

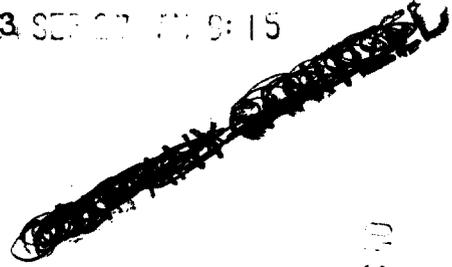
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September 24, 2003 SEP 27 PM 9:15



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Office of Pollution, Prevention and Toxics  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N. W.  
Washington, DC 20460  
Attention: Section 8(e) Coordinator



01 SEP 27 PM 9:17

Re: TSCA Section 8(e) Submissions

Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included a master index for these studies identifying the study title, test substance and CAS number. A Confidential Business Information (CBI) version of this index and the studies also is being submitted today pursuant to EPA procedures. 3M has not provided CBI substantiation with this submission, but would be willing to do so at the Agency's request.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.



Very truly yours,

*Katherine E. Reed (902)*

Dr. Katherine E. Reed, Ph.D  
Staff Vice President  
Environmental Technology and Safety  
Services  
(651) 778-4331  
kereed@mmm.com

2004 DEC 9 PM 4:16



Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on September 24, 2004  
 (Confidential Business Information Redacted)

Primary Eye Irritation Study - Rabbits	20% solids (Ethomeen S/12 1.0M with diethyl sulfate 0.94M); 80% water [Ethomeen S/12 = R-N(EI)+(C2H4OH)2 where R=C18 with 1-2 double bonds]	20% (61791-24-0 with 64-67-5); 80% 7732-18-5	
Guinea Pig Contact Dermal Irritation/Sensitization			
Primary Eye Irritation Study - Rabbits	Butanoic acid, heptfluoro-, calcium salt	2366-98-5	
Acute Oral Toxicity Screen with T-2712CoC in Albino Rabbits	perfluorohexanoic acid	307-24-4	
Primary Skin Irritation Test with T-2725Ec (Repeat Application) in Albino Rabbits			
Acute Ocular Irritation Test with T-2725Ec in Albino Rabbits			
Sensitization Study with T-2741AC in Albino Guinea Pigs			
Oral Range-finder Study of T-3140BS in Pregnant Rats	1-[3-(perfluorooctanesulfonate) anilino amide]-2-potassium 3,4,5,6-tetrachlorophthalate	57589-85-2	
Oral Range-finder Study of T-3139BS in Pregnant Rats	80% 1-[3-(perfluorooctanesulfonate) anilino amide]-2-potassium 3,4,5,6-tetrachlorophthalate; 5% C7 homolog; 5% C5 homolog; 5% C4 homolog; 5% C6 homolog	80% 57589-85-2; 5% 68541-01-5; 5% 68541-02-6; 5% 68568-54-7; 5% 68815-72-5	
Acute Ocular Irritation Test with T-2997CoC in Albino Rabbits	perfluoroethylcyclohexylsulfonic acid diethanol amine salt	salt of 133201-07-7 and 111-42-2	
Sensitization Study with T-3386 in Albino Guinea Pigs			
In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3411			

3M COMPANY SANITIZED

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<p>Acute Oral Toxicity Screen with T-3448 in Albino Rats</p> <p>In Vitro Microbiological Mutagenicity Assays of 3M Company's Compound T-3516</p>	<p>68% poly(oxy-1,2-ethanediyl), alpha-12-ethyl[[heptadecafluorooctyl)sulfonyl]amino[ethyl]-omega-hydroxy-; 12% polyethylene glycol; 7% water; 4.86% poly(oxy-1,2-ethanediyl), alpha-12-ethyl[[pentadecafluorohexyl)sulfonyl]amino[ethyl]-omega-hydroxy-; 4% residual organic fluorochemical; 3% heptadecafluoro-1-octanesulfonic acid; 0.81% poly(oxy-1,2-ethanediyl), alpha-12-ethyl[[undecafluoropentyl)sulfonyl]amino[ethyl]-omega-hydroxy-; 0.3% 1,4-dioxane; 0.2% n-ethylperfluorooctanesulfonamidoethyl alcohol; 0.03% linear n-ethyl perfluorooctanesulfonamide</p>	<p>68% 29117-08-6; 12% 25322-68-3; 7% 7732-18-5; 4.86% 56372-23-7; 4.05% 68298-79-3; 3.24% 68298-81-7; 3% 1763-23-1; 0.81% 68298-80-6; 0.3% 123-91-1; 0.2% 1691-99-2; 0.03% 4151-50-2</p>
<p>Acute Dermal Toxicity Study with T-3451 in Albino Rabbits</p>	<p>C8F17SO2N(CH3)Na</p>	<p>Unknown</p>
<p>Acute Oral Toxicity - Method, Summary, Pathology: Primary Dermal Irritation - Method, Summary: Guinea Pig Maximization - Method, Summary</p>		
<p>Acute Oral Toxicity - Method, Summary, Pathology: Primary Dermal Irritation - Method, Summary: Primary Eye Irritation - Method, Summary:</p>		
<p>Dermal Sensitization Study in Guinea Pigs, Maximization Test - Method, Summary</p>		
<p>4 Hour Acute Aerosol Inhalation Toxicity Study with T-3825 in Rats</p>		
<p>Primary Eye Irritation/Corrosion Study in Rabbits</p>		
<p>4-Hour Acute Aerosol Inhalation Toxicity Study with T-3825 in Rats</p>		

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T-3820: Acute Inhalation Toxicity Test				
T-3821: Acute Inhalation Toxicity Test				
T-3845 Acute Inhalation Toxicity Test	heptafluorobutyl chloride		375-16-6	
Evaluation of the Acute Inhalation Toxicity of T-3920 in the Rat				
Primary Eye Irritation Study in Rabbits - Method, Summary	Decanoic acid, nonadecafluoro- ammonium salt			
Acute Oral Toxicity Study in Rats (OECD Guidelines)	95% ammonium perfluorodecanoate: 5% ammonium perfluorooctanoate	3108-42-7		5% 3825-26-1
Acute Inhalation Toxicity Study with T-4129 in the Rat				
Acute Inhalation Toxicity Study with T-4130 in the Rat				
Acute Oral Toxicity Study in Rats; Acute Dermal Irritation Study in Rabbits; Acute Eye Irritation Study in Rabbits				
Dermal Sensitization Study in Guinea Pigs - Maximization Test				
Mutagenicity Test on T-4413 ( ) Mouse Lymphoma Forward Mutation Assay with Duplicate Cultures				
Acute Inhalation Toxicity Study with T-4354 in the Rat				
Primary Dermal Irritation/Corrosion Study in Rabbits				
Acute Inhalation Toxicity Study in the Rat with T-4397				
Primary Eye Irritation/Corrosion Study of T-5261 in Rabbits	lithium tetrafluoroethane-1,2-disulfonitrile		Unknown	
Acute Inhalation Toxicity Evaluation on T-5231 in Rats				
4-Hour, Acute Inhalation Toxicity Study with T-5305 in Rats				
4-Hour, Acute Inhalation Toxicity Study (Limit Test) with T-5343, 1 in Rats				

Master Index to Studies Submitted Under TSCA 8(e) by 3M Company on September 24, 2004  
 (Confidential Business Information Redacted)

4-Hour, Acute Inhalation Toxicity Study With T-5306 in Rats	[ ]	[ ]	[ ]
4-Hour, Acute Inhalation Toxicity Study (Limit Test) with T-5357-1	[ ]	[ ]	[ ]
Acute Dermal Toxicity Study of T-4201 in Rabbits	[ ]	[ ]	[ ]
Subacute 28-Day Oral Toxicity with T-2816 by Daily Gavage in the Rat Followed by a 14 Day Recovery Period	[ ]	[ ]	[ ]
Subacute 28-Day Oral Toxicity with T-2816 by Daily Gavage in the Rat Followed by a 14-Day Recovery Period	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Evaluation on T-5187 in Rats	[ ]	[ ]	[ ]
T-4240 4-Week Oral Toxicity Study in Rats	[ ]	[ ]	[ ]
Dermal Sensitization Study of T-5473 in Guinea Pigs - Maximization Test	[ ]	[ ]	[ ]
4-Hour, Acute Inhalation Toxicity Study With T-5698 in Rats	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Evaluation on T-5708 in Rats	[ ]	[ ]	[ ]
T-5486 Assessment of Cardiac Sensitization Potential in Dogs	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Evaluation on T-5655 in Rats	[ ]	[ ]	[ ]
T-4201 4 Week Oral Toxicity Study in Rats with 2-Week Recovery Period	[ ]	[ ]	[ ]
T-5658: Eye Irritation to the Rabbit	Lithium Bis(Trifluoromethanesulfonyl)imide	90076-65-6	[ ]
Acute Inhalation Toxicity Evaluation on T-5715 in Rats	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Evaluation on T-5716 in Rats	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Study of T-5724 in Rats	[ ]	[ ]	[ ]
Acute Inhalation Toxicity Study of T-5725 (Resin Solution) in Rats	[ ]	[ ]	[ ]

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Acute Inhalation Toxicity Study (Limit Test) of T-5927 in Rats				
Acute Inhalation Toxicity Study of T-5928 in Rats (LC50)				
Acute Inhalation Toxicity Evaluation on T-5829 in Rats				
Single-Dose Intravenous Pharmacokinetic Study of T-5963 in Rabbits				
Single-Dose Intravenous Pharmacokinetic Study of T-6030 in Rabbits				
5-Daily Dose Dermal Absorption/Toxicity Study of T-6029 and T-6032 in Rabbits	87-93% fluorinated alkyl alkoxylates; 4-10% linear N-ethyl perfluorooctanesulfonamide; 2-4% poly(oxy-1,2-ethanediyl), alpha-[2-ethyl]([pentadecafluorohexyl)sulfonyl]amino]ethyl]-omega-methoxy-; 0-4% residual organic fluorochemicals; 0-2% c8 sulfonamide; 0.1-1% 1-heptanesulfonamide, N-ethyl-			
Single-Dose Intravenous Pharmacokinetic Study of T-6061 in Rabbits				
Single-Dose Intravenous Pharmacokinetic Study of T-6065 in Rabbits				
Single Dose Intravenous Pharmacokinetic Study of T-6063 in Rabbits				
Acute Inhalation Toxicity Study of T-6235 in Rats				
Primary Dermal Irritation/Corrosion Study of T-6402 in Rabbits				
Dermal Sensitization Study of T-6402 in Guinea Pigs- Maximization Test (EC Guidelines)				
Acute Eye Irritation/Corrosion Study with T-6318 in the Rabbit	1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-, Sodium Salt			
		87-93% 68958-61-2; 4-10% 4151-50-2; 2-4% 68958-60-1; 0-2% 31506-32-8; 0.1-1% 68957-62-0		
				102061-82-5

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Primary Skin Irritation / Corrosion Study with T-6567 in the Rabbit (4-Hour Semi-Occlusive Application)				
Assessment of Contact Hypersensitivity to T-6318 in the Albino Guinea Pig (Maximization Test)	1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-, Sodium Salt		102061-82-5	
Single-Dose Intravenous Pharmacokinetic Study of T-6502 in Rabbits				
Single-Dose Intravenous Pharmacokinetic Study of T-6504 in Rabbits				
Single Dose Intravenous Pharmacokinetic Study of T-6506 in Rabbits				
A Study for Effect on Embryofetal Development of the Rat (Inhalation Administration)	20-80% methyl nonafluorobutyl ether; 20-80% methyl nonfluorobutyl ether		20-80% 163702-08-7; 20-80% 163702-07-6	
Bacterial Reverse Mutation Test of T-6695				
5-day Inhalation Toxicity of Perfluorocyclohexene (I); T-6878) in Rats	70% crude perfluorocyclohexene; 30% perfluoromethylcyclopentene		70% 355-75-9	
5-Daily Dose Dermal Absorption/Toxicity Study of T-6502 and T-6503 in Rabbits				
Primary Eye Irritation/Corrosion Study of T-6796 in Rabbits	Lithium Bis(perfluoroethylsulfonyl)imide		132843-44-8	
Primary Dermal Irritation/Corrosion Study of T-6804 in Rabbits	Lithium Bis(perfluoroethylsulfonyl)imide		132843-44-8	
5-Day Inhalation Toxicity Screen of HFE [ ]	c-C6F11OCH3		4943-08-2	
Primary Eye Irritation/Corrosion Study of T-6804 in a Rabbit (OECD Guidelines)	Lithium Bis(perfluoroethylsulfonyl)imide		132843-44-8	
Acute Oral Toxicity Study of T-6804 in Rats (OECD Guidelines)	Lithium Bis(perfluoroethylsulfonyl)imide		132843-44-8	
Dermal Sensitization Study of T-6908 in Guinea Pigs, Mazimization Test (EC Guidelines)				

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Eye Irritation/Corrosion Study of T-4127 in the Rabbit	N-Me Fos Amide-Triphenylbenzyl Phosphonium Chloride Complex; D-1624	31506-32-8	
Single-Dose Intravenous Pharmacokinetic Study of T-6924 in Rabbits			
Dermal Sensitization Study of T-6924 in Guinea Pigs- Maximization Test (EC Guidelines)			
Dermal Sensitization Study of T-7003 in Guinea Pigs - Maximization Test (EC Guidelines)			
Report of Sera and Liver Data for [ ] Monoester - Preliminary ADME Study in Rats	N-ethyl heptadecafluoro-N12-(phosphonoxy)ethyl] octanesulfonamide diammonium salt	67969-69-1	
[ ] Diester-Pharmacokinetic Study in Rats (Study No. T-7043.1, DT-26)	ammonium bis[ethyl(perfluorooctane)sulfonate]phosphate	30381-98-7	
Single Dose Intravenous Pharmacokinetic Study with T-7082 in Rabbits			
[ ] Monoester - Pharmacokinetic Study in Rats (Study No. T-6997.2)	N-ethyl heptadecafluoro-N12-(phosphonoxy)ethyl] octanesulfonamide diammonium salt	67969-69-1	
Determination of PFOS Presence and Concentration in Serum from the Dermal Absorption Studies of T-7106 and T-7107 in Hra:(NZW)SPF Rabbits			
Dermal Sensitization Study of T-7285.5 in Guinea Pigs - Maximization Test (EPA/OECD Guidelines)			
Twenty-eight Day Repeated-Dose Oral Toxicity Study of T-6861 in Rats	Lithium Bis(perfluoroethylsulfonyl)imide	132843-44-8	
Twenty-eight Day Repeated Dose Oral Toxicity Study of T-7005 in Rats			

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Acute (4-Hour) Inhalation Toxicity of Test Atmospheres Obtained after Healing [ ] in Rats			
Toxicokinetic Study of Perfluorooctanesulfonamidoacetate (I); T-7071.2) in Rats	perfluorooctanesulfonamido carboxylic acid	2806-24-8	
Acute Nose-Only Inhalation Toxicity Study of T-7087, T-7088, T-7089 and T-7090 in Rats (Limit Test)			
Acute Ocular Irritation Study of T-7485 Applied to New Zealand White Rabbits			
Toxicokinetic Study of Perfluorooctane Sulfonamide (PFOSA; T-7132.2) in Rats	potassium nonafluorobutanesulfonate	29420-49-3	
Acute Four-Hour Inhalation Study in Rats	perfluorooctanesulfonamide	754-91-6	
Primary Eye Irritation/Corrosion Study of T-7508.2 in Rabbits	Perfluorobutanesulfonyl Fluoride (96-98%) And Perfluorosulfolane (2-4%)	96-98% 375-72-4; 2-4% 42060-64-0	
MV31 K-Salz: Test for Primary Dermal Irritation in the Rabbit			
Assessment of Acute Oral Toxicity with T-7560 in The Rat (Acute Toxic Class Method)			
Acute Eye Irritation/Corrosion Study with T-7560 in the Rabbit			
[ ] Potassium bis-(perfluorobutanesulfonyl)imide			
Repeat Dose ADME Study in Rats	Potassium bis(perfluorobutanesulfonyl)imide	129135-87-1	
Toxicity Study by Repeat Dose Inhalation Administration to CD Rats for 4 Weeks	Perfluorobutanesulfonyl Fluoride (96-98%) And Perfluorosulfolane (2-4%)	96-98% 375-72-4; 2-4% 42060-64-0	
A Sub-acute( 28 Day) Inhalation Toxicity Study, Including a Recovery Study, with T-7479 in Rats	1, 1, 1, 2, 2, 4, 5, 5-nonafluoro-4-(trifluoromethyl)-3-pentanon	756-13-8	
Xenobiochemical Receptor trans-Activation by Perfluorooctane-based Chemicals	perfluorooctanesulfonamide	754-91-6	

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	<p>84% 1-octanesulfonic acid,                  1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-                  heptadecafluoro-, potassium salt; 5.5%                  potassium (perfluorohexyl)sulfonate; 4%                  potassium nonafluorobutanesulfonate; 4%                  potassium perfluoroheptanesulfonate; 2%                  potassium perfluoropentanesulfonate; 0.5%                  unknown</p>	<p>84% 2795-39-3; 5.5% 3871-99-6; 4% 29420-49-3;                  4% 60270-55-5; 2% 3872-25-1</p>
<p>Acute Inhalation Toxicokinetic Study of Perfluorooctanesulfonyl Fluoride (POSF) T-7098.4</p>	<p>perfluorooctanesulfonyl fluoride</p>	<p>307-35-7                  181214-67-5</p>
<p>Five-Day Inhalation Toxicity Study of HFE [ ] in Male CD Rats</p>	<p>c-C6F11-CF2-O-CH3</p>	<p>181214-67-5</p>
<p>Acute Toxicity Screen of Perfluorocyclohexene (T-6878) in Rats</p>	<p>70% crude perfluorocyclohexene; 30% perfluoromethylcyclopentene</p>	<p>70% 355-75-9</p>
<p>Toxicokinetic Study in Rats [ ] (T-7056)</p>	<p>N-Methyl Perfluorobutylsulfonamide = 95% 1-Butanesulfonamide; 1,1,2,2,3,3,4,4,4-Nonafluoro-n-Methyl; 5% N-Methyl-4-Hydro-Perfluorobutylsulfonamide</p>	<p>68298-12-4</p>
<p>Assessment of Acute Oral Toxicity with T-7601.3 in the Rat (Acute Toxic Class Method)</p>	<p>Subchronic 90-Day Oral Toxicity Study with T-7320 By Daily Gavage in the Rat Followed by a 28-Day Recovery Period</p>	<p>84% 1-octanesulfonic acid,                  1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-                  heptadecafluoro-, potassium salt; 5.5%                  potassium (perfluorohexyl)sulfonate; 4%                  potassium nonafluorobutanesulfonate; 4%                  potassium perfluoroheptanesulfonate; 2%                  potassium perfluoropentanesulfonate; 0.5%                  unknown</p>
<p>Protein Binding of Perfluorobutane Sulfonate, Perfluorohexane Sulfonate, Perfluorooctane Sulfonate and Perfluorodecane to Plasma (Human, Rat, and Monkey), and Various Human-Derived Plasma Protein Fractions</p>	<p>potassium nonafluorobutanesulfonate                  potassium (perfluorohexyl)sulfonate</p>	<p>29420-49-3                  3871-99-6</p>

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Five Day Inhalation Toxicity Study of [ ] Monochloride, [ ] and HCF2C25cb in Male CD Rats	potassium perfluorooctanoate	2395-00-8	
	CAF9-OCH2Cl	205367-42-6 (n-isomer) and 221617-86-3 (l-isomer)	
	c-C6F11-CF2-O-CH3	181214-67-5	
	CF2ClCF2CHClF	507-55-1	
Toxicokinetic Screen of [ ] (T-7483) in Rats	C7F15C(O)(N)(H)CH3	89685-56-3	
	84% 1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt; 5.5% potassium (perfluorohexyl)sulfonate; 4% potassium nonafluorobutanesulfonate; 4% potassium perfluorohexanesulfonate; 2% potassium perfluoropentanesulfonate; 0.5% unknown	84% 2795-39-3; 5.5% 3871-99-6; 4% 29420-49-3; 4% 60270-55-5; 2% 3872-25-1	
Low Level Oral Perfluorooctanesulfonate (PFOS) Dose Toxicokinetic Study in Rats: Serum and Liver PFOS			

T-7499

**ACUTE (FOUR-HOUR) INHALATION STUDY IN RATS**

**Data requirement:** US EPA OPPTS Guidelines No. 870.1300  
**Project identity:** MIN 244  
**Study completed on:** 15 September 2000

**Sponsor**

3M Medical Department,  
Toxicology Services,  
3M Center Building 220-2E-02,  
PO Box 33220,  
St. Paul,  
Minnesota 55133-3220,  
USA.

**Research Laboratory**

Huntingdon Life Sciences Ltd.,  
Woolley Road,  
Alconbury,  
Huntingdon,  
Cambridgeshire,  
PE28 4HS,  
ENGLAND.

**COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS**

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid:

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

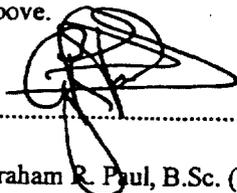
EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No. L 77/8).

United States Environmental Protection Agency, (TSCA) Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

The above GLP standards are considered to be equivalent to the following:

Japan Ministry of International Trade and Industry, Joint Directive, (Kanpogyo No. 39 of Environmental Agency, Yakuhatu No. 229 of Ministry of Health and Welfare; 59 Kikyoku No. 85 of Ministry of International Trade and Industry) of 31 March 1984.

Information regarding test substance characterisation, namely the batch number and expiry date, was not available to Huntingdon Life Sciences for compliance with the Good Laboratory Practice regulations given above.



.....  
Graham R. Paul, B.Sc. (Hons.), M.Sc., C. Biol., M.I. Biol.,  
Study Director,  
Huntingdon Life Sciences Ltd.

.....  
*15 September 2000*  
Date

.....  
*Paul H. Liden*

Sponsor,  
3M Toxicology Services

.....  
*4 October 2000*  
Date

.....  
Submitter

.....  
Date

### QUALITY ASSURANCE STATEMENT

The following have been inspected or audited in relation to this study:

Study Phases Inspected	Date of Inspection	Date of Reporting
<b>Protocol Audit</b>	24 May 2000	24 May 2000
<b>Study Based Inspections</b>		
Study preparation )	6 June 2000	8 June 2000
Test substance control )		
Exposure )		
Sampling )		
Aerosol analysis )		
Clinical signs )		
Records audit )		
Exposure )	8 June 2000	8 June 2000
Clinical signs )		
Records audit )		
<i>Post mortems</i>	20 June 2000	22 June 2000
<i>Post mortems</i> )	22 June 2000	22 June 2000
Records audit )		
<b>Report Audit</b>	5 September 2000	13 September 2000

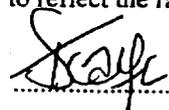
**Protocol Audit:** An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

**Study based inspections:** Inspections of phases of this study were conducted and reported to the Study Director and Company Management as indicated above.

**Process based inspections:** At or about the time this study was in progress inspections and audits of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above.

**Report Audit:** This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.

  
.....

Tracy Scarfe,  
Group Manager,  
Department of Quality Assurance,  
Huntingdon Life Sciences Ltd.

15 September 2000

Date

**CONTRIBUTING SCIENTISTS**

Graham R. Paul, B.Sc. (Hons.), M.Sc., C. Biol., M.I. Biol.,  
Study Director.

Anthony M. Bowden, B.Sc. (Hons.), C.I.A.T.,  
Study Supervisor.

Derek W. Coombs, B.Sc.,  
Toxicologist.

Ian S. Gilkison, M.A., Ph.D.,  
Section Head, Aerosol Technology and Analysis.

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## SUMMARY

### Test substance

A clear colourless liquid identified as T-7499 and estimated to contain 96 - 98% Perfluorobutyl sulfonyl fluoride.

### Test animals

Albino rats (Sprague-Dawley in origin). One control group and 3 test groups, each of 5 male and 5 female rats.

### Route of administration

By inhalation of a test atmosphere containing vapour generated from the test substance.

### Duration of exposure

**Groups 1 to 3** - Four-hour continuous snout-only exposure.

**Group 4** - Terminated during equilibration period.

### Observation period

**Group 1 to 3** - Fourteen days post exposure.

**Group 4** - Discarded on Day 7.

### Exposure levels

**Groups 2 and 3** - The time-weighted average chamber concentrations of T-7499 were 999 ppm and 5,054 ppm for Groups 2 and 3 respectively.

**Group 4** - Exposure of Group 4 (Target 20,000 ppm) was not performed. During the equilibration period the nature and severity of the clinical signs observed justified immediate termination of exposure on humane grounds, in accordance with the UK Home Office guidelines. An atmosphere generation trial, without rats, conducted following cessation of the Group 4 exposure indicated that the chamber concentration of T-7499 was likely to have attained approximately 15,000 ppm before the exposure was stopped.

## **Mortality**

A single Group 3 male rat was sacrificed for humane reasons, on Day 6 of the observation period, due to the deteriorating condition of an eye injury sustained during exposure.

## **Clinical signs**

### **During exposure**

**Groups 2 and 3** - Exaggerated breathing and a decreased breathing rate were evident in Group 3 rats from 30 minutes into exposure and in Group 2 rats from 30 minutes and 3 hours into exposure respectively.

Other clinical signs noted during exposure included initial restless behaviour followed by reduced motor activity, and sudden movements characterised by pronounced jumping. The onset of these signs occurred earlier during exposure of rats at the higher concentration (Group 3). As a consequence of the pronounced jumping, the snout/limbs of Group 3 rats were observed to protrude through the bars of the confinement cage, resulting on occasions in a trapped snout. Fine muscle tremors and vocalising were also noted for a Group 2 male and female respectively.

**Group 4** - Clinical signs evident during the equilibration period included restless behaviour, vocalisation, convulsions and a pronounced jumping, resulting in protrusion of snout/limbs and entrapment of snouts through the bars of the confinement cage. Exposure of Group 4 rats was suspended during the equilibration period due to the nature and severity of these signs.

**During the observation period** - Exaggerated breathing was evident in all test rats immediately following exposure, persisting up to Days 1 and 2 for Groups 2 and 3 respectively. A slow breathing rate was also noted for Group 3 rats following exposure, persisting for at least 2 hours post exposure.

Additional clinical signs noted post exposure included the following: lethargy (all test groups); immobility (Groups 3 and 4); hyperactive when handled (a Group 2 female); sensitive to touch and vocalisation when handled (Groups 2 and 4); extremities cold to touch (Group 3); lacrimation (Group 3 females); yellow staining around uro-genital area (Group 2 and a Group 3 male); yellow substance on fur of snout/jaws (Group 3).

Clinical signs noted following exposure and considered associated with the pronounced jumping evident during exposure included the following: an apparent swelling under the left eye (a Group 2 male); left eye damaged and dark in colour (a Group 3 male); a cut to upper lip (a Group 4 male); apparent swelling of the muzzle (a Group 2 female).

Brown staining on whole body was noted for all Group 3 females on Days 1 and 2 of the observation period.

Rat 45M (Group 3) was sacrificed on Day 6, for humane reasons, due to the deterioration in the condition of an eye injury sustained during exposure. The left eye was damaged, dark in colour and swollen. A clear discharge from the eye was noted and the area around the eye was also swollen. These observations are considered consistent with infection of the eye, 6 days following injury.

There were no treatment-related findings for Groups 2 to 4 from Days 2, 3 and 1 of the observation period respectively.

#### **Bodyweight**

A mean bodyweight loss was evident for Groups 2 and 3 on Day 1 of the observation period and was marked for Group 3 male rats. A higher bodyweight gain was subsequently noted for Group 3 males on Day 2, compared with controls.

#### **Food consumption**

A reduction in food consumption was evident for Groups 2 and 3 on Day 1 and was marked for Group 3 males, persisting for approximately 5 days.

#### **Water consumption**

A reduction in water consumption was evident for Group 2 male rats and Group 3 rats on Day 1 and was marked for Group 3 rats.

#### **Macroscopic pathology**

External findings noted for the rat sacrificed for humane reasons included a damaged left eye. The eye and surrounding area were swollen and a clear discharge was noted from the eye.

#### **Lung weights**

There were no treatment-related effects.

#### **Conclusion**

The  $LC_{50}$  (4-hour) of T-7499 was not determined for humane reasons, due to the nature and severity of the clinical signs evident during exposure of rats at concentrations in excess of approximately 5,000 ppm.

## INTRODUCTION

The acute inhalation toxicity of T-7499 was assessed by exposing 2 groups of rats, for a period up to 4 hours, to a vapour generated from the test substance at target concentrations of 1,000 and 5,000 ppm. An attempt to expose a third test group of rats, at a target concentration of 20,000 ppm, was aborted during the equilibration period due to the nature and severity of clinical signs. A fourth group, acting as a control was exposed to clean air only.

The study was conducted by the Inhalation Studies Group, Huntingdon Research Centre, Huntingdon Life Sciences Ltd., during the period 24 May to 22 June 2000. The protocol for the study was approved by the Study Director and Huntingdon Management on 10 and 11 May 2000 respectively and approved by the Sponsor on 23 May 2000.

This study was designed in compliance with EEC (Annex II, point 5.2.3), OECD (403), US EPA (Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity, 5 August 1998) and J-MAFF test guidelines for acute inhalation studies.

**TEST SUBSTANCE**

**Identity:** T-7499

**Chemical name:** Perfluorobutyl sulfonyl fluoride

**Other name:** PBSF

**Intended use:** None stated

**Appearance:** Clear colourless liquid (presented in a steel pressure vessel)

**Storage conditions:** In the dark at ambient room temperature and in the original container

**Amount received:** Ca. 35 kg

**Batch number:** None stated

**Assay:**

Perfluorobutyl sulfonyl fluoride	96-98%
Perfluorosulfolane	2-4%

**Expiry date:** None stated

**Date received:** 26 April 2000

**Supplier:** Sponsor

The complete description of the chemical and physical properties of the test substance is the responsibility of the Sponsor.

A small sample (1 ml) was sealed in a suitable container and stored in archives at an appropriate temperature.

## MATERIALS AND METHODS

### ANIMALS AND MAINTENANCE

Fifteen male and 15 female albino rats (Sprague-Dawley in origin), approximately 7 and 8 weeks old respectively, were selected from a consignment of rats obtained from Charles River UK Limited, Manston Road, Margate, Kent, England on 31 May 2000. Five male and 5 female rats (same supplier and age) were selected from a second consignment on 7 June 2000.

On arrival the rats were allocated to 1 of 4 groups, each of 5 males and 5 females, and were identified individually by a number tattooed on the ear pinnae. The rats were housed by sex in groups of 5 and acclimatised to laboratory conditions for at least 6 days before the day of exposure.

The holding cages (size 35cm x 53 cm x 25 cm height) were made of stainless steel sheet and wire mesh and were suspended on a movable rack. While in their cages all rats had free access to a measured excess amount of food, SDS rat and mouse diet (RM1 (E) SQC expanded pellet), and tap water supplied by Anglian Water. The water bottles were emptied and re-filled daily. Food and water were analysed routinely to determine the levels of chemical or microbiological contaminants.

The rats remained in a holding room except for the 4-hour exposure and an overnight post exposure period when the rats in the test group were kept in a ventilated cabinet to allow dispersal of any residual test substance.

The temperature and relative humidity of the holding area were recorded using a Kent Clearspan recorder. Air extraction was *via* a balanced system providing at least 15 air changes per hour. The study holding room conditions were generally maintained within the environmental control settings of 22°C ± 3°C and 50% ± 20%. The actual recorded environmental limits were as follows:

Temperature	-	maximum	29.5°C
		minimum	20.5°C
Relative humidity	-	maximum	69%
		minimum	33%

The temperature exceeded the upper setting (25°C), reaching a maximum of 29.5°C on one day only, for a period of 2.5 hours. This was due to a fault with the environmental control system, which was subsequently resolved. This observation and minor deviations of shorter duration are considered not to have affected the integrity of the study.

Room lighting was by artificial light between 06:00 and 18:00 GMT daily and controlled automatically.

## INHALATION EXPOSURES

Two groups, each of 5 male and 5 female rats, were exposed continuously for 4 hours to a vapour generated from T-7499. Exposure of a third test group (Group 4) was aborted during the equilibration phase.

A further group acting as a control received clean air only for 4 hours.

The group identification, target concentrations and date of exposure for the groups were:

Group 1 (Control):	7 June 2000
Group 2 (1,000 ppm):	6 June 2000
Group 3 (5,000 ppm):	8 June 2000
Group 4 (20,000 ppm):	13 June 2000

The achieved chamber concentrations of the vapour for the test groups are presented in the **RESULTS** section of this report.

## EXPOSURE SYSTEM

### Vapour generator

The vapour generator, shown in Figure 1, was designed to produce an atmosphere containing vapour by evaporation of the test substance from a fritted glass disc using a diluent air supply. The air supply to the vaporiser was passed through a metal coil immersed in water, maintained at approximately 50°C for Groups 2 and 3 and at approximately 70°C for Group 4, as an aid to evaporation. The vaporiser was also partly immersed in water maintained, as above, to facilitate vaporisation. All parts of the generator in contact with the test substance were made of glass, except the syringe (polypropylene) and feed line (Teflon®).

The test substance was supplied to the generator from a syringe driven at a constant rate by a syringe pump (Precidor). The compressed air supply to the generator was dried, filtered and oil free.

### Aerosol conditioning

The resultant test vapour was passed through a glass column containing glass wool in order to prevent any condensate entering the exposure chamber.

For Group 4, the test vapour was passed through a condenser located upstream of the 'condensate trap'. A supply of water, maintained at ambient room temperature, was pumped through the condenser to cool the test vapour for exposure of rats.

### Exposure chambers

The whole-body exposure chambers were of square section (size 51 cm x 51 cm x 38 cm height) and made of acrylic polymer. The chambers were fitted with a pyramidal top section and had an enclosed volume of approximately 120 litres. The rats were held for exposure in a stainless steel mesh exposure cage subdivided to provide 10 individual compartments for the rats. The total volume of test rats did not exceed 5% of the volume of the chamber.

The test atmosphere entered the chamber through a port at the top centre of the chamber and was extracted through a port in the centre of the base, below the level of the rats. Each chamber was installed in a large fume cupboard exhausting through an absolute filter.

The configuration of the exposure system is shown in Figure 2.

### PROCEDURE

The test substance, T-7499, was used as supplied.

A supply of pre-heated, clean, dried air was connected to the vapour generator and the supply pressure was adjusted to give a flow rate of 25 litres/minute measured at the generator outlet tube. An in-line flow meter was used to monitor generator airflow during the exposure. The chamber exhaust was calibrated at the point of attachment to the exposure chamber and was adjusted to produce a slightly negative chamber pressure.

A syringe filled with the test substance was fitted to the syringe pump and connected to the generator *via* Teflon® tubing. During preliminary generation trials, bubbles were noted to form in the syringe, enlarging with time. This finding is considered associated with a relatively low boiling point for the test substance and operation of the exposure system at ambient room temperature. The syringes were therefore positioned in a vertical orientation and an 'ice pack' was used to minimise vapour formation in the syringe. Test substance feed rates of 0.18, 0.96 and 3.8 ml/minute were selected for Groups 2 to 4 respectively, as a result of preliminary generation trials. These settings were expected to generate vapour chamber concentrations close to targets of 1,000, 5,000 and 20,000 ppm for Groups 2 to 4 respectively. The test substance feed rate was adjusted as necessary during exposures to maintain the chamber concentration close to target.

The rats to be exposed were placed into separate compartments of the exposure chamber so that males and females were alternately positioned within the chamber.

The syringe pump was switched on and the exposure timed for up to 4 hours, following an 11-minute equilibration period, from the appearance of the test substance on the fritted glass disc. The syringe was replaced with a filled syringe as required during the exposure.

Exposure of Group 4 was stopped during the equilibration period for humane reasons due to the nature and severity of clinical signs.

For Groups 2 and 3, the syringe pump was switched off after 4 hours and the exposure chamber was allowed to clear before the rats were removed for examination.

Following exposure, the rats were returned to the holding cages and food and water supplies were restored. The test rats were kept in a ventilated cabinet overnight and then returned to the holding room for the remainder of the observation period.

The control group was treated similarly but exposed to air only for 4 hours. The control rats were returned to the holding room at the end of the exposure procedure.

#### CHAMBER ATMOSPHERE ANALYSIS

**Groups 2 and 3** - At least 6 air samples were taken from the chamber during exposure in order to determine the concentration of T-7499. In the first instance, samples were obtained following equilibration and then at approximately hourly intervals. Additional samples were obtained as necessary to monitor the chamber concentration in order to ensure satisfactory generation of the test atmosphere usually following adjustments to the exposure system. A time-weighted average was calculated from the individual data in order to prevent undue biasing of repeat samples in the overall mean.

**Group 4** - An atmosphere generation trial, without rats, was conducted following cessation of the exposure. A sample of air was obtained at 7 minutes into the equilibration period in order to estimate the chamber concentration prior to cessation of the exposure. A second sample was obtained following equilibration to confirm the 'final chamber concentration' was close to the target.

The times of sampling are presented in Table 1.

11 minutes is the theoretical time required for the concentration of vapour to reach 90% of its final value under the conditions of exposure employed. The equilibration time ( $t_{eq}$ ) is calculated as follows:

$$t_{eq} \text{ (min)} = \ln \left[ \frac{100}{100 - \% \text{ final conc.}} \right] \times \frac{\text{Chamber volume (litres)}}{\text{Chamber airflow (litres / min)}}$$

Each air sample was withdrawn into a polypropylene syringe fitted with a gas tight valve. The contents of the syringe were injected directly into a gas chromatograph for analysis. The methods of sampling and analysis are described in Appendix 1.

### **NOMINAL CONCENTRATION**

The nominal concentration of the test substance was calculated from the total mass of T-7499 delivered to the vaporiser and the total volume of air flowing through the exposure system during the period of generation.

### **CHAMBER AIR TEMPERATURE AND RELATIVE HUMIDITY**

The air temperature in the exposure chamber was measured using an alcohol-in-glass thermometer and the relative humidity was determined using an Analytical Development Company Ltd infra red water vapour analyser, Model 225. The temperature and humidity were recorded at the start of exposure and then at 30-minute intervals during the 4-hour exposure.

### **OBSERVATIONS**

#### **Mortality**

Throughout the study, all cages were checked at least twice daily, once in the morning and again towards the end of the working day, for dead or moribund animals.

#### **Clinical signs**

The rats were observed intermittently for signs of reaction to the test substance during exposure and at least twice daily throughout the observation period.

The clinical signs were recorded at the end of the chamber equilibration period, at 0.25, 0.5 and 1.0 hours then at hourly intervals during the exposure. Clinical signs were recorded immediately following completion of the exposure and then at 1.0 and 2.0 hours post-exposure.

During the observation period, the clinical signs were recorded once in the morning and then as necessary following a later check for survival.

#### **Bodyweight**

All rats were weighed daily commencing 5 days prior to exposure except Group 2, which commenced measurement 4 days prior to exposure.

### **Food consumption**

The amount of food consumed by each cage of rats was measured daily throughout the study. The daily mean intakes of food for each cage were calculated from the recorded data.

### **Water consumption**

The amount of water consumed by each cage of rats was measured daily throughout the study. The daily mean intakes of water for each cage were calculated from the recorded data.

### **TERMINAL STUDIES**

In view of the minimal exposure of Group 4, aborted during equilibration, rats briefly exposed at ca 15,000 ppm were killed and discarded on Day 7 of the observation period.

At the end of the 14-day observation period, the remaining rats were killed by intraperitoneal injection of pentobarbitone sodium and exsanguinated when clinically dead.

All rats were subjected to a detailed macroscopic examination. The lungs (including the larynx and trachea), liver and kidneys were removed and dissected clear of surrounding tissue. The lungs were weighed and the weights recorded.

Macroscopic abnormalities, lungs (including the larynx and trachea), liver and kidneys were fixed in 10% neutral buffered formalin and were retained for possible histopathological examination but not processed further. Lungs were infused with 10% neutral buffered formalin prior to immersion in fixative.

Tissues were otherwise discarded following necropsy<sup>2</sup>.

### **CALCULATIONS**

In order to minimise the cumulative errors, which result from repeated rounding of numbers, some of the data in this report have been calculated using presented data and only rounded for reporting. Consequently any further calculation using the data as presented will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in this report.

<sup>2</sup> All tissues for Rat 45M, sacrificed on Day 6 for humane reasons, were discarded in accordance with the protocol. The animal was sacrificed prior to issue of protocol amendment 2, which revised requirements for tissue preservation.

### **LOCATION OF STUDY RECORDS**

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

Types of sample and specimen that are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences, Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences in its archive for a period of five years from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

### **DEVIATION FROM PROTOCOL**

For Group 2 only, measurement of bodyweight was initiated 4 days prior to exposure and not 5 days as per protocol.

This deviation from protocol does not affect the scientific objectives or integrity of the study.

## RESULTS

### CHAMBER ATMOSPHERE CONDITIONS

#### Chamber concentration of T-7499

The data are presented in Table 1.

The time-weighted average chamber concentrations of T-7499 were 999 and 5,054 ppm for Groups 2 and 3 respectively and were in good agreement with target (1,000 and 5,000 ppm respectively).

Exposure of Group 4 (Target 20,000 ppm) was not performed. During the equilibration period the nature and severity of the clinical signs observed justified immediate termination of exposure on humane grounds, in accordance with UK Home Office guidelines. An atmosphere generation trial, without rats, conducted following cessation of the Group 4 exposure indicated that the chamber concentration was likely to have attained approximately 15,000 ppm before the exposure was stopped.

#### Nominal concentration

The nominal concentrations were 1,096 and 5,635 ppm for Groups 2 and 3 respectively. For Groups 2 and 3, the time-weighted average chamber concentration of T-7499 is approximately 90% of the nominal concentration and is considered acceptable for generation systems of this design.

#### Chamber air temperature and relative humidity

The mean chamber air temperature, the relative humidity and the standard deviation (sd) of the means during exposure of the groups were:

Group	Temperature (°C)		Relative Humidity (%)	
	Mean	sd	Mean	sd
1 (Control)	22.0	0.35	38	0.8
2 (999 ppm)	24.6	0.39	39	0.9
3 (5,054 ppm)	24.2	0.25	45	4.1
4 (ca 15,000 ppm)*	23.0	-	83	-

\* determined during equilibration period

The mean chamber relative humidity for Group 3 was marginally higher than the control chamber but was considered not to have affected the outcome of the study.<sup>3</sup> The relative humidity for Group 4, determined during the equilibration period, was notably greater than mean values for Groups 1 to 3. This apparent increase in relative humidity with an increase in chamber concentration is considered to reflect, at least in part, interference of the test vapour with the infra red water vapour analyser.

<sup>3</sup> PAULUHN, J. and MOHR, U. (1999) Repeated 4-week inhalation exposure of rats: effect of low, intermediate and high-humidity chamber atmospheres. *Exp. Toxic. Pathol.*, 51, 178-187.

## CLINICAL OBSERVATIONS

### Mortality

A single Group 3 male rat was sacrificed for humane reasons, on Day 6 of the observation period, due to the deteriorating condition of an eye injury sustained during exposure.

### Clinical signs

#### During exposure (Table 2)

**Groups 2 and 3** - Exaggerated breathing and a decreased breathing rate were evident in Group 3 rats from 30 minutes into exposure and in Group 2 rats from 30 minutes and 3 hours into exposure respectively.

Other clinical signs noted during exposure included initial restless behaviour followed by reduced motor activity, and sudden movements characterised by pronounced jumping. The onset of these signs occurred earlier during exposure of rats at the higher concentration (Group 3). As a consequence of the pronounced jumping, the snout/limbs of Group 3 rats were observed to protrude through the bars of the confinement cage, resulting on occasions in a trapped snout. Fine muscle tremors and vocalising were also noted for a Group 2 male and female respectively.

**Group 4** - Clinical signs evident during the equilibration period included restless behaviour, vocalisation, convulsions and a pronounced jumping, resulting in protrusion of snout/limbs and entrapment of snouts through the bars of the confinement cage. Exposure of Group 4 rats was suspended during the equilibration period due to the nature and severity of these signs.

**During the observation period (Table 3)** - Exaggerated breathing was evident in all test rats immediately following exposure, persisting up to Days 1 and 2 for Groups 2 and 3 respectively. A slow breathing rate was also noted for Group 3 rats following exposure, persisting for at least 2 hours post exposure.

Additional clinical signs noted post exposure included the following: lethargy (all test groups); immobility (Groups 3 and 4); hyperactive when handled (a Group 2 female); sensitive to touch and vocalisation when handled (Groups 2 and 4); extremities cold to touch (Group 3); lacrimation (Group 3 females); yellow staining around uro-genital area (Group 2 and a Group 3 male); yellow substance on fur of snout/jaws (Group 3).

Clinical signs noted following exposure and considered associated with the pronounced jumping evident during exposure included the following: an apparent swelling under the left eye (a Group 2 male); left eye damaged and dark in colour (a Group 3 male); a cut to upper lip (a Group 4 male); apparent swelling of the muzzle (a Group 2 female).

Brown staining on whole body was noted for all Group 3 females on Days 1 and 2 of the observation period.

Rat 45M (Group 3) was sacrificed on Day 6, for humane reasons, due to the deterioration in the condition of an eye injury sustained during exposure. The left eye was damaged, dark in colour and swollen. A clear discharge from the eye was noted and the area around the eye was also swollen. These observations are considered consistent with infection of the eye, 6 days following injury.

There were no treatment-related findings for Groups 2 to 4 from Days 2, 3 and 1 of the observation period respectively.

### **Bodyweight**

The data are presented in Figure 3 and Table 4.

A mean bodyweight loss was evident for Groups 2 and 3 on Day 1 of the observation period and was marked for Group 3 male rats. A higher bodyweight gain was subsequently noted for Group 3 males on Day 2, compared with controls. Thereafter, although variable, the mean bodyweight gain of test rats was considered similar to control values.

There were no treatment-related effects on the bodyweights of Group 4 rats, briefly exposed at approximately 15,000 ppm.

### **Food consumption**

The data are presented in Table 5.

A reduction in food consumption was evident for Groups 2 and 3 on Day 1 and was marked for Group 3 males, persisting for approximately 5 days. Thereafter, the food consumption of test rats was similar to control values.

There were no treatment-related effects on the food consumption of Group 4 rats, briefly exposed at approximately 15,000 ppm.

### **Water consumption**

A reduction in water consumption was evident for Group 2 male rats and Group 3 rats on Day 1 and was marked for Group 3 rats. Thereafter, the water consumption of test rats was similar to controls.

There were no treatment-related effects on the water consumption of Group 2 female rats and Group 4 rats.

## **TERMINAL STUDIES**

### **Macroscopic pathology**

The data are presented in Table 6.

External observations noted for the rat sacrificed for humane reasons included a damaged left eye. The eye and surrounding area were swollen and a clear discharge was noted from the eye.

The lungs of most Group 3 rats were congested, generally towards the lower periphery of the lobes. This finding was also evident on the lungs of a proportion of control rats and is considered unlikely to be treatment-related.

Small dark foci were noted on the lungs of 2 test male rats and one control male rat. A 'large volume' of fluid was discharged on removal of the capsule from the right kidney of a control male rat. Small dark foci were noted on the thymus of a control female rat.

### **Lung weights**

The data are presented in Table 7.

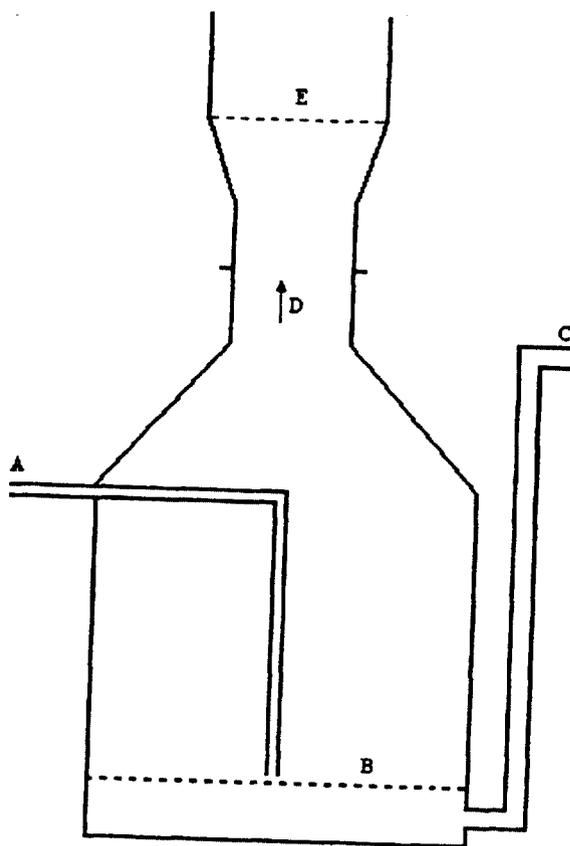
There were no treatment-related effects.

The lung weights of Group 3 male rats were greater than control values. This is consistent with the macroscopic pathology mentioned above.

## **CONCLUSION**

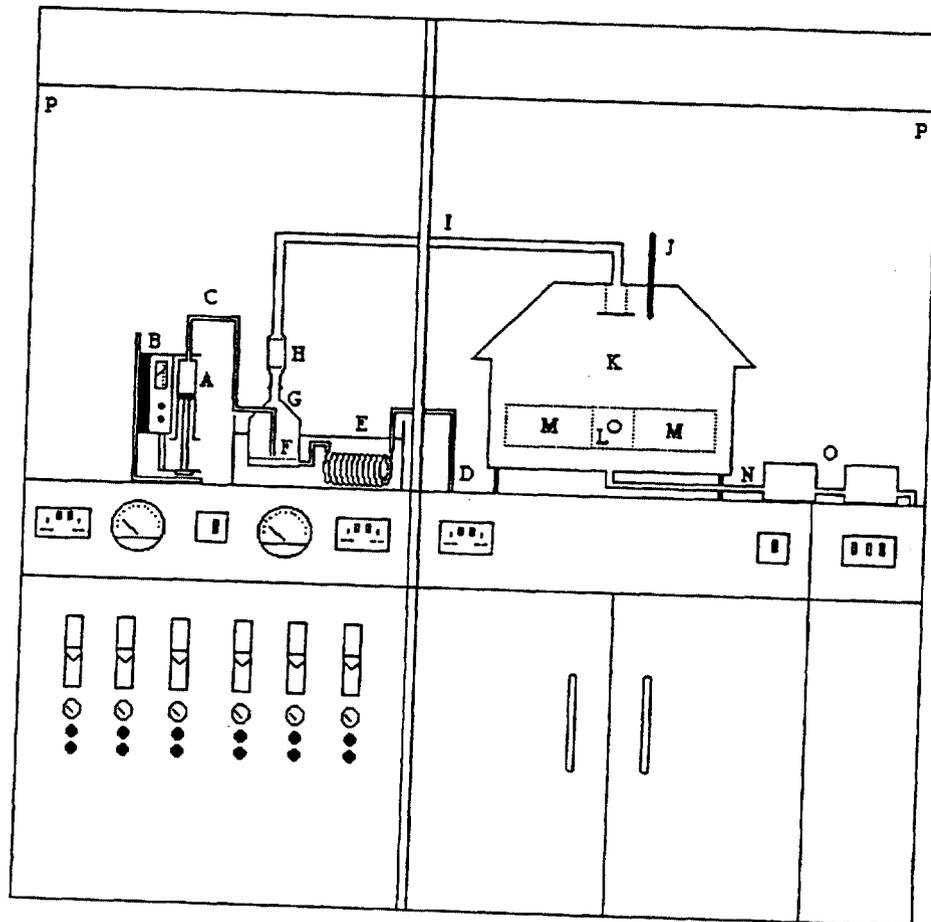
The LC<sub>50</sub> (4-hour) of T-4799 was not determined for humane reasons, due to the nature and severity of clinical signs evident during exposure of rats at concentrations in excess of approximately 5,000 ppm.

**FIGURE 1**  
**Vapour Generator**



- A Test substance feed line
- B Fritted glass disc
- C Air supply
- D Vapour outlet
- E Glass wool trap (to remove any condensate)

**FIGURE 2**  
**Exposure system**  
**Group 2 and 3**

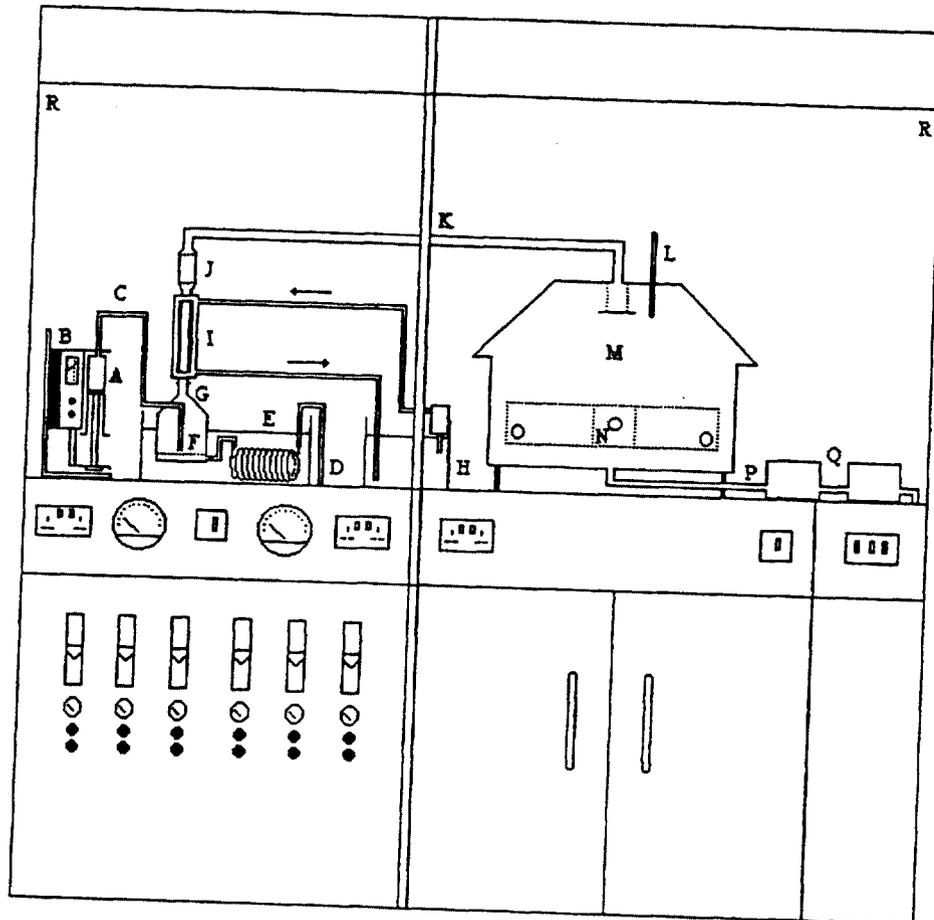


- |  |                                 |
|--|---------------------------------|
| A Test substance in syringe                  | I Glass tubing                  |
| B Syringe pump                               | J Thermometer                   |
| C Teflon® feed line                          | K Exposure chamber (120 litres) |
| D Vaporiser air supply                       | L Sample port                   |
| E Water bath and coiled metal tubing         | M Rat holding cage              |
| F Fritted glass disc                         | N Extract from exposure chamber |
| G Large vaporiser                            | O Vapour absorbers              |
| H Glass wool trap (to remove any condensate) | P Air extraction chamber        |

FIGURE 2

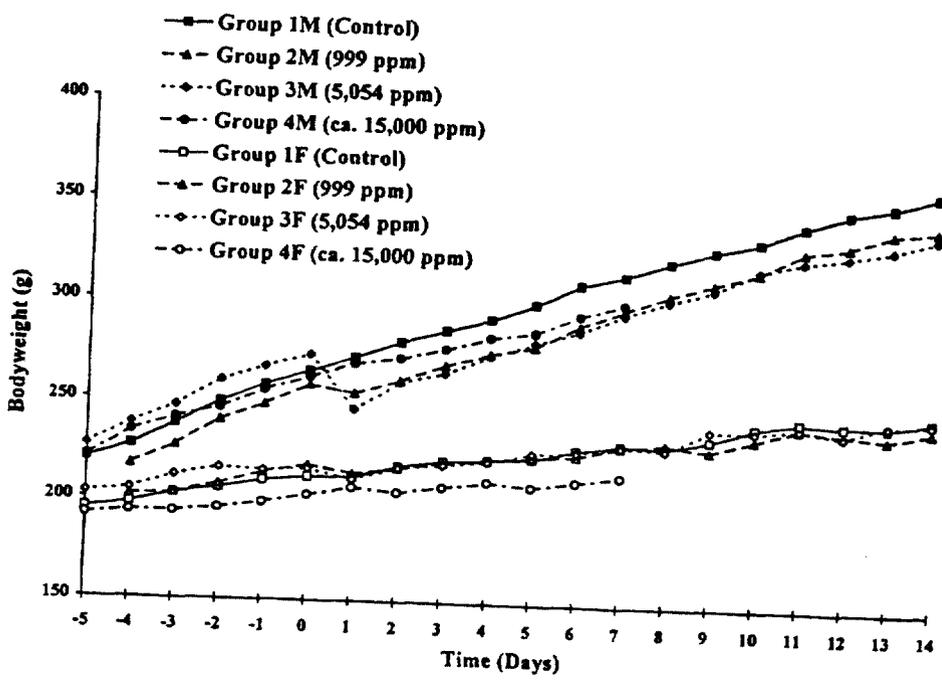
(Exposure system - continued)

Group 4



- |  |  |
|--|--|
| A Test substance in syringe                | J Glass wool trap (to remove any condensate) |
| B Syringe pump                             | K Glass tubing                               |
| C Teflon® feed line                        | L Thermometer                                |
| D Vaporiser air supply                     | M Exposure chamber (120 litres)              |
| E Water bath and coiled metal tubing       | N Sample port                                |
| F Fritted glass disc                       | O Rat holding cage                           |
| G Large vaporiser                          | P Extract from exposure chamber              |
| H Water bath (room temperature) and pump   | Q Vapour absorbers                           |
| I Condenser (to cool the vapour generated) | R Air extraction chamber                     |

**FIGURE 3**  
**Group mean bodyweights**



**TABLE 1**  
**Chamber concentration of T-7499**

Group	Sample	Time taken (h:min)	Analysed concentration (ppm)	Nominal concentration (ppm) †
2 (999 ppm)	1	0:13	856	
	2	0:27	899	
	3	0:46	1023	
	4	2:01	1022	
	5	2:15	1033	
	6	3:00	1011	
	7	3:41	1016	
	TWA		999	
3 (5,054 ppm)	1	0:10	4763	1096
	2	0:20	4905	
	3	1:00	5018	
	4	1:59	4800	
	5	2:44	5234	
	6	3:46	5231	
	TWA		5054	

\* Test substance feed rate increased at 0h:35 min to 0.2 ml/min  
 † Exposure was stopped and chamber was opened at 1h:55 min in order to free animal number 46F, which had trapped its muzzle between the bars of the confinement cage. Atmosphere generation was resumed and the exposure was extended by 16 minutes to compensate for the stoppage (5 minutes) and to allow re-equilibration of the chamber (11 minutes)

† Calculated from the total mass of the test substance dispersed by the generator (85.2 and 440.5 g for Groups 2 and 3 respectively) and the total volume of air supplied to the exposure system (6275 litres) using equations 1 and 2 as follows:

$$\text{Nominal concentration (ppm)} = \frac{V}{V_a + V} \times 10^6 \quad (1)$$

$$V = \frac{W \times R \times T}{M} \quad (2)$$

Where

- V = gaseous volume of T-7499 (ml), assuming atmospheric pressure of 760 mmHg
- W = mass of T-7499 (mg)
- M = molecular weight of PBSF (302.09 g/mole)
- R = 0.08205 ml.atm/mmol.K
- T = temperature (K)
- V<sub>a</sub> = volume of air (ml)

TWA Time weighted average: sum of 'weighted concentrations' (sample concentrations weighted for time) calculated for each sampling occasion as follows:

$$\text{'Weighted concentration' (ppm)} = \frac{\text{Concentration (ppm)} \times \text{'Time weighting' (hours)}}{\text{Exposure duration (hours)}}$$

Where the 'time weighting' is the respective interval between adjustments to the exposure system or the midpoints between consecutive sampling occasions if no adjustment was made

TABLE 1

(Chamber concentration of T-7499 - continued)

Atmosphere generation trial, without rats

Group	Sample	Time taken (h:min)	Analysed concentration (ppm)	Nominal concentration (ppm) †
4 (ca 15,000 ppm)	1 <sup>a</sup>	0:07	15237	22714
	2 <sup>b</sup>	0:22	20881	

- <sup>a</sup> Sample obtained at the point during equilibration at which the animal exposure was stopped to indicate the likely chamber concentration that was attained prior to cessation of the exposure
- <sup>b</sup> Sample obtained following equilibration to confirm the final chamber concentration was close to the expected target
- † Calculated from the total mass of the test substance dispersed by the generator (159 g) and the total volume of air supplied to the exposure system (550 litres) using equations 1 and 2 as follows:

$$\text{Nominal concentration (ppm)} = \frac{V}{V_a + V} \times 10^6 \quad (1)$$

$$V = \frac{W \times R \times T}{M} \quad (2)$$

- Where
- V = gaseous volume of T-7499 (ml), assuming atmospheric pressure of 760 mmHg
  - W = mass of T-7499 (mg)
  - M = molecular weight of PBSF (302.09 g/mole)
  - R = 0.08205 ml.atm/mmol.K
  - T = temperature (K)
  - V<sub>a</sub> = volume of air (ml)

TABLE 2

## Clinical signs during exposure

Group	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1M (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
2M (999 ppm)	Normal appearance and behaviour	5						
	Decreased breathing rate						2	5
	Exaggerated breathing			5	5	5	5	5
	Fine tremor (rapid/low amplitude)					1	1	
	Restless behaviour		5					
	Reduced motor activity			1	5	5	5	5
	Sudden movements - pronounced jump					2	2	2
3M (5,054 ppm)	Normal appearance and behaviour	5						
	Decreased breathing rate				5	5	5	5
	Exaggerated breathing			5	5	5	5	5
	Restless behaviour	5 <sup>a</sup>						
	Reduced motor activity		5	5	5	5	5	5
Sudden movements - pronounced jump	1 <sup>b</sup>					4	5	
4M <sup>c</sup> (ca 15,000 ppm)	Restless	5						
	Sudden movements - pronounced jump	5						
	Pushing snouts/limbs through bars of holding cage, becoming trapped	5						
	Vocalising	5						
	Convulsing	5						

- \* Clinical signs recorded during the 11-minute equilibration period  
<sup>a</sup> Restless behaviour noted following equilibration period but before 15-minute observation point  
<sup>b</sup> Pronounced jump noted approximately 10 minutes into exposure  
<sup>c</sup> Exposure was stopped during equilibration period due to the severity of clinical signs

TABLE 2

(Clinical signs during exposure - continued)

Group	Signs	Number showing signs						
		Time in hours						
		0*	0.25	0.5	1.0	2.0	3.0	4.0
1F (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
2F (999 ppm)	Normal appearance and behaviour	5						
	Decreased breathing rate						3	5
	Exaggerated breathing					5	5	5
	Restless behaviour		5	5	5	5	5	5
	Reduced motor activity				5	5	5	5
	Sudden movements - pronounced jump					2	2	2
	Vocalising					1 <sup>d</sup>	1	
3F (5,054 ppm)	Normal appearance and behaviour	5						
	Decreased breathing rate			5	5	5	5	5
	Exaggerated breathing			5	5	5	5	5
	Restless behaviour	5 <sup>a</sup>						
	Reduced motor activity		5	5	5	5	5	5
	Sudden movements - pronounced jump	2 <sup>b</sup>						
	Muzzle/snout trapped between bars of holding cage					1 <sup>c</sup>	2 <sup>f</sup>	
4F <sup>c</sup> (ca 15,000 ppm)	Restless	5						
	Sudden movements - pronounced jump	5						
	Pushing snouts/limbs through bars of holding cage, becoming trapped	5						
	Vocalising	5						
	Convulsing	5						

- \* Clinical signs recorded during the 11-minute equilibration period
- <sup>a</sup> Restless behaviour noted following equilibration period but before 15-minute observation point
- <sup>b</sup> Pronounced jump noted approximately 10 minutes into exposure
- <sup>c</sup> Exposure was stopped during equilibration period due to the severity of clinical signs
- <sup>d</sup> Vocalising noted following recording of clinical signs at 2-hour observation point
- <sup>e</sup> Sign noted prior to recording of clinical signs at 2-hour observation point
- <sup>f</sup> Sign noted following recording of clinical signs at 3-hour observation point but before 4-hour observation point

**TABLE 3**  
**Clinical signs during observation period**

Group	Signs	Number showing signs																
		Post-exposure*		Day of observation														
		0 hr	1 hr	2 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1M (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2M (999 ppm)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Exaggerated breathing	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Lethargic	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Sensitive to touch	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Vocalisation when handled	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Apparent swelling under eye, left side of head	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Yellow staining around uro-genital area	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

\* Clinical signs recorded after exposure on the day of exposure

**TABLE 3**  
(Clinical signs during observation period - continued)

Group	Signs	Number showing signs															
		Post-exposure*		Day of observation													
		0 hr	2 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3M (5,054 ppm)	Normal appearance and behaviour			1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Exaggerated breathing	5	5														
	Slow breathing rate	5	5														
	Lethargic	5	5														
	Immobile	5	2														
	Extremities cold to touch	5	5														
	Damaged left eye, dark in colour	1	1	1	1	1	1	1	1*								
	Yellow staining around urogenital area		1														
	Yellow substance on fur (snout/jaws)	1	1														
	Dead (total)																
4M (ca 15,000 ppm)	Normal appearance and behaviour										1*	1	1	1	1	1	1
	Exaggerated breathing	5	5	5	5	5	5	5	5	5 <sup>b</sup>							
	Lethargic	5	5														
	Immobile	1															
	Sensitive to touch	5															
	Vocalisation when handled	5															
	Cut to upper lip (left side)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

\* Clinical signs recorded after exposure on the day of exposure  
 \* Animal number 45M sacrificed for humane reasons following recording of clinical signs  
 \* Group 4 rats were discarded on Day 7 of the observation period

**TABLE 3**  
**(Clinical signs during observation period - continued)**

Group	Signs	Number showing signs																
		Post-exposure*			Day of observation													
		0 hr	1 hr	2 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1F (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2F (999 ppm)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Exaggerated breathing																	
	Lethargic																	
	Hyperactive when handled																	
	Sensitive to touch																	
	Vocalisation when handled																	
	Muzzle appears swollen																	
Yellow staining around uro-genital area	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

\* Clinical signs recorded after exposure on the day of exposure

**TABLE 3**  
**(Clinical signs during observation period - continued)**

Group	Signs	Number showing signs																		
		Post-exposure*			Day of observation															
		0 hr	1 hr	2 hr	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
3F (5,054 ppm)	Normal appearance and behaviour																			
	Exaggerated breathing	5	5	5																
	Slow breathing rate	5	5	5																
	Lethargic	5	5	5																
	Immobile	5	2	2																
	Extremities cold to touch		5	5																
	Lacrimation		3	3																
	Yellow substance on fur (snout/jaws)	5	5	5																
	Brown staining whole body																			
						5	5													
4F (ca 15,000 ppm)	Normal appearance and behaviour																			
	Exaggerated breathing	5	5	5																
	Lethargic	5	5	5																
	Immobile	1																		
	Sensitive to touch	5																		
Vocalisation when handled	5																			

\* Clinical signs recorded after exposure on the day of exposure  
 Group 4 rats were discarded on Day 7 of the observation period

TABLE 4

## Individual and group mean bodyweights (g)

Group	Rat	Day of observation									
		-5	-4	-3	-2	-1	0	1	2	3	4
1M (Control)	21	216	221	230	243	253	261	266	274	278	280
	22	208	215	225	236	243	252	259	262	270	273
	23	230	239	253	263	274	280	290	302	306	309
	24	226	235	244	255	263	269	278	286	294	308
	25	222	226	239	249	258	265	267	278	283	288
	Mean	220	227	238	249	258	265	272	280	286	292
2M (999 ppm)	31	nr	227	234	243	254	259	263	270	276	279
	32	nr	189	220	233	245	254	252	257	264	267
	33	nr	224	225	243	245	257	257	265	273	283
	34	nr	222	225	235	244	255	244	251	260	265
	35	nr	221	230	244	253	263	254	262	272	281
	Mean	-	217	227	240	248	258	254	261	269	275

Group	Rat	Day of observation									
		5	6	7	8	9	10	11	12	13	14
1M (Control)	21	293	294	298	305	312	316	326	329	333	337
	22	280	296	299	305	313	317	324	334	337	347
	23	320	328	335	340	332	356	367	371	378	384
	24	312	322	326	336	341	345	349	359	364	368
	25	297	309	315	323	340	330	338	347	348	353
	Mean	300	310	315	322	328	333	341	348	352	358
2M (999 ppm)	31	281	292	301	310	311	319	329	329	332	336
	32	268	280	290	297	307	314	319	325	334	332
	33	290	298	302	314	319	326	341	345	351	356
	34	269	283	291	300	302	309	320	320	332	332
	35	285	298	304	309	319	320	338	339	345	350
	Mean	279	290	298	306	312	318	329	332	339	341

0 Immediately prior to exposure  
nr Not recorded in error

TABLE 4

(Individual and group mean bodyweights (g) - continued)

Group	Rat	Day of observation									
		-5	-4	-3	-2	-1	0	1	2	3	4
3M (5,054 ppm)	41	226	237	245	259	263	269	245	256	259	274
	42	222	233	243	258	266	268	248	261	266	275
	43	224	235	242	254	260	269	241	255	261	265
	44	239	250	261	274	282	286	255	271	276	287
	45	226	235	245	254	265	274	242	257	263	270
	Mean	227	238	247	260	267	273	246	260	265	274
4M (ca 15,000 ppm)	51	216	229	235	241	247	256	256	257	265	273
	52	230	238	243	249	256	264	273	270	277	283
	53	228	245	249	256	275	281	294	300	302	312
	54	223	228	235	238	248	250	258	264	268	273
	55	210	230	241	247	251	258	264	271	272	273
	Mean	221	234	241	246	255	262	269	272	277	283

Group	Rat	Day of observation									
		5	6	7	8	9	10	11	12	13	14
3M (5,054 ppm)	41	279	284	290	299	303	313	323	325	330	339
	42	283	288	298	303	311	321	328	336	338	343
	43	272	276	287	293	300	308	309	313	316	322
	44	296	304	307	316	321	334	335	335	340	344
	45	276	284	- <sup>a</sup>	-	-	-	-	-	-	-
	Mean	281	287	296	303	309	319	324	327	331	337
4M (ca 15,000 ppm)	51	277	291	296	-	-	-	-	-	-	-
	52	288	300	301	-	-	-	-	-	-	-
	53	315	316	333	-	-	-	-	-	-	-
	54	274	282	285	-	-	-	-	-	-	-
	55	278	287	290	-	-	-	-	-	-	-
	Mean	286	295	301	-	-	-	-	-	-	-

0 Immediately prior to exposure

<sup>a</sup> Sacrificed for humane reasons on Day 6 of the observation period<sup>b</sup> Group 4 rats were discarded on Day 7 of the observation period

TABLE 4

(Individual and group mean bodyweights (g) - continued)

Group	Rat	Day of observation									
		-5	-4	-3	-2	-1	0	1	2	3	4
1F (Control)	26	188	194	200	202	210	210	211	219	220	220
	27	191	191	195	198	198	202	203	207	210	212
	28	202	209	214	222	228	229	225	232	238	239
	29	202	200	207	208	209	209	212	218	223	224
	30	193	194	197	202	207	209	207	212	213	213
	Mean	195	198	203	206	210	212	212	218	221	222
2F (999 ppm)	36	nr	200	198	202	209	215	208	215	217	224
	37	nr	208	208	213	220	227	218	222	227	227
	38	nr	203	207	212	215	217	223	222	223	226
	39	nr	207	207	213	218	223	217	221	225	226
	40	nr	193	195	200	207	207	203	206	206	209
	Mean	-	202	203	208	214	218	214	217	220	222

Group	Rat	Day of observation									
		5	6	7	8	9	10	11	12	13	14
1F (Control)	26	223	225	228	223	231	238	241	234	243	243
	27	212	217	221	221	227	235	233	230	234	232
	28	240	249	256	256	250	263	268	271	260	269
	29	224	229	231	229	240	245	249	246	246	251
	30	215	219	220	222	222	226	230	232	231	235
	Mean	223	228	231	230	234	241	244	243	243	246
2F (999 ppm)	36	225	224	228	227	221	230	237	232	228	234
	37	229	232	236	239	229	239	249	245	239	244
	38	228	228	233	236	232	238	243	241	241	244
	39	226	226	235	239	239	237	249	248	246	248
	40	210	215	219	219	226	229	229	228	233	237
	Mean	224	225	230	232	229	235	241	239	237	241

0 Immediately prior to exposure  
 nr Not recorded in error

TABLE 4

(Individual and group mean bodyweights (g) - continued)

Group	Rat	Day of observation									
		-5	-4	-3	-2	-1	0	1	2	3	4
3F (5,054 ppm)	46	205	205	205	220	220	218	222	210	216	220
	47	199	202	206	202	208	215	204	211	212	220
	48	198	203	211	207	204	209	205	214	213	212
	49	211	208	220	224	220	217	219	223	224	224
	50	204	209	217	225	225	223	207	226	230	228
	Mean	203	205	212	216	215	216	211	217	219	221
4F (ca 15,000 ppm)	56	192	198	193	195	204	209	202	208	215	217
	57	189	191	194	196	196	202	207	195	199	205
	58	197	194	197	198	198	202	211	211	209	211
	59	194	194	195	197	197	198	208	193	200	205
	60	186	191	192	193	198	205	209	216	219	219
	Mean	192	194	194	196	199	203	207	205	208	211

Group	Rat	Day of observation									
		5	6	7	8	9	10	11	12	13	14
3F (5,054 ppm)	46	232	231	238	242	247	251	253	247	253	253
	47	226	223	232	236	247	240	246	249	253	251
	48	214	217	218	214	226	227	228	223	230	230
	49	227	231	231	227	236	238	240	237	243	244
	50	232	231	231	227	238	238	238	235	243	248
	Mean	226	227	230	229	239	239	241	238	244	245
4F (ca. 15,000 ppm)	56	214	222	223	-						
	57	202	201	206	-						
	58	213	217	222	-						
	59	199	196	197	-						
	60	219	225	228	-						
	Mean	209	212	215	<sup>b</sup>						

<sup>0</sup> Immediately prior to exposure<sup>b</sup> Group 4 rats were discarded on Day 7 of the observation period

TABLE 5

## Food consumption

Period of consumption (Day)	Food consumption (g/rat/day)			
	1M (Control)	2M (999 ppm)	3M (5,054 ppm)	4M (ca 15,000 ppm)
-5	33	-	33	29
-4	33	30	33	28
-3	33	24	35	28
-2	33	34	35	29
-1	34	34	33	28
1	32	19	6	26
2	32	32	22	29
3	32	33	26	27
4	32	32	28	30
5	33	33	29	29
6	31	33	30	27
7	33	30	32	28
8	34	34	31	.
9	33	33	32	
10	35	33	34	
11	34	37	29	
12	32	31	30	
13	32	30	29	
14	33	32	31	
Cumulative (g/rat) 1 to 14	458	442	389	-

\* Group 4 rats were discarded on Day 7 of the observation period

TABLE 5

(Food consumption - continued)

Period of consumption (Day)	Food consumption (g/rat/day)			
	1F (Control)	2F (999 ppm)	3F (5,054 ppm)	4F (ca 15,000 ppm)
-5	24	-	23	19
-4	23	25	24	22
-3	25	29	23	21
-2	23	25	24	20
-1	22	24	25	22
1	22	15	11	20
2	23	23	22	19
3	25	24	22	22
4	19	26	22	23
5	21	22	24	19
6	23	23	21	19
7	22	23	23	20
8	23	24	20	.
9	21	23	26	
10	26	24	22	
11	25	27	20	
12	17	22	19	
13	21	19	23	
14	22	23	23	
Cumulative (g/rat) 1 to 14	310	318	298	-

\* Group 4 rats were discarded on Day 7 of the observation period

TABLE 6

## Water consumption

Period of consumption (Day)	Water consumption (g/rat/day)			
	1M (Control)	2M (999 ppm)	3M (5,054 ppm)	4M (ca 15,000 ppm)
-5	32	-	29	32
-4	32	33	28	31
-3	30	33	31	29
-2	30	30	32	30
-1	32	32	31	29
1	31	27	10	30
2	31	33	35	31
3	32	30	29	28
4	31	33	30	29
5	30	32	29	30
6	31	32	30	29
7	30	31	29	30
8	31	32	29	.
9	31	32	30	
10	31	32	32	
11	35	37	27	
12	31	32	29	
13	32	30	29	
14	31	31	31	
Cumulative (g/rat) 1 to 14	438	444	399	.

Group 4 rats were discarded on Day 7 of the observation period

TABLE 6

(Water consumption - continued)

Period of consumption (Day)	Water consumption (g/rat/day)			
	1F (Control)	2F (999 ppm)	3F (5,054 ppm)	4F (ca 15,000 ppm)
-5	23	-	24	23
-4	25	24	26	24
-3	23	27	24	22
-2	24	25	25	21
-1	24	27	22	24
1	24	26	13	23
2	26	27	33	19
3	28	29	30	24
4	20	30	25	25
5	24	23	27	20
6	26	25	22	21
7	24	29	24	23
8	22	27	20	.
9	23	24	30	
10	28	27	27	
11	28	31	24	
12	21	27	21	
13	24	22	28	
14	25	27	25	
Cumulative (g/rat) 1 to 14	343	374	349	-

\* Group 4 rats were discarded on Day 7 of the observation period

TABLE 7  
Macroscopic pathology

Group	Rat	Region/organ affected	Observation
1M (Control)	21	Lungs	Minimal congestion towards the lower periphery of right posterior lobe. Small dark foci on left lung and right posterior lobe
	22		No abnormalities detected
	23	Lungs	Severe congestion towards the lower periphery of azygous and right posterior lobes
	24		No abnormalities detected
	25	Kidneys	'Large volume' of fluid discharged on removal of capsule from right kidney
2M (999 ppm)	31		No abnormalities detected
	32		No abnormalities detected
	33		No abnormalities detected
	34	Lungs	Small dark focus on right posterior lobe
	35		No abnormalities detected
3M (5,054 ppm)	41	Lungs	Moderate congestion towards the lower periphery of right posterior lobe
	42	Lungs	Severe congestion towards the lower periphery of left lung, azygous and right middle lobes
	43	Lungs	Severe congestion on the right posterior lobe. Severe congestion towards the lower periphery of azygous lobe
	44	Lungs	Patchy areas of congestion on all lobes. Small dark focus on the right posterior lobe
	45 †	External appearance	Left eye damaged: eye swollen with apparent signs of infection. Area around eye also swollen. Clear discharge from eye

† Sacrificed for humane reasons on Day 6 of the observation period

TABLE 7

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
1F (Control)	26	Thymus	Small dark foci covering whole surface area
	27		No abnormalities detected
	28	Lungs	No abnormalities detected
	29		No abnormalities detected
	30		Severe congestion towards the lower periphery of right posterior lobe and left lung
2F (999 ppm)	36	Lungs	No abnormalities detected
	37		No abnormalities detected
	38		No abnormalities detected
	39		No abnormalities detected
	40		No abnormalities detected
3F (5,054 ppm)	46	Lungs	Minimal congestion towards the lower periphery of right posterior lobe
	47	Lungs	Moderate congestion on the right posterior lobe
	48	Lungs	Moderate congestion towards the lower periphery of right posterior lobe
	49		No abnormalities detected
	50		No abnormalities detected

TABLE 8

Lung weights - individual and group mean values

Group	Rat	Lung weight (g)
1M (Control)	21	1.59
	22	1.57
	23	2.05
	24	1.86
	25	1.64
	Mean sd	1.74 0.207
2M (999 ppm)	31	1.49
	32	1.62
	33	1.46
	34	1.60
	35	1.73
	Mean sd	1.58 0.108
3M (5,054 ppm)	41	1.86
	42	2.48
	43	1.79
	44	2.08
	45 †	1.59
	Mean sd	1.96 0.339

sd Standard deviation

† Sacrificed for humane reasons on Day 6

TABLE 8

(Lung weights - individual and group mean values - continued)

Group	Rat	Lung weight (g)
1F (Control)	26	1.58
	27	1.23
	28	1.39
	29	1.29
	30	1.67
	Mean sd	1.43 0.188
2F (999 ppm)	36	1.29
	37	1.40
	38	1.31
	39	1.51
	40	1.23
	Mean sd	1.35 0.109
3F (5,054 ppm)	46	1.41
	47	1.61
	48	1.26
	49	1.40
	50	1.36
	Mean sd	1.41 0.128

sd Standard deviation

**APPENDIX 1**

**Methods of sample collection and analysis for T-7499**

**SAMPLE COLLECTION**

**Chamber concentration**

A sample of the chamber atmosphere was drawn into a 20 ml polypropylene syringe from a sampling port on the exposure chamber. The syringe was flushed with the test atmosphere prior to sampling. The pressure within the syringe was allowed to equilibrate before the gas tight valve was closed and the syringe removed.

**METHOD OF ANALYSIS**

Chamber atmosphere samples were analysed by gas chromatography. The method of sample analysis is detailed, together with a summary of the method validation, in the Inhalation Analytical Procedure at the end of this appendix.

All references to PBSF in this Appendix refer to the batch of T-7499 supplied.

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 – continued)

## CALCULATIONS

## GC analysis

The samples of chamber atmosphere were injected directly into a gas chromatograph, which was calibrated using vapour standards prepared in gas bags. The method for calculating the concentration of PBSF from the mass used to prepare each vapour standard is given below in equations 1 and 2:

$$\text{Concentration} = \frac{V}{V_a + V} \times 1,000,000 \text{ ppm} \quad (1)$$

$$V = \frac{W \times R \times T}{M} \times \frac{760 \text{ mm Hg}}{\text{Atm}} \quad (2)$$

Where V	=	gaseous volume of PBSF (ml)
W	=	mass of PBSF (mg)
M	=	molecular weight of PBSF (302.09 g/mole)
R	=	0.08205 ml.atm/mmol.K
T	=	temperature (K)
Atm	=	atmospheric pressure (mmHg); assumed as 760 mmHg
V <sub>a</sub>	=	volume of air (ml)

In order to minimise the cumulative errors, which result from repeated rounding of numbers, much of the data in this report have been calculated continuously using unrounded numbers and only rounded for printing. Consequently, any further calculation using these rounded numbers will include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

## COMPOUND SPECIFIC INHALATION ANALYTICAL PROCEDURE FOR PBSF

## The analysis of PBSF in air sample substrate

The method outlined in this document has been validated and is considered fit for the purpose of monitoring test atmospheres and blood headspace in an Inhalation Toxicology study.

This document details the basic procedures for the analysis of PBSF sampled by syringe from test atmospheres. The resulting samples, of approximate concentration 100 to 500 ppm, are analysed by GC. Study specific amendments and additions will be detailed within a supplementary document.

**NOTE** Throughout this document, the symbol § indicates that the relevant information is not available at present, but will be included in a Study specific supplement.

<b>EFFECTIVE DATE:</b>	22 May 2000
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## Test substance

Perfluorobutyl sulfonyl fluoride or PBSF, has the following formula: C<sub>4</sub>F<sub>10</sub>O<sub>2</sub>S.

Appearance	liquid
Subsample Storage of Test mixture	Darkness and a temperature of +4°C. Protected from moisture.

## Equipment

Balance	Sartorius	BP4100
Syringes	Hamilton Hamilton	1000 series gas-tight (100 and 25 ml) 500 series gas-tight (500 ml)
Gas sample bags	SKC INC	Tedlar® 232-series (1 and 3 dm <sup>3</sup> capacity)
Syringe valve	Mininert	Push button valve
Vacuum pump	AEG	ADEB 56 (or equivalent)
Flow meter	J & W Scientific	ADM1000 (acoustic displacement)
General laboratory glassware		

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

**Consumables**

Syringes	Sigma Aldrich	20 ml polypropylene
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**Preparation of samples for analysis**

Samples of the test atmosphere are collected using a 20 ml syringe fitted with a "Mininert" valve. The syringe needle is inserted into the sampling port, the syringe is first flushed with the test atmosphere and 10 ml of test atmosphere is withdrawn into the syringe. The valve is closed and the syringe removed from the sampling port for injection onto the GC.

The gas sampling valve of the GC is set to the "load" position and the syringe is placed into the injection port of the valve. The valve of the syringe is opened and the contents of the syringe are passed into the sampling valve. The valve is then switched to the "inject" position and simultaneously the run start is activated.

**Preparation of calibration standards**

Standards are prepared using the following method. The actual standard concentration ranges used are as detailed in the study specific supplement.

A volume of PBSF is dispensed from the cylinder into a scintillation vial. Weigh approximately 670  $\mu\text{g}$  of PBSF (400  $\mu\text{l}$ ) and inject into a gas bag, mix thoroughly and make up to set volume with air to provide standard S1. The PBSF is vaporised by gentle warming using a hot air blower.

Evacuate a series of gas sample bags of appropriate capacity and introduce by syringe measured volumes of air. Using gas tight syringe(s) fitted with a sealing valve, accurately dispense measured volume(s) of the PBSF vapour into the gas sample bags via the injection port to produce standards covering the concentration range described in the study specific supplement.

**Storage of standards and samples**

The maximum storage periods for the various sample types are detailed below

Sample type	Storage conditions	Storage period
Gas standards	Room temp., light	5 days
Syringe samples	Room temp., ambient	150 minutes

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

**Calibration and quantification**

Calibrate by injecting duplicates of each calibration standard, as detailed in the study specific supplement, at the beginning of each analytical sequence. Measure the peak area response in each injection of the calibration standard solutions and derive the line of best fit using an unweighted least squares method.

For each injection of the sample measure the peak area response and determine the amount present in the sample using the equation below:

$$\text{Amount (ppm)} = \frac{(A - I)}{S}$$

- Where
- A = Peak area response of Perfluorobutyl sulfonyl fluoride (PBSF) in the sample chromatogram
  - S = Slope of calibration line derived from calibration data
  - I = Intercept of calibration line derived from calibration data

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

**Chromatographic conditions**

Analytical column	CP SIL 5CB (100% dimethyl polysiloxane), 30 m x 0.53 mm i.d. 5µm film
Carrier gas	Helium (4.25 ml/min, head pressure 18kPa, 3psig)
Split vent	Helium (20 ml/min)
Septum purge	Helium (1 ml/min)
Split ratio	1:4.7
Make up	Helium (31 ml/min)
Oxidant	Air (450 ml/min)
Fuel	Hydrogen (45 ml/min)
Injection volume	250 µl <i>via</i> gas valve injection loop
Gas valve temperature	60°C
Injector temperature	60°C
Detector temperature	100°C
Column temperature	35°C
Retention time	PBSF approximately 2.5 minutes

**Quality assurance measures**

When the method is established on a chromatographic system six injections of a standard will be used to verify performance of the system. The parameters and acceptance criteria are set out below:

Parameter	Typical value	Acceptable limits
Plate count (USP)	3624	> 80%
Tailing factor (USP)	1.0958	± 20%
Repeatability (CV, n=6)	<1.4%	<5%
QC tolerance	<±2%	<±5%
QC tolerance at LOQ	<±5%	<±10%
	: 51 :	

## APPENDIX 1

## (Methods of sample collection and analysis for T-7499 - continued)

The highest calibration standard will be compared against a standard of similar concentration prepared independently. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

A quality check standard must follow every 6 concentration samples for the analysis to be regarded as valid. The results of the quality check standards must lie within the QC tolerance limits.

A quality check standard of low concentration will be run to verify the LOQ for the run. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

**Summary of method validation**

The raw data for the method validation is located in study MIN/244.

Comparison of test blanks, standards and test samples showed that the analyte was well resolved from any potential interfering peak.

Precision data showed coefficients of variation for PBSF of less than 1.4% with standards in the range of 10,000 to 500 ppm and less than 2.0% to standards to 100 ppm.

Least squares regression analysis, with a  $1/c$  weighted linear weighting, for a peak area response against concentration of standard (100 to 10,000 ppm) produced a correlation coefficient of 0.999991 and relative errors less than 2.1% in the range 10,000 to 100 ppm. The Limit of Quantification (LOQ) for PBSF will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 59.45 and 17.83 ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 100 ppm).

Standards of PBSF in the range 100 to 10,000 ppm stored at room temperature for 5 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal.

Samples of a standard (ca 1,000 ppm) of PBSF stored in the injection syringe for 150 minutes under ambient conditions (room temperature under normal lighting conditions) and subsequently analysed against freshly injected standards showed concentrations within 5% of their nominal concentrations.

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

## GAS CHROMATOGRAPHS IN INHALATION TOXICOLOGY AT 25 SEPTEMBER 1997

System No.	Components of gas chromatography system		
1	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated gas sampling valve, ECD and FID. } 7673 Autosampler } A/D interface } Integration software
	Hewlett Packard	18593B	
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	ThermoQuest*	SP4500	
	ThermoQuest	PC1000	
2	Pye Unicam	PU4550	Chromatograph with gas valve and FID. Autosampler A/D interface Integration software
	Pye Unicam	PU4700	
	ThermoQuest	SP4500	
	ThermoQuest	PC1000	
3	Shimadzu	GC-14A	Chromatograph with FID. Autosampler Autoinjector A/D interface Integration software
	Shimadzu	AOC-1400	
	Shimadzu	AOC-14	
	ThermoQuest	SP4500	
	ThermoQuest	PC1000	
4	Pye Unicam	304	Chromatograph with FID. Autosampler Integrator
	Pye Unicam	PU4700	
	ThermoQuest	SP4400	
5	Pye Unicam	304	Chromatograph with FID. Autosampler Integrator
	Pye Unicam	PU4700	
	ThermoQuest	SP4400	
6	Shimadzu	GC-14A	Chromatograph with FID. Automated gas valve Integrator
	Shimadzu	MGS-4	
	Shimadzu	CR4-A	
7	Shimadzu	GC-14A	Chromatograph with FID. Automated gas valve Integrator
	Shimadzu	MGS-4	
	Shimadzu	CR4-A	
8	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automatic gas sampling valve and FID. } 6890 Series Autosampler } A/D interface } Integration software
	Hewlett Packard	G1513A	
	Hewlett Packard	18596CX	
	Hewlett Packard	G1512AX	
	ThermoQuest	SP4500	
	ThermoQuest	PC1000	
9	Perkin Elmer	Autosystem XL	Chromatograph with programmable capillary inlet, heated automatic gas sampling valve and FID.

\* formerly Spectra-Physics

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

### MIN/244 - STUDY SPECIFIC SUPPLEMENT TO THE INHALATION ANALYTICAL PROCEDURE FOR PBSF

This supplement details additions and amendments to the procedure to be used for the GC assay of PBSF obtained from air samples collected on the above study.

The assay, incorporating the additions and amendments, is suitable for the analysis of PBSF, in air, at concentrations within the range of 100 to 10,000 ppm.

Details given in this supplement supersede those in the compound specific IAP.

<b>EFFECTIVE DATE :</b>	23 May 2000
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#### Analytical standard

Name	Perfluorobutyl sulfonyl fluoride (PBSF)
Batch number	FCS00001822
Purity	96-98% (Perfluorosulfolane 2-4%)
Expiry date	Not Stated
Supplier	Sponsor (filled by Manchester Tank)

#### Preparation of standards

Prepare standards in the nominal range 100 to 10,000 ppm.

#### Calibration and Quantification

Calibration of the instrument is performed using standard gas bags in the range of approximately 100 to 10,000 ppm.

#### Chromatographs

The analysis is performed using chromatograph 1 in Y14.

<b>EFFECTIVE DATE :</b>	5 June 2000
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## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

**Calibration and Quantification**

Calibration of the instrument is performed using standards in the range of approximately 100 to 1,000 ppm.

EFFECTIVE DATE : 7 June 2000

**Calibration and Quantification**

Calibration of the instrument is performed using standards in the range of approximately 2,000 to 8,000 ppm.

**Equipment**

Balance and data printer

Sartorius R200D with YDP-02

EFFECTIVE DATE : 12 June 2000

**Calibration and Quantification**

Calibration of the instrument is performed using standards in the range of approximately 8,000 to 20,000 ppm.

Detector Range 2

EFFECTIVE DATE : 13 June 2000

**Summary of method validation**

A different regression analysis was after the 13 June 2000. The details are given below.

Precision data showed coefficients of variation for PBSF of less than 1.6% with standards in the range of 10,000 to 500 ppm and less than 3.4% to standards to 100 ppm.

## APPENDIX 1

(Methods of sample collection and analysis for T-7499 - continued)

Least squares regression analysis, with a  $1/c^2$  weighted linear weighting, for a peak area response against concentration of standard (100 to 10,000 ppm) produced a correlation coefficient of 0.999984 and relative errors less than 2.6% in the range 10,000 to 100 ppm. The Limit of Quantification (LOQ) for PBSF will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 40.28 and 12.08 ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 100 ppm).

The change in regression analysis was due to a decrease in the baseline noise value produced from the integration. This resulted in a slight increase in peak areas at low concentration, as a result of this, this had the effect of modifying and improving the accuracy of the results obtained.