartment (surface water, sediment and wastewater treatment plant)

3.3.2.1 Estimated aquatic environmental concentrations

The predicted concentrations in the freshwater compartment and for sediment for the seven scenarios are in Tables 3.17 and 3.18.

Table 3.17 Predicted environmental concentrations in freshwater (mg/l)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	4.52x10 ⁻⁵	8.1x10 ⁻⁵	5.45x10 ⁻⁵	5.16x10 ⁻⁵	9.46x10 ⁻⁵	8.63x10 ⁻⁵	4.36x10 ⁻⁵
Photography - formulation - processing	1.21x10 ⁻⁴	1.56x10 ⁻⁴	1.3x10 ⁻⁴	1.27x10 ⁻⁴	1.7x10 ⁻⁴	1.62x10 ⁻⁴	1.19x10 ⁻⁴
	NA	7.48x10 ⁻⁵	NA	NA	NA	NA	NA
Aviation	9.28x10 ⁻⁵	1.29x10 ⁻⁴	1.02x10 ⁻⁴	9.91x10 ⁻⁵	1.42x10 ⁻⁴	1.34x10 ⁻⁴	9.12x10 ⁻⁵
Fire fighting foams -formulation - use A - use B	0.0973	0.0974	0.0973	0.0973	0.0974	NA	NA
	0.0285	0.0286	0.0285	0.0285	0.0286	0.0286	NA
	0.0411	0.0412	0.0411	0.0411	0.0412	0.0412	NA
Photolithography	NA	9.75x10 ⁻⁴	NA	NA	NA	NA	NA
Fabrics - application	NA	5.32x10 ⁻⁴	NA	NA	NA	NA	NA
Paper treatment	NA	0.0613	NA	NA	NA	NA	NA
Coatings	NA	1.6x10 ⁻³	NA	NA	NA	NA	NA
Regional	3.87x10 ⁻⁵	7.45x10 ⁻⁵	4.8x10 ⁻⁵	4.51x10 ⁻⁵	8.81x10 ⁻⁵	7.98x10 ⁻⁵	3.71x10 ⁻⁵

Table 3.18 Predicted environmental concentrations in sediment (mg/kg ww)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	1.21x10 ⁻⁴	2.17x10 ⁻⁴	1.46x10 ⁻⁴	1.38x10 ⁻⁴	2.53x10 ⁻⁴	2.31x10 ⁻⁴	1.17x10 ⁻⁴
Photography - formulation	3.23x10 ⁻⁴	4.18x10 ⁻⁴	3.47x10 ⁻⁴	3.4x10 ⁻⁴	4.55x10 ⁻⁴	4.33x10 ⁻⁴	3.18x10 ⁻⁴
- processing	NA	2.0x10 ⁻⁴	NA	NA	NA	NA	NA
Aviation	2.48x10 ⁻⁴	3.44x10 ⁻⁴	2.73x10 ⁻⁴	2.65x10 ⁻⁴	3.8x10 ⁻⁴	3.58x10 ⁻⁴	2.44x10 ⁻⁴
Fire fighting foams -formulation	0.26	0.261	0.261	0.261	0.261	NA	NA
- use A	0.0764	0.0765	0.0764	0.0764	0.0765	0.0765	NA
- use B	0.11	0.11	0.11	0.11	0.11	0.11	NA
Photolithography	NA	2.61x10 ⁻³	NA	NA	NA	NA	NA
Fabrics - application	NA	1.42x10 ⁻³	NA	NA	NA	NA	NA
Paper treatment	NA	0.164	NA	NA	NA	NA	NA
Coatings	NA	4.28x10 ⁻³	NA	NA	NA	NA	NA
Regional	1.7x10 ⁻⁴	3.27x10 ⁻⁴	2.1x10 ⁻⁴	1.98x10 ⁻⁴	3.86x10 ⁻⁴	3.5x10 ⁻⁴	1.63x10 ⁻⁴

The calculated concentrations in the effluent from waste water treatment do not depend on the regional background and so are not affected by the differences between the scenarios. The resulting concentrations are in Table 3.19.

Table 3.19 Predicted effluent concentrations

Use area	Concentration (µg/l)	Notes
Chromium plating	0.065	PFOS-salt
Photography (formulation)	0.82	PFOS-salt
Photography (processing)	0.003	PFOS-substance, assumed instant conversion
Aviation	0.54	PFOS-salt
Fire fighting foams (formulation)	973	PFOS-salt
Fire fighting foams (use B)	570	PFOS-salt
Photolithography	9.0	PFOS-substance, assumed instant conversion
Fabrics application	4.6	PFOS-substance, assumed instant conversion
Paper treatment	613	PFOS-substance, assumed instant conversion
Coatings	15.2	PFOS-substance, assumed instant conversion

3.3.2.2 Measured aquatic environmental concentrations

Studies have identified the presence of PFOS in surface water and sediment downstream of a production facility, as well as in wastewater treatment plant effluent, sewage sludge and landfill leachate at a number of urban centres in the US (3M Multi City study, various reports reviewed in OECD (2002) and 3M (2003)). Four of the cities (Decatur, Mobile, Columbus, Pensacola) were cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland, Port St. Lucie) were control cities that do not have significant fluorochemical activities. The ranges of PFOS levels in these cities are provided in Table 3.20.

The control cities' samples generally inhabited the lower end of the above ranges, except for the municipal wastewater treatment plant effluent and sludge findings for one of the control cities (Cleveland), which were intermediate in their ranges, and the 'quiet' water samples at control city (Port St. Lucie), which were the highest.

Hansen *et al* (2002) reported concentrations of PFOS measured from surface water samples taken from the Tennessee River upstream and downstream of the outfall from the fluorochemical manufacturing facility of 3M at Decatur. Upstream of the facility the average concentration of PFOS was 32 ± 11 ng/L; the downstream concentrations were observed to increase at a point approximately six miles below the outfall; the average PFOS concentration from that point downstream was 114 ± 19 ng/L.

Table 3.20 Environmental Levels of PFOS in Six US Urban Centres in the US (from OECD, 2002)

Medium	Range of PFOS levels
Municipal wastewater treatment plant effluent	0.041 - 5.29 ppb
Municipal wastewater treatment plant sludge	0.2 - 3,120 ppb (dry weight)
Drinking water	ND - 0.063 ppb
Sediment	ND - 53.1 ppb (dry weight)
Surface water	ND - 0.138 ppb
'Quiet' water	ND - 2.93 ppb

Source: OECD, 2002

Note: ND: not detected

3M (2003) included measured levels from the vicinity of the facility outfall at Decatur for 2001. The mean concentration in water for seven sites (one sample from each) was 61 µg/l. The corresponding sediment concentration was 2,740 µg/kg dw.

The first environmental survey of PFOS and related substances in Japan (which followed a Japanese study that showed measurable levels of PFOS in human blood) found the highest concentration in surface water in Tokyo Bay at 59 ng/L (mean: 26 ng/L). The concentrations of PFOS in surface water were similar to those of polyaromatic hydrocarbons (PAHs) and much higher than those of PCBs, dioxins and furans (Taniyasu *et al*, 2002).

Saito *et al* (2003) determined the PFOS concentrations in surface water samples from 142 locations in Japan (single samples from each location). The geometric mean concentration for river waters (126 samples) was 2.37 ng/l (geometric standard deviation 4.13), the median was 1.68 ng/l, the range 0.3 - 157 ng/l. For coastal water samples, the geometric mean concentration was 1.52 ng/l (SD 4.14), median 1.21 ng/l and range 0.2 - 25.2 ng/l. The authors comment that the levels are much lower than those reported for the US, with the exception of two rivers where 135 and 157 ng/l were measured.

Samples of effluent from fifteen representative industrial branches were analysed for PFOS, perfluoroalkyl carboxylates and perfluoroalkyl sulphonates (Hohenblum *et al*, 2003). The industry branches were printing (1 site), electronics (3), leather, metals, paper (6), photographic, textiles (2). The PFOS levels found ranged from below the detection limit (25 ng/l) to 2,500 ng/l (2,500 ng/l for leather, 120 ng/l for metal, 140-1200 ng/l at four paper sites, 1,200 ng/l for photographic, not found in textiles or electronic).

Groundwater from below the Wurtsmith Air Force Base in Michigan, US was sampled (Moody *et al*, 2003). Fire fighting foams containing PFOS had been used there in training exercises from the 1950s to 1993 when the base was decommissioned. The groundwaters were found to contain PFOS, at levels from 4 to 110 µg/l. The C6 (hexyl) analogue of PFOS was also found, as was PFOA and its C6 analogue.

3.3.2.3 Comparison of measured and estimated aquatic concentrations

There are limited measured data available to compare with the predicted concentrations, and in a number of cases it is not possible to allocate the measured data to the relevant scale, local, regional or continental. Hansen *et al* (2002) reported a concentration of 32 ng/l in an area upstream of a discharge and not considered to be affected by specific sources. The calculated regional background concentrations are in the range 40 – 90 ng/l.

Saito et al (2003) found lower levels in Japan in general, with a median value of 1.2 ng/l. They remarked that the levels were much lower than those found in the United States. This may reflect different patterns of use. Taniyasu et al (2002) found the highest level of PFOS in Tokyo Bay, at 59 ng/l, which is similar to the freshwater background levels but higher by about an order of magnitude than the regional marine concentration (see Section 5.5.2.1). (The value is similar to some of the local concentrations for the marine environment.)

Values up to 2.93 µg/l (ppb) were reported from the 3M Multi-City study. The calculated local values (apart from those for fire fighting foams) are around 0.1 µg/l and so are lower than the highest value. The calculated values are closer to those measured some distance downstream of the production site. The highest calculated concentration is for the formulation of foams, at up to 0.1 mg/l depending on the scenario. This is of a similar order to the levels measured in the vicinity of the production facility outfall, but these are presumably different processes.

The effluent concentrations calculated for chromium plating (65 ng/l) and photography (820 ng/l) agree well with the measured values in effluents reported by Hohenblum et al (2002). The predicted value for paper, 0.6 mg/l, is much higher than the 1200 ng/l reported as the highest measured value. The calculated value is based on the release of PFOS-substance and assumes instant conversion to PFOS before the treatment plant, and so may be expected to over-estimate the concentration of PFOS. Similarly, a concentration of 4.6 µg/l was calculated for fabric treatment, while PFOS was not detected in textile effluents – again the calculated value is based on PFOS-substance release and instant conversion to PFOS. The measurements may indicate a lower presence of PFOS in the effluents from these areas at the point of release. There may have been PFOS-substances present in the effluents, but only one (n-ethylperfluorooctanesulphonamide) was analysed for in the samples – it was not detected in any. The calculated and measured values are therefore not on the same basis and are difficult to compare.

For some of the use areas the calculated effluent concentrations are in reasonable agreement with the range of values reported from the 3M Multi-City study. Higher values are calculated for uses where the PFOS-substances are assumed to be converted instantly to PFOS. Higher values are also calculated for the formulation of PFOS-containing foams (which is a default estimate) and for Use B, which is a use pattern not likely to have been included in the 3M study (use of foams in a fire).

3.3.3 Terrestrial compartment

3.3.3.1 Estimated soil concentrations

The predicted concentrations in the terrestrial compartment for the seven scenarios are in Table 3.21 (concentrations in soil at 30 days after application of sludge) and Table 3.22 (concentrations in groundwater under agricultural soil at 180 days after application of sludge).

Table 3.21 Predicted concentrations in soil (mg/kg wwt, agricultural soil, after 30 days)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	8.52x10 ⁻⁴	8.57x10 ⁻⁴	1.37x10 ⁻³	1.45x10 ⁻³	1.41x10 ⁻³	9.8x10 ⁻⁴	8.47x10 ⁻⁴
Photography - formulation - processing	0.0107	0.0107	0.0112	0.0113	0.0112	0.0108	0.0107
	NA	4.92x10 ⁻⁵	NA	NA	NA	NA	NA
Aviation	7.06x10 ⁻³	7.07x10 ⁻³	7.59x10 ⁻³	7.66x10 ⁻³	7.62x10 ⁻³	7.19x10 ⁻³	7.08x10 ⁻³
Fire fighting foams - formulation - use A - use B	12.7	12.7	12.7	12.7	12.7	NA	NA
	5.05x10 ⁻⁶	1.01x10 ⁻⁵	5.26x10 ⁻⁴	6.03x10 ⁻⁴	5.61x10 ⁻⁴	1.32x10 ⁻⁴	NA
	5.36	5.36	5.37	5.37	5.37	5.36	NA
Photolithography	NA	0.118	NA	NA	NA	NA	NA
Fabrics - application	NA	0.0598	NA	NA	NA	NA	NA
Paper treatment	NA	8.0	NA	NA	NA	NA	NA
Coatings	NA	0.199	NA	NA	NA	NA	NA
Regional (natural)	5.05x10 ⁻⁶	1.01x10 ⁻⁵	5.26x10 ⁻⁴	6.03x10 ⁻⁴	5.61x10 ⁻⁴	1.32x10 ⁻⁴	1.77x10 ⁻⁵

Table 3.22 Predicted concentrations in groundwater under agricultural soil (mg/l, after 180 days)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	3.56x10 ⁻⁵	3.58x10 ⁻⁵	5.74x10 ⁻⁵	6.07x10 ⁻⁵	5.89x10 ⁻⁵	4.09x10 ⁻⁵	3.61x10 ⁻⁵
Photography - formulation - processing	4.48x10 ⁻⁴	4.46x10 ⁻⁴	4.68x10 ⁻⁴	4.71x10 ⁻⁴	4.69x10 ⁻⁴	4.51x10 ⁻⁴	4.47x10 ⁻⁴
	NA	2.05x10 ⁻⁶	NA	NA	NA	NA	NA
Aviation	2.95x10 ⁻⁴	2.45x10 ⁻⁴	3.17x10 ⁻⁴	3.2x10 ⁻⁴	3.18x10 ⁻⁴	3.0x10 ⁻⁴	2.95x10 ⁻⁴
Fire fighting foams - formulation - use A - use B	0.53	0.53	0.53	0.53	0.53	NA	NA
	2.12x10 ⁻⁷	4.24x10 ⁻⁷	2.31x10 ⁻⁵	2.53x10 ⁻⁵	2.35x10 ⁻⁵	5.56x10 ⁻⁶	NA
	0.224	0.224	0.224	0.224	0.224	0.224	NA
Photolithography	NA	4.91x10 ⁻³	NA	NA	NA	NA	NA
Fabrics - application	NA	2.49x10 ⁻³	NA	NA	NA	NA	NA
Paper treatment	NA	0.334	NA	NA	NA	NA	NA
Coatings	NA	8.31x10 ⁻³	NA	NA	NA	NA	NA
Regional	3.28x10 ⁻⁵	6.27x10 ⁻⁵	7.66x10 ⁻⁵	5.91x10 ⁻⁵	2.93x10 ⁻⁴	2.49x10 ⁻⁴	3.4x10 ⁻⁵

3.3.3.2 Measured soil environmental concentrations

No measurements of concentrations of PFOS in soils have been located. Concentrations of PFOS in groundwater under a US Air Force Base are included in Section 3.3.2.2, and range from 4 – 110 µg/l. These are of the same order as those calculated for the use of fire fighting foams in Use B, and for the formulation of foams (default calculation). All other calculated concentrations are much lower than these measurements.

3.3.4 Atmospheric compartment

3.3.4.1 Estimated air concentrations

The predicted concentrations in the atmospheric compartment for the seven scenarios are in Table 3.23.

Table 3.23 Predicted concentrations in air (mg/m³)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	1.1x10 ⁻¹¹	8.13x10 ⁻¹¹	1.09x10 ⁻⁹	1.25x10 ⁻⁹	1.16x10 ⁻⁹	2.75x10 ⁻¹⁰	3.71x10 ⁻¹¹
Photography - formulation - processing	2.51x10 ⁻⁸	2.52x10 ⁻⁸	2.62x10 ⁻⁸	2.64x10 ⁻⁸	2.63x10 ⁻⁸	2.54x10 ⁻⁸	2.52x10 ⁻⁸
	NA	2.09x10 ⁻¹¹	NA	NA	NA	NA	NA
Aviation	1.22x10 ⁻¹¹	2.26x10 ⁻¹¹	1.09x10 ⁻⁹	1.25x10 ⁻⁹	1.16x10 ⁻⁹	2.76x10 ⁻¹⁰	3.84x10 ⁻¹¹
Fire fighting foams - formulation - use A - use B	2.97x10 ⁻⁵	3.04x10 ⁻⁵	2.97x10 ⁻⁵	2.97x10 ⁻⁵	2.97x10 ⁻⁵	NA	NA
	1.05x10 ⁻¹¹	2.09x10 ⁻¹¹	1.09x10 ⁻⁹	1.25x10 ⁻⁹	1.16x10 ⁻⁹	2.74x10 ⁻¹⁰	NA
	1.43x10 ⁻¹¹	2.51x10 ⁻¹¹	1.09x10 ⁻⁹	1.25x10 ⁻⁹	1.17x10 ⁻⁹	2.78x10 ⁻¹⁰	NA
Photolithography	NA	5.39x10 ⁻¹¹	NA	NA	NA	NA	NA
Fabrics - application	NA	3.14x10 ⁻¹¹	NA	NA	NA	NA	NA
Paper treatment	NA	1.89x10 ⁻⁹	NA	NA	NA	NA	NA
Coatings	NA	6.74x10 ⁻¹¹	NA	NA	NA	NA	NA
Regional	1.05x10 ⁻¹¹	2.09x10 ⁻¹¹	1.09x10 ⁻⁹	1.25x10 ⁻⁹	1.16x10 ⁻⁹	2.74x10 ⁻¹⁰	3.66x10 ⁻¹¹

3.3.4.2 Measured air concentrations

Sasaki et al (2003) measured the levels of PFOS in dust samples from two locations in Japan, monthly samples taken over a 12 month period. The PFOS concentration expressed as a concentration in air ranged from zero to 2.12 pg/m³ at one location (geometric mean level 0.6 pg/m³) and from 2.32 to 21.8 pg/m³ (geometric mean 5.3 pg/m³) at the other. The amounts of dust in the air were measured. The mean concentrations of PFOS in dust at the two locations were 19.2 ng/g and 97.4 ng/g.

Moriwaki et al (2003) measured the concentration of PFOS in vacuum cleaner dust in Japan. One sample contained 2,500 ng/g, the other 15 samples were below 140 ng/g.

Shoeib et al (2004) measured the total concentration of the PFOS-substances N-EtFOSE and N-MeFOSE in outdoor air from locations described as semi-urban. They found levels of 31.7 and 16 pg/m³ for N-MeFOSE, and 9.79 and 8.47 pg/m³ for N-EtFOSE. They also separated the contributions from the vapour and particulate phases. They found 75% of N-MeFOSE associated with particulates and ~65% of N-EtFOSE. These values are much higher than would be expected from the measured octanol-air partition coefficients or from the sub-cooled liquid vapour pressure values, both of which give reasonable predictions for many organic substances.

3.3.4.3 Comparison of measured and estimated atmospheric concentrations

The highest calculated concentration in air is around 1 pg/m³, which is similar to some of the measured values. However, the number of measurements is limited and their relation to sources is unknown. Hence a comparison is not particularly meaningful.

3.3.5 Food chain exposure

3.3.5.1 Estimated environmental concentrations

Four different situations for food chain exposure are considered in the evaluation. These are: predator feeding on freshwater fish; marine predator feeding on fish; marine top predator; and terrestrial food chain (feeding on worms). The concentrations in the food organisms in each case are derived from combinations of the local and regional exposures of the organisms to PFOS according to the methods in the Technical Guidance Document. In addition, concentrations have been calculated for food organisms assuming that they are exposed only to the regional background concentration (so without any local contribution). These are

included in the tables as regional concentrations. The calculated concentrations in freshwater fish, marine fish, marine predator and worms are presented in Tables 3.24 to 3.27. (The marine concentrations have been included here to allow comparison with the measured levels.)

Table 3.24 Predicted concentrations in freshwater fish (mg/kg ww)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.217	0.428	0.268	0.252	0.493	0.446	0.208
Photography - formulation - processing	0.405	0.604	0.456	0.44	0.681	0.634	0.396
	NA	0.417	NA	NA	NA	NA	NA
Aviation	0.361	0.541	0.413	0.397	0.638	0.591	0.353
Fire fighting foams -formulation - use A - use B	224	224	224	224	224	NA	NA
	0.435	0.635	0.487	0.47	0.711	0.665	NA
	0.531	0.731	0.583	0.567	0.807	0.761	NA
Photolithography	NA	2.9	NA	NA	NA	NA	NA
Fabrics - application	NA	1.21	NA	NA	NA	NA	NA
Paper treatment	NA	141	NA	NA	NA	NA	NA
Coatings	NA	3.92	NA	NA	NA	NA	NA
Regional	0.214	0.411	0.265	0.249	0.486	0.440	0.205

Table 3.25 Predicted concentrations in marine fish (mg/kg ww)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.0216	0.0432	0.0267	0.0251	0.0484	0.0439	0.0207
Photography - formulation - processing	0.0477	0.0676	0.0528	0.0512	0.0745	0.07	0.0468
	NA	0.0416	NA	NA	NA	NA	NA
Aviation	0.0417	0.0588	0.0468	0.0452	0.0685	0.064	0.0408
Fire fighting foams -formulation - use A - use B	31	31	31	31	31	NA	NA
	0.0434	0.0634	0.0485	0.047	0.0702	0.0657	NA
	0.0653	0.0852	0.0703	0.0688	0.0921	0.0875	NA
Photolithography	NA	0.386	NA	NA	NA	NA	NA
Fabrics - application	NA	0.151	NA	NA	NA	NA	NA
Paper treatment	NA	19.6	NA	NA	NA	NA	NA
Coatings	NA	0.528	NA	NA	NA	NA	NA
Regional	0.021	0.041	0.026	0.025	0.048	0.043	0.020

Table 3.26 Predicted concentrations in marine predators (mg/kg ww)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.0432	0.0838	0.0533	0.0502	0.0968	0.0878	0.0414
Photography - formulation - processing	0.0536	0.0935	0.0638	0.0607	0.107	0.0982	0.0518
	NA	0.0831	NA	NA	NA	NA	NA
Aviation	0.0513	0.090	0.0614	0.0583	0.105	0.0958	0.0495
Fire fighting foams -formulation - use A - use B	12.5	12.5	12.5	12.5	12.5	NA	NA
	0.0519	0.0918	0.0621	0.059	0.106	0.0965	NA
	0.0607	0.101	0.0708	0.0677	0.114	0.105	NA
Photolithography	NA	0.221	NA	NA	NA	NA	NA
Fabrics - application	NA	0.127	NA	NA	NA	NA	NA
Paper treatment	NA	7.9	NA	NA	NA	NA	NA
Coatings	NA	0.277	NA	NA	NA	NA	NA
Regional	0.043	0.082	0.052	0.050	0.096	0.086	0.041

Table 3.27 Predicted concentrations in terrestrial biota (worms) (mg/kg ww)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	1.94×10^{-3}	2.8×10^{-3}	3.8×10^{-3}	3.4×10^{-3}	9.99×10^{-3}	8.22×10^{-3}	1.99×10^{-3}
Photography - formulation - processing	0.0136	0.0144	0.0155	0.0151	0.0216	0.0199	0.0136
	NA	1.84×10^{-3}	NA	NA	NA	NA	NA
Aviation	9.3×10^{-3}	0.0102	0.112	0.0108	0.0173	0.0156	9.35×10^{-3}
Fire fighting foams -formulation - use A - use B	15.1	15.1	15.1	15.1	15.1	NA	NA
	9.38×10^{-4}	1.79×10^{-3}	2.8×10^{-3}	2.4×10^{-3}	8.99×10^{-3}	7.21×10^{-3}	NA
	6.36	6.36	6.36	6.36	6.36	6.36	NA
Photolithography	NA	0.141	NA	NA	NA	NA	NA
Fabrics - application	NA	0.0726	NA	NA	NA	NA	NA
Paper treatment	NA	9.48	NA	NA	NA	NA	NA
Coatings	NA	0.238	NA	NA	NA	NA	NA
Regional ^a	1.86×10^{-3}	3.56×10^{-3}	4.25×10^{-3}	3.36×10^{-3}	0.0166	0.0141	1.93×10^{-3}

Note: the calculation of local soil PECs (from which the local contributions to levels in biota are estimated) use the concentration in natural soil (arising from deposition only) as the regional background. The regional contribution to the biota concentrations is estimated from the concentrations in agricultural soil, which are generally higher than the natural soil levels through sludge application. Hence where the local input is small. The regional contribution to the biota concentration dominates and the purely regional biota concentration can be higher than the local concentrations.

In addition to the above concentrations, EUSES also calculates concentrations in plants (leaf and root), in meat (cattle) and in milk. Rather than present the complete results here, the ranges of values calculated for each use area and for the regional background are presented in Table 3.28. It should be noted that the calculations for these concentrations all involve the use of the log Kow value. A surrogate value has been estimated for PFOS as the actual value cannot be determined for this substance. In this situation the suitability of the methods used is uncertain, and hence the results should be treated as having a high degree of uncertainty.

3.3.5.2 Measured environmental concentrations in biota

PFOS and related fluorochemicals have been traced in animals in a number of studies. These studies (a selection of which are outlined in Table 3.29) have taken place in a variety of locations around the globe and have shown concentrations exceeding 2 ppm in birds and 4 ppm in minks.

Some of the following studies relate to studies already included in part in the OECD hazard assessment (OECD, 2002).

Kannan and Giesy (2002) summarised the results of the analyses on archived samples referred to in the first item in Table 3.29. The tissues analysed came from marine mammals, birds, fish, reptiles and amphibians from around the world, including the Arctic and Antarctic Oceans. Samples collected in the 1990s were used. Around 1,700 samples were analysed, with concentrations in liver, egg yolk, muscle or blood plasma determined. The detection limit varied from 1 to 35 ppb wet weight. A summary of the results is in Table 3.30.

PFOS was detectable in most of the samples, including those from remote marine locations, at concentrations >1 ng/g. The authors compared the results from remote areas with those from more industrial locations. They comment that PFOS is distributed in remote regions, including the polar regions, but that the levels found in more urban and industrial areas (e.g. the Baltic, Great Lakes) are several times higher. The tissues of fish-eating birds in Canada, Italy, Japan and Korea all contained detectable levels of PFOS, suggesting that they are exposed through the fish they consume. The sulphonamide compound, FOSA, was only detected in ~10-15% of samples.

Table 3.28 Calculated concentrations in plants, meat and milk (mg/kg)

	Regional	Use area										
		Chromium plating	Photography – formulation	Photography – processing	Aviation	Fire-fighting foams			Photo-lithography	Fabrics – application	Paper treatment	Coatings
						Formulation	Use A	Use B				
Plant root	0.02-0.18	0.022-0.038	0.28-0.29	1.3×10^{-3}	0.18-0.20	328	$1.3 \times 10^{-4} - 0.016$	139	3.04	1.54	207	5.1
Plant leaf	$(2.5-24) \times 10^{-5}$	$(2.8-54) \times 10^{-5}$	$(5.6-6) \times 10^{-4}$	1.8×10^{-6}	$(2.3-2.6) \times 10^{-4}$	0.67	$(0.26-31) \times 10^{-6}$	0.17	3.8×10^{-3}	1.9×10^{-3}	0.26	6.4×10^{-3}
Meat	$(7.4-67) \times 10^{-6}$	$(5.3-12) \times 10^{-6}$	$(0.95-1.0) \times 10^{-4}$	4.1×10^{-6}	$(4.4-5.1) \times 10^{-5}$	0.11	$(0.62-1.3) \times 10^{-5}$	0.033	7.3×10^{-4}	3.7×10^{-4}	0.05	1.2×10^{-3}
Milk	$(2.3-21) \times 10^{-6}$	$(1.7-3.9) \times 10^{-6}$	$(3-3.2) \times 10^{-5}$	1.3×10^{-6}	$(1.4-1.6) \times 10^{-5}$	0.034-0.036	$(2.0-4.1) \times 10^{-6}$	0.011	2.3×10^{-4}	1.2×10^{-4}	0.011	3.9×10^{-4}

Table 3.29 Monitored Levels of PFOS in Animals (data from selected studies, based on OECD, 2002)

Description	Ref	Highest Reported Concentration	Location of Highest Concentration
Global monitoring survey of marine mammals (Florida, California, Alaska, northern Baltic Sea, Mediterranean Sea, Arctic, Sable Island (Canada))	OECD, 2002	Bottlenose dolphin: 1,520 ng/g wet wt (liver)	Florida
		Ringed seal: 475 ng/mL (blood)	Northern Baltic Sea
US Fish & Wildlife Service survey of piscivorous fish	A	Bald eagle: 1,047 ppb (plasma)	US
		Six bird species: 2,055 ppb (liver)	US
Survey of fish-eating water birds (US, Europe, North Pacific Ocean, Antarctic)	B	Fish: 923 ng/g wet wt. (muscle)	Belgian estuary
		Carp: 296 ng/g wet wt. (muscle)	US Great Lakes
Survey of fish-eating birds (US, Baltic Sea, Mediterranean Sea, Japanese coast, Korean coast)	C	Bald eagle: 2,200 ng/mL (plasma)	Midwest US
		Brandts cormorant: 1,780 ng/g wet wt. (liver)	US
Survey of mink and river otter in the US	D	Mink: 4,800 ng/g wet wt. (liver)	US
		River otter: 994 ng/g wet wt. (liver)	US
Survey of oysters in the US (Chesapeake Bay & Gulf of Mexico)	E	Oysters: 1,225 ng/g dry wt.	US
Clam and fish samples upstream and downstream of 3M facility in Decatur, Alabama, US	F	Fish: 59.1 µg/kg wet wt. (whole body - upstream)	Decatur, US
		Fish: 1,332 µg/kg wet wt. (whole body - downstream)	Decatur, US
		Clam: 15.6 µg/kg wet wt. (upstream) Fish: 14.1 µg/kg wet wt. (downstream)	Decatur, US
First Environmental Survey of PFOS in Japan	G	Fish: 345 ng/mL (average blood levels)	Lake Biwa, Japan
Swedish urban and background fish samples	H	Perch: 3 - 8 ng/g (urban sites in the vicinity of municipal STPs); 20-44 ng/g in Lake Malaren and near Stockholm	Sweden (Lake Malaren)

Sources: A: 3M (2000); B: Giesy and Kannan (2001a); C: Giesy and Kannan (2001b); D: Giesy and Kannan (2001c); E: Giesy and Kannan (2001d); F: Giesy and Kannan (2001e); G: Taniyasu et al (2002); H: Jarnberg and Holmstrom (2003)

Table 3.30 Summary of global archive sample analysis

Species	Maximum concentration ng/g ww	Frequency of detection
Marine mammals	1520	77%
Mink and otter	4900	100%
Birds	2570	60%
Fish	1000	38%

Martin et al (2004) measured the levels of PFOS in liver samples from biota in the Canadian Arctic. PFOS was found in the vast majority of the samples (all except the black guillemot). The highest levels were found in polar bear, with a mean level of 3,100 ng/g from seven animals (maximum value >4,000 ng/g). Generally, higher levels were found in animals higher up the food chain. The sulphonamide FOSA was also found in most of the samples. It was associated with PFOS to some extent. The concentration of FOSA was higher than that of PFOS in fish, but not in mammals. The pattern may be the result of both exposure and metabolism.

A recent conference presentation (personal communication, KEMI, Sweden) reported that the concentration of PFOS in guillemot (*Uria aalge*) eggs collected from the Baltic Sea has increased by more than 30-fold since 1968, with annual average increases of 7-11%.

Van de Vijver et al (2003) measured the concentrations of PFOS in aquatic invertebrates from the Western Scheldt estuary, in starfish (*Asterias rubens*), crab (*Carcinus mainas*) and

shrimp (*Crangon crangon*). Eight locations were sampled. Mean whole body concentrations were 16 ± 3 - 93 ± 34 ng/g in starfish, 40 ± 13 - 319 ± 70 for shrimp and 93 ± 38 - 292 ± 45 ng/g for crab. There were indications of a concentration gradient, with possible sources of PFOS including a fluorochemical manufacturing site and industrial regions drained by a canal entering the estuary.

Hoff et al (2003) sampled fish (bib, *Trisopterus luscus*, and plaice, *Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea, with four separate locations for each fish. Some of the locations were similar to those in the Van de Vijver et al (2003) study above. PFOS was found in the livers of all of the plaice collected (detection limit 10 ng/g ww); levels up to 7,760 ng/g were found at estuarine sites, the values at marine sites were lower. In plaice muscle, PFOS was detected in 20-30% of the marine samples and 75% of the estuarine samples (maximum concentration 87 ng/g). For bib, again all liver samples had concentration above the detection limit, though the highest concentration were lower than those found in plaice, at ~200 ng/g. In bib muscle, marine sites had 50% of samples above 10 ng/g, while the two innermost estuarine sites had all samples above the detection limit. The maximum concentration measured was 111 ng/g.

The first environmental survey of PFOS and related substances in Japan (which followed a Japanese study that showed measurable levels of PFOS in human blood) suggests that PFOS is present in the blood and livers of all fish in surface waters in Japan (Taniyasu *et al*, 2002).

In the 3M Multi City study (see Section 3.3.3.2), measurable quantities of PFOS (up to 0.852 ng/g) were found in four milk samples and one ground beef sample. One of the four milk samples was from a control city, although cities with fluorochemical substances production or use tended to give measurable PFOS levels. The testing included produce such as green beans, apples, pork muscle, cow's milk, chicken muscle, chicken eggs, bread, hot dogs, catfish and ground beef.

3.3.5.3 Comparison of predicted and measured environmental concentrations

The information on measured levels in biota has been largely presented as summaries and ranges, and so the predicted concentrations are also considered as ranges. These are:

Freshwater fish	200 – 800 µg/kg (ng/g)
Salt water fish	20 – 100 µg/kg (ng/g)
Marine predators	40 – 120 µg/kg (ng/g)
Soil organisms	1 – 20 µg/kg (ng/g)

The concentrations predicted in fish appear to agree reasonably well with the summarised measured values in Tables 3.27 and 3.28. If anything the calculated values are generally lower than the upper ends of the ranges, particularly if the predicted concentrations related to the formulation and use of foams are excluded. However, the measured values relate in many cases to specific tissues, whereas the calculated values are whole body concentrations and so would be expected to be lower than tissue concentrations in most cases. Many of the measurements on levels in biota from the environment have been performed on samples taken from relatively remote areas. As such these cannot easily be compared to the calculated levels, which relate to locations closer to sources.

The majority of the calculated concentrations in milk are of the same order or are below the highest reported concentration of 0.852 ng/g (8.5×10^{-4} mg/kg), but data for comparison are limited. The concentrations calculated for three of the use areas are very much higher.

Concentrations in vegetation and fruits were measured as part of the plant toxicity study discussed in Sections 4.2.1 and 3.2.6.2. The actual concentrations are not appropriate for comparison with the calculated levels, as the measurements are not from environmental samples. It is of interest to compare the plant:soil concentration ratios for the measured and calculated concentrations. As described in Section 3.2.6.2, the measured levels in vegetative tissues were 1-2 times those in the soil; for the calculated concentrations, the calculated levels in plant leaf are 0.01-0.05 times those calculated for the soil. The plant root calculated concentrations are not really comparable with the fruit measurements, as most of the fruits were above ground, but the calculated values are 26 times those in soil, while the measured fruit values were less than 10% of those in soil. It thus appears that using the model and values selected, the model may under-estimate the levels in plant leaves but over-estimate those in root crops.

4

EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

The ecotoxicity data relating to PFOS were reviewed for the OECD Hazard Assessment (OECD, 2002). The studies reviewed in the OECD report have not been re-examined for this evaluation. A comparison has been made against the updated 3M report (3M, 2003) to check for any corrections or for new tests. A literature search for studies from 2002 onwards has also been conducted.

4.1 AQUATIC COMPARTMENT (INCLUDING SEDIMENT)

4.1.1 Aquatic studies

4.1.1.1 Fish

The results from the studies on fish toxicity reviewed by the OECD and considered to be of a good or acceptable standard are presented in Tables 4.1 and 4.2. Results are expressed in terms of the potassium salt of PFOS. Where a different salt was used in the study, the result has been converted for this evaluation (and noted in the tables). For further details of the studies see the OECD assessment (OECD, 2002).

Table 4.1 Acute toxicity data for fish (after OECD, 2002)

Species	Protocol	Result (mg/l)	Study standard
Freshwater			
Fathead minnow (<i>Pimephales promelas</i>)	OECD 203 & OPPTS 850.1075 (static)	96-h LC ₅₀ = 9.5	Good
	Not noted (static)	96-h LC ₅₀ = 5.0	Acceptable ^a
	OECD 203 (static)	96-h LL ₅₀ = 133	Acceptable ^b
Bluegill sunfish (<i>Lepomis macrochirus</i>)	OECD 203 & OPPTS 850.1075 (static)	96-h LC ₅₀ = 6.9	Acceptable ^c
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Standard procedures for testing acute lethality of liquid effluents (Environment Canada)	96-h LC ₅₀ = 7.8	Acceptable
	OECD 203 & OPPTS 850.1075 (static)	96-h LC ₅₀ = 22	Acceptable
Seawater			
Sheepshead minnow (<i>Cyprinodon variegatus</i>)	OECD 203 & OPPTS 850.1075 (semi-static, 24 h renewal)	96-h LC ₅₀ > 15	Acceptable
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Standard procedures for testing acute lethality of liquid effluents (Environment Canada)	96-h LC ₅₀ = 13.7	Acceptable

Notes: a – original value 4.7 mg/l, for lithium salt
b – original value 200 mg/l, for didecylmethylammonium salt
c – original value 7.8 mg/l, for DEA salt

Table 4.2 Long-term toxicity data for fish (after OECD 2002)

Species	Protocol	Result (mg/l)	Study standard
Freshwater			
Fathead minnow (<i>Pimephales promelas</i>)	OECD 210 & OPPTS 850.1400 (flow-through)	42-d NOEC _{surv} = 0.30 42-d NOEC _{growth} = 0.30 5-d NOEC _{hatch} = 4.6	Good
	Non-standard (flow-through)	30-d NOEC _{els} = 1	Acceptable
Bluegill sunfish (<i>Lepomis macrochirus</i>)	OECD 305 & OPPTS 850.1731	62-d NOEC _{mortality} = >0.086, <0.87	Good

The long-term study with bluegill sunfish was a bioconcentration study (discussed as such in Section 3.2.6.1). All fish exposed to 0.87 mg/l died by the 35th day of the exposure. At

0.086 mg/l, two fish of the ninety exposed died (2.2%). There were no deaths in the controls. As there were no repeat exposures it is not considered suitable for the determination of a NOEC value. However, the observations in this study are not in contradiction to those in the other long term studies.

Hoff et al (2003) administered a single intraperitoneal injection of PFOS into carp (six concentrations over two experiments) and monitored selected biochemical endpoints. Levels of PFOS in fish liver were measured after one and five days. No mortality was seen, nor inflammation of the liver, peroxisome proliferation, or any effect on serum anti-oxidant levels. The levels of liver enzymes in serum were elevated, indicating a disruption of the liver membrane. In terms of the concentration in the fish, the EC₁₀ values for this effect were determined as 164 ng/g in wet tissue or 258 ng/g in wet liver respectively (concentrations after five days).

4.1.1.2 Invertebrates

The results from the studies on fish toxicity reviewed by the OECD and considered to be of a good or acceptable standard are presented in Tables 4.3 and 4.4. Results are expressed in terms of the potassium salt of PFOS. Where a different salt was used in the study, the result has been converted for this evaluation (and noted in the tables). For further details of the studies see the OECD assessment (OECD, 2002). Results from a more recent publication (Boudreau et al, 2003a) are also included in the tables. These used ASTM methods, and are considered valid for use in the evaluation. As specific details of the tests have not been seen, they have been graded as acceptable in the tables.

Table 4.3 Acute toxicity for invertebrates

Species	Protocol	Result (mg/l)	Study standard
Freshwater			
Water flea (<i>Daphnia magna</i>)	OECD 202 & OPPTS 850.1010 (static)	48-h EC ₅₀ = 61	Good
	ASTM 1981 & OECD 1981 (static)	48-h EC ₅₀ = 27	Acceptable
	Not noted (static)	48-h EC ₅₀ = 223	Acceptable ^b
	OECD 202 (static)	48-h EL ₅₀ = 2.66	Acceptable ^c
	ISO, 1982	48-h EC ₅₀ = 58	Acceptable
	ASTM	48-h EC ₅₀ = 67.2	Acceptable ^a
Water flea (<i>Daphnia pulex</i>)	ASTM	48-h EC ₅₀ = 134	Acceptable ^a
Freshwater mussel (<i>Unio complanatus</i>)	OECD 203, OPPTS 850.1075 & ASTM-E-729-88a (semi-static)	96-h LC ₅₀ = 59	Good
Saltwater			
Mysid shrimp (<i>Mysidopsis bahia</i>)	OPPTS 850.1035 (static)	96-h LC ₅₀ = 3.6	Good
Eastern oyster (<i>Crassostrea virginica</i>)	OPPTS 850.1025 (static)	96-h EC ₅₀ = >3.0	Good
Brine shrimp (<i>Artemia</i> sp.)	Draft ISO, 1981	48-h LC ₅₀ = 8.9	Acceptable

Note: information taken from OECD (2000) except for those marked "a" which are from Boudreau et al (2003a).

b – original value 210 mg/l, for lithium salt

c – original value 4.0 mg/l, for didecyldimethylammonium salt

Table 4.4 Chronic toxicity to invertebrates

Species	Protocol	Result (mg/l)	Study standard
Freshwater			
Water flea (<i>Daphnia magna</i>)	OECD 211, OPPTS 850.1300 & ASTM 1193-87E (semi-static)	21-d NOEC _{repro} = 12 21-d NOEC _{surv} = 12 21-d NOEC _{growth} = 12	Good
	ASTM 1981 & OECD 1981 (semi-static)	28-d NOEC _{repro} = 7	Acceptable
	ASTM	21-d NOEC _{surv} = 5.3	Acceptable ^a
Seawater			
Mysid shrimp (<i>Mysidopsis bahia</i>)	OPPTS 850.1350 (flow-through)	35-d NOEC _{repro} = 0.25 35-d NOEC _{surv} = 0.55 35-d NOEC _{growth} = 0.25	Good

Note: information taken from OECD (2000) except for those marked "a" which are from Boudreau et al (2003a)

4.1.1.3 Aquatic plants

Toxicity data for aquatic plants are presented in Table 4.5. As for invertebrates, these are largely from OECD (2002), with some additional results from Boudreau et al (2003a).

Table 4.5 Toxicity to aquatic plants

Species	Protocol	Result (mg/l)	Study standard
Freshwater			
<i>Selenastrum capricornutum</i> (algae)	OECD 201, OPPTS 850.5400 & ASTM 1218-90E (static)	96-h EC ₅₀ = 71 (cell density) 96-h EC ₅₀ = 126 (growth rate) 96-h NOEC = 44 (cell density, growth rate)	Good
	OECD 201, US EAP 600/9-78-018 & ASTM-E-35.23 (static)	96-h EC ₅₀ = 82 (cell density) 96-h EC ₁₀ = 10 (cell density)	Acceptable
	ASTM	96-h IC ₅₀ = 48.2 (cell density)	Acceptable ^a
<i>Anabaena flos-aquae</i> (algae)	OPPTS 850.5400	96-h EC ₅₀ = 176 (growth rate) 96-h NOEC = 94 (growth rate)	Good
<i>Navicula pelliculosa</i> (algae)	OPPTS 850.5400	96-h EC ₅₀ = 305 (growth rate) 96-h NOEC = 206 (growth rate)	Good
<i>Chlorella vulgaris</i> (algae)	ASTM	96-h IC ₅₀ = 81.6 (cell density)	Acceptable ^a
<i>Lemna gibba</i> (duckweed)	ASTM	7-d IC ₅₀ = 31.1	Acceptable ^a
Seawater			
<i>Skeletonema costatum</i> (algae)	OPPTS 850.5400	96-h EC ₅₀ >3.2 (growth rate) 96-h NOEC >3.2 (growth rate)	Good

Note: information taken from OECD (2000) except for those marked "a" which are from Boudreau et al (2003a)

4.1.1.4 Microcosms

Two microcosm studies have been conducted with PFOS. In the first, indoor microcosms containing zooplankton were exposed to PFOS (potassium salt) for 35 days at three concentrations - 1, 10 and 30 mg/l. The endpoints used were zooplankton species and abundance. Phytoplankton were added as a supplementary food supply at intervals (Sanderson et al, 2002).

Concentrations in the microcosms were measured on days 1, 8 and 35 of the study. Little change in the concentrations was observed, the greatest percentage change being found at the lowest concentration with a 19% reduction over the 35 days. The exposures showed a significant influence of PFOS on the zooplankton community at 10 mg/l after 14 days, with several species markedly reduced or eliminated. The relative susceptibility, assuming all effects due to PFOS exposure, was Copepoda > Cladocera > Rotifera. The statistical power of the study was insufficient to allow a conclusion of no effect at 1 mg/l to be reached.

In the second microcosm study, the effects of PFOS were studied on the zooplankton community and on the floating macrophyte *Lemna gibba* in outdoor microcosms (Boudreau et al, 2003b). The studies were run in triplicate, with PFOS concentrations of 0.3, 3, 10 and 30 mg/l. The potassium salt was used. Concentrations were monitored through the study, and no significant decrease in concentration was noted. The results were based on the nominal concentrations. The zooplankton community was significantly altered from that in the controls at 10 mg/l and 30 mg/l. The NOEC for community effects was 3 mg/l for days 4-28, and 0.3 mg/l for day 35. For *Lemna*, growth was only significantly reduced at 30 mg/l.

4.1.2 Sediment toxicity

No test results on sediment-dwelling organisms have been located.

4.1.3 Wastewater treatment plant (WWTP) micro-organisms

Only one study on activated sludge micro-organisms was considered valid in the OECD assessment. In a 3 hour respiration inhibition test (OECD 209), the highest exposure concentration of 905 mg/l (nominal) resulted in 39% inhibition. The IC₅₀ is therefore >905 mg/l.

4.1.4 Predicted No Effect Concentrations (PNECs) for the aquatic compartment

4.1.4.1 Calculation of a PNEC for surface water

The lowest acute and chronic aquatic toxicity studies from the discussion above are summarised briefly in Table 4.6 The table includes freshwater and marine species.

Table 4.6 Summary of aquatic toxicity data

Acute	Fish	Fathead minnow (<i>Pimephales promelas</i>) (96-h): LC ₅₀ = 4.7 mg/L Rainbow trout (<i>Oncorhynchus mykiss</i> - saltwater) (96-h): LC ₅₀ =13.7 mg/l
	Invertebrates	<i>Daphnia magna</i> (48-h): EC ₅₀ = 27 mg/L Mysid shrimp (<i>Mysidopsis bahia</i> - saltwater) (96-h): LC ₅₀ = 3.6 mg/L
	Algae	<i>Selenastrum capricornutum</i> (96-h): EC ₅₀ =126 mg/l <i>Skeletonema costatum</i> (saltwater) (96-h): EC ₅₀ > 3.2 mg/L
Long-term	Fish	Fathead minnow (<i>Pimephales promelas</i>) (42-day): NOEC _{survival} = 0.3 mg/L
	Invertebrates	<i>Daphnia magna</i> (28-day): NOEC _{reproduction} = 7 mg/L Mysid shrimp (<i>Mysidopsis bahia</i> - saltwater) (35-day): NOEC _{reproduction} = 0.25 mg/L
	Algae	<i>Selenastrum capricornutum</i> (96-h): NOEC=44 mg/l. <i>Skeletonema costatum</i> (saltwater) (96-h): NOEC>3.2 mg/l Duckweed (<i>Lemna gibba</i>) (7-day): NOEC = 15.1 mg/L

Acute toxicity data are available for fish, invertebrates and algae in freshwater and in seawater. The values for freshwater and saltwater fish are close together, those from invertebrates have a greater difference with the salt water value lower. The algal values cannot be compared as the lower, marine, value is a limit value with no effect at the highest concentration achievable in the test medium.

Results from long term tests with species in three taxonomic groups (fish, invertebrates, algae) are available for the freshwater environment. There are also long term results with a salt water invertebrate and a salt water algae (again a limit value). The results of the microcosm tests are similar to the lowest NOEC values obtained in single species tests.

The lowest NOEC from the whole data set is 0.25 mg/l, for Mysid shrimp. This will be used for the PNEC derivation. The next lowest value is 0.3 mg/l, for fathead minnow (and for part of a microcosm test) so a PNEC based on freshwater data alone would be similar. As three taxonomic groups are represented a factor of 10 is used, giving a PNEC for freshwater of 25 µg/l.

For the marine environment, there are three taxonomic groups represented. Although there are saltwater species for two of these, there are no additional marine taxonomic groups in the data set. The TGD indicates a factor of 100 in this situation, giving a PNEC of 2.5 µg/l.

There are some intermittent release scenarios. For these, the PNEC is based on the acute data. The lowest acute value is 3.6 mg/l, again for Mysid shrimp (the saltwater algal value is lower, at 3.2 mg/l, but this is a limit value and no effects were seen at this level). A factor of 100 is used, giving a PNEC of 36 µg/l.

4.1.4.2 Sediment PNEC

In the absence of any toxicity data for sediment organisms, the usual approach would be to use the equilibrium partitioning method. The use of this method may be questioned for substances with the properties of PFOS. However, the log K_{ow} value does not have to be used as a measured value for K_d with sediment is available (8.7 l/kg, see Section 3.2.5.1). Using this gives a freshwater sediment PNEC of 67 µg/kg ww and a marine sediment PNEC of 6.7 µg/kg ww.

4.1.4.3 Calculation of PNEC for WWTP micro-organisms

The IC₅₀ for activated sludge respiration inhibition was determined as >905 mg/l (Section 4.1.3). The appropriate assessment factor for this test is 100, giving a PNEC of >9.05 mg/l.

4.2 TERRESTRIAL COMPARTMENT

4.2.1 Terrestrial toxicity data

No terrestrial toxicity data were available at the time the OECD assessment was produced. A study on earthworms was referred to in the assessment as being planned; this is included in the 3M revised assessment (3M, 2003). The OECD assessment also mentions a planned study on plant toxicity, but this has not been seen.

A 14-day earthworm toxicity test is reported by 3M (2003). A robust study summary for this test is included in the 3M assessment, and only the main points relating to the test are included here. The test protocol was based on OECD Guideline 207. The test was conducted in an artificial soil substrate, at five exposure concentrations (ranging from 78 - 1250 mg/kg nominal) plus control. Concentrations of PFOS in the soil were measured on days 0 and 14, and were found to be within 80% of the nominal concentrations. The results are based on the concentrations at day 0. The contents of the test chambers were removed at 7 days for observations and returned to the chambers without the addition of further substance. The endpoints observed were mortality, burrowing behaviour, body weight and clinical signs of toxicity. The results of the test are in Table 4.7.

Table 4.7 Toxicity results for earthworms (3M, 2003)

Endpoint	Time	Value (mg/kg dwt)	95% CI
LC ₅₀	7 days	398	289 - 488
	14 days	373	316 - 440
NOEC	7 days	289	
	14 days	77	

The toxicity of PFOS to plants through soil exposure has been investigated using a method based on the OECD 21-day test guideline (3M, 2004). The endpoints examined were emergence, survival, shoot height and shoot weight. These endpoints were measured after 21 days of exposure. Where possible, the plants were allowed to continue growing after this time until they produced fruit or sufficient leaf for analysis. The results of these later analyses are included in Section 3.2.6.2.

The seven plant species included in the study were: lettuce (*Lactuca sativa*); ryegrass (*Lolium perenne*); tomato (*Lycopersicon esculentum*); onion (*Allium cepa*); alfalfa (*Medicago sativa*); flax (*Linum usitatissimum*); and soybean (*Glycine max*). The nominal concentrations used in the tests were 3.91, 15.6, 62.5, 250 and 1000 mg/kg. Concentrations measured in the soil at the beginning of the exposures were all within 70% of the nominal values. Concentrations were also measured at the final termination of the study after 205 days, when the concentrations ranged from 22.8% to 62.8% of nominal. Samples were taken at 21 days, but the results were considered to be artefacts due to the irrigation and sampling methods used. The results presented are based on the nominal concentrations. The lowest no effect concentrations for each endpoint were as follows:

Emergence	62.5 mg/kg	onion, ryegrass
Survival	15.6 mg/kg	onion, tomato
Shoot height	<3.91 mg/kg	lettuce (23% reduced compared to control)
Shoot weight	<3.91 mg/kg	lettuce (35% reduced compared to control)

4.2.2 Calculation of PNEC for the soil compartment

One short term test result is available for earthworms. A factor of 1000 is applied to the LC₅₀ from this test, 373 mg/kg dwt, giving a PNEC of 373 µg/kg dwt.

The plant test available can be considered to be a chronic study. As there is only one such study, an assessment factor of 100 is indicated according to the TGD. Effects were seen on lettuce at the lowest exposure concentration, so that the PNEC is <39 µg/kg wwt.

When terrestrial toxicity data are limited, the Technical Guidance document recommends that a PNEC be calculated from the aquatic PNEC using the equilibrium partitioning method. This method may not be appropriate for a substance with the properties of PFOS, but a measured K_d value is available for soil. A value of 26.9 is used in this evaluation (see Section 3.2.5.1), which corresponds to a K_{soil-water} of 40.6. Using this method gives a PNEC of 597 µg/kg wwt, or 680 µg/kg dwt.

The values obtained from the worm toxicity test and the partitioning method are similar, but the value from the plant test is much lower.. The value derived from the toxicity test for plants, <39 µg/kg wwt (or 46 µg/kg dwt based on 15% moisture in the soil) will be used in the evaluation, recognising that a PNEC derived from the actual effect level is expected to be below this.

4.3 ATMOSPHERIC COMPARTMENT

No data on effects of PFOS through exposure via the atmosphere were located, either for biotic or abiotic effects. The low volatility of the substance suggests that significant exposure through the vapour phase would not be expected.

4.4 NON-COMPARTMENT SPECIFIC EFFECTS RELEVANT TO THE FOOD CHAIN (SECONDARY POISONING)

4.4.1 Mammalian toxicity data

Mammalian toxicity data were reviewed for the OECD assessment (OECD, 2002). Results from acute, sub-chronic and chronic exposures to rats, sub-chronic exposures to monkeys, and a two-generation study on rats are available. Details of these studies are not included here, they can be found in the OECD assessment.

In a two year carcinogenicity assay using rats, effects on the liver were monitored. From this study the NOAEL for PFOS was considered to be 0.5 ppm in food in male rats and 2 ppm in food in female rats. The corresponding LOAELs were 2 ppm for males and 5 ppm for females. These values are taken from the main text of the OECD assessment. In the summary of the OECD assessment the LOAEL for male rats is said to be 0.5 ppm, with no NOAEL established. The main text indicates that the effects seen on male rats at 0.5 ppm were considered to be due to old age and were not treatment related. For comparison, a level in food of 0.5 ppm is equivalent to a dose of 0.025 mg/kg bw/day using the conversion factors in the Technical Guidance document. The range of doses estimated from the study was 0.015 to 0.057 mg/kg bw/day, so this fits into the observed range. The value required for the risk evaluation is the concentration in food.

Doses of 4.5 mg/kg bw/day were lethal to Rhesus monkeys over a seven week exposure.

In the two generation study on rats, the NOAEL for reductions in pup weights in the second generation was 0.1 mg/kg bw/day, with a LOAEL of 0.4 mg/kg bw/day.

Adult female Sprague-Dawley rats were injected intraperitoneally daily for 14 days (Austin et al, 2003). The doses were 1 and 10 mg/kg bw/day. Food intake and body weight were monitored daily. The oestrus cycle of the rats was also monitored. At the end of the exposures, the levels of leptin and corticosterone in the serum were measured, as were monoamines in the hypothalamus. PFOS levels were measured in a range of tissues at the end of the exposures.

PFOS was found in all of the tissues sampled after exposure, including blood, liver, serum, kidneys, heart, ovaries, adrenal, brain and spleen. Levels in liver were 26,627 ng/g at the low dose and 97,358 ng/g at the high dose. There were marked changes in body weight and food uptake at the higher dose. There was a significant decrease in serum leptin levels by the end of two weeks exposure at the higher dose, and also a significant increase in serum corticosterone levels. All of the animals in the control groups had regular oestrus cycles, compared to 66% in the lower dose group and 42% in the higher dose group.

4.4.2

Avian toxicity

Two studies on the acute toxicity of PFOS to birds are included in the OECD assessment (2002) and the 3M (2003) assessment. Both were 5-day studies, followed by 3 or 17 days observation. The concentration of the PFOS potassium salt was measured in the food. The LC₅₀ values derived from the studies were 628 mg/kg food for mallard duck (*Anas platyrhynchos*) and 220 mg/kg food for northern bobtail quail (*Colinus virginianus*).

The effects of PFOS administered in the diet on mallard ducks (*Anas platyrhynchos*) and northern bobwhite quail (*Colinus virginianus*) have been investigated in 21-week dietary reproduction studies (3M, 2004). Adult birds were brought into the reproductive phase at week 11 (mallard) or week 7 (quail). After hatching, the chicks were fed an untreated diet for 14 days. The exposure levels for both species were 0, 10, 50 and 150 ppm in food. In both cases, overt toxicity at the two highest dose levels led to early termination of these exposures.

For mallard, a NOAEC of 10 ppm was established for mortality, body weight, liver weight, and reproduction in adults, and for survivability, bodyweight and liver weight in offspring. Gross pathology in females also had a NOAEL of 10 ppm. In males, there was an increase in the incidence of small testis size and decreased spermatogenesis at 10 ppm. Although these effects are normal in the early post-reproductive phase, PFOS may have accelerated the process compared to untreated birds. Based on these effects the LOAEC was determined to be 10 ppm.

The results for northern bobwhite were similar. The NOAEC was 10 ppm for mortality, body weight, food consumption, liver weight (males) and gross pathology (females). Effects were seen on liver weight in females at 10 ppm, and similar effects on the testes and sperm in males as for mallard. Again, the exposure to PFOS appeared to accelerate the post-reproductive phase regression, and a LOAEC of 10 ppm was determined for this endpoint. A statistically significant reduction in offspring survival was seen at 10 ppm, together with slight, not statistically significant, reductions in fertility and hatchability. A LOAEC of 10 ppm was determined for offspring survival.

4.4.3

Derivation of PNEC_{oral}

The lowest no effect level is 0.5 ppm, for liver effects in male rats. This is from a chronic study, so an assessment factor of 30 is appropriate. This gives a PNEC of 0.0167 mg/kg in food.

In comments from the consultation on the draft evaluation report, it was suggested that the above may be an over-conservative choice of endpoint for the assessment of secondary poisoning. Alternatives proposed were the 2 ppm LOAEC from the same study, or the NOAEL of 0.1 mg/kg bw/day from the reproduction study. The 2 ppm level from the carcinogenicity study gives a PNEC of 0.067 mg/kg using the same assessment factor of 30 as above. For the NOAEL of 0.1 mg/kg bw/day, the conversion factor from dose to concentration in food is 20 from the Technical Guidance Document, so that the NOEC from this study is 2 mg/kg. With an assessment factor of 30 for a chronic study this also gives a PNEC of 0.067 mg/kg. This value will also be considered in the risk evaluation.

The chronic studies on birds show an NOAEC of 10 ppm for most of the endpoints, but a LOAEC for effects on males and on survival of offspring. A PNEC derived from this figure would be 0.33 mg/kg using an assessment factor of 30. Although the no effect level is not

fully defined, this result suggests that the PNECs derived from the mammalian data can be considered to cover the risk to birds as well.

4.5 CLASSIFICATION FOR ENVIRONMENTAL HAZARD

PFOS is not listed as a substance on EINECS and has no classification. The acid form of PFOS is not classified on Annex I of Directive 67/548/EEC, and neither are any of the usual salts (potassium, ammonium, lithium).

From Section 4.1.1, there are a number of species for which the L(E)C₅₀ values are in the range 1 – 10 mg/l for the potassium salt. This substance is not readily biodegradable. On the basis of this information the substance should be classified as dangerous to the environment, N R51-53 (toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment)

There are also invertebrate test results for the lithium salt, DEA salt and didecyldimethylammonium salt below 10 mg/l. Although these substances have not been specifically tested for biodegradation, on the basis of the evidence it is unlikely that the PFOS moiety will be degraded. Hence these should also be classified as N R51-53.

5 RISK EVALUATION

The exposure estimates in Section 3.3 considered seven possible scenarios for combinations of releases from different uses and with different degradation rates for PFOS-substances. The scenarios were:

- 1 - PFOS-salt releases only (all relevant uses in Table 3.8)
- 2 - PFOS-salt plus PFOS-substances, assuming instant degradation to PFOS (all relevant uses in Table 3.8).
- 3 - PFOS-salt plus PFOS-substances, assuming a 20 day half life for degradation to PFOS (all relevant uses in Table 3.8).
- 4 - PFOS-salt plus PFOS-substances, assuming a 1 year half life for degradation to PFOS (all relevant uses in Table 3.8).
- 5 - PFOS-salt, PFOS-substances with 20 day half life, PFOS-polymer with 30 year half life degradation to PFOS (all uses in Table 3.8).
- 6 - PFOS-salt, PFOS-substances with 20 day half life, PFOS-polymer with 30 year half life degradation to PFOS (uses in Table 3.8 excluding foam formulation, fabric treatment, paper treatment and coatings).
- 7 - PFOS-salt, PFOS-substances with 20 day half life degradation to PFOS (chromium plating, photographic, aviation, photolithography only)

As well as calculating the risk characterisation ratios, back-calculations have been performed to estimate the amount of PFOS which if released into waste water would result in a concentration equal to the PNEC value. The standard assumptions for water flows, dilutions, sludge applications etc have been used in the calculations. For the aquatic and terrestrial compartments, these calculations assume that there is no regional background contribution, this seems a reasonable assumption as the background concentrations calculated in this evaluation are low compared to the PNECs for these end points (see below). The same is not true for the secondary poisoning endpoints, where the regional background concentrations are significant and the exposure concentrations depend on both the local and the regional contributions. For these, a range of concentrations is presented. The upper value is the release which would lead to a risk with no regional contribution, the lower value would give a risk if the regional contribution were the same as the local contribution.

5.1 AQUATIC COMPARTMENT

5.1.1 Surface water and sediment

The risk characterisation is based on the predicted environmental concentrations from Section 3.3.2.1. The predicted no effect concentration (PNEC) is 25 µg/l, from Section 4.1.4.1.

Table 5.1 shows the risk characterisation ratios for freshwater for the seven scenarios and the various uses. As both the exposure and effect concentrations for sediment are derived by the equilibrium partition method, and the sorption coefficient is below the threshold for the extra factor of 10, the ratios for sediment are the same as for water, and are not presented separately.

Table 5.1 Risk characterisation ratios for freshwater

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.002	0.003	0.002	0.002	0.004	0.003	0.002
Photography - formulation	0.005	0.006	0.005	0.005	0.007	0.006	0.005
- processing	NA	0.003	NA	NA	NA	NA	NA
Aviation	0.004	0.005	0.004	0.004	0.006	0.005	0.004
Fire fighting foams - formulation	3.89	3.89	3.89	3.89	3.9	NA	NA
- use A	0.793	0.794	0.793	0.793	0.794	0.794	NA
- use B	1.14	1.14	1.14	1.14	1.14	1.14	NA
Photolithography	NA	0.039	NA	NA	NA	NA	NA
Fabrics - application	NA	0.021	NA	NA	NA	NA	NA
Paper treatment	NA	2.45	NA	NA	NA	NA	NA
Coatings	NA	0.084	NA	NA	NA	NA	NA
Regional	0.002	0.003	0.002	0.002	0.004	0.003	0.001

NA – local concentration not calculated for this use pattern in the particular scenario

Two of the use patterns have ratios above one for all scenarios. The calculation of emissions from the formulation of fire fighting foams uses a large site and default emission factors, and so could be refined. (The site in fact no longer uses PFOS-based chemicals in the production of foams.) Foam use B is the situation where spent foam is collected and discharged to a waste water treatment plant. This is probably only likely in an industrial setting. The other release pattern for foams, use A, is where the release is split 50:50 between surface water (no treatment) and soil, and this does not give a risk. The emission scenario for foam use in both cases considers an arbitrary release of 1% of the annual use in one fire, and the actual distribution of foam emissions between the different compartment will be different for every fire location. Hence the ratios for these two scenarios are only indicative. They do suggest that the release of foams from fires may have effects on aquatic organisms. The revised calculation for foam use based on different assumptions (in Appendix 4) shows no risks for direct effects on aquatic organisms.

One other use pattern gives a ratio above one – this is for paper treatment, and assumes the complete conversion of PFOS-substance to PFOS before release. As such it is likely that the PFOS concentration is over-estimated. Against this, there are no data on the toxicity of the PFOS-substances themselves.

The ratios for the regional aquatic environment are all well below one, the highest being 0.004. Hence the risk ratios for the use patterns are governed by the local emissions. The regional concentration in water does not vary greatly between the scenarios, a factor of three covering all of the results. The variation in degradation rate for the PFOS substances between the scenarios has little effect on the water concentration, and so it may not be necessary to have precise information about this rate. The yield of PFOS from the breakdown of the substances (and polymers) may have more influence on the concentrations – this is considered in Appendix 3.

The measured levels in surface water are below the PNEC value.

The PNEC for the aquatic compartment is derived from three long-term NOEC values, and so is not likely to be increased by further testing. The calculations of emissions, and hence the exposure estimates, could be revised.

The release to waste water calculated to give a risk for this endpoint is 0.69 kg/day.

5.1.2 Wastewater treatment plant (WWTP) micro-organisms

The estimated effluent concentrations are included in Section 3.3.2.1, with the highest value being 0.97 mg/l. The PNEC for micro-organisms is >9.05 mg/l (Section 4.1.4.3). Hence all ratios are below one, and no risks are indicated.

5.2 TERRESTRIAL COMPARTMENT

The risk characterisation is based on the predicted environmental concentrations from Section 3.3.3.1. The predicted no effect concentration (PNEC) is 25 µg/l, from Section 4.2.2.

Table 5.2 shows the risk characterisation ratios for the terrestrial compartment for the seven scenarios and the various uses.

Table 5.2 Risk characterisation ratios for terrestrial compartment

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.022	0.022	0.035	0.037	0.036	0.025	0.022
Photography - formulation - processing	0.274	0.274	.0287	0.290	.0287	0.277	0.274
	NA	0.001	NA	NA	NA	NA	NA
Aviation	0.181	0.181	0.195	0.196	0.195	0.184	0.182
Fire fighting foams - formulation - use A - use B	326	326	326	326	326	326	326
	<0.001	<0.001	0.013	0.015	0.014	0.003	NA
	137	137	138	138	138	137	NA
Photolithography	NA	3.03	NA	NA	NA	NA	NA
Fabrics - application	NA	1.53	NA	NA	NA	NA	NA
Paper treatment	NA	205	NA	NA	NA	NA	NA
Coatings	NA	5.1	NA	NA	NA	NA	NA
Regional	0.017	0.042	0.05	0.033	0.17	0.15	0.017

NA – local concentration not calculated for this use pattern in the particular scenario

The risk characterisation ratios for soil follow a similar pattern to those for the aquatic compartment. The formulation of fire-fighting foams gives ratios above one for all scenarios. As noted above, the emissions estimates use default emission factors and are based on a large site which no longer uses PFOS-based substances. The foam use pattern B also indicates a risk. This use pattern has release via a waste water treatment plant, and sludge from the plant after treatment of the foam is applied to soil. The calculation assumes that sludge is applied once a year, containing the same level of PFOS from the release. As the release is considered to be intermittent, it is unlikely that the same waste water treatment plant will receive the same foam release each year and the sludge be applied to the same soil. Hence this is not considered to be a realistic scenario. However, the soil concentration following a single application of sludge for this scenario would be enough to give a risk characterisation ratio of 36.

Risks are also indicated for the use areas of photolithography, fabrics, paper treatment and coatings in Scenario 2. In this scenario, instant transformation of PFOS-substance to PFOS is assumed. In addition, default emission factors are used for some of these areas (mainly paper treatment), so the emission estimates for these use patterns could be revised.

The highest ratio for the regional environment is 0.17, so there are no risks at this scale. Note that the concentrations used for this risk characterisation are the regional concentrations in agricultural soil, not the natural soil values included in Section 3.3.3.1. The regional soil concentrations are affected much more by the differences between the scenarios, the natural soil concentrations varying over two orders of magnitude. The risk characterisation ratios

show the influence of the release from polymers, with the two highest ratios being those for Scenarios 5 and 6 which include the polymer emissions.

The PNEC for the terrestrial compartment is derived from one long-term toxicity test result and so could be refined. As noted above the emission estimates could also be revised.

The calculated emission to waste water which would give rise to a risk for the terrestrial compartment is 7.4 g/day. This is for applications of sludge over 10 years, the emission which would give rise to a risk following a single application is 0.67 kg/day.

5.3 ATMOSPHERIC COMPARTMENT

No data on effects on organisms from exposure through the air were located. The estimated concentrations are very low, so PFOS is not expected to contribute significantly to abiotic effects in the atmosphere. No assessment is carried out.

5.4 NON-COMPARTMENT SPECIFIC EFFECTS RELEVANT TO THE FOOD CHAIN (SECONDARY POISONING)

5.4.1 Freshwater food chain

The concentrations for exposure through the freshwater food chain are in Section 3.3.5.1. The PNEC for secondary poisoning is 0.0167 mg/kg ww in food, from Section 4.4.3.

The risk characterisation ratios for the aquatic food chain are presented in Table 5.3.

Table 5.3 Risk characterisation ratios for freshwater food chain exposure

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	13	25.7	16.1	15.1	29.6	26.8	12.5
Photography - formulation - processing	24.3	36.3	27.4	26.4	40.8	38.1	23.7
	NA	25	NA	NA	NA	NA	NA
Aviation	21.7	32.4	24.8	23.8	38.3	35.5	21.2
Fire fighting foams - formulation - use A - use B	13400	13400	13400	13400	13400	NA	NA
	26.1	38.1	29.2	28.2	42.7	39.9	NA
	31.9	43.9	35	34	48.4	45.7	NA
Photolithography	NA	174	NA	NA	NA	NA	NA
Fabrics - application	NA	72.3	NA	NA	NA	NA	NA
Paper treatment	NA	8470	NA	NA	NA	NA	NA
Coatings	NA	235	NA	NA	NA	NA	NA
Regional	12.8	24.6	15.9	14.9	29.1	26.3	12.2

NA – local concentration not calculated for this use pattern in the particular scenario

For the freshwater food chain all of the use areas indicate a risk for all of the scenarios for which they are relevant. Consumption of prey exposed only to the regional background concentrations is also indicated as a risk for all scenarios. As noted above the regional water concentrations are only affected a little by the different release patterns in the scenarios. However, in most cases the specific uses also make a significant contribution to the exposure through this route, similar to or greater than that from the regional concentration. The exceptions to this are chromium plating and the processing life stage for photography.

None of the emission estimates are based on specific information about the releases of PFOS from the specific industry area. Hence they could all be refined. Considering only the

possible continuing uses (those included in Scenario 7), for chrome plating the local calculation is based on a scenario for the industry, but the overall emissions assume complete release of the amount used each year in the absence of information on its fate. Assumptions have been made on the nature of the substances released in the production of film and its developing. Data on the releases from photolithography come from the industry, but there may be more specific treatment of waste waters than assumed here. The aviation emissions are based on an emission scenario for hydraulic fluids, but do not relate specifically to the aviation industry.

The PNEC value is based on the results of a 2-year study with rats, so it is unlikely that the PNEC would be changed significantly in the light of further tests. An alternative PNEC of 0.067 mg/kg was also derived in Section 4.4.3. If this PNEC were used, all of the ratios would still be above one.

A comparison of the PNEC value with measured concentrations for freshwater fish shows that the highest measured concentrations reported exceed the PNEC of 0.0167 mg/kg. A number of values also exceed the alternative PNEC value of 0.067 mg/kg. It should be noted that this comparison is based on the highest values. In some cases these relate to specific tissues rather than whole body levels. Also, from the global archive sample analysis, PFOS was only detected in 38% of the samples. Nevertheless, measurements such as those in the Scheldt Estuary may indicate the presence of PFOS in aquatic biota at levels expected to have effects.

The emissions to waste water giving rise to a risk for this food chain are estimated to be 83 – 166 mg/day. Assuming a similar pattern of releases to air, water and soil, the regional emissions would need to be reduced by a factor of over twelve times (for the alternative PNEC, by three times) to remove the risk at the regional level.

5.4.2 Terrestrial food chain

The concentrations for exposure through the terrestrial food chain are in Section 3.3.5.1. The PNEC for secondary poisoning is 0.0167 mg/kg ww in food, from Section 4.4.3.

The risk characterisation ratios for the terrestrial food chain are presented in Table 5.4.

Table 5.4 Risk characterisation ratios for exposure through the terrestrial food chain

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.117	0.168	0.228	0.204	0.599	0.493	0.119
Photography - formulation - processing	0.816	0.867	0.927	0.903	1.3	1.19	0.818
	NA	0.11	NA	NA	NA	NA	NA
Aviation	0.558	0.609	0.67	0.646	1.04	0.934	0.561
Fire fighting foams - formulation	9.03	9.03	9.03	9.03	9.04	NA	NA
- use A	0.056	0.108	0.168	0.144	0.539	0.433	NA
- use B	381	381	382	381	382	382	NA
Photolithography	NA	8.47	NA	NA	NA	NA	NA
Fabrics - application	NA	4.36	NA	NA	NA	NA	NA
Paper treatment	NA	569	NA	NA	NA	NA	NA
Coatings	NA	14.3	NA	NA	NA	NA	NA
Regional	0.11	0.21	0.26	0.20	0.99	0.84	0.12

NA – local concentration not calculated for this use pattern in the particular scenario

Most use patterns indicate a risk in one or more of the scenarios for the terrestrial food chain. Exceptions are chromium plating, and the fire fighting foams use pattern Use A. No risks

through regional exposure are indicated, although one scenario has a ratio just below one. This is the scenario involving the greatest uncertainty, as it includes releases from the breakdown of polymeric material, about which there is no specific information. The scenario assumes eventual breakdown of polymer to PFOS in the environment, which is a worst case assumption; it is therefore considered unlikely that this scenario will actually lead to a risk. The comments on these uses in the section above on the freshwater food chain are relevant here, as the route to the terrestrial environment is through sludge application and hence depends on the emissions to water treatment. As above, the PNEC is unlikely to be changed by new tests. Using the alternative PNEC value of 0.067 mg/kg, photography and aviation would not show a risk.

The emissions to waste water giving rise to a risk for the terrestrial food chain are estimated to be 1.65 – 3.30 g/day. These are for 10 years of application; the emissions giving a risk from a single application of sludge are estimated to be 15 – 30 g/day.

5.5 MARINE ENVIRONMENT

5.5.1 PBT assessment

The PBT assessment included here relates to PFOS itself, and uses data from tests on PFOS salts. It does not apply directly to PFOS-substances. However, the potential for the substances to degrade to PFOS in the environment makes this assessment relevant to the general consideration of the group of substances.

5.5.1.1 Persistence

PFOS (as the potassium salt) has been tested for biodegradability in a series of tests commissioned by 3M and reported in the risk assessment of PFOS (3M, 2003). The following tests were conducted:

- activated sludge;
- acclimated activated sludge (including added soil and sediment materials) in both aerobic and closed vial exposures;
- aerobic soil and sediment cultures;
- anaerobic sludge from sludge digester; and
- pure microbial cultures.

None of these studies showed any evidence for the biodegradation of PFOS. 3M also reported the results of standard studies on hydrolysis (at 50°C and a range of pHs) and photolysis. Neither study showed any evidence for degradation of PFOS. The OECD hazard assessment (OECD, 2002) includes the results of a MITI-I study (ready biodegradability) which showed no evidence for ultimate or primary degradation (removal of the parent compound). The conclusion is that PFOS meets the screening criteria for P (Persistent) or vP⁹ (very Persistent) criteria.

5.5.1.2 Bioaccumulation

There are a limited number of studies available on bioaccumulation of PFOS. A flow-through study on bluegill sunfish (*Lepomis macrochirus*) is cited in both 3M and the OECD

⁹ Classification as a vP is on the basis that no degradation has been observed in any study to date, which makes it likely that the substance would meet this criterion.

assessment. The bioconcentration factors for edible tissues, non-edible tissues and whole fish were calculated from the rates of uptake and depuration because steady state had not been reached after 56 days of exposure.¹⁰ The values obtained were 1124 (edible), 4103 (non-edible) and 2796 (whole fish). The exposure concentration was 0.086 mg/l.

A flow-through study on carp (*Cyprinus carpio*) resulted in lower values of 720 at 20 µg/l exposure and 200-1500 at 2 µg/l exposure. Higher values of 6,300 - 125,000 have been reported (for bioaccumulation factors) for in situ measurements at the scene of a spill of fire fighting foam, but these were considered to be due to the uptake of derivatives which were then metabolised to PFOS, hence the values were over-estimated.

In summary, BCF values up to 2800 have been measured in laboratory studies, and this meets the B or 'Bioaccumulative' criterion.

The occurrence in a range of biota supports this; PFOS has been found in a wide range of higher organisms in Europe, including seals, dolphins, whales, cormorants, eagles, swordfish, tuna and salmon. The Global Biophase Monitoring Programme found PFOS in livers, blood and other tissues of animals, especially in fish-eating animals.

5.5.1.3 Toxicity

According to the assessment criteria laid out in the EU TGD, toxicity criteria is based on either aquatic toxicity or on classification.

The lowest aquatic NOEC value (from the OECD and 3M reports) is 0.25 mg/l, which is above the criterion level of 0.01 mg/l for the T criterion. On this basis, PFOS does not meet the T criterion. The NOAEC from chronic bird studies is 10 ppm for most endpoints, which is below the criterion value of <30 mg/kg in food. The level of 10 ppm was also considered a LOAEC for some effects in the same studies. Hence PFOS meets the T criterion on this basis.

PFOS is not listed as a substance on EINECS and has no classification. The acid form of PFOS is not classified on Annex I of Directive 67/548/EEC, and neither are any of the usual salts (potassium, ammonium, lithium). The mammalian toxicity data included in the OECD and 3M assessments has thus been used to consider what classification would be appropriate for PFOS.

Various toxicity studies have been conducted (as highlighted in earlier sections) to determine the toxicity of PFOS related substances, with the tests conducted in:

- a 90-day repeat dose toxicity study on rats showing that all rats died when fed on diets containing 300ppm PFOS and above (equivalent to 18 mg/kg bw/day and above). Some deaths (5 out of 10 animals) were also noted in rats fed diets containing 100 ppm PFOS (6 mg/kg bw/day)¹¹. All rats receiving diets containing 30 ppm PFOS

¹⁰ The robust summary in the OECD hazard assessment has different values to those used in the main OECD text (which are those cited here). The 3M (2003) report explains that the original study used an inappropriate method to estimate the kinetic BCF values, and that those were revised in a later amended study report. This is assumed to explain the different values in the OECD robust summary, as the BCF values in the main report and the 3M report agree.

¹¹ The criteria for the classification of a substance as 'Toxic' with a risk phrase R48 are based on observations of serious damage to health at concentrations ≤5.0 mg/kg bw/day in a 90-day study, while the classification of a substance as 'Harmful' with a risk phrase R48 is based on observations of serious damage to health at concentrations of the order of ≤50 mg/kg bw/day in a 90-day study.

(2.0 mg/kg/day) survived until the end of the study, but small changes in body and organ weights were reported. The effects seen in rats receiving 6.0 mg/kg/day suggest that PFOS fulfils the criteria for classification as Toxic, with the risk phrase R48, and hence meets the PBT criteria for T;

- a two year carcinogenicity study showing significant increases in hepatocellular adenomas observed in both male and female rats at 1 mg/kg bw/day. In view of the lack of effects in a number of genotoxicity test systems, the 3M report concluded that the carcinogenic effect was due to a threshold mediated non-genotoxic mechanism. This test could result in a Category 3 classification for carcinogenicity or could even provide insufficient evidence to warrant classification as a carcinogen, and thus may not fulfill the PBT criteria for T;
- a two generation rat study with PFOS showing significant reductions in the viability of pups in the F1 generation at exposure levels of 1.6 and 3.2 mg/kg bw/day. A subsequent study determined a NOAEL for pup mortality and growth of 1.2 mg/kg bw/day. No effects on mortality were observed over the whole study at 0.4 mg/kg bw/day. This may not fulfill the criteria for classification as “Toxic for Reproduction”, and hence not meet the T criterion for PBT. The view of Sweden in their comments on the draft RER is that the data fulfill the criteria for reproductive toxicity as category 3, and possibly also for category 2. Comments from the Norwegian Institute of Public Health also support classification as a reproductive toxicant category 3;
- developmental and reproductive toxicity studies on rabbits showing effects on the development of the foetus at doses of 5 and 10 mg/kg bw/day. These are largely maturational delays and reduced foetal body weight. A NOAEL of 1 mg/kg bw/day has been determined for most of these effects in rats. Signs of maternal toxicity are also observed at similar levels, and in some cases the NOAEL for maternal effects is lower than that for developmental effects. The 3M RAR concluded that there was no indication of specific teratogenic effects. These may not meet the requirements for classification as “Toxic for Reproduction” and hence not meet the T criterion for PBT; and
- tests on rhesus monkeys showing that all animals died at 10 mg/kg bw/day, the lowest dose tested, with a follow up study showing deaths at 4.5 mg/kg bw/day. There were no deaths at 1.5 mg/kg bw/day, but there were signs of gastrointestinal toxicity. The results of this test show that PFOS fulfils the criteria for classification as Toxic, with the risk phrase R48, and hence meets the T criterion for PBT.

PFOS has been shown to cause death in both rats and monkeys at doses of 6.0 and 4.5 mg/kg/day respectively in repeat-dose 90-day toxicity studies, although significant signs of toxicity were not seen in groups of rats or monkeys receiving lower doses of PFOS. Despite this apparent steep dose-response relationship in its toxicity, the severity of the effects seen at doses around 5.0 mg/kg/day warrant classification as “Toxic” and assigned the Risk Phrase R48.

It is therefore concluded that PFOS should be classified as ‘Toxic’ and carry the Risk Phrase R48. There is also support for classification as toxic for reproduction, category 3. It therefore meets the T or ‘Toxicity’ criterion on this basis, as well as on the basis of the bird feeding studies.

5.5.1.4 PBT conclusion

PFOS meets the vP, B and T criteria and hence is considered as a PBT substance. It should be noted that this conclusion does not apply directly to PFOS-substances, but the potential for the substances to be degraded to PFOS means that it is of relevance in the consideration of the substances.

5.5.2 Marine risk evaluation

As PFOS meets the PBT criteria, there is strictly no requirement for a risk evaluation. However the relevant results are generated as part of the modelling and so are included here.

5.5.2.1 Marine water

The concentrations of PFOS in marine waters have been calculated as part of the modelling described in Section 3. The values are presented in Table 5.5.

The risk characterisation ratios for the marine aquatic compartment are presented in Table 5.6. The PNEC is 2.5µg/l. As for freshwater, the exposure and effect concentrations are derived from the aquatic values by equilibrium partitioning, and so the ratios are the same as those for the aquatic compartment.

Table 5.5 Predicted environmental concentrations in marine water

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	4.76x10 ⁻⁶	8.33x10 ⁻⁶	5.67x10 ⁻⁶	5.39x10 ⁻⁶	9.56x10 ⁻⁶	8.75x10 ⁻⁶	4.6x10 ⁻⁶
Photography - formulation - processing	1.52x10 ⁻⁵	1.88x10 ⁻⁵	1.61x10 ⁻⁵	1.58x10 ⁻⁵	2.0x10 ⁻⁵	1.92x10 ⁻⁵	1.51x10 ⁻⁵
	NA	7.47x10 ⁻⁶	NA	NA	NA	NA	NA
Aviation	1.14x10 ⁻⁵	1.49x10 ⁻⁵	1.23x10 ⁻⁵	1.2x10 ⁻⁵	1.62x10 ⁻⁵	1.53x10 ⁻⁵	1.12x10 ⁻⁵
Fire fighting foams - formulation - Use A - Use B	0.0135	0.0135	0.0135	0.135	0.135	NA	NA
	2.85x10 ⁻³	2.86x10 ⁻³	2.85x10 ⁻³	2.85x10 ⁻³	2.86x10 ⁻³	2.86x10 ⁻³	NA
	5.7x10 ⁻³	5.71x10 ⁻³	5.7x10 ⁻³	5.7x10 ⁻³	5.71x10 ⁻³	5.71x10 ⁻³	NA
Photolithography	NA	1.32x10 ⁻⁴	NA	NA	NA	NA	NA
Fabrics - application	NA	7.09x10 ⁻⁵	NA	NA	NA	NA	NA
Paper treatment	NA	8.51x10 ⁻³	NA	NA	NA	NA	NA
Coatings	NA	2.19x10 ⁻⁴	NA	NA	NA	NA	NA
Regional	3.86x10 ⁻⁶	7.43x10 ⁻⁶	4.77x10 ⁻⁶	4.51x10 ⁻⁶	8.68x10 ⁻⁶	7.87x10 ⁻⁶	3.71x10 ⁻⁶

NA – local concentration not calculated for this use pattern in the particular scenario

The pattern of the risk characterisation ratios for the marine aquatic compartment is similar to that for freshwater (Section 5.1.1) and the same comments and conclusions apply. In addition to the freshwater areas indicating risks, the other use of foam (Use A) also shows a risk for the marine compartment. This use is also discussed in Section 5.1.1. The scenarios for the use of foams could be considered to be relevant for the offshore use of foams in the first instance.

The emission to waste water calculated to give rise to a risk for the marine aquatic compartment is 0.5 kg/day.

Table 5.6 Risk characterisation ratios for the marine aquatic compartment

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	0.002	0.003	0.002	0.002	0.004	0.004	0.002
Photography - formulation	0.006	0.008	0.006	0.006	0.008	0.008	0.006
- processing	NA	0.003	NA	NA	NA	NA	NA
Aviation	0.005	0.006	0.005	0.005	0.006	0.006	0.004
Fire fighting foams - formulation	5.4	5.4	5.4	5.4	5.4	NA	NA
- Use A	1.14	1.14	1.14	1.14	1.14	1.14	NA
- Use B	2.28	2.28	2.28	2.28	2.28	2.28	NA
Photolithography	NA	0.053	NA	NA	NA	NA	NA
Fabrics - application	NA	0.028	NA	NA	NA	NA	NA
Paper treatment	NA	3.4	NA	NA	NA	NA	NA
Coatings	NA	0.088	NA	NA	NA	NA	NA
Regional	0.002	0.003	0.002	0.002	0.004	0.003	0.002

NA – local concentration not calculated for this use pattern in the particular scenario

5.5.2.2 Secondary poisoning in the marine environment

There are two calculations for secondary poisoning in the marine environment, to include two levels of predator. These are a fish-eating bird or mammal, similar to that in the freshwater environment, and a top predator. The concentrations in the food organisms for these endpoints were included in Section 3.3.5.1. The risk characterisation ratios for these two are presented in Tables 5.7 and 5.8.

Table 5.7 Risk characterisation ratios for fish-eating bird or mammal (marine)

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	1.3	2.59	1.6	1.51	2.91	2.63	1.24
Photography - formulation	2.86	4.06	3.17	3.07	4.47	4.2	2.81
- processing	NA	2.5	NA	NA	NA	NA	NA
Aviation	2.5	3.53	2.81	2.71	4.11	3.84	2.45
Fire fighting foams - formulation	1860	1860	1860	1860	1860	NA	NA
- Use A	2.61	3.8	2.91	2.82	4.21	3.94	NA
- Use B	3.92	5.11	4.22	4.13	5.52	5.25	NA
Photolithography	NA	23.2	NA	NA	NA	NA	NA
Fabrics - application	NA	9.08	NA	NA	NA	NA	NA
Paper treatment	NA	1170	NA	NA	NA	NA	NA
Coatings	NA	31.7	NA	NA	NA	NA	NA
Regional	1.26	2.46	1.56	1.50	2.87	2.57	1.20

NA – local concentration not calculated for this use pattern in the particular scenario

Table 5.8 Risk characterisation ratios for marine top predator

Use area	Scenario number						
	1	2	3	4	5	6	7
Chromium plating	2.59	5.03	3.2	3.01	5.81	5.27	2.48
Photography - formulation - processing	3.22	5.61	3.83	3.64	6.44	5.89	3.11
	NA	4.99	NA	NA	NA	NA	NA
Aviation	3.08	5.4	3.68	3.5	6.29	5.75	2.97
Fire fighting foams - formulation - Use A - Use B	747	749	748	747	750	NA	NA
	3.12	5.51	3.72	3.54	6.33	5.79	NA
	3.64	6.03	4.25	4.06	6.86	6.31	NA
Photolithography	NA	13.3	NA	NA	NA	NA	NA
Fabrics - application	NA	7.61	NA	NA	NA	NA	NA
Paper treatment	NA	474	NA	NA	NA	NA	NA
Coatings	NA	16.6	NA	NA	NA	NA	NA
Regional	2.57	4.91	3.11	2.99	5.75	5.15	2.46

NA – local concentration not calculated for this use pattern in the particular scenario

The ratios for the marine food chain exposures are lower than those from the freshwater food chain, but are all above one. Risks are also indicated at the regional level for all scenarios. The same comments as for the freshwater food chain (Section 5.4) are relevant here. Using the alternative PNEC, most of the use areas would have a ratio above one for at least one of the scenarios, although there would be no ratios above one for the marine food chains for Scenario 7.

The emissions to waste water estimated to give rise to a risk for these endpoints are 0.6 – 1.2 g/d for the fish-eating predator, and 0.3 – 3.0 g/day for the marine predator.

5.6 UNCERTAINTIES

It is to be expected in a risk evaluation that there will be some areas of uncertainty due to a lack of sufficiently detailed information in some areas. In the case of PFOS, the nature of the substance and the use of a large number of derivatives or related substances add further to the usual types of uncertainty. These areas have been indicated in the evaluation as they have arisen, but are discussed again here, with in some cases additional consideration of the effect of the uncertainty on the outcome of the evaluation.

5.6.1 PFOS-salt properties

The unusual properties of PFOS mean that the usual approach of estimating a number of properties from the log Kow value cannot be taken here. To circumvent this, the values for properties such as soil-water partition have been taken as far as possible from direct measurements. It is recognised that these properties are likely to depend to some extent on the characteristics of the environment. There is no information on this dependence, and therefore no calculations have been carried out to examine this variation. As the values used come from measurements on particular soils, sediment or sludge then they can be considered to relate at least to these materials. The standard approach assumes a fixed composition for these materials, and so in that sense the current approach is not very different.

The effect of considering the range of solubility values determined for PFOS salts, and the uncertainty in the air-water partition coefficient, have been considered in Appendix 8. These changes have no impact on the levels or distribution calculated in the model.

For one specific use area, hydraulic fluids in aircraft, the substance used is not PFOS but a related substance. This evaluation has assumed that the properties of this substance are similar to those of PFOS, which is a further area of uncertainty with regard to this use.

5.6.2 PFOS releases

These comments relate to the releases of PFOS-substance as well as PFOS-salt. The emission estimates made in the risk evaluation are based on the most specific information available. There are no specific measurements of the releases of PFOS at specific locations and relating to particular processes. Information on the amounts used in each area is available, and in some cases there are estimates of the total amounts released on an EU basis. Where possible, information has been taken from emission scenarios related to the particular use area, although it is not possible in all cases to find information related to the specific use of the PFOS-related substance. There is only one area in which the Technical Guidance Document default values have been used, and that is for fire-fighting foams. Here the formulation step is based on defaults. The nature of the use of such foams means that complete release to the environment is possible. Alternative estimates for the amount used at a single incident have been made in Appendix 4. For other areas, it is recognised that better information could allow the assessment of emission (and hence the calculation of environmental concentrations) to be refined. No extra calculations have been performed in the absence of more specific information, with the exception of photolithography, where the cessation of use for a specific purpose has been considered (Appendix 3).

5.6.3 PFOS-substance properties

The property values used in the evaluation are those for N-EtFOSE. There are a large number of substances which come under this grouping, and clearly some will have properties which differ from those of the chosen substance. However, it is felt that having selected one substance to represent the group it is better to use data only for this substance rather than vary the values to try to cover the possible range within the group. There is one exception to this, which is the degradation of the substances, and this is considered below.

5.6.4 PFOS-substance degradation

This is one of the key areas of uncertainty in the evaluation. Information on the breakdown of PFOS-substances was discussed in Sections 3.2.2.2.1 and 3.3.1.1.2. The data available are limited, but they do appear to show that N-EtFOSE can be broken down by aerobic biodegradation in a series of steps, the eventual products from which appear to be PFOS and PFOA. At least one estimate of the yield of PFOS from this process has been presented in the literature, but both the yields and the rates of the steps are uncertain.

The main calculations in the evaluation include scenarios in which the PFOS-substances are degraded to PFOS on or before release, and scenarios where the half life for degradation to PFOS is 20.5 days or 1 year. Comments from the consultation argued that the overall rate may be much longer than this. Appendix 5 considers a longer half life of 10 years as well as the two shorter half lives. These calculations show a reduction in the amount degraded in the region at steady state as the half lives in water and soil increase. It should be noted that most of the PFOS-substance released in the model is removed from the regional model and the continental mode by water flow and air movement before it can be degraded. This has the potential to degrade in more remote areas, and so contribute to the overall global burden. The longer the half lives used for the regional model, the greater the proportion of releases which are moved to more remote areas. It should also be borne in mind that the biodegradation half

lives used here are for the overall process, from PFOS-substance to PFOS. There will be intermediate products along this process, some of which may have significant lifetimes in the environment. As such they may behave differently to the example PFOS-substance used in the calculations. There is at present no way to address this aspect.

The second aspect is the degree to which the degradation of PFOS-substances leads to PFOS itself. The effect of a lower yield of PFOS from this degradation is investigated in Appendix 3, using the scenario for photolithography in isolation. Where the assumptions relate to the fraction of PFOS-substance released as PFOS from waste water treatment, the effect on concentration is essentially linear. Hence the assumption of a 20% yield leads to local concentrations which are 20% of those when complete conversion to PFOS is assumed.

The calculations on the behaviour of PFOS-substance show that degradation in air is also important in terms of the production of PFOS from PFOS-substance. The value for the half life of this process is calculated from the structure of the PFOS-substance, and strictly relates to the initial reaction of the substance with hydroxyl radicals, rather than the formation of PFOS. There is no real information on the products of the reactions of PFOS-substances in air. Appendix 5 considers the effect of a longer overall lifetime for degradation in air, but with the process leading to PFOS. The calculations are performed on the emissions estimated for Scenario 7. The appendix also includes the situation where the degradation does not lead to PFOS.

The effect of a longer half life is a reduced contribution to the overall production of PFOS from the air compartment. The concentration of PFOS calculated for a scenario will also be reduced where the degradation in air made a significant contribution to the inputs of PFOS. For the Scenario 7 emissions (considering the PFOS-substance emissions only), the regional concentration in surface water is reduced by ~4 times when the half life in air is increased by two orders of magnitude. The same effect would be seen if only a proportion of the degradation of PFOS-substance in air lead to PFOS.

The key areas which affect the predicted concentrations appear to be: the degree to which PFOS-salt is produced from PFOS-substances in waste water treatment, and to a lesser extent the degree to which this occurs in the environment; and the production of PFOS-salts from PFOS-substances through degradation in air, and the rate of this process. Nevertheless, even when a number of less conservative assumptions are made, risks are still indicated from the releases of very low concentrations of PFOS-substances.

5.7 SUMMARY OF CONCLUSIONS

The major area of concern is for secondary poisoning, in particular for water. All of the use patterns considered in the evaluation lead to a risk for secondary poisoning in the relevant scenarios, for the freshwater, marine predator and marine top predator endpoints. This is true of the uses releasing only small amounts overall, such as photography and aviation, as well as for the uses with larger emissions. Calculations for each of the uses considered to be continuing, carried out individually and not shown in this evaluation, indicate that four (chromium plating, photography (formulation), aviation and photolithography) could lead to a risk based on the assumptions made in this evaluation (and assuming instant conversion of PFOS-substance to PFOS where relevant). The only use pattern not leading to a risk is the use in photography (developing). Risks are indicated at the regional level for secondary poisoning through the freshwater and marine food chains for all scenarios considered. Some measured values in aquatic biota are above the PNEC for secondary poisoning. For

freshwater, the regional emissions would need to be reduced to less than one twelfth of the estimated values in order to remove the risk at the regional level (assuming a similar distribution of emissions). In the case of the alternative PNEC, the reduction would need to be by over two-thirds.

The PNEC value for secondary is derived from a two-year feeding study, and is not likely to be changed by further tests. The alternative PNEC also comes from long term testing. The bioconcentration factor for fish comes from valid measurements, and PFOS has been measured in fish. The biomagnification factor used in the calculations, a value of two, is the default value from the Technical Guidance Document, and so could be revised through measurement, but reducing this to a factor of one would not remove the concerns in most cases. The main scope for revising the evaluation would appear to be through better estimates of emissions.

There are also indications of risk for the aquatic and terrestrial compartments for direct effects on organisms. These relate mostly to the fire-fighting foams, where releases on use may be sufficient to cause concern. These calculations are very dependent on the assumptions about how much foam is used at an individual fire. The calculation for the formulation of the foams also shows a risk, but the exposure estimate is based on defaults and on a site which no longer uses PFOS-related substances.

N.B. No assessment of risk to humans has been carried out.

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ABBREVIATIONS

ASTM	American Society for Testing and Materials
B	Bioaccumulative, within the meaning of the PBT criteria
BCF	Bioconcentration Factor
bw	body weight / <i>Bw</i> , <i>b.w.</i>
CAS	Chemical Abstract Services
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
EINECS	European Inventory of Existing Commercial Chemical Substances
EPA	Environmental Protection Agency (USA)
EPCI	European Photographic Chemicals Industry
ESD	Emission Scenario Document
ESIA	European Semi-Conductor Industry Association
ESR	Existing Substances Regulation
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
FOSA	Perfluorooctanesulphonamide
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ISO	International Organisation for Standardisation
Kow	octanol/water partition coefficient
Kd	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LC50	median Lethal Concentration
LOAEL	Lowest Observed Adverse Effect Level
MITI	Ministry of International Trade and Industry, Japan
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
N-EtFOSE	N-ethylperfluorooctanesulphonamidoethanol (structure in Section 1.1)
N-MeFOSE	N-methylperfluorooctanesulphonamidoethanol
NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
nt.	Normal, Temperature and Pressure
OECD	Organisation for Economic Cooperation and Development
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent, within the meaning of the PBT criteria
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PFOA	Perfluorooctane carboxylic acid
PFOS	Perfluorooctane sulphonate (used to refer to the anion, or the moiety in other substances) (structure in Section 1.1)
PNEC	Predicted No Effect Concentration
QSAR	(Quantitative) Structure-Activity Relationship
RAR	Risk Assessment Report
SEMI	Semiconductors Equipment and Materials International
SIDS	Screening Information Data Set, OECD
SNUR	Significant New Use Rule (US EPA)

STP	Sewage Treatment Plant
T	Toxic, within the meaning of the PBT criteria
TG	Test Guideline
TGD	Technical Guidance Document
US EPA	Environmental Protection Agency, USA
vP	very Persistent, within the meaning of the PBT criteria
w/w	weight per weight ratio
WWTP	Waste Water Treatment Plant

Appendix 1 - Draft List of Compounds Potentially Degrading to PFOS in the Environment

Table A1.1: Draft List of Compounds Potentially Degrading to PFOS in the Environment		
Ref No.	CAS Number	PFOS related substance
1	307-35-7	1-Octanesulphonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
2	376-14-7	2-Propenoic acid, 2-methyl-, 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl ester
3	383-07-3	2-Propenoic acid, 2-[butyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl ester
4	423-82-5	2-Propenoic acid, 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl ester
5	423-86-9	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -2-propenyl-
6	754-91-6	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
7	1652-63-7	1-Propanaminium, 3-[[[(heptadecafluorooctyl)sulphonyl]amino]- <i>N,N,N</i> -trimethyl-, iodide
8	1691-99-2	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-
9	1763-23-1	1-Octanesulphonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
10	1869-77-8	Glycine, <i>N</i> -ethyl- <i>N</i> -[(heptadecafluorooctyl)sulphonyl]-, ethyl ester
11	2250-98-8	1-Octanesulphonamide, <i>N,N,N</i> ''- [phosphinylidynetris(oxy-2,1-ethanediyl)]tris[<i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
12	2263-09-4	1-Octanesulphonamide, <i>N</i> -butyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-
13	2795-39-3	1-Octanesulphonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt
14	2991-50-6	Glycine, <i>N</i> -ethyl- <i>N</i> -[(heptadecafluorooctyl)sulphonyl]-
15	2991-51-7	Glycine, <i>N</i> -ethyl- <i>N</i> -[(heptadecafluorooctyl)sulphonyl]-, potassium salt
16	3820-83-5	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -[2-(phosphonoxy)ethyl]-
17	3871-50-9	Glycine, <i>N</i> -ethyl- <i>N</i> -[(heptadecafluorooctyl)sulphonyl]-, sodium salt
18	4151-50-2	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
19	13417-01-1	1-Octanesulphonamide, <i>N</i> -[3-(dimethylamino)propyl]-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
20	14650-24-9	2-Propenoic acid, 2-methyl-, 2- [[(heptadecafluorooctyl)sulphonyl]methylamino]ethyl ester
21	24448-09-7	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)- <i>N</i> -methyl-
22	24924-36-5	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -2-propenyl-
23	25268-77-3	2-Propenoic acid, 2-[[[(heptadecafluorooctyl)sulphonyl]methylamino]ethyl ester
24	29081-56-9	1-Octanesulphonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt
25	29117-08-6	Poly(oxy-1,2-ethanediyl), .alpha.-[2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl]-.omega.-hydroxy-

Table A1.1: Draft List of Compounds Potentially Degrading to PFOS in the Environment		
Ref No.	CAS Number	PFOS related substance
26	29457-72-5	1-Octanesulphonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, lithium salt
27	30295-51-3	1-Octanesulphonamide, N-[3-(dimethyloxidoamino)propyl]-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
28	30381-98-7	1-Octanesulphonamide, <i>N,N'</i> -[phosphinicobis(oxy-2,1-ethanediyl)]bis[<i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt
29	31506-32-8	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -methyl-
30	38006-74-5	1-Propanaminium, 3-[[[(heptadecafluorooctyl)sulphonyl]amino]- <i>N,N',N''</i> -trimethyl-, chloride
31	50598-29-3	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(phenylmethyl)-
32	52550-45-5	Poly(oxy-1,2-ethanediyl), a-[2-[[[(heptadecafluorooctyl)sulphonyl]propylamino]ethyl]-? -hydroxy-
33	56773-42-3	Ethanaminium, <i>N,N',N''</i> -triethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulphonic acid (1:1)
34	57589-85-2	Benzoic acid, 2,3,4,5-tetrachloro-6-[[[3- [[(heptadecafluorooctyl)sulphonyl]oxy]phenyl]amino]carbonyl]-, monopotassium salt
35	58920-31-3	2-Propenoic acid, 4-[[[(heptadecafluorooctyl)sulphonyl]methylamino]butyl ester
36	61577-14-8	2-Propenoic acid, 2-methyl-, 4-[[[(heptadecafluorooctyl)sulphonyl]methylamino]butyl ester
37	61660-12-6	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -[3-(trimethoxysilyl)propyl]-
38	67939-42-8	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -[3-(trichlorosilyl)propyl]-
39	67969-69-1	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -[2-(phosphonoxy)ethyl]-, diammonium salt
40	67939-88-2	1-Octanesulphonamide, <i>N</i> -[3-(dimethylamino)propyl]- 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, monohydrochloride
41	68081-83-4	Carbamic acid, (4-methyl-1,3-phenylene)bis-, bis[2-[ethyl[(perfluoro-C4-8-alkyl)sulphonyl]amino]ethyl] ester
42	68298-11-3	1-Propanaminium, 3-[[[(heptadecafluorooctyl)sulphonyl](3-sulphopropyl)amino]- <i>N</i> -(2-hydroxyethyl)- <i>N,N</i> -dimethyl-, hydroxide, inner salt
43	68329-56-6	2-Propenoic acid, eicosyl ester, polymer with 2-[[[(heptadecafluorooctyl)sulphonyl] methylamino]ethyl 2-propenoate, hexadecyl 2-propenoate, 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-propenoate and octadecyl 2-propenoate
44	68239-73-6	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(4-hydroxybutyl)- <i>N</i> -methyl-
45	68310-75-8	1-Propanaminium, 3-[[[(heptadecafluorooctyl)sulphonyl]amino]- <i>N,N',N''</i> -trimethyl-, iodide, ammonium salt
46	68541-80-0	2-Propenoic acid, polymer with 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate and octadecyl 2-propenoate
47	68555-90-8	2-Propenoic acid, butyl ester, polymer with 2-[[[(heptadecafluorooctyl)sulphonyl]methylamino]ethyl 2-propenoate, 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-propenoate and 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-propenoate

Table A1.1: Draft List of Compounds Potentially Degrading to PFOS in the Environment		
Ref No.	CAS Number	PFOS related substance
48	68555-91-9	2-Propenoic acid, 2-methyl-, 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl ester, polymer with 2-[ethyl[(nonafluorobutyl)sulphonyl]amino] ethyl 2-methyl-2-propenoate, 2-[ethyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate and octadecyl 2-methyl-2-propenoate
49	68555-92-0	2-Propenoic acid, 2-methyl-, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl ester, polymer with 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate and octadecyl 2-methyl-2-propenoate
50	68608-14-0	Sulphonamides, C4-8-alkane, perfluoro, <i>N</i> -ethyl- <i>N</i> -(hydroxyethyl), reaction products with 1,1'-methylenebis[4-isocyanatobenzene]
51	68649-26-3	1-Octanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-, reaction products with <i>N</i> -ethyl-1,1,2,2,3,3,4,4,4-nonafluoro- <i>N</i> -(2-hydroxyethyl)-1-butanedisulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-heptanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro- <i>N</i> -(2-hydroxyethyl)-1-hexanesulphonamide, <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,5-undecafluoro- <i>N</i> -(2-hydroxyethyl)-1-pentanesulphonamide, polymethylenepolyphenyleneisocyanate and stearyl alc.
52	68867-60-7	2-Propenoic acid, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl ester, polymer with 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-propenoate and .alpha.-(1-oxo-2-propenyl)-.omega.-methoxypoly(oxy-1,2-ethanediyl)
53	68877-32-7	2-Propenoic acid, 2-methyl-, 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl ester, polymer with 2-[ethyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(tridecafluoro-hexyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(undecafluoro-pentyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate and 2-methyl-1,3-butadiene
54	68891-96-3	Chromium, diaquatetrachloro[.mu.-[<i>N</i> -ethyl- <i>N</i> -[(heptadecafluorooctyl)sulphonyl] glycinate-.kappa.O:.kappa.O']]-.mu.-hydroxybis(2-methylpropanol)di-
55	68909-15-9	2-Propenoic acid, eicosyl ester, polymers with branched octylacrylate, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl acrylate, 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl acrylate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl acrylate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl acrylate, 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl acrylate, polyethylene glycol acrylate Me ether and stearyl acrylate
56	68958-61-2	Poly(oxy-1,2-ethanediyl), .alpha.-[2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl]-.omega.-methoxy-
57	70225-14-8	1-Octanesulphonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1)
58	70776-36-2	2-Propenoic acid, 2-methyl-, octadecyl ester, polymer with 1,1-dichloroethene, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl 2-propenoate, <i>N</i> -(hydroxymethyl)-2-propenamamide, 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-propenoate and 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-propenoate
59	71463-78-0	Phosphonic acid, [3-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]propyl]-
60	71463-80-4	Phosphonic acid, [3-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]propyl]-, diethyl ester
61	71487-20-2	2-Propenoic acid, 2-methyl-, methyl ester, polymer with ethenylbenzene, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl 2-propenoate, 2-[methyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-propenoate, 2-[methyl[(undecafluoropentyl)sulphonyl]amino]ethyl 2-propenoate and 2-propenoic acid

Table A1.1: Draft List of Compounds Potentially Degrading to PFOS in the Environment		
Ref No.	CAS Number	PFOS related substance
62	91081-99-1	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -(hydroxyethyl)- <i>N</i> -methyl, reaction products with epichlorohydrin, adipates (esters)
63	92265-81-1	Ethanaminium, <i>N,N,N</i> -trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]-, chloride, polymer with 2-ethoxyethyl 2-propenoate, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl 2-propenoate and oxiranylmethyl 2-methyl-2-propenoate
64	94133-90-1	1-Propanesulphonic acid, 3-[[3-(dimethylamino)propyl][(heptadecafluorooctyl) sulphonyl]amino]-2-hydroxy-, monosodium salt
65	94313-84-5	Carbamic acid, [5-[[[2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethoxy]carbonyl]amino]-2-methylphenyl]-, 9-octadecenyl ester, (<i>Z</i>)-
66	98999-57-6	Sulphonamides, C ₇₋₈ -alkane, perfluoro, <i>N</i> -methyl- <i>N</i> -[2-[(1-oxo-2-propenyl)oxy]ethyl], polymers with 2-ethoxyethyl acrylate, glycidyl methacrylate and <i>N,N,N</i> -trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]ethanaminium chloride
67	127133-66-8	2-Propenoic acid, 2-methyl-, polymers with Bu methacrylate, lauryl methacrylate and 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl methacrylate
68	129813-71-4	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -methyl- <i>N</i> -(oxiranylmethyl)
69	148240-78-2	Fatty acids, C ₁₈ -unsatd., trimers, 2-[[heptadecafluorooctyl)sulphonyl]methylamino]ethyl esters
70	148684-79-1	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -(hydroxyethyl)- <i>N</i> -methyl, reaction products with 1,6-diisocyanatohexane homopolymer and ethylene glycol
71	160901-25-7	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -ethyl- <i>N</i> -(hydroxyethyl), reaction products with 2-ethyl-1-hexanol and polymethylenepolyphenylene isocyanate
72	178094-69-4	1-Octanesulphonamide, <i>N</i> -[3-(dimethyloxidoamino)propyl]-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-,potassium salt
73	178535-22-3	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -ethyl- <i>N</i> -(hydroxyethyl)-, polymers with 1,1'-methylenebis[4-isocyanatobenzene] and polymethylenepolyphenylene isocyanate, 2-ethylhexyl esters, Me Et ketone oxime-blocked
74	182700-90-9	1-Octanesulphonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -methyl-, reaction products with benzene-chlorine-sulphur chloride (S ₂ Cl ₂) reaction products chlorides
75	L-92-0151 (US Pre-manufacture notice)	2-Propenoic acid, 2-methyl-, butyl ester, polymer with 2-[ethyl[(heptadecafluorooctyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(nonafluorobutyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(pentadecafluoroheptyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate, 2-[ethyl[(tridecafluorohexyl)sulphonyl]amino]ethyl 2-methyl-2-propenoate and 2-propenoic acid
76	P-94-2205 (US Pre-manufacture notice)	Polymethylenepolyphenylene isocyanate and bis(4-NCO-phenyl)methane reaction products with 2-ethyl-1-hexanol, 2-butanone, oxime, <i>N</i> -ethyl- <i>N</i> -(2-hydroxyethyl)-1-C ₄ -C ₈ perfluoroalkanesulphonamide
77	192662-29-6	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -[3-(dimethylamino)propyl], reaction products with acrylic acid
78	251099-16-8	1-Decanaminium, <i>N</i> -decyl- <i>N,N</i> -dimethyl-, salt with 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulphonic acid (1:1)
79	306973-46-6	Fatty acids, linseed-oil, dimers, 2- [[heptadecafluorooctyl)sulphonyl]methylamino]ethyl esters
80	306973-47-7	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -(hydroxyethyl)- <i>N</i> -methyl, reaction products with 12-hydroxystearic acid and 2,4-TDI, ammonium salts

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Ref No.	CAS Number	PFOS related substance
81	306974-19-6	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N</i> -methyl- <i>N</i> -[(3-octadecyl-2-oxo-5-oxazolidinyl)methyl]
82	306974-28-7	Siloxanes and Silicones, di-Me, mono[3-[(2-methyl-1-oxo-2-propenyl)oxy]propyl]group]-terminated, polymers with 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl acrylate and stearyl methacrylate
83	306974-45-8	Sulphonic acids, C ₆₋₈ -alkane, perfluoro, compounds with polyethylene-polypropylene glycol bis(2-aminopropyl) ether
84	306974-63-0	Fatty acids, C ₁₈ -unsatd., dimers, 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino] ethyl esters
85	306975-56-4	Propanoic acid, 3-hydroxy-2-(hydroxymethyl)-2-methyl-, polymer with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol and <i>N,N'</i> ,2-tris(6-isocyanatohexyl)imidodicarbonic diamide, reaction products with <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-octanesulphonamide and <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-heptanesulphonamide, compounds with triethylamine
86	306975-57-5	Propanoic acid, 3-hydroxy-2-(hydroxymethyl)-2-methyl-, polymer with 1,1'-methylenebis[4-isocyanatobenzene] and 1,2,3-propanetriol, reaction products with <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-octanesulphonamide and <i>N</i> -ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro- <i>N</i> -(2-hydroxyethyl)-1-heptanesulphonamide, compounds with morpholine
87	306975-62-2	2-Propenoic acid, 2-methyl-, dodecyl ester, polymers with 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl acrylate and vinylidene chloride
88	306975-84-8	Poly(oxy-1,2-ethanediyl), .alpha.-hydro-.omega.-hydroxy-, polymer with 1,6-diisocyanatohexane, <i>N</i> -(hydroxyethyl)- <i>N</i> -methyl perfluoro C ₄₋₈ -alkane sulphonamides-blocked
89	306975-85-9	2-Propenoic acid, 2-methyl-, dodecyl ester, polymers with <i>N</i> -(hydroxymethyl)-2-propenamide, 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl methacrylate, stearyl methacrylate and vinylidene chloride
90	306976-25-0	1-Hexadecanaminium, <i>N,N</i> -dimethyl- <i>N</i> -[2-[(2-methyl-1-oxo-2-propenyl)oxy]ethyl]-, bromide, polymers with Bu acrylate, Bu methacrylate and 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl acrylate
91	306976-55-6	2-Propenoic acid, 2-methyl-, 2-methylpropyl ester, polymer with 2,4-diisocyanato-1-methylbenzene, 2-ethyl-2-(hydroxymethyl)-1,3-propanediol and 2-propenoic acid, <i>N</i> -ethyl- <i>N</i> -(hydroxyethyl)perfluoro-C ₄₋₈ -alkanesulphonamides-blocked
92	306977-58-2	2-Propenoic acid, 2-methyl-, 3-(trimethoxysilyl)propyl ester, polymers with acrylic acid, 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl acrylate and propylene glycol monoacrylate, hydrolysed, compounds with 2,2'-(methylimino)bis[ethanol]
93	306978-04-1	2-Propenoic acid, butyl ester, polymers with acrylamide, 2-[methyl[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]amino]ethyl acrylate and vinylidene chloride
94	306978-65-4	Hexane, 1,6-diisocyanato-, homopolymer, <i>N</i> -(hydroxyethyl)- <i>N</i> -methyl perfluoro-C ₄₋₈ -alkane sulphonamides- and stearyl alc.-blocked
95	306979-40-8	Poly(oxy-1,2-ethanediyl), .alpha.-[2-(methylamino)ethyl]-.omega.-[(1,1,3,3-tetramethylbutyl)phenoxy]-, <i>N</i> -[(perfluoro-C ₄₋₈ -alkyl)sulphonyl]
96	306980-27-8	Sulphonamides, C ₄₋₈ -alkane, perfluoro, <i>N,N'</i> -[1,6-hexanediylbis[(2-oxo-3,5-oxazolidinediyl)methylene]]bis[<i>N</i> -methyl-
Sources: US EPA (2002a); US EPA (2002b); Environment Agency (2001); Consultation with Environment Canada (note that RPA reviewed a confidential draft list of PFOS precursors that was provided by Environment Canada. This was developed from the 182 PFAS that were listed in a June 2000 survey to Canadian industry (Environment Canada, 2000). The draft list will be available as an appendix in the draft screening assessment report on PFOS and its Precursors that the Departments of Environment and Health Canada will release for public comment in early 2004 (Windle, 2003)).		

Appendix 2 – Indirect emissions of PFOS from PFOS-substances and PFOS-polymers

The emissions in the tables below are used in Scenarios 3 to 7 to allow for the production of PFOS in the environment through the degradation of PFOS-substances or PFOS-polymers. For each scenario the EUSES model was run with the properties of PFOS-substance or PFOS-polymer from Section 3.3.1.1 of the evaluation and the relevant emissions of the substance or polymer for that scenario (emissions in Table 3.8). From the results of this modelling for the regional scale, the rate of degradation of PFOS-substance or polymer in each compartment was determined - these values are in the columns labelled “Degradation rate” in the tables. These rates were then converted into formation rates for PFOS (as 94% in the case of PFOS-substance, 30% for polymers). These values are in the columns labelled “PFOS formation”. These rates were added to the direct emissions of PFOS-salt for the scenario to give the total emissions.

Scenario 2 is not included, because in this case the PFOS-substance emissions were converted directly to PFOS before release to the environment, and added directly to the PFOS-salt releases.

Scenario 3 - PFOS-substance, half life 20.5 days.

PFOS-substance

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Direct release of PFOS (kg/year)	Total (kg/year)
Air	353	332	0.034	332
Waste water	-	-	1000	1000
Surface water	15.9	14.9	28.5	43.4
Agricultural soil	200	188	-	188
Industrial soil	32	30	29.5	59.5

Scenario 4 - PFOS-substance, half life 1 year.

PFOS-substance

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Direct release of PFOS (kg/year)	Total (kg/year)
Air	406	382	0.034	383
Waste water	-	-	1000	1000
Surface water	1.16	1.09	28.5	29.6
Agricultural soil	11.8	11.1	-	11.1
Industrial soil	11.8	11.1	29.5	40.6

Scenario 5 - PFOS-substance, half life 20.5 days plus PFOS-polymer, half life 30 years.

Polymer

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Combined release of PFOS from Scenario 3 (kg/year)	Total (kg/year)
Air	-	-	332	332
Waste water	-	-	1000	1000
Surface water	4.66	1.4	43.4	44.8
Agricultural soil	6130	1840	188	2028
Industrial soil	2020	606	59.5	665.5

Scenario 6 - PFOS-substance, half life 20.5 days, PFOS-polymer, half life 30 years, from selected use areas.

PFOS-substance

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Direct release of PFOS (kg/year)	Total (kg/year)
Air	63.2	59.4	0.034	59.4
Waste water	-	-	1000	1000
Surface water	6.08	5.72	28.5	34.2
Agricultural soil	25	23.5	-	23.5
Industrial soil	32	30.1	29.5	59.6

Polymer

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Combined releases of PFOS from above (kg/year)	Total (kg/year)
Air	-	-	59.4	59.4
Waste water	-	-	1000	1000
Surface water	4.59	1.38	34.2	35.6
Agricultural soil	5920	1776	23.5	1800
Industrial soil	2020	606	59.6	666

Scenario 7 - PFOS-substance, half life 20.5 days, from selected use areas.

PFOS-substance

Compartment	Degradation rate (kg/year)	PFOS formation (kg/year)	Direct release of PFOS (kg/year)	Total (kg/year)
Air	8.84	8.31	0.034	8.34
Waste water	-	-	1000	1000
Surface water	0.302	0.284	-	0.28
Agricultural soil	5.35	5.03	-	5.03
Industrial soil	-	-	1.02	1.02

Appendix 3 - Alternative calculation for releases from photolithography

The calculation of releases from photolithography in the main evaluation report includes the use of PFOS-related substances in developers in this industry. SEMI and ESIA have commented that this is not a critical use, and that such use could be phased out within the next five years. The calculations below reflect the emissions expected if this use were phased out, with no concomitant increase in the use in resists. These revised emission estimates are also used to explore the possible effect of different assumptions about the degree to which PFOS-substances degrade to PFOS-salts in the environment.

Emissions

ESIA-SEMI provided a mass balance for the use of PFOS-related substances in this area in October 2003. The key steps in this for this alternative calculation are as follows. All quantities relate to annual use in the EU.

Resist materials – 7% of substance used (46 kg) remains on wafer = 3.2 kg.

TARC materials – 40% of substance used (136 kg) remains on wafer = 54.4 kg.

BARC materials – 7% of substance used (8 kg) remains on wafer = 0.56 kg.

The wafers are then treated with developer, which for this calculation is assumed to no longer contain any PFOS-related substance. Removal of PFOS-related substance by the developer is as follows:

100% of TARC material is removed, hence 54.4 kg.

50% of resist material is removed, hence 1.6 kg.

No removal of BARC material by developer.

The revised estimate of releases to waste water is therefore 56 kg per year.

The calculation in Section 3.1.3 of the main report assumes the use of 13 kg of PFOS-related substance per year at a representative site; this amount is 4.7% of the total EU usage of 276 kg in this area (this total excludes developer). Taking the same percentage the annual release for a site would be 2.63 kg, and for 360 days operation the release would be 7.3 g/day. The regional emissions would be 10% of the total, or 5.6 kg/year, and the continental emission 50.4 kg/year. These are in terms of PFOS-substance.

Predicted environmental concentrations

The worst case assumption for the local situation is that the PFOS-substances are converted to PFOS-salt before release. The above emissions of PFOS-substances correspond to emissions of PFOS-salt as follows: local, 6.9 g/day; regional 5.3 kg/year; continental, 47.4 kg/year.

These revised emission figures have been used to examine the effect of different assumptions about the extent to which PFOS-substances break down to give PFOS. (The effects of considering different rates for the breakdown process are considered elsewhere).

Two different assumptions have been made. The first is as in the main evaluation report, so that the PFOS-substance breaks down to PFOS-salt on release. This is recognised as likely to

be an over-estimation, but provides a worst case estimate. For the second case, it is assumed that 20% of the PFOS-substance is degraded to PFOS under aerobic biodegradation treatment. The remaining 80% is released as PFOS-substance and is distributed in the environment according to the properties in Section 3.3.1.1.2. The half life in water, sediments and soil is assumed to be one year. At steady state, it is assumed that 50% of the PFOS-substance is converted to PFOS. This production of PFOS is added to the 20% emitted directly.

The results of these calculations are presented in Table A3.1. As the main area of potential concern from the main assessment is for secondary poisoning, only the concentrations relevant to this endpoint have been included here. Also included in the table are the equivalent concentrations calculated using the full releases estimated for this use area in Scenario 7 of the main report, assuming instant conversion to PFOS, but without any input from other uses.

Table A3.1 Calculated concentrations for secondary poisoning from photolithography (mg/kg)

	Main report releases, instant conversion	Reduced releases, instant conversion	Reduced releases, 20% instant, 80% indirect
Local			
Freshwater fish	2.53	0.687	0.14
Marine fish	0.35	0.095	0.019
Marine predator	0.14	0.038	7.8×10^{-3}
Earthworms	0.14	0.039	7.8×10^{-3}
Regional			
Freshwater fish	4.9×10^{-3}	1.1×10^{-3}	3.1×10^{-4}
Marine fish	4.8×10^{-4}	1.1×10^{-4}	3.1×10^{-5}
Marine predator	9.6×10^{-4}	2.2×10^{-4}	6.2×10^{-5}
Earthworms	4.4×10^{-5}	9.7×10^{-6}	6.3×10^{-6}

The assumption of reduced emissions reduces the predicted concentrations as expected. The alternative assumptions regarding the degradation of PFOS-substance to PFOS-salt also result in reduced concentrations. The calculated concentrations in freshwater and marine fish are above the PNEC of 0.0167 mg/kg in all three cases. Those for marine predators and earthworms are below the PNEC for the less conservative assumptions. All of the regional concentrations are below the PNEC.

If the less conservative PNEC of 0.067 mg/kg is used, the concentration in freshwater fish is above the PNEC for the local scenario.

Appendix 4 – Alternative calculations for fire fighting foam use

The estimation of emissions to the environment from the use of PFOS-containing fire fighting foams in the main evaluation assumes that 15% of the amount stockpiled is used in one year. This is in line with the overall level of use of foams in the UK for 2002. However, the level of use of PFOS-containing foams for this period was only 0.5%. The higher value was used for a number of reasons. It was felt that the low use may be due to holders trying to minimise the use of this type of foam, or due to a limited occurrence of the type of fire requiring their use. If the level of use was only 0.5%, then for a 20-year shelf life around 90% of the foam would be disposed of at the end of its life without being used, which seemed high. However, a calculation has been performed assuming the lower rate of use to apply.

For the UK stockpile of 76,000 litres of PFOS-containing foam concentrates, use of 0.5% per year is 380 litres of foam, or 380 kg assuming a density of 1 kg/l. At a content of 1% in the concentrate, this contains 3.8 kg of PFOS-salt. The main report considers the use of 1% of the annual total at a single fire. In this calculation that would be only 3.8 litres of foam, which seems too small. Therefore in this case use of 10% at one (worst case) fire is assumed, giving a release of 0.38 kg. As in the main report, this is assumed to go either to drain and to soil (50% each, so 0.19 kg each), or to waste water treatment (100%, so 0.38 kg) for calculation of local concentrations.

Assuming the UK emissions to be 20% of those in the EU, the total EU emissions are 19 kg. The regional emission is 0.95 kg/year, with half to surface water and half to soil. The continental emissions are 8.55 kg/year, again split equally between surface water and soil.

These revised emissions have been used to replace those from fire fighting foams in Scenario 1 and the calculations of concentration repeated. The resulting concentrations are included in Table A4.1 Only the local and regional concentrations in surface water and freshwater fish for secondary poisoning have been included as the most significant for the evaluation. The relevant PEC/PNEC ratios are also included.

Table A4.1 Revised concentrations for fire fighting foam use

Use area	Freshwater (mg/l)	PEC/PNEC	Fish (mg/kg)	PEC/PNEC
Use A	9.54×10^{-3}	0.38	0.279	16.8
Use B	0.0137	0.55	0.312	18.7
Regional	3.7×10^{-5}	0.001	0.207	12.4

The revised scenario has no risks to the freshwater environment from direct effects for either use pattern. However, both use patterns and the regional concentration indicate a possible risk through secondary poisoning. The regional concentration in water is reduced by less than 1% from that in the main report.

Appendix 5 - Effects of the half life for PFOS-substance degradation

Scenarios 3 and 4 in the main report explore the effect of differing degradation rates for PFOS-substances. The degradation rates used in these scenarios are 20.5 days and 1 year respectively. The effect of the change is masked to some degree in the main report as there are also direct releases of PFOS-salts in these scenarios, although Appendix 2 shows the contribution from PFOS-substance degradation for the two scenarios. To make the effect clearer and to consider other aspects of degradation, calculations have been performed on the emission estimates for Scenario 7 with different values for the degradation rates in the various compartments.

For water, sediment and soil, half lives of 20.5 days and 1 year, as in the main assessment, and 10 years have been used. As in the main report, the model has been run using the properties for PFOS-substances in Section 3.3.1.1.2, and the PFOS-substance emissions relevant for Scenario 7 from Table 3.8. From the results of the modelling, the degradation rates for PFOS-substance (as kg/year) have been calculated. In this case they have not been converted to the equivalent production rate of PFOS. The results are in Table A5.1 .

Table A5.1 Degradation rates for PFOS-substances assuming different half lives in water, sediment and soil.

Compartment	Half life		
	20.5 days	1 year	10 years
Air	8.84	9.92	10.7
Surface water	0.3	0.02	0.002
Soil	5.35	3.14	1.4
Total	16.5	13.1	12.1

As the half life in the three compartments increases, the amount degraded in the air compartment increases, that in water decreases significantly, and that in soil decreases more slowly. The overall total input is reduced, but only by ~25% over the range of half lives.

From the above, degradation in the air compartment is a significant route for PFOS-substance in the model, and if conversion to PFOS is assumed then it is a significant source of PFOS in terms of indirect production. The half-life used above is 16 hours, calculated using the AOP program. This half-life is strictly for the initial reaction of the substance with OH radicals rather than for the production of PFOS or other species. To investigate the effect of variation in this half-life, calculations were performed with values of 160 and 1600 hours in addition to the original 16 hour value. These were performed with the half-life of 1 year for water, sediment and soil and the other values the same as above. The results are in Table A5.2 .

Table A5.2 Degradation rates for PFOS-substances assuming different half lives in air (kg/year)

Compartment	Half life		
	16 hours	160 hours	1600 hours
Air	9.92	2.08	0.30
Surface water	0.02	0.02	0.02
Soil	3.14	3.14	3.14
Total	13.08	5.24	3.46

In this case, the increasing half-life in air results in a lower amount being degraded in air but no change in the amounts in other compartments. Overall the amount degraded in the system (and potentially producing PFOS) decreases.

The amounts degraded were assumed to be converted into PFOS on a 1:1 basis. Taking these as the only emissions the resulting regional concentrations in freshwater were 0.21, 0.085 and

0.057 ng/l for 16, 160 and 1600 hours respectively. Hence a change of two orders of magnitude in the half life results in a decrease of ~4 times in the concentration.

Appendix 6 - Effect of choice of log Kow value for polymer

Calculations were performed with EUSES using the releases of polymer for Scenario 6 from the main report. The data from the main report (Table 3.16) were used, but the value for the log Kow was 6, 8 or 10. The concentrations in the compartments for the regional scale are presented in Table A6.1.

Table A6.1 Concentrations of PFOS-polymer in regional model

Compartment (units)	Log Kow = 6	Log Kow = 8	Log Kow = 10
Surface water (mg/l)	5.4×10^{-5}	2.45×10^{-5}	2.26×10^{-5}
Sea water (mg/l)	5.4×10^{-6}	2.22×10^{-6}	1.89×10^{-6}
Air (mg/m ³)	4.5×10^{-7}	1.24×10^{-7}	4.33×10^{-9}
Agricultural soil (mg/kg)	0.0314	0.0421	0.044
Natural soil (mg/kg)	0.0112	7.29×10^{-3}	3.55×10^{-4}
Industrial soil (mg/kg)	0.257	0.59	0.815
Freshwater sediment (mg/kg)	0.187	0.603	0.649
Marine sediment (mg/kg)	0.0199	0.125	0.161

The calculated rates of PFOS-salt formation from the degradation of the polymer are in Table A6.2.

Table A6.2 Production of PFOS-salt from PFOS-polymer (in kg/year):

Compartment	Log Kow = 6	Log Kow = 8	Log Kow = 10
Surface water	1.38	0.62	0.58
Agricultural soil	1776	2382	2490
Industrial soil	606	1389	1920

The regional concentrations of PFOS-salt resulting from the polymer emissions for Scenario 6 and log Kow values of 6 or 10 are shown in Table A6.3.

Table A6.3 Regional PFOS-salt concentrations from polymer emissions

Compartment (units)	Log Kow = 6	Log Kow = 10
Surface water (mg/l)	7.98×10^{-5}	1.13×10^{-4}
Sea water (mg/l)	7.87×10^{-6}	1.11×10^{-5}
Air (mg/m ³)	2.74×10^{-10}	3.74×10^{-10}
Natural soil (mg/kg)	1.32×10^{-4}	1.61×10^{-4}
Soil pore water (mg/l)	2.49×10^{-4}	3.33×10^{-4}

The change in concentration in all compartments is less than a factor of two for the four order of magnitude change in the log Kow value.

Appendix 7 – Contribution of use areas to overall emissions

Table A7.1 presents the estimated emissions to the regional model from each of the use areas according to the various scenarios. All emissions in the table are total emissions, i.e. the combined value for all compartments. For PFOS-salts these are the direct emissions. For PFOS-substances, these are the emissions estimated based on the various assumptions about the rate of degradation to PFOS-salt. The same is true for the PFOS-polymer contributions where relevant. The percentage which each source contributes to the total emission is also given.

Table A7.1 Summary of Emissions based on Scenarios

Use	Regional release (kg/year)	Percentage
<i>Scenario 1 – PFOS-salt</i>		
Chrome plating	1000	94.4
Photography (film production)	0.71	0.07
Aviation	1.46	0.14
Fire fighting foams	57	5.4
<i>Scenario 2 – PFOS-salt plus instant release of PFOS-substance</i>		
Chrome plating	1000	48.8
Photography (film production)	0.71	0.03
Aviation	1.46	0.05
Fire fighting foams	57	2.8
PFOS-substances (total) of which:	991	48.3
Photolithography	23.5	1.2
Photography (developing)	0.71	0.03
Textiles (treatment)	3.2	0.16
Textiles (service life)	169.5	8.3
Paper treatment	753	36.7
Coatings	42.5	2.1
<i>Scenario 3 – PFOS-salt plus PFOS-substance with 20.5 day half life</i>		
Chrome plating	1000	61.5
Photography (film production)	0.71	0.04
Aviation	1.46	0.09
Fire fighting foams	57	3.5
PFOS-substances (total) of which:	568	34.9
Photolithography	13.6	0.84
Photography (developing)	0.4	0.02
Textiles (treatment)	1.8	0.11
Textiles (service life)	97.1	6.0
Paper treatment	431	26.5
Coatings	24.4	1.5
<i>Scenario 4 – PFOS-salt plus PFOS-substance with 1 yr half life</i>		
Chrome plating	1000	67.1
Photography (film production)	0.71	0.05
Aviation	1.46	0.10
Fire fighting foams	57	3.8
PFOS-substances (total) of which:	431	28.9
Photolithography	10.3	0.7
Photography (developing)	0.3	0.02
Textiles (treatment)	1.38	0.09
Textiles (service life)	73.7	5.0
Paper treatment	327	21.9
Coatings	18.5	1.2

<i>Scenario 5 - PFOS-salt plus PFOS-substance with 20.5 day half life plus PFOS-polymer</i>		
Chrome plating	1000	24.1
Photography (film production)	0.71	0.02
Aviation	1.46	0.04
Fire fighting foams	57	1.4
PFOS-substances (total) of which:	568	13.7
Photolithography	13.6	0.33
Photography (developing)	0.4	0.01
Textiles (treatment)	1.8	0.04
Textiles (service life)	97.1	2.3
Paper treatment	431	10.4
Coatings	24.4	0.6
PFOS-polymer (total) of which:	2517	60.7
Textile (treatment)	46	1.1
Textiles (service life)	2471	59.6
<i>Scenario 6 – PFOS-salt plus PFOS-substances with 20.5 day half life plus PFOS-polymers for current uses</i>		
Chrome plating	1000	27.5
Photography (film production)	0.71	0.02
Aviation	1.46	0.04
Fire fighting foams	57	1.6
PFOS-substances (total) of which:	119	3.3
Photolithography	14.5	0.4
Photography (developing)	0.43	0.01
Textiles (treatment)	104	2.9
PFOS-polymer (textile service life)	2454	67.6
<i>Scenario 7 – PFOS-salts plus PFOS-substances with 20.5 day half life for continuing uses</i>		
Chrome plating	1000	98.4
Photography (film production)	0.71	0.07
Aviation	1.46	0.14
PFOS-substances (total) of which:	14.5	1.4
Photolithography	14.1	1.4
Photography (developing)	0.4	0.04

Appendix 8 - Effect of varying solubility and air-water partitioning values for PFOS-salt

The following calculations were performed using the emissions from Scenario 1, i.e. emissions of PFOS-salt only. The property values from Table 3.14 were used for all properties except solubility, where the two values of 519 mg/l (as in Table 3.14) and 680 mg/l were used. For these two calculations the air-water partition coefficient was $3.19 \times 10^{-4} \text{ Pa m}^3 \text{ mole}^{-1}$ (1.35×10^{-7} dimensionless). A further calculation using a Henry's law constant of 2×10^{-6} (dimensionless) and the solubility of 680 mg/l was carried out. The resulting concentrations in water are presented in the table.

Use area	Concentration in water (mg/l)		
	sol = 519 mg/l	sol = 680 mg/l	Ka-w = 2×10^{-6}
Chromium plating	4.52×10^{-5}	4.52×10^{-5}	4.52×10^{-5}
Photography - formulation	1.21×10^{-4}	1.21×10^{-4}	1.21×10^{-4}
Aviation	9.28×10^{-5}	9.28×10^{-5}	9.28×10^{-5}
Fire fighting foams - formulation	0.0973	0.0973	0.0973
- use A	0.0285	0.0285	0.0285
- use B	0.0411	0.0411	0.0411
Regional	3.87×10^{-5}	3.87×10^{-5}	3.87×10^{-5}

Variation in the values of these properties over these ranges has no impact on the predicted concentrations.