CONFIDENTIAL

FINAL REPORT

Test Facility Study No. 190300, Report No. 30572

The Excretion and Tissue Distribution of [^{14}C]-Ammonium Perflourohexanoate in the Mouse and the Rat Following a Multiple Oral Administration of 50 mg/kg

Report Amendment 1

DATA REQUIREMENTS:
OTTPS 870.7485

TEST FACILITY:
Charles River
Tranent
Edinburgh
EH33 2NE
UK

SPONSOR:
Daikin Industries Limited
Umeda Center Building
4-12 Nakazaki-Nishi 2-chrome,
Kita-ku, Osaka
Japan

STUDY COMPLETION DATE
27 November 2009

REPORT ISSUE DATES
Date of Original report completion: 27 November 2009
Date of Report Amendment 1 completion: 04 December 2009

1 STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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2 COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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 amendment represents a true and accurate record of the results obtained.

_____ Date: 04 DECEMBER 2009

Study Director
Charles River

The compliance statement in the original final report was signed on 27 November 2009

_____ Date: 08 DEC 2009

Sponsor

Daikin Industries, LTD.

_____ Date: _____

Submitter

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3 REASON FOR ISSUE OF AMENDMENT

This report was re-issued due to formatting errors present in the original final report. The page numbering was incorrect and has therefore been updated in agreement with the Sponsor.

As a result of the above amendment, the report pagination and section numbering has changed from that in the original report. This page has been added, the total number of pages has been altered, and there have been corresponding changes in the Table of Contents.

A new Compliance Statement (page 3) and Quality Assurance Statement (page 11) are also included, as required for the issue of this report amendment. Report issue dates have also been included on Page 1 of the report for clarification purposes.

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4 QUALITY ASSURANCE STATEMENT

Test Facility Study No: 190300

Study Title: The Excretion and Tissue Distribution of [^{14}C]-Ammonium Perfluorohexanoate in the Mouse and the Rat Following a Multiple 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
23 February 2009	Protocol Amendment 1 Review	Not Applicable
25 March 2009	Protocol Amendment 2 Review	Not Applicable
05 May 2009	Protocol Amendment 3 Review	05 May 2009
25 June 2009	Dosing Preparation Review/ Dosing/Protocol Compliance	26 June 2009
08 July 2009	Dosing Preparation Review/ Dosing/Protocol Compliance	09 July 2009
07-11 September 2009	Draft Report Audit	11 September 2009
19 November 2009	Final Report Audit	19 November 2009
01 December 2009	Final Report Amendment 1 Audit	01 December 2009

* Protocol amendment reviews before 27 April 2009 were not reported to management if no observations were noted.

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.

_____ 04 December 2009
Date

Quality Assurance

The Quality Assurance statement in the original final report was signed on 27 November 2009

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5 RESPONSIBLE PERSONNEL

Study Director:

Report Compilation:

Scientific Staff:

Quality Assurance:

6 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 dissociates in the same situations to the same C6 ion, is an alternative to the acid, avoiding the possible issues of acid toxicity and allowing introduction of a significant amount of the ion.

The objective of this study was to examine excretion patterns and rates following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by 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.

Following multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by 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 80.7 and 77.8% of the dose in males and females, respectively. Faecal elimination accounted for 12.9 and 12.6% of the dose in males and females respectively. Excretion of the C6 ion was rapid, with means of 93.7 and 90.4% (equivalent to 98.5 and 96.5 % of the ultimately recovered material) recovered by 24 h post dose, in males and females, respectively.

At 168 h post dose, mean recoveries of total radioactivity were 95.1 and 93.7% of the administered dose for males and females, respectively. Excretion was almost complete with approximately 0.2% of the dose still remaining in the gastrointestinal tract and carcass.

Following multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by 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 81.1 and 83.4% of the dose in males and females, respectively. Faecal elimination accounted for 10.6 and 9.6% of the dose in males and females respectively. Excretion of total radioactivity was rapid with means of 93.5 and 92.2% (equivalent to 96.4 and 95.6 % of the ultimately recovered material) recovered by 24 h post dose, in males and females, respectively.

At 168 h post dose mean recoveries of total radioactivity were 97.0 and 96.4% of the administered dose for males and females, respectively. Excretion was almost complete with approximately 0.1% of the dose still remaining in the gastrointestinal tract and carcass.

At 168 h post dose in rats and mice, circulating radioactivity in most tissues were below blood concentrations, with the exception of liver.

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At 12 h post dose in rats, mean plasma concentrations were to 0.8 and 0.4 µg/mL in males and females, respectively. By 24 h post dose, mean plasma values decreased to 0.5 and 0.3 µg/mL in males and females, respectively.

At 12 h post dose in mice, mean plasma concentrations were to 1.3 and 1.0 µg/mL in males and females, respectively. By 24 h post dose, mean plasma values decreased to 1.0 and 0.5 µg/mL in males and females, respectively.

7 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 dissociates 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. 190300 and amendments 1-3 and the following timetable:

Study Initiation:	16 December 2008
Experimental Start Date	12 June 2009
Experimental Completion Date	21 September 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.

8 EXPERIMENTAL PROCEDURE

8.1 Test Item

Carbon 14 labelled Ammonium Perfluorohexanoate (Batch No. 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. LOT 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.

8.2 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.

8.3 Animals and Husbandry

Eight male and 8 female Sprague Dawley (Crl:CD(SD)) rats, age approximately 7-10 weeks at dosing (body weights 182-362 g), were supplied by Charles River (UK) Limited. Sixteen male and 17 female CD-1 mice, age approximately 7-10 weeks at dosing (body weights 23-34 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. Mice were housed in solid floored polypropylene and stainless steel caging.

During on-study periods, animals used for collection of excreta samples were housed singly in all glass metabolism cages specially designed for the separate, quantitative, collection of urine and faeces. Rats not used for collection of excreta samples were housed in pairs by sex in suitable polycarbonate and stainless steel caging with raised wire mesh floors. Male mice were housed singly and females in pairs in polypropylene and stainless steel caging with raised wire mesh floors.

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 4 hours before dosing and approximately 2 hours after dosing.

8.4 Radiochemical Purity

The radiochemical purity of [^{14}C]-Ammonium Perfluorohexanoate was assessed prior to dose preparation and prior to dosing. The stability was confirmed in a trial preparation under study number 189541 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) R1

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 (initial confirmation and predose) 254 nm (post dose)
Scintillant:	Flowlogic™

8.5 Dose Preparation

8.5.1 Dose Preparation: Non radiolabelled Formulation

An appropriate volume of Ammonium Perfluorohexanoate (10.55 mL, equivalent to 5000.23 mg of Ammonium Perfluorohexanoate) was added to a dose jar. The required volume (*ca* 990 mL) of sterile water was then added to the dose jar and mixed to give a solution. The final concentration was 5.02 mg/mL, with a final formulation weight of 995.41g.

8.5.2 Dose Preparation: Radiolabelled Formulation

An appropriate volume (1.74 mL, equivalent to 4.86 mg and 32.03 MBq) of [^{14}C]-Ammonium Perfluorohexanoate prepared stock solution was added into the dose jar. An appropriate volume of Ammonium Perfluorohexanoate (834 µl, equivalent to 395.32 mg of Ammonium Perfluorohexanoate) was then added to the dose jar. The required volume (*ca* 77.4 mL)of sterile water was then added and mixed to give a solution. The final concentration was 5.06 mg/mL and 0.440 MBq/mL, with a final formulation weight of 79.15 g.

8.5.3 Dose Preparation: Replacement animal 041F

For the unlabelled preparation an appropriate volume of Ammonium Perfluorohexanoate (105 µL, equivalent to 49.77 mg) was added to a dose jar. The required volume (*ca* 10 mL) of sterile water was then added to the dose jar and mixed to give a solution. The final concentration was 5.05 mg/mL, with a final formulation weight of 9.87g.

For the radioactive formulation an appropriate volume (189 µL, equivalent to 0.303 mg and 2 MBq) of [^{14}C]-Ammonium Perfluorohexanoate prepared stock solution was added into the dose jar. An appropriate volume of Ammonium Perfluorohexanoate (52 µl, equivalent to 24.65 mg) was then added to the dose jar. The required volume (*ca* 4.76 mL) of sterile water was then added and mixed to give a solution. The final concentration was 5.02 mg/mL and 0.434 MBq/mL, with a final formulation weight of 4.98 g.

8.6 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.

Animal 013F had to be prematurely terminated prior to the last dosing occasion due to poor wellbeing of the animal. At post mortem this was found to be a result of a ruptured oesophagus and not to be a result of the test item. Animal 037F from Phase 2 was used in its place for the purpose of mass balance collections. A replacement mouse (041F) was dosed in order to obtain a 24 h plasma sample and meet the requirements of the protocol.

The dose received by each animal is presented in Appendices 3-6.

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8.7 Sample Collection

Eight male and 8 female rats, and twelve male and 13 female mice each received a daily oral administration of Ammonium Perfluorohexanoate for 13 consecutive days followed by a single oral dose of [^{14}C]-Ammonium Perfluorohexanoate (Day 14), all at a target daily dose level of 50 mg/kg.

8.7.1 Phase 1

After the last dose administration (Day 14), urine samples were collected into containers cooled by solid carbon dioxide from each animal for the periods 0-6, 6-24 then at 24 h intervals to 168 h post dose.

Faeces samples were collected into containers cooled by solid carbon dioxide at 24 h intervals to 168 h post dose.

Cages were washed with water at the time of each faeces collection.

At the end of the 168 h collection period, each animal was humanely killed by CO_2 narcosis. A terminal blood sample was taken (approximately 5-10 mL for rats and 0.5-1 mL for mice) from the *vena cava* and the heart, respectively, into heparinised tubes. The gastrointestinal tract, selected tissues and residual carcass from each animal were retained.

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

8.7.2 Phase 2

All animals were humanely killed by CO_2 narcosis

Serial blood samples were taken from the rats (approximately 0.4 mL) from the tail vein predose and at 12 h post dose. A terminal blood sample was taken (approximately 5-10 mL) from the vena cava at 24 h post dose. All blood samples were collected into heparinised tubes.

Terminal blood samples were taken from the mice (approximately 0.5-1 mL) from the heart predose (taken from 4 male and 4 female control undosed mice) at 12 and 24 h post dose. All blood samples were collected into heparinised tubes.

Plasma was separated from all blood samples by centrifugation. The levels of total radioactivity were determined in each sample collected.

8.8 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.

8.9 Preparation of Samples For Total Radioactivity Analysis

8.9.1 Liquid Samples

Duplicate aliquots of liquid samples were made up to 1 mL with water (if necessary) and mixed with scintillation fluid.

Duplicate aliquots of each blood sample were combusted using a PerkinElmer 307 Sample Oxidiser

8.9.2 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* 0.2-0.3g) were combusted using a PerkinElmer 307 Sample Oxidiser. Carcass samples were minced, then analysed as described for faeces. All gastrointestinal tract and tissue samples were finely scissor chopped, then analysed as described for faeces.

All aliquots were combusted using a PerkinElmer 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%.

8.10 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. At the specific activity used, the limit of reliable measurement of *ca* 0.06 µg equiv/g for tissue and blood weight of *ca* 0.1 g. The calculated limit of reliable measurement is 0.1 µg equiv/g for a mean plasma weight of *ca* 0.05 g. Where results have arisen from data below the limit of reliable determination, the fact is noted.

9 RESULTS

9.1 Radiochemical Purity and Stability

[^{14}C]-Ammonium Perfluorohexanoate was shown by chromatography with Ammonium Perfluorohexanoate to be authentic and 99.1% radiochemically pure. An example radiochromatogram is presented in Appendix 7.

The post dose radiochemical purity of [^{14}C]-Ammonium Perfluorohexanoate in the dose formulation was 99.2% pure. Due to technical difficulties, the predose radiochemical purity could not be determined. However, the post dose radiochromatogram was satisfactory and demonstrated that the test item was stable in the formulation over the dosing period. An example radiochromatogram of [^{14}C]-Ammonium Perfluorohexanoate in the dose formulation is presented in Appendix 8.

A further purity of the [^{14}C]-Ammonium Perfluorohexanoate stock was shown by chromatography with Ammonium Perfluorohexanoate to be authentic and 99.4% radiochemically pure. This purity was taken prior to dosing the replacement animal. The predose and post dose radiochemical purities of [^{14}C]-Ammonium Perfluorohexanoate in the dose formulation were 99.1 and 98.8% pure, respectively.

9.2 Excretion Kinetics Following Oral Administration to Male and Female Rats

The excretion of total radioactivity following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by 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 the radiolabelled dose administration, the major route of elimination of radioactivity was *via* the urine with means of 80.7 and 77.8% of the dose in males and female respectively. Faecal elimination accounted for 12.9% in males and 12.6% in females. Excretion of total radioactivity was rapid with means of 93.7 and 90.4% recovered by 24 hours post dose (equivalent to 98.5 and 96.5 % of the ultimately recovered material).

By 168 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 95.1 and 93.7% of the dose administered in males and females respectively.

9.3 Excretion Kinetics Following Oral Administration to Male and Female Mice

The excretion of total radioactivity following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by 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.

Following the radiolabelled dose administration, the major route of elimination was *via* the urine with means of 81.1 and 83.4% of the dose in males and female respectively. Faecal elimination accounted for 10.6% in males and 9.6% in females. Excretion of total radioactivity was rapid with means of 93.5 and 92.2% recovered by 24 hours post dose (equivalent to 96.4 and 95.6 % of the ultimately recovered material).

By 168 h post dose, approximately 0.1% 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 97.0 and 96.4% of the dose administered in males and females respectively.

9.4 Tissue Concentrations Following Oral Administration to Male and Female Rats

Individual and mean group tissue concentrations of radioactivity following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female rats are presented in Tables 5-6.

At 168 h post dose, the mean blood concentrations of radioactivity were 0.15 and 0.16 µg equiv/g, in males and females, respectively. The only tissue concentration above circulating blood level was noted in the liver, with values of 1.16 and 0.85 µg equiv/g in males and females, respectively. All other tissues were lower than the blood level or below the limit of quantification, with mean concentrations above the limit of quantification ranging from 0.10-0.13 µg equiv/g.

9.5 Tissue Concentrations Following Oral Administration to Male and Female Mice

Individual and mean group tissue concentrations of radioactivity following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice are presented in Tables 7-8.

At 168 h post dose, the mean blood concentrations of radioactivity were 0.17 µg equiv/g, in both males and females. The only tissue concentration above circulating blood level was noted in the liver, with values of 0.70 and 0.61 µg equiv/g in males and females, respectively. All other tissues were lower than the circulating blood level and below the limit of quantification.

9.6 Plasma Kinetics Following Oral Administration to Male and Female Rats

Individual animal data and mean plasma concentrations are tabulated in Tables 9-10 for males and females respectively.

Following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female rats, at the 2 timepoints examined, the mean concentrations of radioactivity in plasma at 12 h post dose were 0.8 and 0.4 µg/mL in males and females respectively. Thereafter, plasma concentrations declined to 0.5 and 0.3 µg/mL in males and females, respectively at 24 h post dose.

Analysis of the predose plasma confirmed the radioactive levels were at background level.

9.7 Plasma Kinetics Following Oral Administration to Male and Female Mice

Individual animal data and mean plasma concentrations are tabulated in Tables 11-12 for males and females respectively.

Following a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female mice, the mean concentrations of radioactivity in plasma at 12 h post dose were 1.3 and 1.0 µg/mL in males and females respectively. Thereafter, mean plasma concentrations declined to 1.0 and 0.5 µg/mL in males and females, respectively at 24 h post radiolabelled dose.

Analysis of the predose (control mice) plasma confirmed the radioactive levels were at background level.

10 DISCUSSION AND CONCLUSION

This study was designed to examine excretion patterns and rates following multiple (13 daily doses) oral administrations of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate to male and female rats and mice.

Irrespective of sex or species, a multiple (13 daily doses) oral administration of Ammonium Perfluorohexanoate followed by a single oral administration of [^{14}C]-Ammonium Perfluorohexanoate, total radioactivity excretion was rapid, with mean recoveries of over 90% of the dose administered (and with mean values >95% of the ultimately recovered material) at 24 h post dose. The major route of elimination was *via* the urine (means of 77.8-83.4% of the dose), followed by the faeces (mean of 9.6-12.9%), indicating that the majority of the administered dose had been absorbed.

At 168 h post dose in rats, mean recoveries of total radioactivity were 95.1 and 93.7% in males and females respectively, indicating that the dose was almost completely excreted, with only approximately 0.2% remaining in the gastrointestinal tract and carcass.

At 168 h post dose in mice, mean recoveries of total radioactivity were 97.0 and 96.4% in males and females respectively, indicating that the dose was almost completely excreted, with only approximately 0.1% remaining in the gastrointestinal tract and carcass.

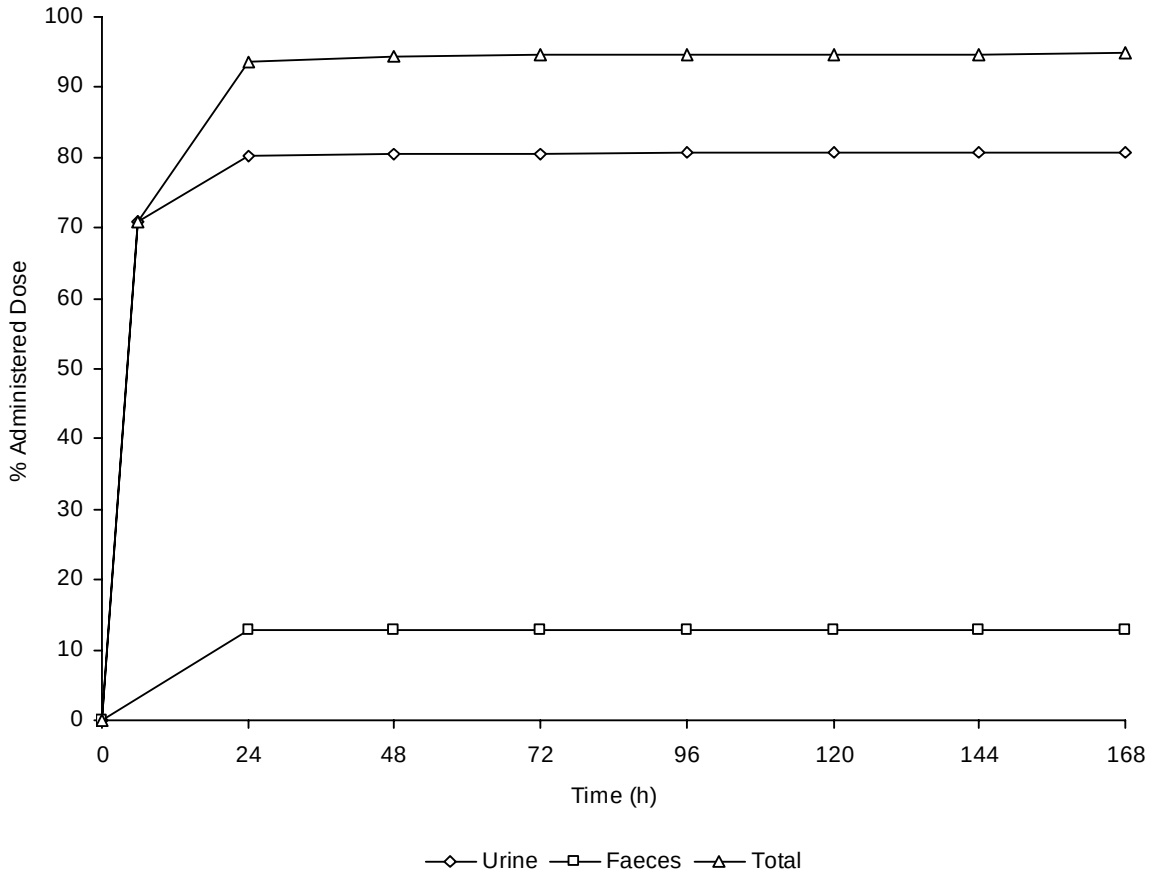
At 168 h post dose in rats and mice, radioactivity was generally very low or below the limit of detection in most tissues. Tissue concentrations were below blood concentrations with the exception of liver, which was approximately 4-8 times higher than the circulating blood level. Elevated levels of measurable radioactivity in the liver are consistent with its role in metabolism and excretion.

At 12 h post dose in rats, mean plasma concentrations were to 0.8 and 0.4 µg/mL in males and females, respectively. By 24 h post dose, mean plasma values decreased to 0.5 and 0.3 µg/mL in males and females, respectively.

At 12 h post dose in mice, mean plasma concentrations were to 1.3 and 1.0 µg/mL in males and females, respectively. By 24 h post dose, mean plasma values decreased to 1.0 and 0.5 µg/mL in males and females, respectively.

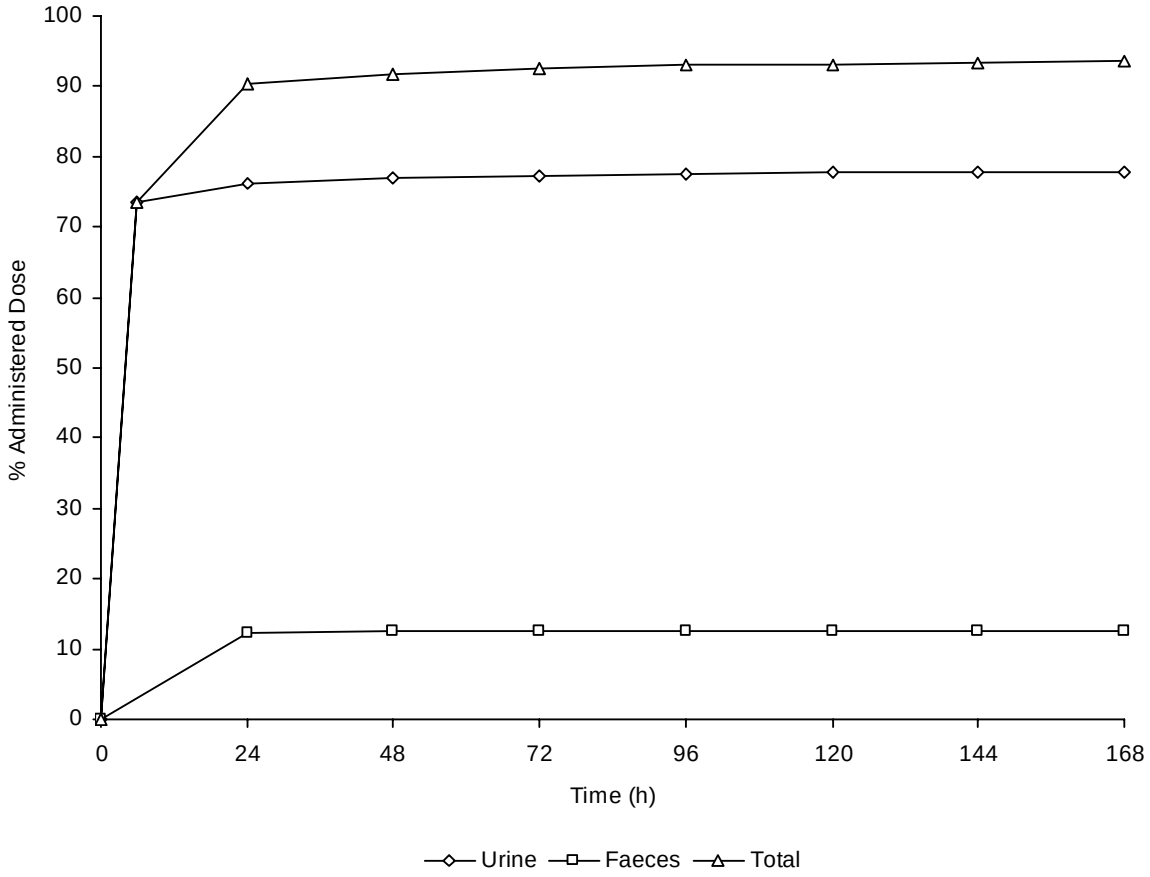
11 FIGURES

Figure 1 **Mean Cumulative Excretion of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Daily Target Dose level of 50 mg/kg**



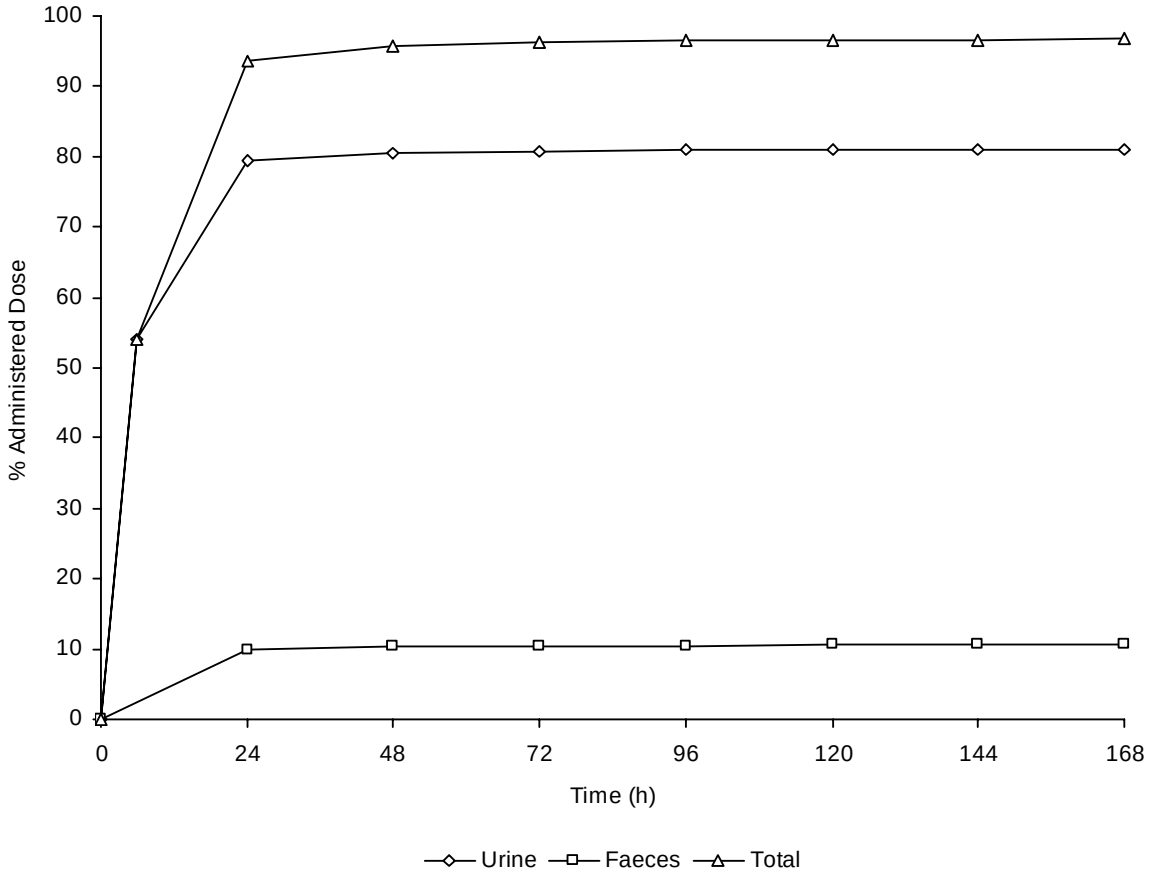
Total includes cagewash

Figure 2 **Mean Cumulative Excretion of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Daily Target Dose level of 50 mg/kg**



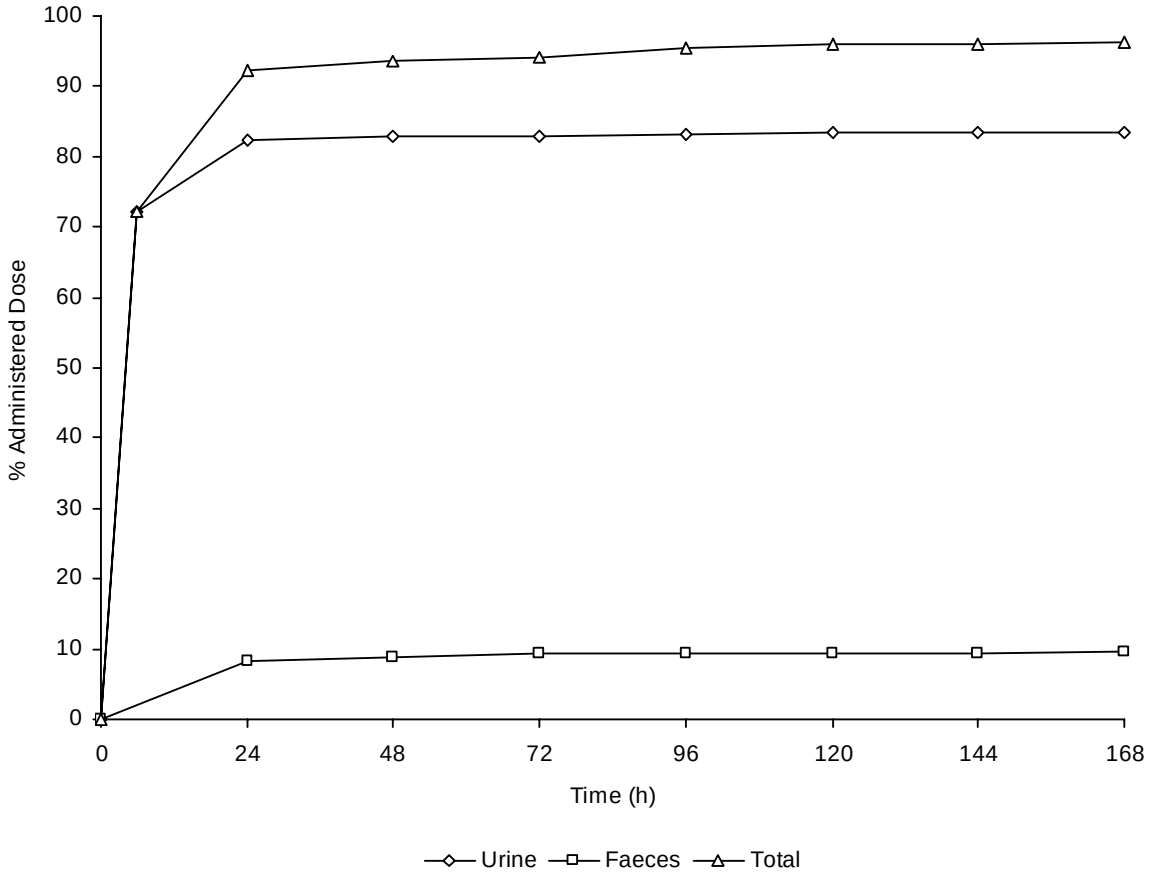
Total includes cagewash

Figure 3 **Mean Cumulative Excretion of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Daily Target Dose level of 50 mg/kg**



Total includes cagewash

Figure 4 **Mean Cumulative Excretion of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Daily Target Dose level of 50 mg/kg**



Total includes cagewash

12 TABLES

Table 1 **Recovery of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	001M	002M	003M	004M	Mean	SD
Urine	6 h	66.69	66.55	68.75	81.78	70.94	7.30
	24 h	6.64	9.30	14.00	6.90	9.21	3.41
	48 h	0.36	0.18	0.38	0.44	0.34	0.11
	72 h	0.16	0.04	0.12	0.01	0.08	0.07
	96 h	0.09	0.03	0.07	0.11	0.08	0.03
	120 h	0.04	0.02	0.04	0.11	0.05	0.04
	144 h	0.03	0.01	0.03	0.03	0.02	0.01
	168 h	0.02	0.01	0.02	0.02	0.02	0.01
Subtotal		74.03	76.13	83.40	89.40	80.74	7.03
Faeces	24 h	22.70	13.42	11.58	3.22	12.73	7.99
	48 h	0.03	0.07	0.24	0.10	0.11	0.09
	72 h	0.01	0.01	0.09	0.11	0.06	0.06
	96 h	*0.00	*0.00	0.05	0.02	º0.02	º0.02
	120 h	*0.00	0.00	0.01	0.01	º0.01	º0.00
	144 h	0.01	*0.00	0.00	0.02	º0.01	º0.01
	168 h	*0.00	*0.00	*0.01	0.01	º0.00	º0.00
Subtotal		22.75	13.50	11.98	3.49	12.93	7.89
Cage Wash	24 h	1.07	0.78	0.60	0.69	0.78	0.20
	48 h	0.08	0.06	0.17	0.48	0.20	0.19
	72 h	0.03	*0.02	0.06	0.20	º0.08	º0.08
	96 h	*0.01	*0.00	0.03	0.07	º0.03	º0.03
	120 h	*0.01	*0.00	0.02	0.05	º0.02	º0.02
	144 h	*0.01	*0.00	*0.01	0.04	º0.01	º0.02
	168 h	*0.01	*0.00	*0.01	0.02	º0.01	º0.01
Subtotal		1.22	0.87	0.91	1.54	1.14	0.31
Tissues	168 h	0.09	0.10	0.12	0.12	0.11	0.01
G.I. Tract	168 h	*0.00	0.00	*0.01	*0.00	º0.00	º0.00
Carcass	168 h	0.15	0.14	0.13	0.25	0.16	0.05
Total		98.25	90.75	96.54	94.80	95.08	3.21

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 2 **Recovery of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	005F	006F	007F	008F	Mean	SD
Urine	6 h	75.58	74.05	62.28	81.83	73.44	8.17
	24 h	4.40	3.51	1.37	1.74	2.75	1.44
	48 h	1.50	0.69	0.63	0.53	0.84	0.45
	72 h	0.25	0.49	0.31	0.40	0.36	0.11
	96 h	0.17	0.19	0.22	0.23	0.20	0.03
	120 h	0.08	0.09	0.17	0.09	0.11	0.04
	144 h	0.04	0.04	0.06	0.06	0.05	0.01
	168 h	0.02	0.04	0.05	0.03	0.04	0.01
Subtotal		82.05	79.09	65.08	84.92	77.79	8.80
Faeces	24 h	9.48	11.04	23.59	5.46	12.39	7.82
	48 h	0.14	0.12	0.08	0.10	0.11	0.03
	72 h	0.02	0.07	0.03	0.05	0.04	0.03
	96 h	0.01	0.06	0.03	0.02	0.03	0.02
	120 h	0.01	0.01	0.02	0.02	0.01	0.00
	144 h	*0.00	*0.01	0.01	0.01	º0.01	º0.00
	168 h	*0.00	0.01	0.02	0.01	º0.01	º0.01
Subtotal		9.66	11.32	23.77	5.67	12.61	7.81
Cage Wash	24 h	1.86	2.01	1.95	1.48	1.82	0.24
	48 h	0.36	0.41	0.61	0.40	0.44	0.11
	72 h	0.18	0.31	0.25	0.36	0.27	0.08
	96 h	0.22	0.38	0.15	0.30	0.26	0.10
	120 h	0.06	0.10	0.09	0.09	0.08	0.01
	144 h	0.08	0.05	0.03	0.08	0.06	0.02
	168 h	0.15	0.20	0.05	0.08	0.12	0.07
Subtotal		2.90	3.45	3.13	2.78	3.07	0.30
Tissues	168 h	0.09	0.08	0.08	0.08	0.08	0.00
G.I. Tract	168 h	*0.00	0.01	0.01	*0.01	º0.01	º0.00
Carcass	168 h	*0.12	*0.13	0.29	*0.12	º0.17	º0.08
Total		94.82	94.08	92.35	93.59	93.71	1.03

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 3 **Recovery of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Daily Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	009M	010M	011M	012M	Mean	SD
Urine	6 h	70.44	65.29	33.11	46.96	53.95	17.16
	24 h	11.77	26.78	20.48	43.25	25.57	13.30
	48 h	0.99	0.66	0.98	1.34	0.99	0.28
	72 h	0.36	0.31	0.25	0.28	0.30	0.05
	96 h	0.20	0.13	0.18	0.15	0.17	0.03
	120 h	0.04	0.05	0.08	0.06	0.06	0.02
	144 h	0.04	0.02	0.04	0.05	0.04	0.01
	168 h	0.02	*0.02	0.05	0.03	º0.03	º0.02
Subtotal		83.86	93.25	55.18	92.12	81.10	17.78
Faeces	24 h	7.92	1.44	26.36	3.47	9.80	11.37
	48 h	0.23	0.33	1.67	0.07	0.58	0.74
	72 h	0.04	0.12	0.27	0.02	0.11	0.11
	96 h	0.05	*0.02	0.07	0.03	º0.04	º0.02
	120 h	0.05	*0.01	0.06	0.04	º0.04	º0.02
	144 h	*0.00	*0.01	0.07	*0.01	º0.02	º0.03
	168 h	0.03	*0.01	0.04	*0.01	º0.02	º0.02
Subtotal		8.33	1.93	28.53	3.65	10.61	12.25
Cage Wash	24 h	2.38	0.96	12.78	0.65	4.19	5.78
	48 h	0.30	1.32	0.41	0.22	0.56	0.51
	72 h	0.11	*0.07	0.15	*0.11	º0.11	º0.03
	96 h	*0.02	0.09	*0.07	*0.06	º0.06	º0.03
	120 h	*0.03	*0.01	*0.04	*0.05	º0.03	º0.02
	144 h	*0.03	*0.00	*0.07	*0.02	º0.03	º0.03
	168 h	*0.11	*0.01	*0.09	*0.07	º0.07	º0.04
Subtotal		2.97	2.46	13.60	1.18	5.05	5.75
Tissues	168 h	0.10	0.07	0.10	0.10	0.09	0.02
G.I. Tract	168 h	0.01	*0.01	0.01	0.02	º0.01	º0.01
Carcass	168 h	*0.09	*0.06	*0.13	*0.07	º0.09	º0.03
Total		95.34	97.78	97.55	97.14	96.95	1.11

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 4 **Recovery of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Daily Target Dose level of 50 mg/kg**

Results expressed as % administered dose

Sample	Timepoint	037F	014F	015F	016F	Mean	SD
Urine	6 h	78.22	78.25	56.97	75.01	72.11	10.21
	24 h	8.99	5.91	18.54	7.55	10.25	5.67
	48 h	0.34	0.37	0.62	0.73	0.52	0.19
	72 h	0.07	0.14	0.20	0.17	0.15	0.06
	96 h	0.06	0.13	0.22	0.23	0.16	0.08
	120 h	0.13	0.10	0.14	0.21	0.15	0.04
	144 h	0.05	0.03	0.08	0.10	0.06	0.03
	168 h	0.03	*0.02	0.02	0.03	º0.03	º0.00
Subtotal		87.90	84.97	76.79	84.02	83.42	4.72
Faeces	24 h	5.93	8.52	12.47	6.72	8.41	2.92
	48 h	0.16	0.68	0.28	0.81	0.48	0.31
	72 h	0.03	0.11	1.33	0.21	0.42	0.61
	96 h	0.02	0.17	0.10	0.06	0.09	0.06
	120 h	0.03	0.04	0.04	0.02	0.03	0.01
	144 h	0.01	0.06	0.06	0.04	0.04	0.02
	168 h	*0.01	0.07	0.03	0.22	º0.08	º0.10
Subtotal		6.20	9.65	14.31	8.08	9.56	3.47
Cage Wash	24 h	1.48	0.68	2.32	1.25	1.43	0.68
	48 h	0.12	*0.03	0.57	0.39	º0.28	º0.25
	72 h	*0.06	*0.04	0.31	0.20	º0.15	º0.13
	96 h	0.21	*0.10	2.90	1.24	º1.11	º1.30
	120 h	*0.06	*0.06	0.16	0.31	º0.15	º0.12
	144 h	*0.03	*0.08	*0.06	*0.07	º0.06	º0.02
	168 h	*0.11	*0.08	*0.11	0.18	º0.12	º0.04
Subtotal		2.08	1.08	6.44	3.66	3.31	2.34
Tissues	168 h	0.07	0.08	0.07	0.07	0.07	0.00
G.I. Tract	168 h	0.01	*0.01	*0.01	*0.01	º0.01	º0.00
Carcass	168 h	*0.07	*0.07	*0.08	*0.02	º0.06	º0.02
Total		96.32	95.86	97.70	95.86	96.43	0.87

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 5 **Concentration of Total Radioactivity in Tissues at 168 h Post Dose Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/g

Sample	001M	002M	003M	004M	Mean	SD
Fat-White	*0.01	*0.04	*0.03	*0.03	º0.03	º0.01
Kidneys	0.09	0.15	0.13	0.08	0.11	0.03
Liver	0.83	1.54	1.05	1.22	1.16	0.30
Spleen	*0.02	*0.03	*0.03	*0.03	º0.03	º0.01
G.I. Tract	*0.02	0.05	*0.02	*0.02	º0.03	º0.01
Carcass	0.08	0.09	0.08	0.14	0.10	0.03
Wh Blood	0.14	0.19	0.16	0.12	0.15	0.03

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 6 **Concentration of Total Radioactivity in Tissues at 168 h Post Dose Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/g

Sample	005F	006F	007F	008F	Mean	SD
Fat-White	*0.03	*0.04	*0.03	*0.03	º0.03	º0.00
Kidneys	0.12	0.11	0.15	0.13	0.13	0.02
Liver	0.90	0.76	0.90	0.84	0.85	0.06
Spleen	*0.04	*0.03	*0.04	*0.03	º0.04	º0.00
G.I. Tract	*0.02	0.03	0.03	*0.02	º0.03	º0.00
Carcass	*0.07	*0.08	0.17	*0.07	º0.10	º0.05
Wh Blood	0.17	0.14	0.17	0.15	0.16	0.02

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 7 **Concentration of Total Radioactivity in Tissues at 168 h Post Dose Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/g

Sample	009M	010M	011M	012M	Mean	SD
Fat-White	*0.02	*0.02	*0.01	*0.07	º0.03	º0.03
Kidneys	*0.04	*0.03	*0.05	0.08	º0.05	º0.02
Liver	0.75	0.55	0.70	0.79	0.70	0.11
Spleen	*0.00	*0.00	*0.00	*0.09	º0.02	º0.05
G.I. Tract	0.03	*0.02	0.05	0.06	º0.04	º0.02
Carcass	*0.06	*0.04	*0.08	*0.05	º0.06	º0.02
Wh Blood	0.14	0.21	0.17	0.18	0.17	0.03

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 8 **Concentration of Total Radioactivity in Tissues at 168 h Post Dose Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/g

Sample	037F	014F	015F	016F	Mean	SD
Fat-White	*0.07	*0.04	*0.03	*0.02	º0.04	º0.02
Kidneys	*0.08	*0.06	*0.03	*0.05	º0.05	º0.02
Liver	0.66	0.56	0.53	0.68	0.61	0.07
Spleen	*0.04	*0.02	*0.01	*0.01	º0.02	º0.02
G.I. Tract	0.04	*0.02	*0.02	*0.02	º0.03	º0.01
Carcass	*0.05	*0.04	*0.05	*0.02	º0.04	º0.02
Wh Blood	0.19	0.15	0.18	0.16	0.17	0.02

*=Results calculated from data less than 30 d.p.m. above background
°=Mean includes results calculated from data less than 30 d.p.m above background

Table 9 **Plasma Concentrations of Total Radioactivity following a Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/ml

Sample	Timepoint	017M	018M	019M	020M	Mean	SD
Plasma	12 h	0.8	0.7	0.7	0.8	0.8	0.1
	24 h	0.5	0.5	0.5	0.6	0.5	0.0

Table 10 **Plasma Concentrations of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Rats at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/ml

Sample	Timepoint	021F	022F	023F	024F	Mean	SD
Plasma	12 h	0.3	0.4	0.5	0.5	0.4	0.1
	24 h	0.3	0.4	0.4	0.3	0.3	0.1

Table 11 **Plasma Concentrations of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Male Mice at a Daily Target Dose level of 50 mg/kg**

Results expressed as µg equiv/ml

Sample	Timepoint	1	2	3	4	Mean	SD
Plasma	12 h	0.8	2.4	1.0	1.0	1.3	0.7
	24 h	0.8	1.4	0.9	0.7	1.0	0.3

1= Ans 025M and 033M for 12 & 24h plasma samples respectively
2= Ans 026M and 034M for 12 & 24h plasma samples respectively
3= Ans 027M and 035M for 12 & 24h plasma samples respectively
4= Ans 028M and 036M for 12 & 24h plasma samples respectively

Table 12 **Plasma Concentrations of Total Radioactivity Following a Multiple (13 Daily Doses) Oral Administration of Ammonium Perfluorohexanoate Followed by a Single Oral Administration of [^{14}C]-Ammonium Perfluorohexanoate to Female Mice at a Daily Target Dose level of 50 mg/kg**

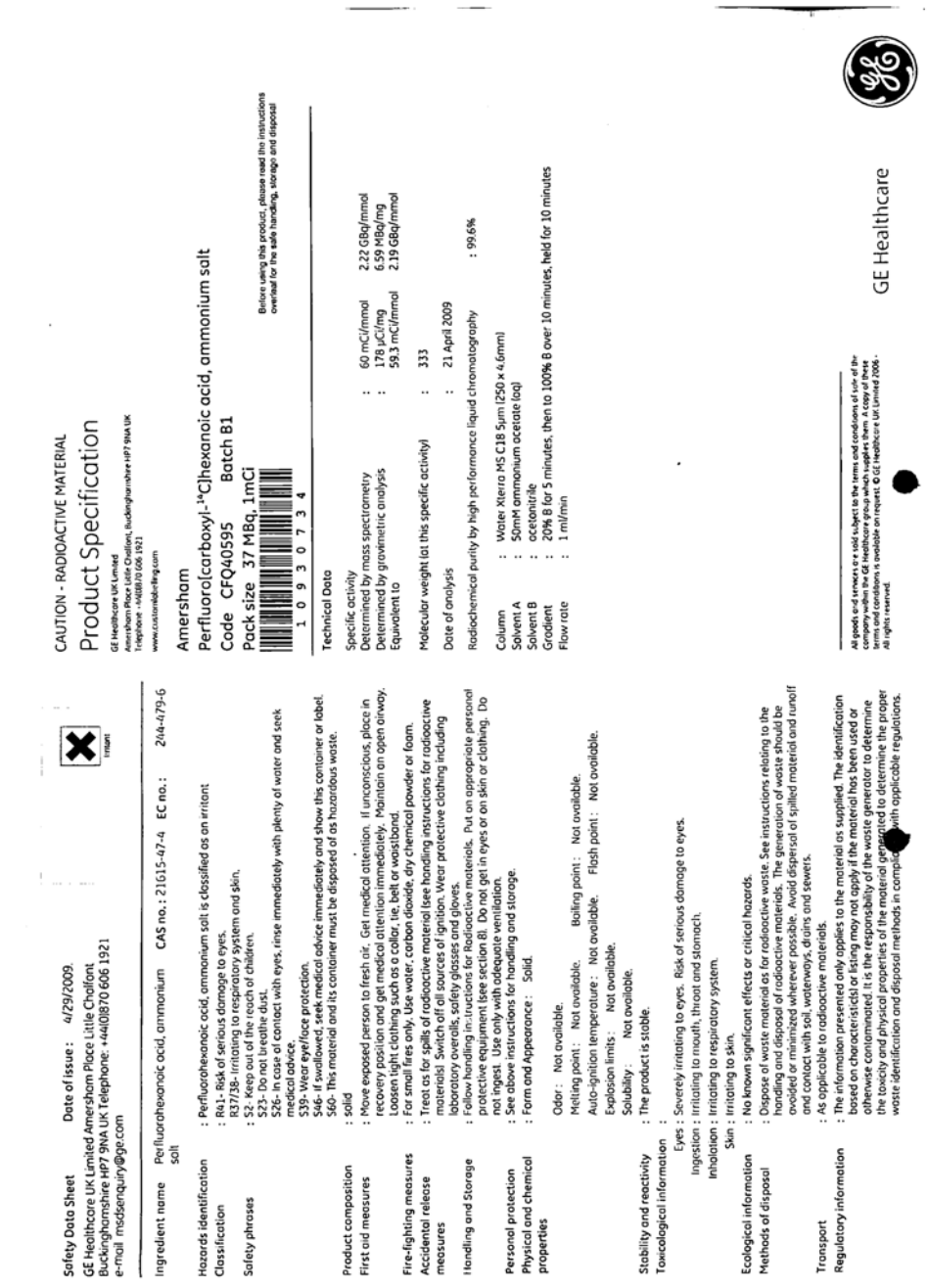
Results expressed as µg equiv/ml

Sample	Timepoint	1	2	3	4	Mean	SD
Plasma	12 h	0.6	0.7	0.9	1.7	1.0	0.5
	24 h	0.5	0.4	0.5	0.5	0.5	0.1

1= An 029F and 041F for 12 & 24h plasma samples respectively
2= Ans 030F and 038F for 12 & 24h plasma samples respectively
3= Ans 031F and 039F for 12 & 24h plasma samples respectively
4= Ans 032F and 040F for 12 & 24h plasma samples respectively

13 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.customerservice@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.

Report Amendment 1

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)

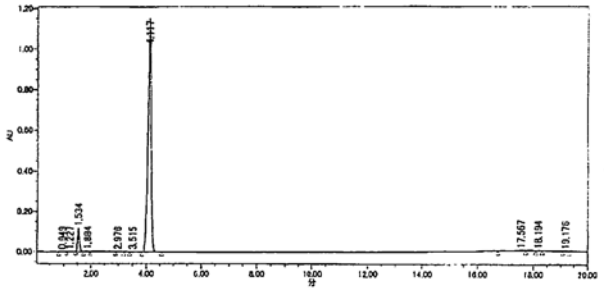
Chemical R&D Center
Unidyne Group
Senior Researcher

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		

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Appendix 3 Dosing Data for the Administration of Ammonium Perfluorohexanoate to Rats

Animal No.	Dose (mg/kg) Received on Day No.						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
001M	51.1	49.2	50.2	50.1	49.4	49.1	45.2
002M	49.3	49.5	50.9	50.2	49.1	48.0	51.0
003M	49.4	49.8	50.6	49.1	50.0	49.8	51.0
004M	49.2	48.6	50.3	50.5	48.9	49.7	50.9
005F	51.2	51.8	49.4	49.6	50.2	51.6	50.3
006F	51.3	49.2	51.6	49.6	50.4	50.2	49.2
007F	50.5	49.8	50.5	50.6	50.1	50.3	51.2
008F	50.1	51.2	52.3	50.8	48.6	51.0	50.4
017M	49.5	49.1	49.7	50.6	50.1	49.0	50.8
018M	50.1	48.7	49.7	48.7	49.6	50.6	50.4
019M	50.0	49.1	48.9	48.9	50.7	49.6	51.0
020M	50.5	49.4	49.6	49.7	50.0	50.0	50.6
021F	50.1	49.8	50.6	48.5	50.8	51.3	49.5
022F	50.5	49.0	50.3	48.7	49.7	48.5	48.5
023F	49.2	51.8	50.3	48.5	50.9	49.3	51.2
024F	51.3	52.0	49.1	50.0	50.6	49.3	48.9

Appendix 3 Dosing Data for the Administration of Ammonium Perfluorohexanoate to Rats (Continued)

Animal No.	Dose (mg/kg) received on Day No.						
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Average (Day 1-13) mg/kg Received (± SD)
001M	48.2	49.7	50.0	49.7	49.4	47.8	49.2 ± 1.5
002M	48.4	48.8	50.5	49.9	48.7	49.6	49.5 ± 0.9
003M	49.5	48.5	50.3	49.0	50.4	48.1	49.7 ± 0.8
004M	49.3	48.3	50.5	48.7	48.7	49.7	49.5 ± 0.9
005F	50.7	50.5	48.9	48.6	49.5	49.2	50.1 ± 1.0
006F	50.2	50.6	49.3	49.6	50.9	51.2	50.3 ± 0.8
007F	51.2	48.7	49.9	50.6	50.1	49.6	50.2 ± 0.7
008F	48.2	48.9	50.4	50.0	49.0	50.0	50.1 ± 1.2
017M	49.7	49.4	49.3	49.3	49.0	48.3	49.5 ± 0.7
018M	48.0	49.1	50.3	49.6	49.1	49.0	49.5 ± 0.8
019M	48.9	48.7	49.2	49.9	49.4	48.5	49.4 ± 0.8
020M	49.3	49.1	50.0	49.6	49.5	48.4	49.7 ± 0.6
021F	49.9	49.2	48.4	49.0	50.3	48.5	49.7 ± 0.9
022F	47.7	49.5	50.3	49.3	48.6	50.1	49.3 ± 0.9
023F	48.8	48.8	49.4	50.0	50.7	51.6	50.0 ± 1.1
024F	49.5	49.7	48.4	49.9	50.0	52.1	50.1 ± 1.1

Appendix 4 Dosing Data for the Administration of Ammonium Perfluorohexanoate to Mice

Animal No.	Dose (mg/kg) Received on Day No.						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
009M	50.9	51.3	51.1	50.5	52.1	52.0	50.2
010M	49.8	49.6	51.0	50.3	50.8	52.4	51.8
011M	51.3	52.2	51.4	51.0	49.5	52.0	51.3
012M	51.1	50.7	50.6	50.9	50.6	50.8	51.7
013F	50.0	50.0	49.9	50.9	52.7	52.3	51.7
014F	49.2	50.7	50.4	49.8	52.0	52.7	50.5
015F	51.1	53.2	50.2	49.9	50.5	50.2	51.0
016F	40.5	48.9	51.0	49.0	53.1	51.6	52.1
025M	50.4	52.7	49.3	50.6	50.7	51.6	53.4
026M	52.1	50.6	48.4	50.8	51.3	51.0	50.7
027M	51.1	52.9	49.5	50.4	49.8	51.4	52.5
028M	49.9	52.9	51.4	49.7	51.3	51.5	50.5
029F	50.5	52.9	50.0	52.4	49.9	49.6	52.4
030F	51.5	52.7	50.5	51.3	51.9	51.4	50.8
031F	50.8	52.3	52.4	52.1	49.8	49.5	50.9
032F	52.8	51.8	52.0	50.9	50.2	52.7	50.9
033M	52.5	51.3	51.0	52.1	51.9	52.3	51.9
034M	53.4	50.6	50.7	52.0	50.1	51.0	48.1
035M	52.0	50.6	50.4	53.0	50.6	52.7	51.1
036M	49.4	51.1	51.4	50.8	51.1	49.6	50.4
037F	52.1	50.6	50.5	51.2	50.0	51.9	51.8
038F	53.0	51.8	51.9	51.1	50.3	52.5	52.5
039F	52.8	52.6	51.5	45.6	50.9	51.8	51.5
040F	51.9	51.7	41.5	50.3	49.9	51.2	51.4
041F	48.5	49.6	51.6	51.2	50.2	51.7	50.5

Appendix 4 Dosing Data for the Administration of Ammonium Perfluorohexanoate to Mice (Continued)

Animal No.	Dose (mg/kg) Received on Day No.						
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Average (Day 1-13) mg/kg Received (± SD)
009M	49.8	48.2	50.1	48.9	49.8	49.7	50.4 ± 1.1
010M	49.7	50.1	49.0	49.2	49.3	49.7	50.2 ± 1.0
011M	49.3	49.7	51.0	50.2	50.0	50.5	50.7 ± 0.9
012M	49.8	49.3	50.4	49.3	50.0	49.1	50.3 ± 0.8
013F	50.0	50.1	49.8	50.8	48.6	43.9	50.1 ± 2.2
014F	50.1	50.2	51.1	50.3	48.4	48.2	50.3 ± 1.3
015F	49.8	49.7	49.8	49.1	47.6	49.8	50.1 ± 1.3
016F	49.3	48.9	50.2	51.1	49.4	51.0	49.7 ± 3.1
025M	51.5	48.7	50.4	50.9	51.6	51.1	51.0 ± 1.3
026M	50.5	49.3	49.8	50.7	49.8	49.2	50.3 ± 1.0
027M	50.6	49.5	51.2	50.7	50.7	49.8	50.8 ± 1.1
028M	50.5	50.1	51.3	51.3	49.8	51.3	50.9 ± 0.9
029F	49.9	49.8	51.3	49.9	51.2	52.5	50.9 ± 1.2
030F	49.3	49.6	49.9	49.4	51.0	49.6	50.7 ± 1.1
031F	50.3	48.9	50.6	47.8	50.6	49.7	50.4 ± 1.3
032F	49.2	50.5	50.3	48.9	50.4	49.7	50.8 ± 1.2
033M	50.7	49.3	50.2	49.4	52.0	49.2	51.1 ± 1.2
034M	50.1	49.9	50.0	50.4	48.8	50.2	50.4 ± 1.3
035M	49.6	49.4	49.5	48.9	47.5	49.9	50.4 ± 1.5
036M	50.9	50.3	49.7	48.0	50.9	49.8	50.3 ± 0.9
037F	47.0	48.7	50.1	49.3	50.7	49.0	50.2 ± 1.5
038F	49.7	49.2	51.3	48.8	50.0	49.3	50.9 ± 1.4
039F	50.9	50.6	49.5	50.3	50.9	49.4	50.6 ± 1.8
040F	49.5	51.2	50.4	49.7	49.8	50.2	49.9 ± 2.6
041F	49.7	50.8	52.9	49.8	49.9	51.0	50.6 ± 1.1

Appendix 5 Dosing Data for the Administration of [^{14}C]-Ammonium Perfluorohexanoate to Rats

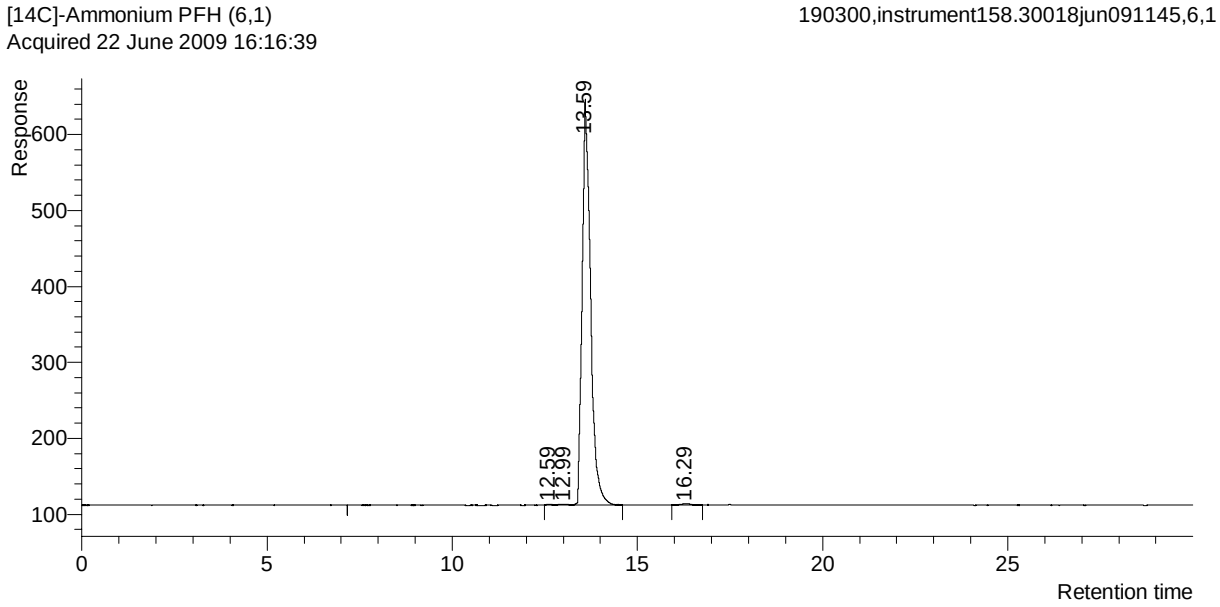
Phase	Animal Number	Animal Weight (g)	Dose Recieved			
			MBq	mg	mg /kg	MBq/kg
1- Rat	001M	296	1.31	15.1	50.9	4.42
	002M	325	1.45	16.6	51.2	4.45
	003M	352	1.54	17.7	50.2	4.36
	004M	343	1.50	17.3	50.4	4.38
	005F	206	0.92	10.6	51.2	4.45
	006F	215	0.94	10.8	50.2	4.37
	007F	220	0.96	11.0	50.1	4.35
	008F	223	0.96	11.1	49.7	4.32
2- Rat	017M	306	1.35	15.6	50.9	4.42
	018M	334	1.46	16.8	50.2	4.36
	019M	362	1.59	18.2	50.4	4.38
	020M	342	1.51	17.3	50.7	4.41
	021F	209	0.86	9.9	47.5	4.13
	022F	226	0.99	11.4	50.3	4.37
	023F	217	0.96	11.0	50.9	4.42
	024F	217	0.92	10.5	48.6	4.22

Appendix 6 Dosing Data for the Administration of [^{14}C]-Ammonium Perfluorohexanoate to Mice

Phase	Animal Number	Animal Weight (g)	Dose Recieved			
			MBq	mg	mg /kg	MBq/kg
1- Mouse	009M	31	0.13	1.52	48.9	4.25
	010M	32	0.14	1.58	49.1	4.27
	011M	31	0.13	1.48	47.1	4.10
	012M	30	0.13	1.49	48.9	4.25
	037F	28	0.12	1.41	50.2	4.36
	014F	28	0.12	1.36	48.8	4.24
	015F	27	0.12	1.37	51.3	4.46
	016F	25	0.10	1.19	46.9	4.07
2- Mouse	025M	33	0.14	1.58	48.4	4.21
	026M	31	0.13	1.53	50.2	4.36
	027M	32	0.14	1.56	48.7	4.24
	028M	25	0.11	1.24	50.3	4.37
	029F	29	0.12	1.41	48.0	4.17
	030F	28	0.12	1.32	48.3	4.20
	031F	29	0.11	1.31	45.5	3.96
	032F	27	0.12	1.35	50.6	4.40
	033M	31	0.13	1.48	47.9	4.17
	034M	32	0.13	1.51	47.5	4.13
	035M	31	0.13	1.48	47.1	4.10
	036M	27	0.11	1.27	47.1	4.10
	013F	[A]	[A]	[A]	[A]	[A]
	038F	29	0.13	1.44	50.3	4.37
	039F	27	0.12	1.33	49.6	4.31
	040F	29	0.13	1.45	50.6	4.40
	041F	26	0.11	1.29	49.5	4.29

[A]= An 013F sacrificed prematurely due to ill health

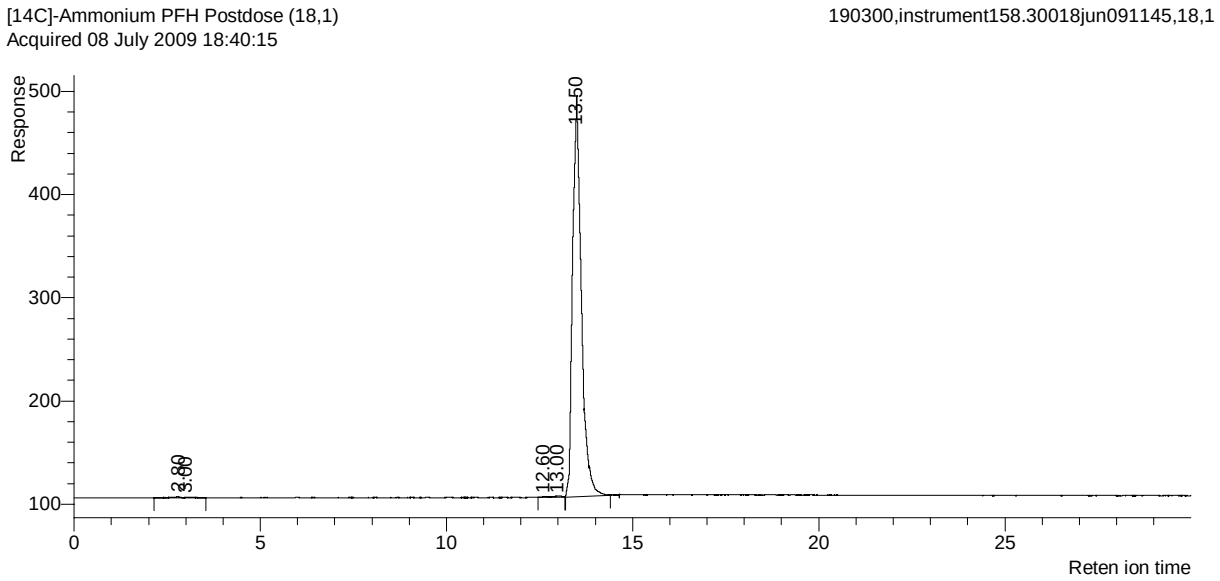
Appendix 7 Representative Radio-HPLC Chromatogram for the Radiochemical Purity of [^{14}C]-Ammonium Perfluorohexanoate



Peak No.	Retention Time (min)	Peak Name	% Area
1	12.59	-	0.1
2	12.99	-	0.3
3	13.59	[^{14}C]- Ammonium Perfluorohexanoate *	99.1
4	16.29	-	0.5

* = Assigned by co-chromatography with unlabelled Ammonium Perfluorohexanoate

Appendix 8 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	2.80	-	0.3
2	3.00	-	0.1
3	12.60	-	0.1
4	13.00	-	0.3
5	13.50	[^{14}C]- Ammonium Perfluorohexanoate*	99.2

* = Assigned by co-chromatography with unlabelled Ammonium Perfluorohexanoate