CONFIDENTIAL

FINAL REPORT

Test Facility Study No. 189541, Report No. 30417

The Excretion of [^{14}C]-Ammonium Perfluorohexanoate in the Mouse and the Rat Following a Single Oral Administration at 50 mg/kg

DATA REQUIREMENTS:
OTTPS 870.7485

TEST FACILITY:
Charles River
Tranent
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SPONSOR:
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STUDY COMPLETION DATE
27 November 2009

1 STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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2 COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Test Facility Study No: 189541

The study described in this report was conducted in accordance with the OECD Principles of Good Laboratory Practice as incorporated into the United Kingdom Statutory Instrument for GLP and as acceptable to the United States of America (EPA) as per 40 CFR 160 and Japan (MHLW, MAFF, METI). The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.

_____ Date: 27 NOVEMBER 2009

Study Director
Charles River

_____ Date: 08 DEC 2009

Sponsor

Daikin Industries, LTD.

_____ Date: _____

Submitter

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

3 QUALITY ASSURANCE STATEMENT

Test Facility Study No: 189541

Study Title: The Excretion of [^{14}C]-Ammonium Perfluorohexanoate in the Mouse and the Rat Following a Single Oral Administration at 50 mg/kg

The Charles River Quality Assurance Unit conducted a protocol review, protocol amendment review (s), study-based inspections and report audits on this study, as detailed below.

Date(s) of QA Activity	Activity	Date of Report to Management and Study Director*
19 December 2008	Protocol Review	19 December 2008
25 March 2009	Protocol Amendment 1 Review	NA
22 May 2009	Dosing Preparation Review/ Dosing/ Protocol Compliance	22 May 2009
03-04 August 2009	Report Audit	04 August 2009
19 November 2009	Final Report Audit	19 November 2009

* Protocol amendment reviews before 27 April 2009 were not reported to management.

Process-based inspections relevant to this study are scheduled once every quarter. The outcome of each inspection is reported to Management and, where relevant, the Study Director.

Facilities relevant to this study are included in Charles River's annual facility inspection programme. The outcome of each inspection is reported to Management.

This report is considered to describe accurately and completely the procedures used in the study and the results obtained.

_____ 27 November 2009

Quality Assurance Date

4 RESPONSIBLE PERSONNEL

Study Director:

Report Compilation:

Senior Technician:

Quality Assurance:

5 SUMMARY

Perfluorohexanoic acid is the ultimate degradation product of a number of new compounds that Daikin Industries Limited is introducing to the market. In aqueous conditions, including in-vivo situations, the acid readily dissociates the C6 ion, which is the moiety of interest. Ammonium Perfluorohexanoate, which also readily disasociates in the same situations to the same C6 ion, is an alternative to the acid, avoiding the possible issues of toxicity and allowing introduction of a significant amount of the ion.

The objective of this pilot study was to examine excretion patterns and rates following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice and rats at a target dose level of 50 mg/kg. The results from this study were used to determine the study parameters for the main Tier study (Charles River Study No. 190300).

Following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female rats, the major route of elimination was *via* the urine with means of 73.0 and 90.2% of the dose in males and females, respectively. Faecal elimination accounted for 15.5 and 7.3% of the dose in males and females respectively. Elimination *via* expired air was negligible, accounting for 0.05% of the dose in both sexes. Excretion of total radioactivity was rapid, with means of 95.6 and 99.2% recovered by 24 h post dose.

At 72 h post dose, mean recoveries of total radioactivity were 97.4 and 100.8% of the administered dose for males and females, respectively. Excretion was almost complete with only *ca.* 0.2% of the dose still remaining in the gastrointestinal tract and carcass.

Following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice, the major route of elimination was *via* the urine with means of 80.3 and 84.0% of the dose in males and females, respectively. Faecal elimination accounted for 10.5 and 7.0% of the dose in males and females respectively. Elimination *via* expired air was negligible, accounting for approximately 0.1% of the dose in both sexes. Excretion of total radioactivity was rapid with means of 90.9 and 94.1% recovered by 24 h post dose.

At 72 h post dose mean recoveries of total radioactivity were 95.4 and 97.3% of the administered dose for males and females, respectively. Excretion was almost complete with only *ca.* 0.6-0.9% of the dose still remaining in the gastrointestinal tract and carcass.

6 INTRODUCTION

Perfluorohexanoic acid is the ultimate degradation product of a number of new compounds that Daikin Industries Limited is introducing to the market. In aqueous conditions, including in-vivo situations, the acid readily dissociates the C6 ion, which is the moiety of interest. Ammonium Perfluorohexanoate, which also readily disasociates in the same situations to the same C6 ion, is an alternative to the acid, avoiding the possible issues of toxicity and allowing introduction of a significant amount of the ion.

This study was designed to fulfil the EEC, EPA and JMAFF requirements for toxicokinetic studies. This study design is in accordance with the OPPTS Guideline for Testing of Chemicals 870.7485.

This study was carried out at Charles River Preclinical Services, Tranent, Edinburgh, EH33 2NE, UK according to Study No. 189541 and amendment 1 and the following timetable:

Study Initiation:	16 December 2008
Experimental Start Date	27 March 2009
Experimental Completion Date	04 June 2009
Study Completion Date	27 November 2009

All raw data generated and recorded during this study, will be stored in the Scientific Archive of Charles River, Preclinical Services Edinburgh for 2 years after the issue of the final report. After the 2 year period the Sponsor will be consulted regarding the disposal, transfer or continued storage of the raw data.

The original signed copy of the final report will be stored indefinitely in the Scientific Archives of Charles River, Preclinical Services Edinburgh.

Biological samples generated during the course of this study will be held deep frozen for a period of 16 weeks following the date of issue of the final report. Samples will then be disposed of unless Charles River receives prior written instructions regarding shipment of the samples to the Sponsor or continued storage at Charles River.

7 EXPERIMENTAL PROCEDURE

7.1 Test Item

Carbon 14 labelled Ammonium Perfluorohexanoate (Batch CFQ40595 Batch B1) was supplied by GE Healthcare Ltd and was stored at -20°C in the dark. The radiolabelled material was supplied as a powder with a stated specific activity of 6.59 MBq/mg. The Certificate of Analysis is presented in Appendix 1.

Non-radiolabelled Ammonium Perfluorohexanoate (also known as C-1500N: Batch No. 7005) was supplied by the Sponsor as an aqueous solution at a concentration of 474 mg/mL. It was used as a reference for chromatographic purposes and for radiodilution of [^{14}C]-Ammonium Perfluorohexanoate in the dose formulations. The non-radiolabelled material was stored at ambient in the dark. The Certificate of Analysis is presented in Appendix 2.

7.2 General Materials

Sterile water was obtained from Hameln Pharmaceuticals Ltd, UK.

Aquasafe 500 Plus® liquid scintillation fluid was obtained from Zinsser Analytic, Maidenhead, UK.

Carbo-Sorb® CO_2 absorbing solution and Permafluor® E^+ scintillation fluid were used in conjunction with the PerkinElmer Model 307 Sample Oxidiser and were supplied by PerkinElmer Life Science and Analytical Instruments Inc, Sears Green, UK.

Spec-Check™-^{14}C was used to estimate efficiencies of combustion and was also obtained from PerkinElmer.

Flowlogic ™-M scintillant was obtained from PerkinElmer Analytical Instruments, UK.

All other materials and chemicals used were of analytical grade where available.

7.3 Animals and Husbandry

Two male and 2 female Sprague Dawley (Crl:CD(SD)) rats, age approximately 7 weeks at dosing (body weights 172-253 g), were supplied by Charles River (UK) Limited. Two male and 2 female CD-1 mice, age approximately 7 weeks at dosing (body weights 26-36 g), were also supplied by Charles River (UK) Limited The animals were acclimatised to the experimental unit for at least 5 days before use on the study. During this acclimatisation

period, the animals were carefully observed to ensure that they were in good health and suitable for inclusion in the study.

During the pre-trial holding period, rats were multiply housed by sex in suitable polycarbonate and stainless steel caging with bedding and chewsticks. Male mice were housed singly and females multiply in solid floored polypropylene and stainless steel caging.

During on-study periods, animals were housed singly in all glass metabolism cages specially designed for the separate, quantitative, collection of urine and faeces.

A standard laboratory diet of known formulation (SDS Rat and Mouse Diet No. 1, Special diets Services, stepfield, Witham, UK) and domestic mains tap water, were available *ad libitum*. Each batch of diet is routinely analysed for composition and for the presence of contaminants. No contaminants were found to be present in the diet or water at levels considered to be capable of interfering with the purpose or outcome of the study. Representative analytical data for typical diet and water available in the study are retained in the study data.

Food was withheld from the rats for 10-12 hours before dosing and approximately 3-4 hours after dosing.

7.4 Radiochemical Purity

The radiochemical purity of [^{14}C]-Ammonium Perfluorohexanoate was assessed prior to dose preparation and stability confirmed in the trial preparation at 3 h and 24 h.

Equipment

HPLC Model:	Agilent 1100
Radiodetector Model:	Radiomatic™ Flo-one®, Flow Scintillation Analyser (Model 150TR)
Data Handling:	Atlas 2002 (Thermo Labsystems) Product Release 1

Conditions

Column:	Waters Xterra MS C18 (MP 162) (250cm x 4.6mm, 5µm)
Column Temperature:	25 °C

Auto-sampler Temperature	4 °C
Mobile Phase:	A: 50mM Ammonium Acetate B: Acetonitrile
Mobile Phase conditions:	Gradient

Gradient:

Time (min)	%A	%B
0	80	20
5	80	20
15	0	100
25	0	100
30	80	20

Flow rate:	1 mL/min
UV Detector wavelength:	220 nm
Scintillant:	Flowlogic™

7.5 Dose Preparation and Stability

7.5.1 Dose Preparation: Trial Formulation

An appropriate amount (1.22 mg) of [^{14}C]-Ammonium Perfluorohexanoate was weighed into a glass vial. An appropriate volume of Ammonium Perfluorohexanoate (98 µl, equivalent to 49 mg of Ammonium Perfluorohexanoate) was then added to the vial. The required volume of sterile water was then added to the vial and mixed to give a solution. The final concentration was 5.127 mg/ml, with a final formulation weight of 9.79 g.

Aliquots of the dose formulation were analysed by radio-HPLC at 3 and 24 h after preparation to confirm the stability of the test material in the formulation and over the dosing period. The results of the stability check were also used for subsequent dosing occasions.

7.5.2 Dose Preparation: Rat Formulation

An appropriate amount (0.73 mg) of [^{14}C]-Ammonium Perfluorohexanoate was weighed into a glass vial. An appropriate volume of Ammonium Perfluorohexanoate (156 µl, equivalent to 73.94 mg of Ammonium Perfluorohexanoate) was then added to the vial. The required

volume of sterile water was then added to the vial and mixed to give a solution. The final concentration was 4.90 mg/mL, with a final formulation weight of 15.23 g.

7.5.3 Dose Preparation: Mouse Formulation

An appropriate amount (1.69 mg) of [^{14}C]-Ammonium Perfluorohexanoate was weighed into a glass vial. An appropriate volume of Ammonium Perfluorohexanoate (246 µl, equivalent to 123.1 mg of Ammonium Perfluorohexanoate) was then added to the vial. The required volume of sterile water was then added to the vial and mixed to give a solution. The final concentration was 4.74 mg/mL, with a final formulation weight of 24.58 g.

7.6 Dose Administration

The formulations were administered by gastric gavage at a target dose volume of 10 mL/kg to achieve a target dose level of 50 mg/kg (target radioactive dose level: 3-5 MBq/kg).

Each animal was accurately weighed prior to dosing. The syringes were weighed prior to and following each dosing. The actual dose received by each animal was determined with reference to the radioactive concentration, the weight of dose administered and the calculated specific activity of the dose formulation.

The dose received by each animal is presented in Appendix 4.

7.7 Sample Collection

Two male and two female rats, and two male and two female mice each received a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate at a target dose level of 50 mg/kg.

Urine and faeces samples were collected into containers cooled by solid carbon dioxide from each animal for the periods 0-6 (urine only), 6-24 then at 24 h intervals to 72 h post dose. Cages were washed with water at the time of each faeces collection. Expired air was collected over 0-24 and 24-48 h post dose.

At the end of the 72 h collection period, each animal was humanely killed by CO_2 narcosis. The gastrointestinal tract and residual carcass from each rat was retained.

The levels of total radioactivity were determined in each sample collected.

7.8 Sample Storage

All samples not analysed immediately were stored at ca -20°C until taken for analysis. After analysis, samples were returned to storage at ca -20°C.

Cage wash samples were stored at ambient temperature.

7.9 Preparation of Samples For Total Radioactivity Analysis

7.9.1 Liquid Samples

Duplicate aliquots of liquid samples (*ca* 0.1 mL for urine and 1 mL for cage wash) were made up to 1 mL with water (if necessary) and mixed with scintillation fluid.

Duplicate aliquots of expired air samples (*ca* 1 mL) were mixed with scintillant using a PerkinElmer Tri-Carb 307 Sample Oxidiser.

7.9.2 Solid Samples

Faeces samples were weighed, an appropriate amount of water added and the total weight recorded prior to homogenisation. Duplicate aliquots of each (*ca* 0.2-0.3 g) were combusted using a PerkinElmer Tri-Carb 307 Sample Oxidiser. Carcass samples were minced, then analysed as described for faeces. All gastrointestinal tract samples were finely scissor chopped, then analysed as described for faeces.

All aliquots were combusted using a PerkinElmer Tri-Carb 307 Sample Oxidiser. The [^{14}C]-carbon dioxide generated was absorbed and mixed with scintillant, prior to analysis by liquid scintillation counting. The efficiency of oxidation of test samples relative to [^{14}C]-standard oxidation efficiencies, was determined at regular intervals during each series of oxidations. Combustion of standards showed that recovery efficiencies were all greater than 97%.

7.10 Quantification of Radioactivity

All samples prepared in scintillation fluid were subjected to liquid scintillation counting for 5 mins, together with representative blanks samples, using a Parkard TR 2100 Liquid Scintillation Analyser with automatic quench correction by an external method. Where possible, samples were analysed in duplicate and allowed to heat and light stabilise prior to analysis. Prior to calculation of each result, a background count was determined and subtracted from each sample count rate.

For scintillation counting, a limit of reliable determination of 30 d.p.m above background has been instituted in these laboratories. Where results have arisen from data below the limit of reliable determination, the fact is noted.

8 RESULTS

8.1 Radiochemical Purity and Dose Stability

[^{14}C]-Ammonium Perfluorohexanoate was shown by chromatography with Ammonium Perfluorohexanoate to be authentic and 99.6% radiochemically pure. An example radiochromatogram is presented in Appendix 4.

[^{14}C]-Ammonium Perfluorohexanoate in the dose formulation was shown to be stable at 3 and 24 hours (purity values of 99.6 and 99.8, respectively) after a trial dose preparation, covering the duration of the dosing procedure. An example radiochromatogram is presented in Appendix 5.

8.2 Excretion Kinetics Following Oral Administration to Male and Female Rats

The excretion of total radioactivity following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female rats are shown in Tables 1-2, with mean cumulative results presented graphically in Figures 1-2.

Following a single oral administration, the major route of elimination was *via* the urine with means of 73.0 and 90.2% of the dose in males and female respectively. Faecal elimination accounted for 15.5% in males and 7.3% in females. Elimination *via* expired air was minimal accounting for only 0.04% of the dose in both sexes. Excretion of total radioactivity was rapid with means of 95.6 and 99.2% recovered by 24 hours post dose.

By 72 h post dose, approximately 0.2% of the dose remained in the gastrointestinal tract and carcass, indicating that excretion was almost complete. Mean recoveries of total radioactivity (including residual radioactivity in the gastrointestinal tract and carcass) were 97.4 and 100.8% of the dose administered in males and females respectively.

8.3 Excretion Kinetics Following Oral Administration to Male and Female Mice

The excretion of total radioactivity following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice are shown in Tables 3-4, with mean cumulative results presented graphically in Figures 3-4. Animal 008F had to be prematurely terminated at 48 h post dose due to poor wellbeing of the animal. This was considered not to be a result of the test item. This is not considered to have significant impact on the results of this study, as the majority of the radioactivity was excreted by this endpoint.

Following a single oral administration, the major route of elimination was *via* the urine with means of 80.3 and 84.0% of the dose in males and female respectively. Faecal elimination

accounted for 10.5% in males and 7.0% in females. Elimination *via* expired air was minimal accounting for only 0.07-0.08% of the dose. Excretion of total radioactivity was rapid with means of 90.9 and 94.1% recovered by 24 hours post dose.

By 72 h post dose, approximately 0.6-0.9% of the dose remained in the gastrointestinal tract and carcass, indicating that excretion was almost complete. Mean recoveries of total radioactivity (including residual radioactivity in the gastrointestinal tract and carcass) were 95.4 and 97.3% of the dose administered in males and females respectively.

9 DISCUSSION AND CONCLUSION

This study was designed to examine excretion patterns and rates following a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice and rats.

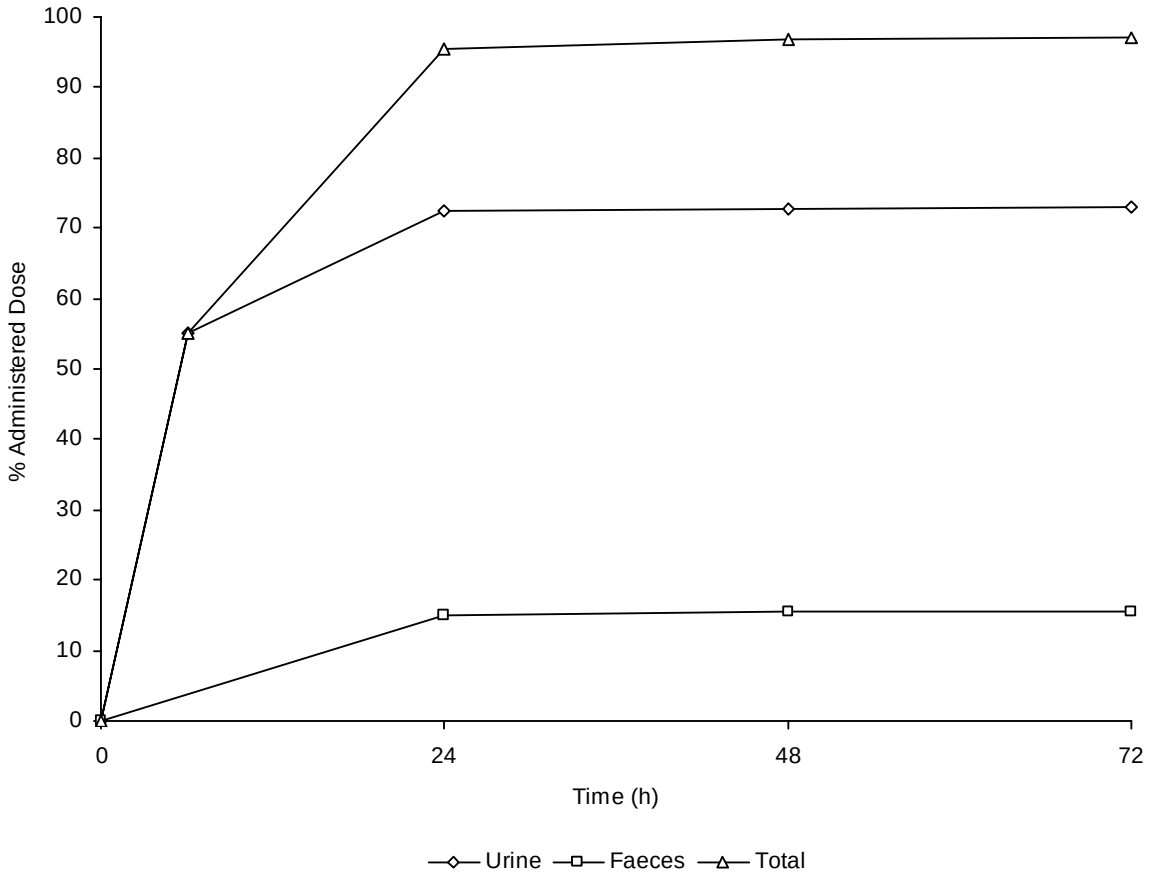
Irrespective of sex or species, following a single oral administration, total radioactivity excretion was rapid, with mean recoveries of over 90% of the dose at 24 h post dose. The major route of elimination was *via* the urine (means of 73.0-90.2% of the dose), followed by the faeces (mean of 7.0-15.5%). Elimination *via* expired air was negligible.

At 72 hours post dose in rats, mean recoveries of total radioactivity were 97.4 and 100.8% in males and females respectively, with approximately 0.2% remaining in the gastrointestinal tract and carcass.

At 72 hours post dose in mice, mean recoveries of total radioactivity were 95.4 and 97.3% in males and females respectively, with approximately 0.6-0.9% remaining in the gastrointestinal tract and carcass.

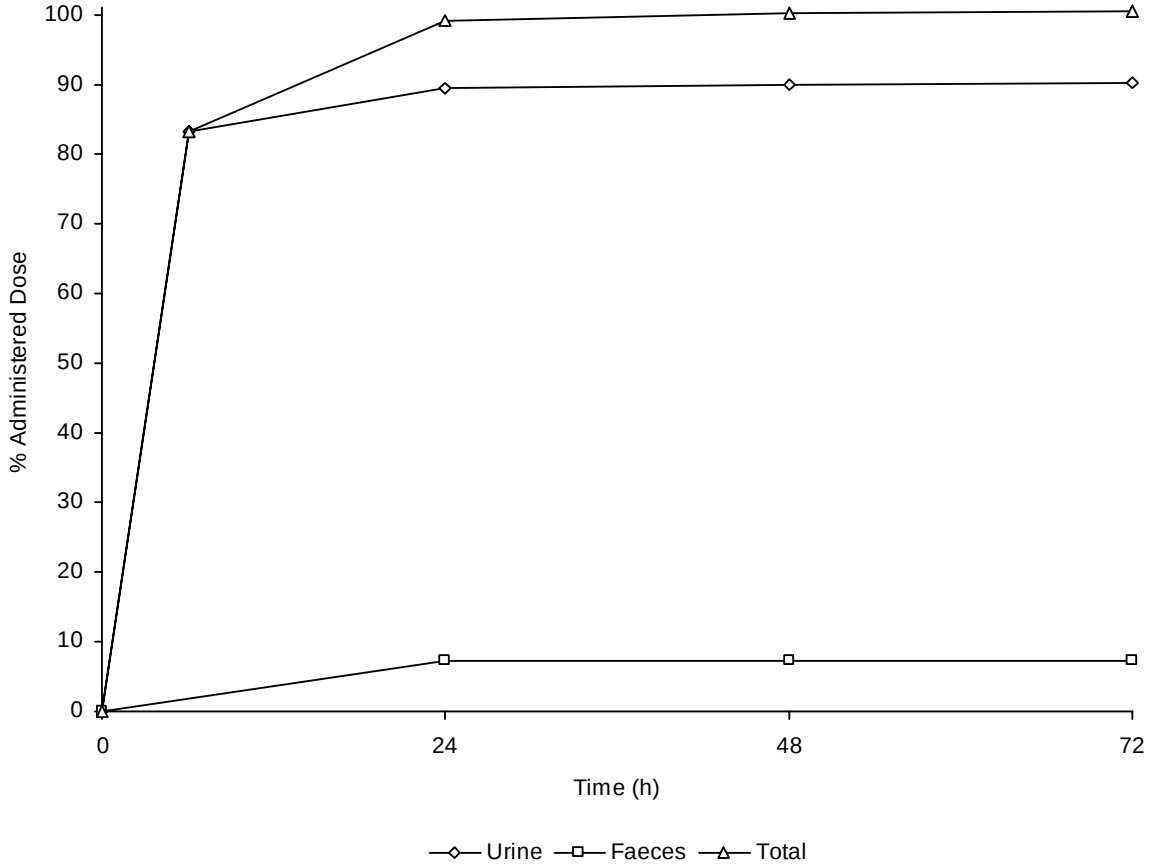
10 FIGURES

Figure 1 **Mean Cumulative Excretion of Total Radioactivity Following a Single Oral administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Target Dose level of 50 mg/kg**



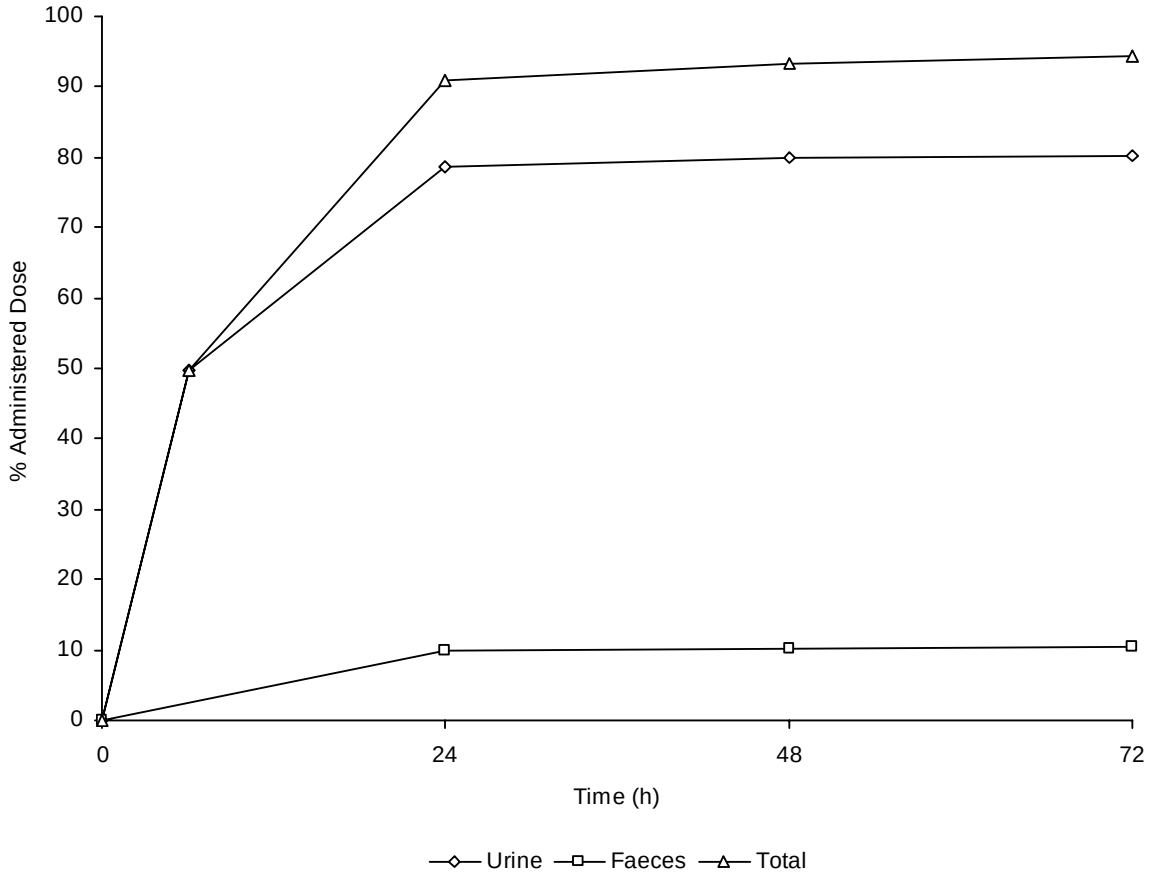
Total includes cagewash and expired air

Figure 2 **Mean Cumulative Excretion of Total Radioactivity Following a Single Oral administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Target Dose level of 50 mg/kg**



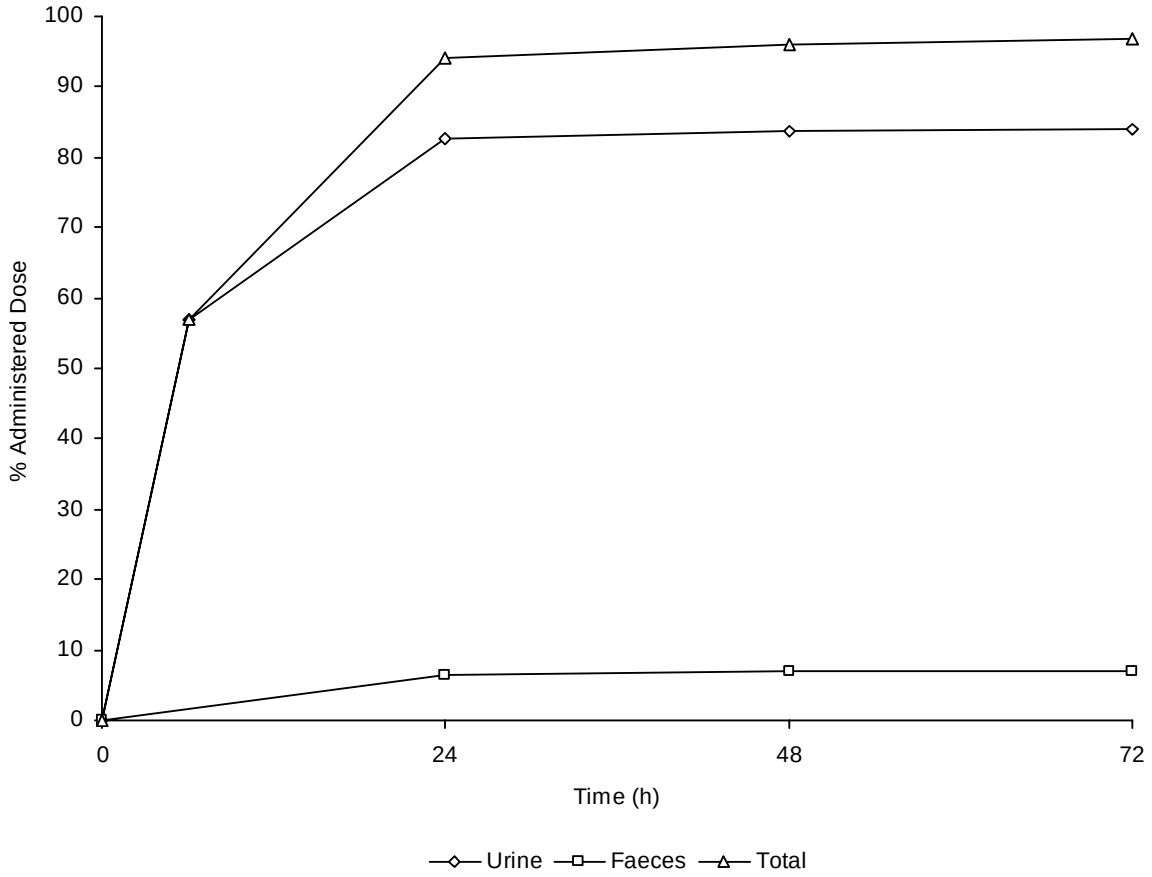
Total includes cagewash and expired air

Figure 3 **Mean Cumulative Excretion of Total Radioactivity Following a Single Oral administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Target Dose level of 50 mg/kg**



Total includes cagewash and expired air

Figure 4 **Mean Cumulative Excretion of Total Radioactivity Following a Single Oral administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Target Dose level of 50 mg/kg**



Total includes cagewash and expired air

11 TABLES

Table 1 Recovery of Total Radioactivity Following a Single Oral Dose of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Target Dose level of 50 mg/kg

Results expressed as % administered dose

Sample	Timepoint	001M	002M	Mean	SD
Urine	6 h	52.53	57.41	54.97	N.A.
	24 h	19.85	14.91	17.38	N.A.
	48 h	0.48	0.37	0.43	N.A.
	72 h	0.11	0.23	0.17	N.A.
Subtotal		72.98	72.92	72.95	N.A.
Faeces	24 h	22.30	7.38	14.84	N.A.
	48 h	1.12	0.17	0.64	N.A.
	72 h	0.02	0.01	0.01	N.A.
Subtotal		23.43	7.55	15.49	N.A.
Cage Wash	24 h	3.27	13.39	8.33	N.A.
	48 h	0.24	0.21	0.22	N.A.
	72 h	0.15	0.10	0.12	N.A.
Subtotal		3.65	13.70	8.67	N.A.
Exp Air-1	24 h	0.05	0.03	0.04	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.05	0.03	0.04	N.A.
Exp Air-2	24 h	*0.01	*0.01	º0.01	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.01	0.01	0.01	N.A.
G.I. Tract	72 h	*0.00	0.00	º0.00	N.A.
Carcass	72 h	*0.20	0.25	º0.22	N.A.
Total		100.31	94.47	97.39	N.A.

Table 2 **Recovery of Total Radioactivity Following a Single Oral Dose of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	003F	004F	Mean	SD
Urine	6 h	85.33	80.76	83.05	N.A.
	24 h	6.07	6.57	6.32	N.A.
	48 h	0.40	0.72	0.56	N.A.
	72 h	0.31	0.17	0.24	N.A.
Subtotal		92.11	88.23	90.17	N.A.
Faeces	24 h	5.45	9.08	7.27	N.A.
	48 h	0.07	0.07	0.07	N.A.
	72 h	*0.01	*0.00	º0.01	N.A.
Subtotal		5.53	9.15	7.34	N.A.
Cage Wash	24 h	2.36	2.72	2.54	N.A.
	48 h	0.44	0.21	0.32	N.A.
	72 h	0.22	0.08	0.15	N.A.
Subtotal		3.02	3.01	3.02	N.A.
Exp Air-1	24 h	*0.03	0.04	º0.03	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.03	0.05	0.04	N.A.
Exp Air-2	24 h	*0.01	*0.01	º0.01	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.01	0.01	0.01	N.A.
G.I. Tract	72 h	*0.00	*0.00	º0.00	N.A.
Carcass	72 h	*0.18	*0.18	º0.18	N.A.
Total		100.90	100.63	100.76	N.A.

Table 3 **Recovery of Total Radioactivity Following a Single Oral Dose of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	005M	006M	Mean	SD
Urine	6 h	55.93	43.65	49.79	N.A.
	24 h	18.24	39.29	28.76	N.A.
	48 h	1.43	1.38	1.40	N.A.
	72 h	0.16	0.60	0.38	N.A.
Subtotal		75.76	84.92	80.34	N.A.
Faeces	24 h	13.88	5.77	9.82	N.A.
	48 h	0.51	0.11	0.31	N.A.
	72 h	0.56	0.12	0.34	N.A.
Subtotal		14.95	6.00	10.48	N.A.
Cage Wash	24 h	1.06	3.82	2.44	N.A.
	48 h	0.65	0.67	0.66	N.A.
	72 h	0.48	0.48	0.48	N.A.
Subtotal		2.18	4.97	3.58	N.A.
Exp Air-1	24 h	*0.05	0.08	º0.07	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.05	0.08	0.07	N.A.
Exp Air-2	24 h	*0.01	*0.01	º0.01	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.01	0.01	0.01	N.A.
G.I. Tract	72 h	0.02	0.19	0.11	N.A.
Carcass	72 h	0.66	0.97	0.81	N.A.
Total		93.63	97.14	95.38	N.A.

Table 4 **Recovery of Total Radioactivity Following a Single Oral Dose of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Target Dose level of 50 mg/kg**

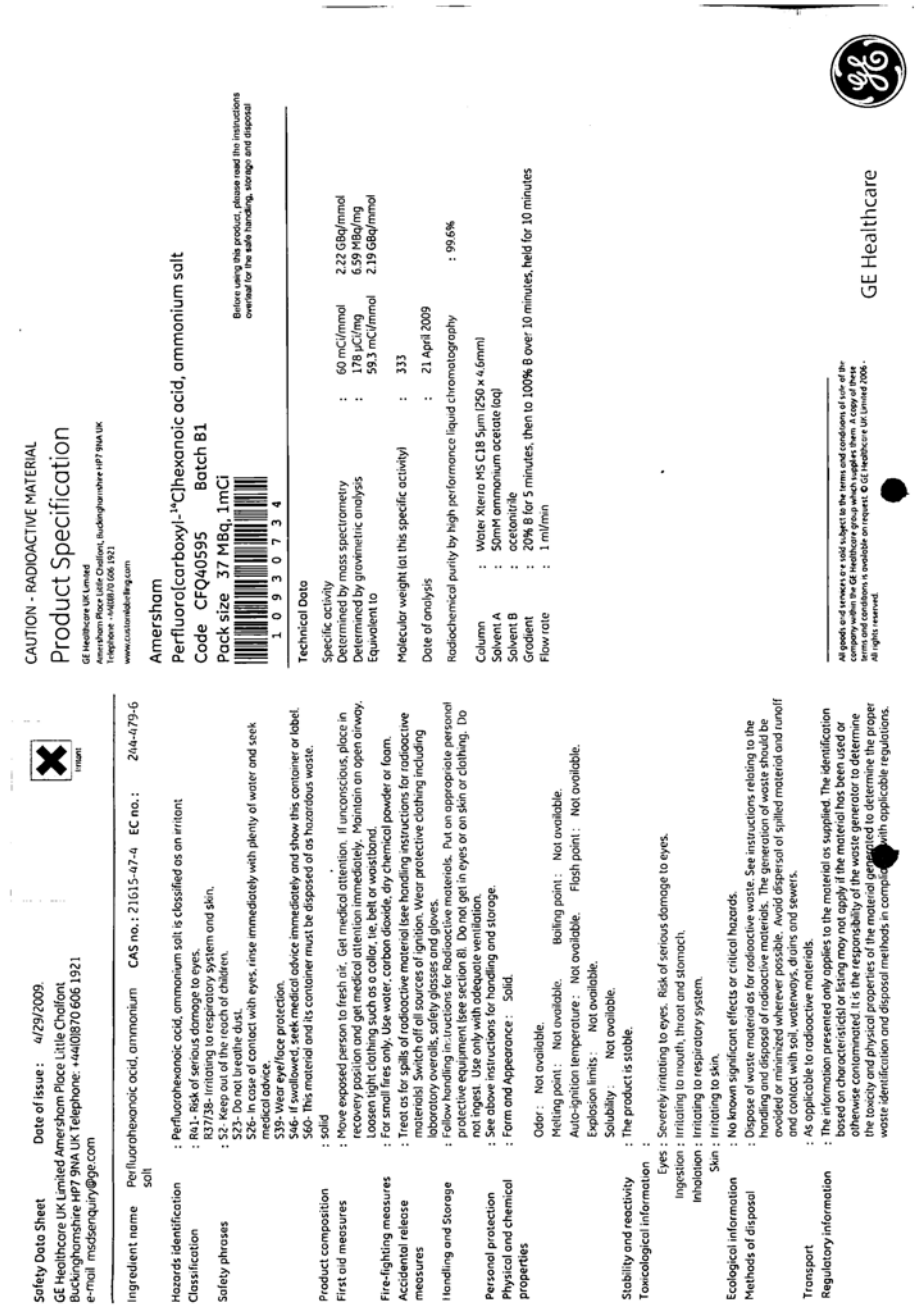
Results expressed as % administered dose

Sample	Timepoint	007F	008F	Mean	SD
Urine	6 h	56.56	57.40	56.98	N.A.
	24 h	25.69	25.46	25.58	N.A.
	48 h	1.20	1.30	1.25	N.A.
	72 h	0.34	N.S.	0.34	N.A.
Subtotal		83.80	84.17	83.98	N.A.
Faeces	24 h	4.84	8.18	6.51	N.A.
	48 h	0.78	0.07	0.43	N.A.
	72 h	0.14	N.S.	0.14	N.A.
Subtotal		5.76	8.25	7.01	N.A.
Cage Wash	24 h	5.76	4.04	4.90	N.A.
	48 h	0.42	0.37	0.40	N.A.
	72 h	0.64	N.S.	0.64	N.A.
Subtotal		6.82	4.41	5.61	N.A.
Exp Air-1	24 h	0.08	*0.06	º0.07	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.08	0.07	0.07	N.A.
Exp Air-2	24 h	*0.01	*0.01	º0.01	N.A.
	48 h	*0.00	*0.00	º0.00	N.A.
Subtotal		0.01	0.01	0.01	N.A.
G.I. Tract	[A]72 h	0.05	0.26	0.15	N.A.
Carcass	[A]72 h	0.52	0.45	0.49	N.A.
Total		97.03	97.61	97.32	N.A.

[A]: Animal 008F sacrificed at 48h post dose due to poor health, refer to section 7.3

12 APPENDICES

Appendix 1 Certificate of Analysis of [^{14}C]-Ammonium Perfluorohexanoate



CAUTION - RADIOACTIVE MATERIAL

Product Specification

GE Healthcare UK Limited
Amersham Place Little Chalfont, Buckinghamshire HP7 9NA UK
Telephone +44(0)870 606 1921
www.customisation.ge.com

Amersham

Perfluoro[carboxyl-^{14}C]hexanoic acid, ammonium salt

Code CFQ40595 Batch B1

Pack size 37 MBq, 1mCi

1 0 9 3 0 7 3 4

Before using this product, please read the instructions overleaf for the safe handling, storage and disposal

Technical Data

Specific activity		
Determined by mass spectrometry	: 60 mCi/mmol	2.22 GBq/mmol
Determined by gravimetric analysis	: 178 µCi/mg	6.59 MBq/mg
Equivalent to	: 59.3 mCi/mmol	2.19 GBq/mmol
Molecular weight (at this specific activity)	: 333	
Date of analysis	: 21 April 2009	
Radiochemical purity by high performance liquid chromatography	: 99.6%	
Column	: Waters Xterra MS C18 5µm (250 x 4.6mm)	
Solvent A	: 50mM ammonium acetate (aq)	
Solvent B	: acetonitrile	
Gradient	: 20% B for 5 minutes, then to 100% B over 10 minutes, held for 10 minutes	
Flow rate	: 1 ml/min	

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GE Healthcare

Safety Data Sheet Date of issue : 4/29/2009.
GE Healthcare UK Limited Amersham Place Little Chalfont
Buckinghamshire HP7 9NA UK Telephone: +44(0)870 606 1921
e-mail msdsenquiry@ge.com

Ingredient name	Perfluorohexanoic acid, ammonium salt CAS no.: 21615-47-4 EC no.: 244-479-6
Hazards identification	: Perfluorohexanoic acid, ammonium salt is classified as an irritant
Classification	: R41- Risk of serious damage to eyes. R37/38- Irritating to respiratory system and skin.
Safety phrases	: S2- Keep out of the reach of children. S23- Do not breathe dust. S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S39- Wear eye/face protection. S46- If swallowed, seek medical advice immediately and show this container or label. S60- This material and its container must be disposed of as hazardous waste.
Product composition	: solid
First aid measures	: Move exposed person to fresh air. Get medical attention. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.
Fire-fighting measures	: For small fires only. Use water, carbon dioxide, dry chemical powder or foam.
Accidental release measures	: Treat as for spills of radioactive material (see handling instructions for radioactive materials). Switch off all sources of ignition. Wear protective clothing including laboratory overalls, safety glasses and gloves.
Handling and Storage	: Follow handling instructions for Radioactive materials. Put on appropriate personal protective equipment (see section 8). Do not get in eyes or on skin or clothing. Do not ingest. Use only with adequate ventilation.
Personal protection	: See above instructions for handling and storage.
Physical and chemical properties	: Form and Appearance : Solid. Odor : Not available. Melting point : Not available. Boiling point : Not available. Auto-ignition temperature : Not available. Flash point : Not available. Explosion limits : Not available. Solubility : Not available.
Stability and reactivity	: The product is stable.
Toxicological information	: Eyes : Severely irritating to eyes. Risk of serious damage to eyes. Ingestion : Irritating to mouth, throat and stomach. Inhalation : Irritating to respiratory system. Skin : Irritating to skin.
Ecological information	: No known significant effects or critical hazards.
Methods of disposal	: Dispose of waste material as for radioactive waste. See instructions relating to the handling and disposal of radioactive materials. The generation of waste should be avoided or minimized wherever possible. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.
Transport	: As applicable to radioactive materials.
Regulatory information	: The information presented only applies to the material as supplied. The identification based on characteristics or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Appendix 1 (Continued) Certificate of Analysis of [^{14}C]-Ammonium Perfluorohexanoate

Chemical identity
The material co-chromatographs with customer supplied material in the chromatographic system overleaf.
The mass spectrum is consistent with the proposed structure and a non-labelled reference.

Packaging and storage of Perfluoro[carboxyl-^{14}C]hexanoic acid, ammonium salt
Perfluoro[carboxyl-^{14}C]hexanoic acid, ammonium salt is supplied as a solid in a borosilicate multidose vial with additional screw-cap ('Dimple vial').

Storage at -20°C in the absence of moisture, light and air is recommended.

Preparation of Perfluoro[carboxyl-^{14}C]hexanoic acid, ammonium salt
Manufactured to GE Healthcare Life Sciences procedures, which are certified to ISO9001:2000
Perfluoro[carboxyl-^{14}C]hexanoic acid, ammonium salt is prepared from barium ^{14}C-carbonate by a method developed by GE Healthcare.

Safety warnings and precautions
USE IN HUMANS - WARNING. This product is NOT suitable or intended for use in humans in the form in which it is supplied. Further modification, alteration, preparation and/or testing of this product by the user is required prior to use in applications involving humans, including any use in clinical trials, and is subject to an Investigational New Drug ('IND') application from the United States Food and Drug Administration ('FDA') and/or equivalent applications in other countries. Any such use of this product is the sole responsibility of the user, and the user must ensure compliance with all international, national and local regulations.

Caution: Radioactive material For professional users only

Instructions relating to the handling, use, storage and disposal of radioactive materials.

1 Upon receipt, vials or ampoules containing radioactive material should be checked for contamination. All radioactive materials should be stored in specially designated areas and suitable shielding should be used where appropriate. Access to these areas should be restricted to authorized personnel only.

2 Radioactive material should be used by responsible persons only in authorized areas. Care should be taken to prevent ingestion or contact with skin or clothing. Protective clothing, such as laboratory overalls, safety glasses and gloves should be worn whenever radioactive materials are handled. Where this is appropriate, the operator should wear personal dosimeters to measure radiation dose to the body and fingers.

3 No smoking, drinking or eating should be allowed in areas where radioactive materials are used. Avoid actions that could lead to the ingestion of radioactive materials, such as the pipetting of radioactive solutions by mouth.

4 Vials containing radioactive materials should not be touched by hand; wear suitable protective gloves as normal practice. Use forceps when handling vials containing 'hard' beta emitters such as phosphorus-32 or gamma emitting labelled compounds. Ampoules likely to contain volatile radioactive compounds should be opened only in a well ventilated fume cabinet.

5 Work should be carried out on a surface covered with absorbent material or in enamel trays of sufficient capacity to contain any spillage. Working areas should be monitored regularly.

6 Any spills of radioactive material should be cleaned immediately and all contaminated materials should be decontaminated or disposed of as radioactive waste via an authorized route. Contaminated surfaces should be washed with a suitable detergent to remove traces of radioactivity.

7 After use, all unused radioactive materials should be stored in specifically designated areas. Any radioactive product not required or any materials that have come into contact with radioactivity should be disposed of as radioactive waste via an authorized route.

8 Hands should be washed after using radioactive materials. Hands and clothing should be monitored before leaving the designated area, using appropriate instruments to ensure that no contamination has occurred. If radioactive contamination is detected, hands should be washed again and rechecked. Any contamination persisting on hands and clothing should be reported to the responsible person so that suitable remedial actions can be taken.

9 Certain national/international organisations and agencies consider it appropriate to have additional controls during pregnancy. Users should check local regulations. Most countries have legislation governing the handling, use, storage, disposal and transportation of radioactive materials. The instructions set out above complement local regulations or codes of practice. Such regulations may require that a person be nominated to oversee radiological protection. Users of radioactive products must make themselves aware of and observe local regulations or codes of practice which relate to such matters.

CAUTION - Substance not yet fully tested.
The full chemical and toxicological properties of this compound are unknown to GE Healthcare.
The safety precautions given above will generally provide adequate protection from any non-radioactive hazards associated with this material in the form and quantity supplied.

The users of this product should also refer to any information they have available on the properties and hazards of this product.

Appendix 2 Certificate of Analysis of Ammonium Perfluorohexanoate

DAIKIN

Certificate of Analysis

Daikin Industries,LTD.

Name of Sample *PFH Ammonium Salt (C-1500N)*
Lot. *7005*
Date of Analysis *May 14, 2009*
Purify *47.4% (Effective component in Water)*
**50.8*0.934%=47.4%*

COMPOSITION

identity		Conc.
#1	Ammonium Perfluorohexanoate CAS RN. 21615-47-4	93.4%
#2	Unknown	6.6%
	Total	100%

Analysis system (HPLC)
Equipment : Waters Alliance2695
Detector : Waters 2487UV
Detection wavelength : 210nm
Analysis condition
Column : TOSOH TSKGel ODS120T 4.6mm×150mm
Temp. : 40 ℃
Mobile phase : A=acetonitrile 、 B=Solution of 0.6% perchloric acid in water
Gradient : A:B=50:50(mass%) (0-10min.) →90:10(mass%) (15-20min.)
Injection volume : 20μL
Injection Concentration : 1% (dilute 50times with water)

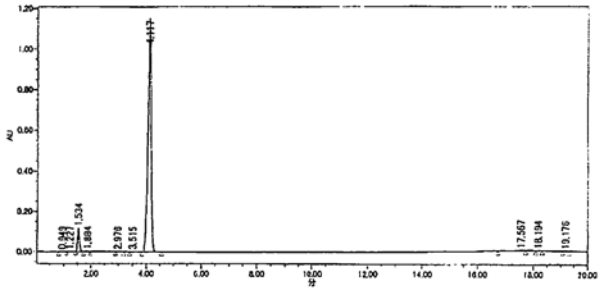
Chemical R&D Center
Unidyne Group
Senior Researcher

SIGNATURE
DATE : May 18, 2009

Appendix 2 Certificate of Analysis of Ammonium Perfluorohexanoate (Continued)

Analysis

サンプル名:	C1500N	分析担当者:	System
サンプルの種類:	未知試料	分析日:	2009/05/14 11:49:44
バイアル:	82	取り込みメソッドセット:	090514S
注入#:	1	解析日:	2009/05/14 12:55:17
注入量:	20.00 µl	解析メソッド:	C1500N
分析時間:	20.00 分	チャンネル名:	2487チャンネル 1
サンプルセット名:		解析チャンネルの説明:	



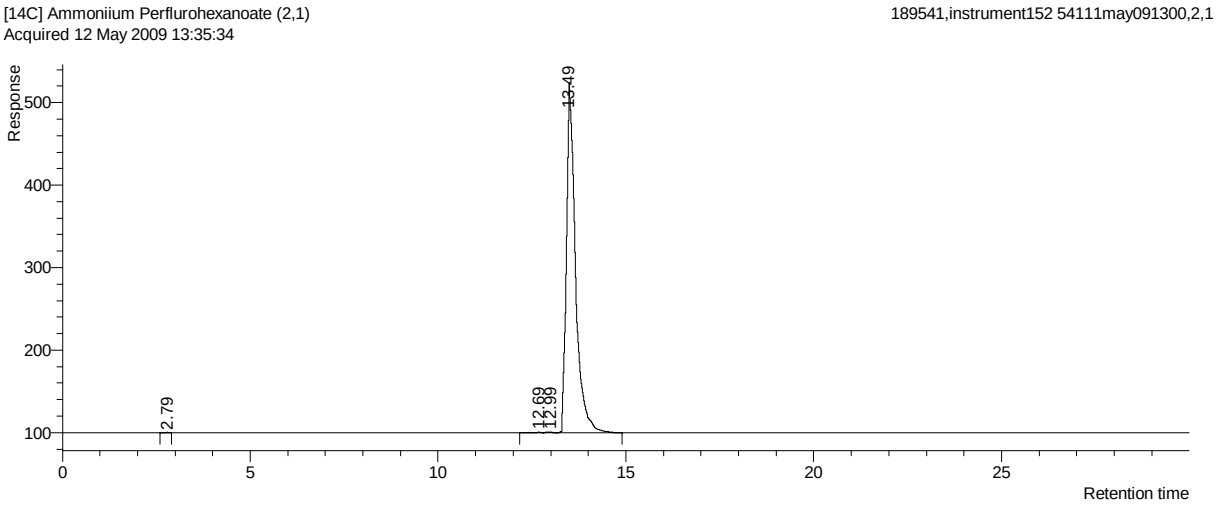
	成分名	Retention Time(min.)	Area (µVsec.)	Area (%)	Height (µV)		
1		0.949	17634	0.18	2554		
2		1.227	20551	0.20	1927		
3		1.534	574060	5.71	116134		
4		1.884	5543	0.06	710		
5		2.976	2424	0.02	414		
6		3.515	4940	0.05	361		
7		4.117	9390042	93.38	1144218		
8		17.567	29475	0.29	984		
9		18.194	6956	0.07	1088		
10		19.176	3881	0.04	592		

1/1 ページ

Appendix 3 Dosing Data for the Administration of [^{14}C]-Ammonium Perfluorohexanoate to Rats and Mice

Phase	Animal Number	Animal Weight (g)	Dose Recieved			
			MBq	mg	mg /kg	MBq/kg
Male Rat	001M	253	0.776	12.03	47.56	3.07
	002M	235	0.745	11.56	49.18	3.17
Female Rat	003F	174	0.532	8.26	47.45	3.06
	004F	172	0.535	8.30	48.25	3.11
Male Mice	005M	36	0.184	1.92	53.46	5.11
	006M	35	0.177	1.85	52.88	5.05
Female Mice	007F	26	0.138	1.44	55.39	5.29
	008F	26	0.138	1.45	55.61	5.31

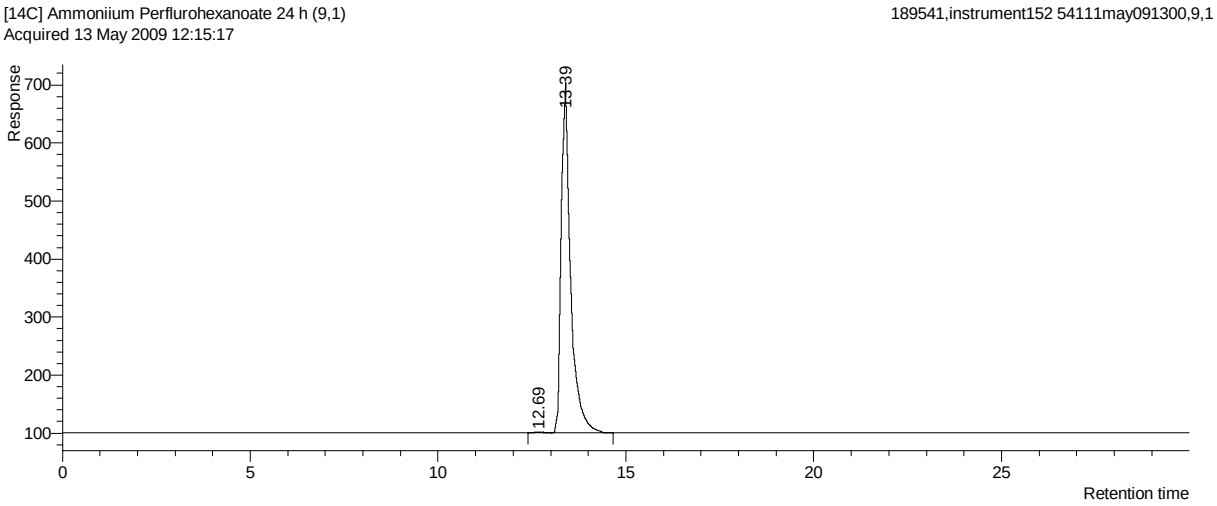
Appendix 4 Representative Radio-HPLC Chromatogram for the Radiochemical Purity of [^{14}C]-Ammonium Perfluorohexanoate



Peak No.	Retention Time (min)	Peak Name	% Area
1	2.79	-	0.1
2	12.69	-	0.2
3	12.99		0.2
4	13.49	[^{14}C]- Ammonium Perfluorohexanoate*	99.6

* = Assigned by co-chromatography with unlabelled Ammonium Perfluorohexanoate

Appendix 5 Representative Radio-HPLC Chromatogram for the Radiochemical Purity of [^{14}C]-Ammonium Perfluorohexanoate in the Formulation



Peak No.	Retention Time (min)	Peak Name	% Area
1	12.69	-	0.2
2	13.39	[^{14}C]- Ammonium Perfluorohexanoate*	99.8

* = Assigned by co-chromatography with unlabelled Ammonium Perfluorohexanoate