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TSCA NON-CONFIDENTIAL BUSINESS INFORMATION

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Mexichem
Fluor

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December 20, 2010

8EHQ-09-17629

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428. Attn: Section 8(e)
US Environmental Protection Agency
1201 Constitution Avenue NW
Washington, DC 20004-3302
Phone: 202-564-8940

8911000056

Toxic Substances Control Act (TSCA) Section 8(e) Substantial Risk Notification

Re: 8EHQ-09-17629, Supplemental Information

Subject Chemical: 3,3,3-trifluoro-1-propene (CAS No. 677-21-4)
Alternative Name: Refrigerant 1243zf (R-1243zf)

Dear Sir or Madam:

On August 26, 2009 INEOS Fluor Americas LLC submitted a notice under Section 8(e) of the Toxic Substances Control Act (TSCA) regarding preliminary findings from a 28-day inhalation study in the rat employing 0, 5,000, 15,000 and 50,000 ppm levels of R-1243zf. INEOS Fluor Americas LLC also indicated that the full results of the 28-day study would be submitted to the TSCA 8(e) Coordinator. The final report is enclosed.

On April 1, 2010, the INEOS group sold its fluorochemicals group, which included INEOS Fluor Americas LLC, to Mexichem S.A.B. de C.V. As part of that transaction, INEOS Fluor Americas LLC, and its associated refrigerant development component, became Mexichem Fluor Inc.

Mexichem Fluor Inc., previously INEOS Fluor Americas LLC, hereby withdraws its requests for confidentiality regarding all information previously submitted regarding R-1243zf.

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MEXCHEM

CONTAINS NO CBI

Mexichem
Fluor

Sincerely,

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MEXCHEM

Huntingdon
Life Sciences
Working for a better tomorrow

3, 3, 3-Trifluoroprop-1-ene (HFO 1243zf): Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks

HLS study number:	ATS0031
Version ID:	Final
Issue date:	24 November 2010

Details of Sponsor, Test Facilities and Test Site

Sponsor	Ineos Fluor Ltd PO Box 13 The Heath Runcorn Cheshire WA7 4QX UK
Test facility (Primary location)	Huntingdon Life Sciences Huntingdon Research Centre Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS UK
Test facility (Histology)	Huntingdon Life Sciences Eye Research Centre Eye Suffolk IP23 7PX UK
Test site (Urinary fluoride)	Butterworth Laboratories Limited 54-56 Waldegrave Road Teddington Middlesex TW11 8LG UK

Table of Contents

Details of Sponsor, Test Facilities and Test Site 2
Compliance with Good Laboratory Practice 6
Quality Assurance Statement 7
Contributing Scientists 8
Summary 10
1. Introduction 12
1.1 Objective 12
1.2 Regulatory compliance 12
1.3 Test system 12
1.4 Route of administration 12
1.5 Treatment groups and doses 12
2. Experimental Procedure 13
2.1 Study schedule and structure 13
2.1.1 Duration of treatment 13
2.1.2 Time schedule 13
2.1.3 Identity of treatment groups 13
2.2 Test substance 14
2.3 Animal management 15
2.3.1 Animal supply, acclimatisation and allocation 15
2.3.2 Animal housing, diet and water supply 15
2.3.3 Administration 16
2.4 Serial observations 17
2.4.1 Clinical observations 17
2.4.2 Bodyweight 17
2.4.3 Food consumption 17
2.4.4 Ophthalmoscopy 18
2.4.5 Haematology, peripheral blood 18
2.4.6 Blood chemistry 19
2.4.7 Urinalysis 20
2.4.8 Urinary fluoride 21
2.5 Necropsy and Histology 21
2.5.1 Method of kill 21
2.5.2 Macroscopic pathology 21
2.5.3 Organ weights 21
2.5.4 Fixation 22
2.5.5 Histology 22
2.6 Pathology 23
2.6.1 Light microscopy 23
2.7 Computer Systems 23
2.8 Data treatment 24
2.8.1 Definition of "Week" 24
2.8.2 Signs 24
2.8.3 Bodyweight 24
2.8.4 Ophthalmoscopy 24
2.8.5 Haematology 25
2.8.6 Blood chemistry 25
2.8.7 Urinalysis 25
2.8.8 Organ weights 25

2.8.9 Pathology 25
2.8.10 Statistical analysis 26
2.9 Quality assurance and archiving procedures 27
2.9.1 Quality assurance 27
2.9.2 Archives 27
2.10 Deviations from protocol 28

3. Results 29

3.1 Chamber atmosphere conditions 29
3.2 Signs and mortality 29
3.3 Bodyweight 29
3.4 Food consumption 30
3.5 Ophthalmoscopy 30
3.6 Haematology 30
3.7 Blood chemistry 30
3.8 Urinalysis 31
3.9 Urinary fluoride 31
3.10 Organ weights 31
3.11 Macropathology 31
3.12 Histopathology 32

4. Discussion 33

5. Conclusion 34

6. References 35

List of Figures

Figure 1 Bodyweight 36

List of Tables

Table 1 Bodyweight 37
Table 2 Haematology 39
Table 3 Blood chemistry 45
Table 4 Urinalysis 49
Table 5 Urinary fluoride 51
Table 6 Organ weights 53
Table 7 Macropathology 55
Table 8 Histopathology 56

List of Appendices

Appendix 1 Clinical signs 59
Appendix 2 Dose observations 64
Appendix 3 Bodyweight 80
Appendix 4 Food consumption 82
Appendix 5 Ophthalmoscopy 84
Appendix 6 Haematology 86
Appendix 7 Blood chemistry 94
Appendix 8 Urinalysis 98
Appendix 9 Urinary fluoride 104
Appendix 10 Organ weights 106
Appendix 11 Macropathology and histopathology 108

List of Annexes

Annex 1 Administration of HFO 1243zf to rats 149
Annex 2 Urinary fluoride report 164
Annex 3 Pathology report 173
Annex 4 Certificates of analysis 178
Annex 5 GLP compliance statements 187

Compliance with Good Laboratory Practice

3, 3, 3-Trifluoroprop-1-ene (HFO 1243zf): Toxicity study by inhalation administration to CD rats for 4 weeks

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

> The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).
>
> OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
>
> EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

The urinary fluoride phase of the study was conducted by the Principal Investigator in accordance with the UK Good Laboratory Practice Regulations stated above.

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

Jeannette A Blair BSc (Hons) PhD Study Director Huntingdon Life Sciences	24 November 2010 Date

Quality Assurance Statement

3, 3, 3-Trifluoroprop-1-ene (HFO 1243zf): Toxicity study by inhalation administration to CD rats for 4 weeks

The following inspections and audits have been carried out in relation to this study:

Study Phase	Date(s) of Inspection	Date of Reporting to Study Director and Management
Protocol	09 Apr 2009	09 Apr 2009
Study preparation, Test Item Control and Disposition, Generation of test atmospheres and exposure/post exposure signs, atmosphere sampling	20 Apr 2009	20 Apr 2009
Protocol Amendment No.1	23 Apr 2009	23 Apr 2009
Protocol Amendment No.2	24 Apr 2009	24 Apr 2009
Necropsy	18 May 2009	18 May 2009
Protocol Amendment No.3	02 Jul 2009	02 Jul 2009
Protocol Amendment No.4	09 Sep 2010	09 Sep 2010
Report audit	04 Aug 2009-05 Aug 2009	11 Aug 2009

In addition, process based inspections were conducted of other routine and repetitive procedures employed on this type of study at or about the time this study was in progress. Similarly an inspection of the facility where this study was conducted was carried out on an annual basis. These inspections were reported to Company Management.

Details of Quality Assurance inspections and audits relating to the urinary fluoride phase are indicated in the Test Site QA statement within the Principal Investigator's report included as Annex 2.

D. Smith
Dilys Smith MRQA
Lead Auditor
Department of Quality Assurance
Huntingdon Life Sciences

24 November 2010
Date

Contributing Scientists

3, 3, 3-Trifluoroprop-1-ene (HFO 1243zf): Toxicity study by inhalation administration to CD rats for 4 weeks

Study management

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Study Director

Colin J Hardy BSc (Hons) PhD MIBiol CBiol DipRCPath (Toxicology)
Monitoring Toxicologist

Aerosol technology

Simon A Moore BSc (Hons) PhD MRSC
Head of Aerosol Technology

Formulation and inhalation analysis

Keith Laing BSc (Hons)
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Head of Statistics

Summary

The systemic toxic potential of 3, 3, 3-trifluoroprop-1-ene (HFO 1243zf), a development refrigerant, to Crl:CD® (SD)IGS BR rats by inhalation administration, was assessed over a period of 4 weeks.

Three groups, each comprising five male and five female rats, were exposed to HFO 1243zf at target concentrations of 5000, 15000 or 50000 ppm and a similarly constituted Control group comprising 5 male and 5 female rats received air only for 6 hours a day, 5 days a week for 28 days.

During the study, clinical condition, bodyweight, food consumption, ophthalmic examination, haematology, blood chemistry, urinalysis, urinary fluoride, organ weight, macropathology and histopathology investigations were undertaken.

Results

The achieved mean exposure concentrations were 4967, 14953, and 49686 (ppm) HFO 1243zf after 4 weeks of exposure.

There were no treatment-related clinical signs, effects on food consumption, ophthalmic findings, organ weight changes or macropathology findings considered to be related to exposure to HFO 1243zf.

An incidence of myocardial vacuolation/myofibre degeneration was noted in all groups of male and female rats exposed to HFO 1243zf, but not in controls. An incidence of myocardial fibrosis was observed in all groups of male rats, and in groups of females exposed at 14953 or 49686 ppm, but not in controls. Myocardial congestion was seen in some male rats exposed at 4967 or 14953 ppm and in some females exposed at 49686 ppm.

Urinary fluoride concentrations were higher (up to 6.4X control in males and 10.1X control in females) in all groups exposed to HFO 1243zf.

Increased urine volume (up to 1.9X control), together with a decrease in specific gravity (SG) and urinary protein (up to 0.58X control) were evident for all treated male groups. Total sodium levels were higher (up to 2.1X control) in animals exposed to 49686 ppm HFO 1243zf.

Lower group mean bodyweight gains were evident for female groups (up to 0.33X control).

Reticulocyte counts were lower (up to 0.56X control) in all female groups exposed to HFO 1243zf and in males (up to 0.79X control) exposed at 14953 or 49686 ppm. White blood cell counts were lower (up to 0.71X control) in all female groups exposed to HFO 1243zf.

Aspartate aminotransferase levels were higher (up to 1.31X control) in both sexes exposed at 49686 ppm HFO 1243zf.

Conclusion

Exposure of male and female rats by inhalation to HFO 1243zf for 28 days over a 4 week period at exposure levels ≥4967 ppm caused myocardial vacuolation, fibrosis and congestion.

A no observed adverse effect level (NOAEL) for HFO 1243zf was not established in this study.

1. Introduction

1.1 Objective

The objective of this study was to assess the systemic toxic potential of 3, 3, 3-trifluoroprop-1-ene (HFO 1243zf), a development refrigerant, when administered by inhalation to CD rats for 4 weeks.

1.2 Regulatory compliance

The study was designed to meet the requirements of the following guidelines:

> OECD Guideline for the Testing of Chemicals, No.412: Repeated Dose Inhalation Toxicity 28-day or 14-day Study (Adopted: 12 May 1981).
>
> EC Methods for the determination of toxicity. Annex to Commission Directive 92/69/EC (Official Journal No. L 383 A/140), Part B, Method B.8: Repeated dose (28 days) toxicity (inhalation).

The study was conducted in accordance with the requirements of current, internationally recognised Good Laboratory Practice Standards, and the applicable sections of the United Kingdom Animals (Scientific Procedures) Act 1986.

1.3 Test system

The rat was chosen as the test species because of its acceptance as a model of toxicity in man. The Crl:CD® (SD)IGS BR strain was used because of the historical control data available in this laboratory.

1.4 Route of administration

The inhalation route of administration was chosen to simulate the conditions of potential human exposure.

1.5 Treatment groups and doses

Previous studies have shown that HFO 1243zf has a low level of acute toxicity, with the 4-hour LC_{50} in the rat being >367,000 ppm. A previous repeat dose toxicity study on HFO 1243zf in the rat showed no adverse effects following exposure to 987 ppm, 6 hours/day, 5 days/week, for 2 weeks. This data, coupled with knowledge of the toxicity of structurally-related analogues in 4-week repeat dose inhalation toxicity studies in the rat, suggested that the proposed exposure levels were appropriate.

2. Experimental Procedure

2.1 Study schedule and structure

2.1.1 Duration of treatment

The test substance, 3, 3, 3-trifluoroprop-1-ene (HFO 1243zf), was administered by inhalation over a period of 4 consecutive weeks. The necropsy procedures were completed in one day. The duration of treatment is reported as four weeks.

2.1.2 Time schedule

Study initiation: (Protocol signed by Study Director)	06 April 2009
Experimental start date:	09 April 2009
Animal arrival:	09 April 2009
Treatment commenced:	20 April 2009
Necropsy completed:	18 May 2009
Experimental completion date:	13 August 2010
Study completion:	24 November 2010

2.1.3 Identity of treatment groups

The study consisted of one Control and three treated groups of rats, identified as follows:

Group	Treatment	Exposure level (ppm)	No. of animals		Animal numbers		Cage numbers	
			Male	Female	Male	Female	Male	Female
1	Control	0	5	5	1-5	21-25	1	5
2	HFO 1243zf	5000	5	5	6-10	26-30	2	6
3	HFO 1243zf	15000	5	5	11-15	31-35	3	7
4	HFO 1243zf	50000	5	5	16-20	36-40	4	8

2.2 Test substance

Information supplied by the Sponsor regarding the test substance is contained in the test substance data sheet, which is retained in study records, and the Certificate of Analysis, which is presented in Annex 4.

The following information is given in summary:

Identification:	HFO 1243zf
Chemical name:	3, 3, 3-trifluoroprop-1-ene
Action:	Development refrigerant
Description:	Liquefied gas
Storage conditions:	Ambient temperature
Supplier:	Sponsor

Batch number:	Pures1	Pures2	Pures3
Date of receipt:	10 March 2009	22 April 2009	22 April 2009
Quantity received:	9.86 kg	10.08 kg + 9.82 kg	10.18 kg +8.74 kg
Expiry date:	05 March 2010	10 March 2010	10 March 2010
Purity:	99.955 %wt	99.968 %wt	99.944 %wt

Batch number:	Pures4	Pures5
Date of receipt:	22 April 2009	22 April 2009
Quantity received:	9.96 kg + 9.02 kg	3.12 kg
Expiry date:	10 March 2010	10 March 2010
Purity:	99.979 %wt	99.991 %wt

The Sponsor was responsible for the characterisation of the test substance and the documentation of the methods of synthesis, fabrication or derivation and stability.

It was not practical for a small sample to be taken and stored in Archives since the test substance was contained in a pressurised cylinder.

2.3 Animal management

2.3.1 Animal supply, acclimatisation and allocation

A total of 25 male and 25 female Crl:CD® (SD)IGS BR rats were received from Charles River (UK) Ltd. The rats were ordered at 36 to 42 days of age and within a weight range of 150-175 g for males and 142-166 g for females.

On arrival, the animals were removed from the transit boxes and allocated to study cages. Using the sequence of cages in the battery, one animal at a time was placed in each cage with the procedure being repeated until each cage held the appropriate number of animals. Each sex was allocated separately.

The cages constituting each group were blocked together by sex and the groups were dispersed in batteries so that possible environmental influences arising from their spatial distribution were equilibrated, as far as was practicable.

Each animal was assigned a number and identified uniquely within the study by a tail tattoo. Each cage label was colour-coded according to group and was numbered uniquely with cage and study number, as well as the identity of the occupants.

The animals were allowed to acclimatise to the conditions described below for 11 days before treatment commenced. Each group of animals (including spares) were acclimated to the restraint procedure used for inhalation snout-only exposure on 5 occasions prior to the commencement of the first test substance exposure. For those animals selected for this study, their age at the start of treatment was 47 to 53 days and their bodyweights were in the range of 223 to 265 g for males and 179 to 210 g for females.

The spare animals were removed from the study room after treatment commenced.

2.3.2 Animal housing, diet and water supply

Animals were housed inside a restricted entry rodent facility (Building Y11, Room 010). The facility was designed and operated to minimise the entry of external biological and chemical agents and to minimise the transference of such agents between rooms. The room was cleaned and disinfected before the study commenced.

Each animal room was kept at positive pressure with respect to the outside by its own supply of filtered fresh air, which was passed to atmosphere and not re-circulated. The temperature and relative humidity controls were maintained within the range of 19 to 23°C and 40 to 70% respectively. Artificial lighting was controlled to give a cycle of 12 hours continuous light and 12 hours continuous dark per 24 hours.

Temperature and humidity were monitored continuously. Although humidity was occasionally outside the indicated range, deviations were minor, of short duration and were not considered to have influenced the health of the animals or the outcome of the study.

Alarms were activated if there was any failure of the ventilation system, or temperature limits were exceeded. A stand-by electricity supply was available to be automatically brought into operation should the public supply fail.

The animals were housed five of one sex per cage. The cages were made of a polycarbonate body with a stainless steel mesh lid. Wood shavings (Lignocel 3/4) were used as bedding and were sterilised by autoclaving and changed at appropriate intervals each week. Cages, food hoppers and water bottles were changed at appropriate intervals.

The animals were allowed free access to a standard rodent diet (Rat and Mouse No. 1 Maintenance Diet), except during exposure, when urine was being collected, and overnight before routine blood sampling. This diet contained no added antibiotic or other chemotherapeutic or prophylactic agent.

Potable water taken from the public supply was freely available via polycarbonate bottles fitted with sipper tubes, except when urine was being collected or during the dosing procedure.

Each cage of animals was provided with an Aspen chew block for environmental enrichment. Chew blocks were provided throughout the study, except during exposure, when urine was being collected or overnight, before routine blood sampling, and were replaced when necessary.

Each batch of diet was analysed routinely by the supplier for various nutritional components and chemical and microbiological contaminants. Supplier's analytical certificates were scrutinised and approved before any batch of diet was released for use. The quality of the water supply is governed by regulations published by the Department for Environment, Food and Rural Affairs. Certificates of analysis were received routinely from the water supplier. Certificates of analysis were received routinely from the supplier of the wood shavings and Aspen chew blocks. Since the results of these various analyses did not provide evidence of contamination that might have prejudiced the study, they are not presented.

No other specific contaminants that were likely to have been present in the wood shavings, chew blocks, diet or water were analysed, as none that may have interfered with or prejudiced the outcome of the study was known.

2.3.3 Administration

The gaseous test substance was administered by snout-only inhalation 6 hours a day, for 5 consecutive days a week, over a 4 week period (total 20 exposures). Control animals were similarly exposed to air only. The test atmosphere was produced by metering the test gas from a pressure resistant cylinder, followed by dilution with clean air, prior to the resultant test atmosphere passing into the snout-only inhalation exposure chamber.

Details of the administration and analysis of the test gas, together with the results obtained are presented in Annex 1.

2.4 Serial observations

Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit, as well as to the group observations and examinations outlined in this experimental procedure, were recorded in the Study Day Book. In addition, observations relating to individual animals made throughout the day were recorded.

All observations described below were performed in cage number sequence except where otherwise indicated.

2.4.1 Clinical observations

Animals were inspected visually at least twice daily for evidence of ill-health or reaction to treatment. Cages were inspected daily for evidence of ill-health amongst the occupant(s). Any deviation from normal was recorded at the time in respect of nature and severity, date and time of onset, duration and progress of the observed condition, as appropriate.

On days of exposure, detailed observations were recorded daily at the following times in relation to dose administration:

Pre-exposure
As each animal was returned to the home cage.
As late as possible in the working day

Observation during exposure was severely restricted due to the fact that the rats were restrained in a tube.

On days of non-exposure, detailed observations were made daily according to the following frequency:

Early in the working day (equivalent to pre-exposure observation)
As late as possible in the working day

In addition, a more detailed weekly physical examination was performed on each animal to monitor general health.

During the acclimatisation period, observations of the animals and their cages were recorded at least once per day.

2.4.2 Bodyweight

The weight of each rat was recorded one week before treatment commenced (Week -1), on the day that treatment commenced (Week 0), weekly throughout the treatment period and before necropsy.

2.4.3 Food consumption

The weight of food supplied to each cage, that remaining and an estimate of any spilled was recorded for the week before treatment started (Week -1), and each week throughout the treatment period. From these records, the mean weekly consumption per animal (g/animal/week) was calculated for each cage.

2.4.4 Ophthalmoscopy

Before treatment commenced, the eyes of all animals allocated to the study (including spare animals) were examined by means of a binocular indirect ophthalmoscope. During Week 4 of treatment, the eyes of all animals were similarly examined.

Prior to each examination, the pupils of each animal were dilated using a 0.5% tropicamide ophthalmic solution (Mydriacyl). The adnexae, conjunctiva, cornea, sclera, anterior chamber, iris (pupil dilated), lens, vitreous and fundus were examined.

2.4.5 Haematology, peripheral blood

During Week 4 of treatment (before dosing), blood samples were obtained from all animals after overnight withdrawal of food and chew blocks. Animals were held under light general anaesthesia induced by isoflurane and blood samples were withdrawn from the sublingual vein.

Blood samples (nominally 0.5 mL) were collected into tubes containing EDTA as anticoagulant and examined for the following characteristics:

The following were measured using a Bayer Advia 120 haematology analyser

- Haematocrit (Hct)
- Haemoglobin concentration (Hb)
- Erythrocyte count (RBC)
- Reticulocyte counts (Retic)
- Mean cell haemoglobin (MCH)
- Mean cell haemoglobin concentration (MCHC)
- Mean cell volume (MCV)
- Total white cell count (WBC)
- Differential WBC count
  - Neutrophils (N)
  - Lymphocytes (L)
  - Eosinophils (E)
  - Basophils (B)
  - Monocytes (M)
  - Large unstained cells (LUC)
- Platelet count (Plt)

Morphology flags were generated by the Advia 120 analyser. The most common morphological changes, anisocytosis, micro/macrocytosis and hypo/hyperchromasia were recorded as follows:

- \- = no abnormalities detected

Blood film (prepared for all samples) - Romanowsky stain, examined for abnormalities by light microscopy, in the case of flags from the Advia 120 analyser. Confirmation or a written description from the blood film was made where appropriate.

Additional blood samples (nominally 0.5 mL) were taken into tubes containing citrate anticoagulant and examined in respect of:

Prothrombin time (PT) - using an ACL 9000 analyser and IL PT-Fibrinogen reagent.

Activated partial thromboplastin time (APTT) - using an ACL 9000 Analyser and IL APTT reagent.

2.4.6 Blood chemistry

At the same time and using the same animals as for peripheral haematology, further blood samples (nominally 0.7 mL) were collected into tubes containing lithium heparin as anticoagulant. All tubes were mechanically agitated for at least two minutes and the sample subsequently centrifuged at 2000 g for 10 minutes in order to separate the plasma. After separation, the plasma was examined in respect of:

Using a Roche P Modular Analyser:

Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Gamma-glutamyl transpeptidase (gGT)
Total bilirubin (Bili)
Urea
Creatinine (Creat)
Glucose (Gluc)
Total cholesterol (Chol)
Triglycerides (Trig)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Calcium (Ca)
Inorganic phosphorus (Phos)
Total protein (Total Prot)
Albumin (Alb)

Albumin/globulin ratio (A/G Ratio) was calculated from total protein concentration and analysed albumin concentration.

2.4.7 Urinalysis

During Week 4 of treatment, overnight urine samples were collected from all animals. Animals were placed in an individual metabolism cage without food or water at approximately 16.05 hours; urine was collected until approximately 07.40 hours the following day.

The individual samples were examined for the following characteristics:

Appearance (App) - by visual assessment using the following abbreviations:

PY	Pale yellow	CPY	Cloudy pale yellow
MY	Medium yellow		

Volume (Vol)
pH - using a Radiometer PHM 92 pH meter
Specific gravity (SG) - using Atago UR-1 digital refractometer
Protein (Prot), urinary sodium (U-Na), urinary potassium (U-K), urinary chloride (U-Cl) and total sodium (T-Na), total potassium (T-K) and total chloride (T-Cl) - using Roche P Modular Clinical Chemistry Analyser.

Glucose (Gluc), ketones (Keto), bile pigments (Bili), blood pigments (UBld) by Multistix, diagnostic reagents used as qualitative indicators of analyte concentration. The reagent strips were interpreted using the Clinitek®500 instrument and results are reported according to the following convention:

NEG	=	negative
TRA	=	trace levels
1+	=	'small amount' of analyte
2+	=	'moderate amount' of analyte
3+	=	'large amount' of analyte

A microscopic examination of the urine sediment was performed. An aliquot of the urine sample was centrifuged, stained with Kova stain and the resulting deposit spread on a microscope slide. The number of elements seen in nine high power fields (HPF) was recorded in the raw data and entered onto the database and the number seen /HPF was derived from these data as described below.

Casts
Leucocytes (CLEU)
Erythrocytes (CURB)
Crystals (CCRY)
Sperm (CSP)
Epithelial cells/HPF (CEPI)
Abnormalities (A)

Following the analysis of urine samples collected in Week 4, the residual urine was frozen immediately over solid carbon dioxide or in a freezer at approximately -20°C or below, prior to despatch to a Principal Investigator (described in section 2.4.8).

2.4.8 Urinary fluoride

Residual urine samples from the Week 4 urinalysis investigations (described in section 2.4.7) were transported on dry ice to a Principal Investigator.

The method of analysis, further details on test procedures and results are presented in the Urinary Fluoride Report (Annex 2).

2.5 Necropsy and Histology

2.5.1 Method of kill

Animals were killed by an intra-peritoneal injection of sodium pentobarbitone followed by exsanguination. The sequence in which the animals were killed after completion of treatment was selected to allow satisfactory inter-group comparison.

2.5.2 Macroscopic pathology

All animals were subject to a detailed necropsy.

After a review of the history of each animal, a full macroscopic examination of the tissues was performed. All external features and orifices were examined visually. The cranial roof was removed to allow observation of the brain, pituitary gland and cranial nerves. After ventral mid-line incision, the neck and associated tissues and the thoracic, abdominal and pelvic cavities and their viscera were exposed and examined in situ. Any abnormal position, morphology or interaction was recorded.

The requisite organs were weighed and external and cut surfaces of the organs and tissues were examined as appropriate. Any abnormality in the appearance or size of any organ and tissue was recorded and the required tissue samples preserved in appropriate fixative.

For animals of Groups 1 (Control) and 4 (49686 ppm), samples of liver (approximately 1.0 g), heart (that in excess of histology requirements) and kidney (that in excess of histology requirements) were weighed, placed in separate cryovials (labelled with animal identity and name of tissue) and frozen rapidly in liquid nitrogen prior to storage at approximately -70°C or below. Samples were stored frozen pending possible future examination.

The retained tissues were checked before disposal of the carcass.

2.5.3 Organ weights

The following organs, taken from each animal killed after 4 weeks of treatment, were dissected free of adjacent fat and other contiguous tissue and the weights recorded:

Adrenals	Lungs with mainstem bronchi
Heart	Spleen
Kidneys	Testes
Liver	

Bilateral organs were weighed together. Organ weights were also adjusted for terminal bodyweight, using the weight recorded immediately before necropsy.

2.5.4 Fixation

Testes were fixed in modified Davidson's fluid and eyes were fixed in Davidson's fluid. Samples (or the whole) of the other tissues listed below from all animals were preserved in 10% neutral buffered formalin:

Abnormalities	Optic nerves
Adrenals	Ovaries
Aorta - thoracic	Pancreas
Brain	Pituitary
Caecum	Prostate
Colon	Rectum
Duodenum	Salivary glands - submandibular
Epididymides	- sublingual
Eyes	Sciatic nerves
Femur including joint	Seminal vesicles
Harderian glands	Skeletal muscle
Head#	Skin with mammary glands (inguinal area)
Heart	Spinal cord
Ileum	Spleen
Jejunum	Sternum
Kidneys	Stomach
Lachrymal glands	Testes
Larynx	Thymus
Liver	Thyroid with parathyroids
Lungs including bronchi	Tongue
Lymph nodes - mandibular	Trachea (including bifurcation)
- mesenteric	Urinary bladder
- tracheo-bronchial	Uterus and cervix
Nasal turbinates	Vagina
Oesophagus	

Including nasal cavity, paranasal sinuses and nasopharynx

2.5.5 Histology

For those animals specified in the Pathology section, the relevant tissues were subject to histological processing.

Tissue samples were dehydrated, embedded in paraffin wax, sectioned at approximately four to five micron thickness and stained with haematoxylin and eosin.

Those tissues subject to histological processing included the following regions:

Adrenals	- cortex and medulla
Heart	- included auricular and ventricular regions
Kidneys	- included cortex, medulla and papilla regions
Larynx	- two levels
Liver	- section from two lobes
Lungs	- section from all major lobes, including bronchi
Nasal turbinates	- three levels

For bilateral organs, sections of both organs were prepared. A single section was prepared from each of the remaining tissues required for microscopic pathology.

2.6 Pathology

2.6.1 Light microscopy

Microscopic examination was performed as follows:

> The respiratory tract (nasal turbinates, larynx, trachea and lungs), liver, kidneys, adrenals, testes and spleen were examined for all animals of Groups 1 (Control) and 4 (49686 ppm) and heart was examined for all animals sacrificed on completion of the scheduled treatment period.

Findings were either reported as "present" or assigned a severity grade. In the latter case one of the following five grades was used - minimal, slight, moderate, marked or severe. A reviewing pathologist undertook a peer review of the microscopic findings. An additional (external) peer review was performed on behalf of the Sponsor. The external peer review pathologist and study pathologist concurred on the histopathologic diagnoses and the interpretation of the pathology data.

Macroscopic and microscopic findings are presented in the Pathology Report (Annex 3).

2.7 Computer Systems

The computer systems with version numbers that were used on this study to acquire and quantify data include:

> ClinAxys II (version 2.0.2) used for haematology, blood chemistry and urinalysis.
>
> Xybion Path/Tox System (version 4.2.2) used for clinical signs, bodyweights, food consumption, ophthalmoscopy, necropsy and pathology data.
>
> Liberate (version 2.0) in-house system used for reporting clinical signs, bodyweights, food consumption, necropsy and pathology data, statistics.
>
> Waters Empower 2, Build 2154, Service Pack A used for Formulation and Inhalation Analysis data.
>
> Sample Registry System (version 1.0.2) used for Formulation and Inhalation Analysis data.
>
> Quasar (version 1.0) in-house statistical analysis package used for bodyweight and organ weight data.
>
> StarTox (version 3.2) in-house statistical analysis package used for haematology, clinical chemistry and urinalysis data.

2.8 Data treatment

This report contains serial observations pertaining to all weeks of treatment completed, together with signs data collected during the necropsy period. The only serial observations relating to the acclimatisation period included in this report relate to the detailed physical examination, Week -1 bodyweights, food consumption and ophthalmic examination.

Summary statistics (e.g. means and standard deviations) presented in this report were calculated from computer-stored individual raw data. The summary statistics and the individual data were stored in the computer to a certain number of decimal places, different for each parameter. For presentation purposes, however, they were usually rounded to fewer places. It is, therefore, not generally possible to reproduce the presented means and standard deviations exactly using the presented individual data.

Throughout the report the following abbreviations are used:

M	Male
F	Female
N	Number of animals examined
SD	Standard deviation

2.8.1 Definition of "Week"

The first week of treatment started at midnight prior to treatment commencing and ended at midnight on the seventh day following. Subsequent experimental weeks of treatment were of the same duration.

2.8.2 Signs

Signs are considered in two parts: observations made in association with treatment, classified as "dose observations" and extended changes in condition, classified as "clinical signs". Both are presented for each animal that showed signs, providing detail of type of sign, day or week of occurrence and information on the duration of the sign applicable.

The death codes in Appendix 1 have the following meaning:

T	Scheduled kill

2.8.3 Bodyweight

Group mean weight changes were calculated from the weight changes of individual animals.

2.8.4 Ophthalmoscopy

For each animal that showed findings, observations are presented in the final report. Observations were bilateral unless otherwise indicated:

L	Left eye
R	Right eye

2.8.5 Haematology

The abbreviations used in Appendix 6 have the following meanings:

NVR	No valid result

2.8.6 Blood chemistry

Albumin to globulin ratios (A/G Ratio) were calculated as:

$$\text{A/G Ratio} = \frac{\text{Albumin concentration}}{\text{Total protein} - \text{albumin concentration}}$$

2.8.7 Urinalysis

The abbreviations used in Appendix 8 have the following meanings:

INS	Insufficient sample

Group means and standard deviations are presented for volume, pH, specific gravity, protein and electrolytes only.

2.8.8 Organ weights

The weights of the samples of liver, heart and kidney that were taken from animals of Groups 1 (Control) and 4 (49686 ppm) and frozen in liquid nitrogen for possible future examinations are not presented in this report, but are retained in the raw data.

2.8.9 Pathology

The abbreviations used in Appendix 11 have the following meanings:

Ln/LN	Lymph nodes
Bifurc.	Bifurcation

Tissues which could not be examined are specified in Appendix 11. The absence of a comment for a tissue scheduled for examination therefore indicates that the tissue was considered to be normal. Tissues recorded as abnormal macroscopically but found to be normal microscopically are described as 'No significant lesion' in the microscopic pathology appendix. In all tabular presentations of data the tissues specified in the protocol for histopathological examination precede other tissues.

2.8.10 Statistical analysis

All statistical analyses were carried out separately for males and females using the individual animal as the basic experimental unit.

The following data types were analysed at each timepoint separately:

Bodyweight, using change over an appropriate study period
Blood chemistry, haematology and urinalysis
Urinary fluoride
Organ weights, both absolute and adjusted for terminal bodyweight

The following sequence of statistical tests was used for bodyweight, organ weight and clinical pathology data:

A parametric analysis was performed if Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level. The F_1 approximate test was applied. This test is designed to detect significant departure from monotonicity of means when the main test for the comparison of the means is a parametric monotonic trend test, such as Williams' test (Williams 1971, 1972). The test statistic compares the mean square, NMS, for the deviations of the observed means from the maximum likelihood means, calculated under a constraint of monotonicity with the usual error mean square, EMS. The null hypothesis is that the true means are monotonically ordered. The test statistic is F_1 = NMS/EMS which can be compared with standard tables of the *F* distribution with 1 and EMS degrees of freedom. If the F_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F_1 approximate test was significant, suggesting that the dose response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.

A non-parametric analysis was performed if Bartlett's test was still significant at the 1% level following both logarithmic and square-root transformations. The H1 approximate test, the non-parametric equivalent of the F_1 test described above, was applied. This test is designed to be used when the main test for comparison of the means is a non-parametric monotonic trend test, such as Shirley's test (Shirley 1977). The test statistic compares the non-monotonicity sums of squares, NRSS, for the deviations of the observed mean ranks from the maximum likelihood mean ranks with the non-parametric equivalent of the error sums of squares, ERSS = N(N+1)/12. The test statistic is H_1 = NRSS/ERSS which can be compared to standard tables of the $\chi 2$-distribution with 1 degree of freedom. If the H_1 approximate test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H_1 approximate test was significant, suggesting that the dose-response was not monotone, Steel's test (Steel 1959) was performed instead.

For clinical pathology data, if 75% of the data (across all groups) were the same value, for example c, Fisher's Exact tests (Fisher 1973) were performed. Treatment groups were compared using pairwise comparisons of each dose group against the control both for i) values <c versus values ≥c, and for ii) values ≤c versus values >c, as applicable.

For organ weight data, analysis of covariance was performed using terminal bodyweight as covariate (Angervall and Carlstrom, 1963). The treatment comparisons were made on adjusted group means in order to allow for differences in bodyweight which might influence the organ weights.

Key to tables

Significant differences between Control and treated groups were expressed at the 5% ($p<0.05$) or 1% ($p<0.01$) level. The key to the annotation used on the tables that contain statistical results is given below.

l	Data were log transformed for the statistical analysis.
Du	Treated groups compared to Control using Dunnett's test.
Fe	Treated groups compared to Control using Fisher's Exact test.
Sh	Treated groups compared to Control using Shirley's test.
Wi	Treated groups compared to Control using Williams' test.

Codes placed above adjusted means indicate that the comparisons were based on adjusted means.

*	$p<0.05$
**	$p<0.01$

2.9 Quality assurance and archiving procedures

2.9.1 Quality assurance

Details of the Quality Assurance inspections and audits (undertaken at Huntingdon Life Sciences) are presented on the Quality Assurance Statement.

Details of the Quality Assurance inspections and audits undertaken by the Principal Investigator are presented in the urinary fluoride report (Annex 2).

2.9.2 Archives

Following completion of this study all raw data, specimens and samples, except those generated or used during any Sponsor's or supplier's analysis, were stored in the archives of Huntingdon Life Sciences. Types of sample and specimen which are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences Standard Operating Procedures.

A copy of the final report and all Quality Assurance inspection records will be retained indefinitely. All other appropriate specimens and records will be retained for a minimum period of one year from the date of issue of the final report. At the end of the one year retention period the Sponsor will be contacted and advice sought on the above requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

All documentation and data pertaining to the analytical aspects of the study undertaken by the Principal Investigator will be transferred to the archive at Huntingdon Life Sciences for storage. Specimens/samples that were unsuitable for long term retention and archiving will be disposed of in accordance with the Principal Investigator's local procedures.

2.10 Deviations from protocol

There were no deviations from protocol.

3. Results

3.1 Chamber atmosphere conditions

Annex 1

Summary data are presented below:

Group	Chamber concentration HFO 1243zf (ppm)	
	Target	Analysed
2	5000	4967
3	15000	14953
4	50000	49686

The achieved exposure levels were 99, 100 and 99% of the target concentrations for Groups 2, 3, 4, respectively.

3.2 Signs and mortality

Appendix 1 and Appendix 2

There were no unscheduled deaths.

There were no treatment-related clinical signs.

Wet fur and red staining predominantly around the eyes were observed in relation to exposure in animals from all groups during the study. These signs are considered to be a non-specific effect associated with the method of restraint used and to be of no toxicological importance.

The incidence and distribution of all other clinical signs were low and varied and therefore, were considered to be of no toxicological importance.

3.3 Bodyweight

Figure 1, Table 1 and Appendix 3

Lower group mean bodyweight gains were evident in all exposed groups of female rats after four weeks of treatment (0.77, 0.79 and 0.34X control for females exposed at 4967, 14953 and 49686 ppm HFO 1243zf, respectively). Statistical significance was attained for all affected groups, with some evidence of a dose-relationship.

Slightly higher group mean bodyweight gains (1.06 to 1.09X control) were evident for groups of male rats after four weeks of exposure. However, this difference did not attain statistical significance and was not exposure level-related.

The marked reduction in bodyweight gain evident for all groups between Weeks 3 and 4 can be attributed to the overnight food deprivation prior to Week 4 investigations.

3.4 Food consumption

Appendix 4

There were no treatment-related effects.

3.5 Ophthalmoscopy

Appendix 5

There were no treatment-related findings.

3.6 Haematology

Table 2 and Appendix 6

Reticulocyte counts were statistically significantly lower (as low as 0.56X Control) for all exposed groups of female rats, when compared with controls. Levels were also lower (as low as 0.79X control) in males exposed at 14953 or 49686 ppm HFO 1243zf. In males there was no statistical significance. This change was not related to the level of exposure.

White blood cell counts were statistically significantly lower (as low as 0.71X control) in all exposed groups of females rats, when compared with controls. This difference was primarily due to lower (as low as 0.68X control) group mean numbers of lymphocytes in all groups of exposed females, attaining statistical significance in females exposed to 14953 or 49686 ppm HFO 1243zf) when compared with controls. Monocyte counts were also lower (as low as 0.52X control) for all groups of exposed females. These changes were not seen in males and were not related to the level of exposure.

Other differences seen were generally small, largely inconsistent between the sexes, showed no dose-relationships and were considered to be of no toxicological importance.

3.7 Blood chemistry

Table 3 and Appendix 7

Mean aspartate aminotransferase levels were higher (up to 1.31X control) in groups of both males and females exposed at 49686 ppm HFO 1243zf. In males the effects were not statistically significant.

Other differences seen were generally small, largely inconsistent between the sexes, showed no dose-relationships and were considered to be of no toxicological importance.

3.8 Urinalysis

Table 4 and Appendix 8

An increase in urine volume (up to 1.9X control) associated in most cases with a small decrease in specific gravity (SG), was evident for all exposed groups of male rats.

Total protein levels were statistically significantly lower (as low as 0.58X control) for all exposed male groups in an exposure-related manner when compared with controls.

Total sodium levels were higher (up to 2.1X control) in both male and female rats exposed to 49686 ppm HFO 1243zf, attaining statistical significance in males.

Other differences seen were generally small, largely inconsistent between the sexes, showed no dose-relationships and were considered to be of no toxicological importance.

3.9 Urinary fluoride

Table 5, Appendix 9 and Annex 2

After 4 weeks of exposure, urinary fluoride concentrations were higher (from 6.2 to 6.4X control in males and from 6.1 to 10.1X control in females) in all groups exposed to HFO 1243zf. There was some evidence of a dose-relationship in both sexes, but the increases were not proportional to increases in the exposure level.

3.10 Organ weights

Table 6 and Appendix 10

Mean body weight adjusted testes weights were lower in all groups of exposed male rats (as low as 0.9X control) in an exposure-dependent manner.

Mean adjusted lungs + bronchi, kidney and heart weights were higher for all groups of exposed female rats (up to 1.19X, 1.15X and 1.30X control) generally in an exposure-dependent manner; however, these differences were not apparent in the treated males and were considered likely to be due to the reduced bodyweight as absolute organ weights were similar for all groups.

3.11 Macropathology

Table 7, Appendix 11 and Annex 3

The macroscopic examination performed after 4 weeks of treatment revealed no intergroup differences of note.

The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes.

3.12 Histopathology

Table 8 and Appendix 11, Annex 3

Treatment-related findings

Histopathological changes related to exposure to HFO 1243zf occurred in the heart.

Focal or multifocal myocardial inflammation, vacuolisation and myofibre degeneration was seen in rats from all treated groups. In some of the rats, there was also myocardial fibrosis and congestion. Control rats showed only presence of minimal inflammatory cell foci which was considered an incidental background finding.

Summary of treatment related findings in the heart for animals sacrificed after 4 weeks of treatment

Group/sex		1M	2M	3M	4M	1F	2F	3F	4F
Exposure level ppm		0	4967	14953	49686	0	4967	14953	49686
Focal / Multifocal Inflammation Vacuolation, Myofibre Degeneration									
	Minimal	0	0	1	0	0	2	4	1
	Slight	0	4	2	5	0	1	0	4
	Moderate	0	0	1	0	0	0	0	0
	Total	0	4	4	5	0	3	4	5
Myocardial Fibrosis									
	Minimal	0	0	2	2	0	0	1	0
	Slight	0	1	0	0	0	0	0	1
	Total	0	1	2	2	0	0	1	1
Congestion									
	Minimal	0	2	2	0	0	0	0	0
	Moderate	0	0	0	0	0	0	0	1
	Total	0	2	2	0	0	0	0	1
Number of animals examined		5	5	5	5	5	5	5	5

Incidental findings

All other findings were considered incidental and unrelated to the test substance.

4. Discussion

In this study, 3 groups of male and 3 groups of female rats were exposed by snout-only inhalation to test atmospheres containing HFO 1243zf for 6 hours daily for 5 consecutive days each week for 4 consecutive weeks. A fourth group of rats of each sex acting as controls was exposed to air only using a snout-only exposure system.

The achieved exposure levels were 4967, 14953, or 49686 (ppm) HFO 1243zf after 4 weeks of exposure. These exposure levels were clinically well tolerated. There were no unscheduled deaths, or test article-related clinical signs, effects on food consumption, or ophthalmic or macroscopic findings.

Changes considered related to treatment with HFO 1243zf were confined to the heart.

Focal/multifocal inflammation was present in the heart of control and treated animals. However, in all groups of animals exposed to HFO 1243zf, the inflammation was accompanied by degenerative changes of myofibres of a minimal to moderate degree. Myofibre degeneration presented as fine vacuolation of the peripheral (subsarcolemmal) region of cardiac muscle cells. A clear dose-relationship could not be observed histologically, but myocardial changes were more pronounced in male animals than in females. Aspartate aminotransferase (AST) plasma activity was higher in both males and females exposed to HFO 1243zf at 49686 ppm. AST can be released from hepatocytes in the liver and from skeletal or cardiac muscle cells. In the absence of any effect on alanine aminotransferase (ALT) and in view of the severity of myocardial damage seen, the heart musculature is considered the most likely source of the AST.

Higher levels of urinary fluoride observed in animals exposed at 49686 ppm is consistent with the metabolic breakdown of fluorine-containing compounds, such as HFO 1243zf. A common observation in animals exposed to such compounds is polyuria (Whitford and Taves, 1973). The lower urinary potassium concentrations, specific gravity and total protein levels were probably related to the increased urine volumes evident in treated males.

Treatment-related reductions in bodyweight gain were seen at all levels of exposure in females. Reductions in weight gain were not clearly related to the level of exposure, but were considered to be treatment-related and were considered not to be adverse.

Lower reticulocyte counts were seen in both sexes exposed to HFO 1243zf at all levels (with the exception of males receiving 4967 ppm). The differences from control were statistically significant in females only, and there was no evidence of a dose relationship. In the absence of any dose relationship or associated pathology, the aetiology of this change is unknown.

Adjusted lungs and bronchi, kidney and heart weights were higher in all groups of exposed female rats. Absolute and adjusted testes weights were lower in all groups of exposed male rats. In the absence of any pathological correlates, these findings were considered not related to treatment.

5. Conclusion

Exposure of male and female rats by inhalation to HFO 1243zf for 28 days over a 4 week period at exposure levels ≥4967 ppm caused myocardial vacuolation, fibrosis and congestion.

A no observed adverse effect level (NOAEL) for HFO 1243zf was not established in this study.

6. References

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FIGURE 1

Bodyweight - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

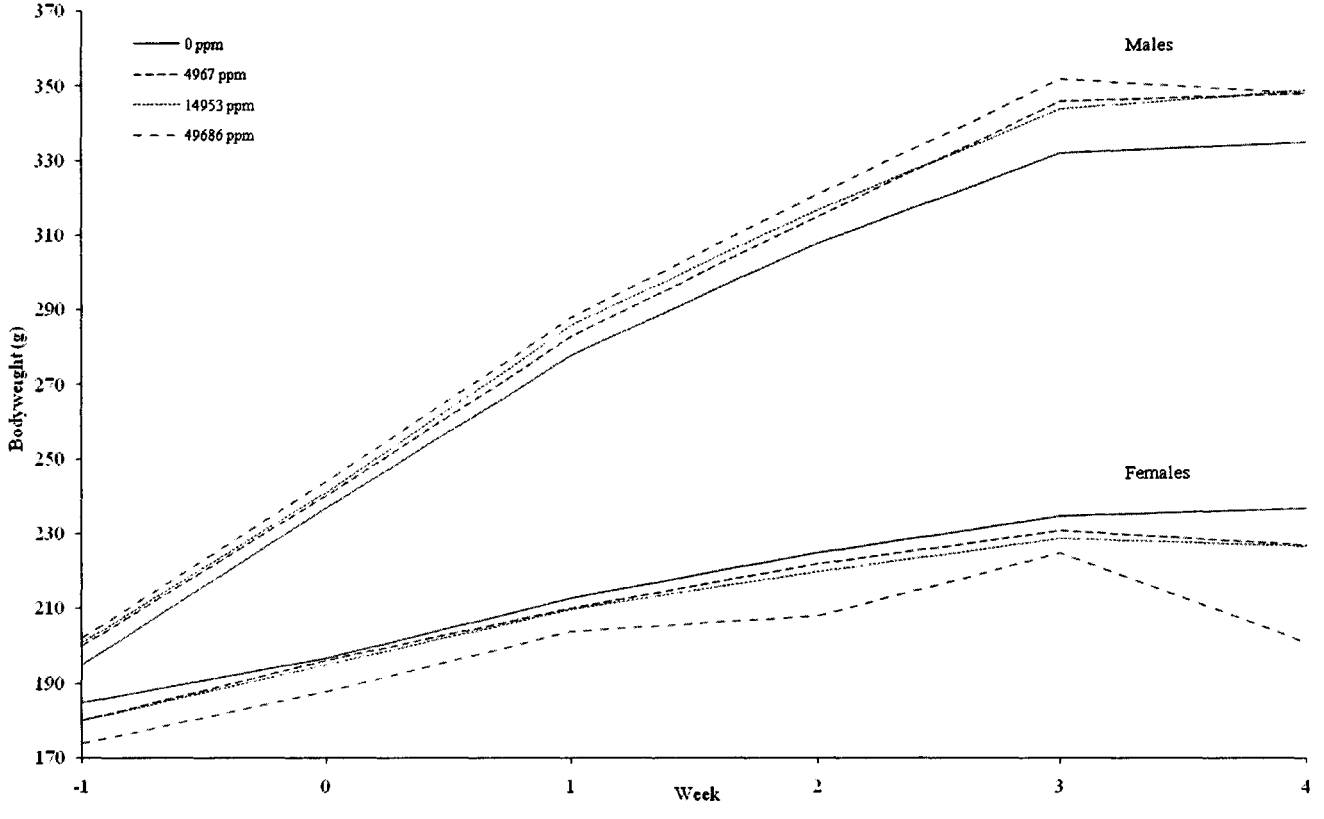

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TABLE 1

Request ID: 167334

Bodyweight - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Week -1	Week 0	Week 1	Week 2	Week 3	Week 4	Change 0-4	As % of Control
Statistical test:								Wi	
1M	Mean	195	237	278	308	332	335	99	-
	SD	8.0	8.4	11.2	13.0	11.5	12.8	14.6	
	N	5	5	5	5	5	5	5	
2M	Mean	200	240	283	315	346	348	108	109
	SD	7.5	12.3	20.6	27.9	32.8	37.6	27.0	
	N	5	5	5	5	5	5	5	
3M	Mean	201	241	286	317	344	349	108	109
	SD	10.4	15.5	19.0	24.2	33.5	34.2	22.1	
	N	5	5	5	5	5	5	5	
4M	Mean	202	244	288	321	352	348	104	106
	SD	12.6	14.4	15.8	12.3	15.2	14.0	4.9	
	N	5	5	5	5	5	5	5	

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ATS0031

TABLE 1 - continued

Request ID: 167334

Bodyweight - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Week -1	Week 0	Week 1	Week 2	Week 3	Week 4	Change 0-4	As % of Control
Statistical test:								Wi	
1F	Mean	185	197	213	225	235	237	40	-
	SD	11.5	5.8	8.3	7.3	10.0	8.4	7.9	
	N	5	5	5	5	5	5	5	
2F	Mean	180	196	210	222	231	227	31*	77
	SD	10.6	11.9	13.6	15.9	15.3	14.5	3.6	
	N	5	5	5	5	5	5	5	
3F	Mean	180	195	210	220	229	227	32*	79
	SD	9.3	8.7	10.5	14.5	11.9	12.4	5.2	
	N	5	5	5	5	5	5	5	
4F	Mean	174	188	204	208	225	201	13**	34
	SD	4.1	4.7	7.2	7.1	6.7	6.7	2.3	
	N	5	5	5	5	5	5	5	

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TABLE 2

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Hct L/L	Hb g/dL	RBC $x10^{12}/L$	Retic %	MCH pg	MCHC g/dL	MCV fL
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	0.464	16.0	8.58	1.81	18.7	34.5	54.2
	SD	0.0102	0.34	0.263	0.348	0.68	0.29	2.04
	N	5	5	5	5	5	5	5
2M	Mean	0.455	15.8	8.44	2.02	18.7	34.7	53.9
	SD	0.0161	0.49	0.476	0.565	0.51	0.22	1.55
	N	5	5	5	5	5	5	5
3M	Mean	0.475	16.5	8.98	1.43	18.4	34.7	53.0
	SD	0.0247	0.94	0.545	0.536	0.27	0.27	1.03
	N	5	5	5	5	5	5	5
4M	Mean	0.484	16.7	8.91	1.46	18.7	34.5	54.3
	SD	0.0146	0.42	0.142	0.250	0.36	0.22	1.28
	N	5	5	5	5	5	5	5

TABLE 2 - continued

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		WBC $x10^9/L$	N $x10^9/L$	L $x10^9/L$	E $x10^9/L$	B $x10^9/L$	M $x10^9/L$	LUC $x10^9/L$
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	15.52	1.89	12.95	0.10	0.10	0.34	0.13
	SD	2.868	0.987	2.004	0.035	0.086	0.120	0.043
	N	5	5	5	5	5	5	5
2M	Mean	14.55	2.29	11.67	0.12	0.10	0.30	0.09
	SD	1.727	1.614	1.794	0.060	0.029	0.127	0.023
	N	5	5	5	5	5	5	5
3M	Mean	13.70	1.51	11.55	0.11	0.11	0.30	0.11
	SD	1.960	0.535	1.706	0.028	0.046	0.157	0.066
	N	5	5	5	5	5	5	5
4M	Mean	13.54	1.81	11.12	0.14	0.09	0.28	0.10
	SD	2.220	1.249	2.932	0.029	0.040	0.080	0.043
	N	5	5	5	5	5	5	5

TABLE 2 - continued

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Plt $x10^9/L$	PT sec	APTT sec
Statistical test:		Wi	Wi	Wi
1M	Mean	1268	14.6	17.4
	SD	103.5	0.87	2.72
	N	5	5	5
2M	Mean	1338	14.1	19.8
	SD	124.9	0.39	2.21
	N	5	5	5
3M	Mean	1314	15.0	19.2
	SD	70.0	0.30	1.94
	N	5	5	5
4M	Mean	1316	14.6	19.3
	SD	84.1	0.23	1.88
	N	5	5	5

TABLE 2 - continued

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Hct L/L	Hb g/dL	RBC $x10^{12}/L$	Retic %	MCH pg	MCHC g/dL	MCV fL
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	0.423	15.1	8.15	1.79	18.5	35.6	52.0
	SD	0.0108	0.47	0.394	0.474	0.57	0.25	1.85
	N	5	5	5	5	5	5	5
2F	Mean	0.429	15.3	8.49	1.01**	18.0	35.7	50.6
	SD	0.0134	0.38	0.331	0.306	0.55	0.56	1.02
	N	5	5	5	5	5	5	5
3F	Mean	0.443	15.9	8.60	1.17**	18.5	35.8	51.6
	SD	0.0109	0.42	0.534	0.374	0.83	0.38	2.34
	N	5	5	5	5	5	5	5
4F	Mean	0.434	15.4	8.37	1.14**	18.5	35.6	51.9
	SD	0.0101	0.25	0.178	0.215	0.50	0.52	1.88
	N	5	5	5	5	5	5	5

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TABLE 2 - continued

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		WBC $x10^9/L$	N $x10^9/L$	L $x10^9/L$	E $x10^9/L$	B $x10^9/L$	M $x10^9/L$	LUC $x10^9/L$
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	11.23	1.04	9.68	0.12	0.05	0.25	0.08
	SD	2.136	0.183	2.217	0.050	0.022	0.065	0.045
	N	5	5	5	5	5	5	5
2F	Mean	9.11*	0.86	7.89	0.11	0.03	0.14*	0.07
	SD	0.490	0.577	1.010	0.027	0.013	0.031	0.028
	N	5	5	5	5	5	5	5
3F	Mean	8.01**	1.08	6.62**	0.09	0.03	0.13*	0.05
	SD	0.781	0.970	0.939	0.034	0.011	0.049	0.023
	N	5	5	5	5	5	5	5
4F	Mean	8.54**	0.75	7.42**	0.10	0.03	0.18*	0.06
	SD	0.960	0.277	0.717	0.024	0.016	0.069	0.027
	N	5	5	5	5	5	5	5

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TABLE 2 - continued

Haematology - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Plt $x10^9/L$	PT sec	APTT sec
Statistical test:		Wi	Sh	Wi
1F	Mean	1360	14.6	17.8
	SD	130.7	0.99	2.05
	N	5	5	5
2F	Mean	1291	15.3	18.7
	SD	111.1	0.08	1.72
	N	5	5	5
3F	Mean	1293	15.5	18.0
	SD	51.6	0.30	1.02
	N	4	5	5
4F	Mean	1286	15.6*	16.6
	SD	158.7	0.55	0.51
	N	5	5	5

TABLE 3

Blood chemistry - group mean values - during Week 4 of treatment

Group :	1	2	3	4
Compound :	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm) :	0	4967	14953	49686

Group /Sex		ALP U/L	ALT U/L	AST U/L	gGT U/L	Bili µmol/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	Trig mmol/L
Statistical test:		Wi	Wi	Wi	Fe	Fe	Wi	Wi	Wi	Wi	Wi
1M	Mean	182	55	100	3	2	5.09	29	7.37	1.66	0.61
	SD	38.1	7.7	10.8	6.3	0.0	0.523	1.9	1.233	0.385	0.181
	N	5	5	5	5	5	5	5	5	5	5
2M	Mean	162	50	100	3	2	4.82	30	7.59	1.75	0.63
	SD	26.4	5.9	33.3	7.2	0.4	0.551	2.6	1.232	0.436	0.210
	N	5	5	5	5	5	5	5	5	5	5
3M	Mean	181	51	99	0	2	5.39	28	7.86	1.66	0.50
	SD	42.4	7.2	32.8	0.4	0.5	1.021	3.0	0.969	0.334	0.166
	N	5	5	5	5	5	5	5	5	5	5
4M	Mean	154	51	128	3	2	5.41	30	8.20	1.33	0.81
	SD	20.1	7.3	23.5	4.8	0.5	0.938	2.8	2.062	0.100	0.232
	N	5	5	5	5	5	5	5	5	5	5

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TABLE 3 - continued

Blood chemistry - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Na mmol/L	K mmol/L	Cl mmol/L	Ca mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	A/G Ratio
Statistical test:		Wi	Wi	Wi	Wi	Du	Wi	Wi	Wi
1M	Mean	144	4.0	101	2.71	2.42	65	36	1.20
	SD	1.3	0.26	1.3	0.061	0.088	3.0	1.5	0.083
	N	5	5	5	5	5	5	5	5
2M	Mean	143	4.0	101	2.71	2.64*	68	36	1.16
	SD	0.7	0.15	0.5	0.054	0.133	6.1	0.7	0.195
	N	5	5	5	5	5	5	5	5
3M	Mean	143	4.5	102	2.67	2.41	64	35	1.20
	SD	0.8	0.73	1.8	0.068	0.136	2.7	1.5	0.083
	N	5	5	5	5	5	5	5	5
4M	Mean	143	4.3	102	2.67	2.35	66	36	1.16
	SD	1.0	0.49	0.4	0.033	0.078	4.2	1.7	0.115
	N	5	5	5	5	5	5	5	5

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ATS0031

TABLE 3 - continued

Blood chemistry - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		ALP U/L	ALT U/L	AST U/L	gGT U/L	Bili µmol/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	Trig mmol/L
Statistical test:		Wi	Wi	Wi	Fe	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	91	40	86	0	2	6.24	37	6.72	1.56	0.83
	SD	15.7	7.9	13.9	0.0	0.4	1.840	3.0	0.931	0.188	0.306
	N	5	5	5	5	5	5	5	5	5	5
2F	Mean	108	40	83	0	2	5.67	35	6.68	1.66	0.68
	SD	12.5	5.1	15.8	0.5	0.5	0.358	4.7	0.603	0.444	0.366
	N	5	5	5	5	5	5	5	5	5	5
3F	Mean	99	35	88	0	2	6.40	34	6.34	1.45	0.77
	SD	28.0	3.8	20.1	0.9	0.8	1.008	1.9	1.032	0.215	0.205
	N	5	5	5	5	5	5	5	5	5	5
4F	Mean	92	38	113*	0	2	5.86	33	6.34	1.41	0.56
	SD	9.1	6.8	24.6	0.0	0.7	0.500	3.0	0.677	0.215	0.192
	N	5	5	5	5	5	5	5	5	5	5

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ATS0031

TABLE 3 - continued

Blood chemistry - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Na mmol/L	K mmol/L	Cl mmol/L	Ca mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	A/G Ratio
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	143	3.6	101	2.68	1.86	68	37	1.22
	SD	1.0	0.22	1.5	0.061	0.136	3.0	2.2	0.058
	N	5	5	5	5	5	5	5	5
2F	Mean	143	3.7	102	2.64	1.92	68	36	1.15
	SD	1.1	0.43	1.3	0.085	0.152	1.3	1.3	0.089
	N	5	5	5	5	5	5	5	5
3F	Mean	143	3.7	102	2.64	1.88	68	37	1.18
	SD	1.3	0.27	1.3	0.031	0.158	3.9	0.5	0.147
	N	5	5	5	5	5	5	5	5
4F	Mean	143	3.8	102	2.63	1.99	64	35	1.23
	SD	0.8	0.11	1.5	0.068	0.110	1.9	1.1	0.047
	N	5	5	5	5	5	5	5	5

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TABLE 4

Urinalysis - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Vol mL	pH	SG g/L	Prot g/L	U-Na mmol/L	U-K mmol/L	U-Cl mmol/L	T-Na mmol	T-K mmol	T-Cl mmol
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	4.6	7.0	1035	1.12	86.4	196.4	80.8	0.421	0.875	0.392
	SD	1.43	0.48	4.7	0.271	45.47	32.18	33.30	0.2667	0.1667	0.2310
	N	5	5	5	5	5	5	5	5	5	5
2M	Mean	7.8	7.1	1030	0.81*	93.2	170.0	86.8	0.723	1.276	0.644
	SD	2.81	0.16	3.6	0.103	11.45	26.12	14.92	0.3046	0.3454	0.1644
	N	5	5	5	5	5	5	5	5	5	5
3M	Mean	6.7	6.7	1032	0.68**	91.2	172.7	75.4	0.622	1.102	0.517
	SD	2.76	0.26	3.4	0.084	12.21	33.52	8.02	0.3023	0.3071	0.2399
	N	5	5	5	5	5	5	5	5	5	5
4M	Mean	8.9*	6.7	1028*	0.65**	98.6	126.9**	76.0	0.894*	1.094	0.692
	SD	2.78	0.50	5.0	0.235	13.41	21.06	14.65	0.3782	0.2253	0.2884
	N	5	5	5	5	5	5	5	5	5	5

TABLE 4 - continued

Urinalysis - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Vol mL	pH	SG g/L	Prot g/L	U-Na mmol/L	U-K mmol/L	U-Cl mmol/L	T-Na mmol	T-K mmol	T-Cl mmol
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	3.7	6.6	1030	0.17	71.4	142.8	58.6	0.250	0.489	0.209
	SD	2.53	0.42	5.7	0.035	23.97	34.59	15.84	0.1633	0.3132	0.1308
	N	5	5	5	5	5	5	5	5	5	5
2F	Mean	4.2	6.4	1037	0.20	81.6	162.0	47.8	0.328	0.571	0.182
	SD	2.45	0.73	16.8	0.074	17.84	81.25	11.78	0.1513	0.1576	0.0681
	N	5	5	5	5	5	5	5	5	5	5
3F	Mean	4.4	5.9	1039	0.18	91.6	145.0	72.8	0.407	0.597	0.304
	SD	1.75	0.23	12.1	0.051	19.82	52.12	16.54	0.1838	0.1789	0.0810
	N	5	5	5	5	5	5	5	5	5	5
4F	Mean	4.9	6.1	1035	0.16	99.4*	134.3	69.8	0.483	0.616	0.326
	SD	2.02	0.32	6.6	0.019	10.43	32.31	12.28	0.1801	0.0899	0.0658
	N	5	5	5	5	5	5	5	5	5	5

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TABLE 5

Urinary fluoride - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Fluoride expressed as F (mg/L)
Statistical test:		lWi
1M	Mean	1.2
	SD	0.16
	N	5
2M	Mean	7.4**
	SD	1.69
	N	5
3M	Mean	7.5**
	SD	2.22
	N	5
4M	Mean	7.7**
	SD	0.72
	N	5

TABLE 5 - continued

Urinary fluoride - group mean values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Fluoride expressed as F (mg/L)
Statistical test:		lWi
1F	Mean	<0.8
	SD	<0.26
	N	5
2F	Mean	4.9**
	SD	2.81
	N	5
3F	Mean	5.1**
	SD	1.29
	N	5
4F	Mean	8.1**
	SD	0.96
	N	5

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TABLE 6

Request ID: 167328

Organ weights - group mean values - unadjusted and adjusted (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Terminal Bodyweight	Adrenals	Heart	Kidneys	Liver	Lungs + Bronchi	Spleen	Testes
Unadjusted Means									
Statistical test:		Wi							
1M	Mean	335	0.053	1.291	2.18	14.09	1.300	0.669	3.37
	SD	14	0.002	0.207	0.06	1.39	0.071	0.104	0.17
	N	5	5	5	5	5	5	5	5
2M	Mean	348	0.057	1.346	2.45	14.96	1.331	0.718	3.35
	SD	37	0.010	0.154	0.23	2.28	0.174	0.130	0.12
	N	5	5	5	5	5	5	5	5
3M	Mean	349	0.060	1.250	2.21	14.78	1.343	0.685	3.15
	SD	34	0.012	0.134	0.19	1.71	0.185	0.152	0.36
	N	5	5	5	5	5	5	5	5
4M	Mean	348	0.054	1.234	2.20	14.53	1.279	0.668	2.97
	SD	14	0.004	0.079	0.12	2.38	0.137	0.087	0.27
	N	5	5	5	5	5	5	5	5
Adjusted Means									
Statistical test:			Wi	Wi	Du	Wi	Wi	Wi	Wi
1M	Mean		0.053	1.299	2.22	14.69	1.340	0.687	3.41
2M	Mean		0.057	1.344	2.43*	14.76	1.317	0.712	3.33
3M	Mean		0.060	1.247	2.19	14.56	1.328	0.678	3.14
4M	Mean		0.054	1.231	2.19	14.35	1.267	0.662	2.96**

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ATS0031

TABLE 6 - continued

Request ID: 167328

Organ weights - group mean values - unadjusted and adjusted (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex		Terminal Bodyweight	Adrenals	Heart	Kidneys	Liver	Lungs + Bronchi	Spleen
Unadjusted Means								
Statistical test:		Wi						
1F	Mean	237	0.059	0.878	1.38	9.19	1.053	0.567
	SD	8	0.008	0.082	0.07	1.05	0.053	0.092
	N	5	5	5	5	5	5	5
2F	Mean	227	0.062	0.905	1.44	7.79	1.079	0.464
	SD	15	0.007	0.094	0.09	0.68	0.082	0.039
	N	5	5	5	5	5	5	5
3F	Mean	226	0.062	0.870	1.48	8.74	1.096	0.468
	SD	13	0.007	0.128	0.10	1.09	0.123	0.053
	N	5	5	5	5	5	5	5
4F	Mean	202**	0.058	0.908	1.45	7.30	1.047	0.494
	SD	7	0.011	0.078	0.06	0.51	0.025	0.041
	N	5	5	5	5	5	5	5
Adjusted Means								
Statistical test:			Wi	Wi	Wi	Wi	Wi	Du
1F	Mean		0.055	0.794	1.33	8.45	0.977	0.531
2F	Mean		0.060	0.883	1.42	7.59	1.058*	0.454
3F	Mean		0.061	0.849	1.46*	8.55	1.077*	0.459
4F	Mean		0.064	1.036**	1.53*	8.43	1.163**	0.548

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TABLE 7

Request ID: 167319

Macropathology - group distribution of findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Tissue and finding	Group/sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number Examined:	5	5	5	5	5	5	5	5
Kidneys									
Pelvic dilatation		0	0	0	0	1	0	0	1
Liver									
Median cleft pale area(s)		1	0	0	0	0	0	0	0
Ln mandibular									
Congested		2	0	0	0	0	0	0	0
Enlarged		2	2	0	2	1	0	2	0
Ln bronchial									
Not visible		0	0	1	0	0	0	0	0
Stomach									
Antrum white nodule(s)		1	0	1	0	0	0	1	0
Forestomach depression(s)		0	0	0	0	0	0	1	0
Uterus									
Fluid distension		-	-	-	-	3	2	1	1

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TABLE 8

Request ID: 174454

Histopathology - group distribution of findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
	:	0	4967	14953	49686

Tissue and Finding	Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number:	5	5	5	5	5	5	5	5
Lungs + Bronchi	Number Examined:	5	0	0	5	5	0	0	5
Aggregations of Alveolar Macrophages		1	0	0	2	1	0	0	2
Alveolar Osseous Metaplasia		1	0	0	0	0	0	0	0
Arterial Mural Mineralisation		1	0	0	0	0	0	0	1
Perivascular Inflammatory Cells		0	0	0	2	0	0	0	1
Liver	Number Examined:	5	0	0	5	5	0	0	5
Inflammation, Focal		2	0	0	2	2	0	0	1
Single Cell Necrosis		0	0	0	0	1	0	0	3
Kidneys	Number Examined:	5	0	0	5	5	0	0	5
Cortical Cyst(s)		0	0	0	0	1	0	0	0
Mineralisation, Corticomedullary		0	0	0	0	1	0	0	0
Heart	Number Examined:	5	5	5	5	5	5	5	5
Congestion		0	2	2	0	0	0	0	1
Focal/Multifocal Inflammation		3	0	0	0	4	0	0	0
Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration		0	4	4	5	0	3	4	5
Myocardial Fibrosis		0	1	2	2	0	0	1	1
Spleen	Number Examined:	5	0	0	5	5	0	0	5
Haemosiderosis		0	0	0	0	1	0	0	0

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ATS0031

TABLE 8 - continued

Request ID: 174454

Histopathology - group distribution of findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
	:	0	4967	14953	49686

Tissue and Finding	Group/Sex: Number:	1M 5	2M 5	3M 5	4M 5	1F 5	2F 5	3F 5	4F 5
Testes	Number Examined:	5	0	0	5	-	-	-	-
Seminiferous Tubular Atrophy		2	0	0	1	-	-	-	-
Seminiferous Tubular Vacuolation		2	0	0	4	-	-	-	-
Larynx	Number Examined:	5	0	0	5	5	0	0	5
Inflammation		5	0	0	4	4	0	0	5
Tracheal Bifurc.	Number Examined:	5	0	0	5	5	0	0	5
Subepithelial Inflammatory Cell Aggregates		0	0	0	1	0	0	0	1
Nasal turbinates	Number Examined:	5	0	0	5	5	0	0	5
Olfactory Epithelium - Degeneration		0	0	0	0	1	0	0	0
Olfactory Epithelium - Rosette Formation		0	0	0	1	1	0	0	0
Olfactory Epithelium Cyst		0	0	0	0	0	0	0	1
Olfactory Epithelium Mineralisation		0	0	0	0	1	0	0	1
Respiratory Epithelium - Pseudogland Formation		0	0	0	1	1	0	0	0
Respiratory Epithelium Mineralisation		0	0	0	0	0	0	0	1
LN Mandibular	Number Examined:	3	2	0	2	1	0	2	0
Lymphoid Hyperplasia		0	2	0	0	0	0	2	0
Plasmacytosis		3	2	0	2	0	0	2	0
Sinus Erythrocytosis/Erythrophagocytosis		2	0	0	0	0	0	0	0

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ATS0031

TABLE 8 - continued

Request ID: 174454

Histopathology - group distribution of findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
	:	0	4967	14953	49686

Tissue and Finding	Group/Sex:	1M	2M	3M	4M	1F	2F	3F	4F
	Number:	5	5	5	5	5	5	5	5
Stomach	Number Examined:	0	0	0	0	0	0	1	0
Epithelial Hyperplasia - Nonglandular Region		0	0	0	0	0	0	1	0
Hyperkeratosis - Nonglandular Region		0	0	0	0	0	0	1	0
Submucosal Inflammation - Nonglandular Region		0	0	0	0	0	0	1	0
Uterus	Number Examined:	-	-	-	-	3	2	1	1
Luminal Dilatation		-	-	-	-	3	2	1	0

Huntingdon Life Sciences
ATS0031

APPENDIX 1

Request ID: 167320

Clinical signs - individual observations

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Death Code	Death Day	Category	Observation	Week(s)
2M	6	T	29	Coat	Hair loss, Head	-1-2
	9	T	29	Coat	Hair loss, Forelimb (Left)	-1
					Hair loss, Forelimbs	1

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ATS0031

APPENDIX 1 - continued

Request ID: 167320

Clinical signs - individual observations

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Death Code	Death Day	Category	Observation	Week(s)
4M	17	T	29	Staining	Abnormal Colour, Red, Head	4
	18	T	29	Staining	Abnormal Colour, Red, Head	4

APPENDIX 1 - continued

Request ID: 167320

Clinical signs - individual observations

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Death Code	Death Day	Category	Observation	Week(s)
1F	21	T	29	Staining	Abnormal Colour, Red, Head	4-5
	23	T	29	Behaviour	Vocalisation	-1

Huntingdon Life Sciences
ATS0031

Clinical signs - individual observations

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Death Code	Death Day	Category	Observation	Week(s)
3F	32	T	29	Staining	Abnormal Colour, Red, Head	4-5
	35	T	29	Behaviour	Vocalisation	1-2
				Staining	Abnormal Colour, Red, Head	2

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ATS0031

APPENDIX 1 - continued

Request ID: 167320

Clinical signs - individual observations

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Death Code	Death Day	Category	Observation	Week(s)
4F	36	T	29	Behaviour	Vocalisation	4-5
	38	T	29	Behaviour	Vocalisation	4-5
	39	T	29	Staining	Abnormal Colour, Red, Head	4
					Abnormal Colour, Red, Dorsal Body Surface	4

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ATS0031

APPENDIX 2

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
1M	1	Coat			
		Wet fur, Moderate		2-3, 9, 11-12, 16	
		Staining			
		Red, around both eyes		2-3, 9, 18, 22	
	2	Coat			
		Wet fur, Moderate		2-3, 5, 11-12, 15, 17, 22-23, 25	
		Wet fur, Marked		18-19	
		Staining			
		Red, around both eyes		2-5, 9-12, 15-16, 18, 25-26	
	3	Coat			
		Wet fur, Moderate		1-2, 9-10, 23	
		Wet fur, Marked		16	
		Staining			
		Red, around both eyes		2-5, 8, 11, 15, 18, 23, 26	

Huntingdon Life Sciences
ATS0031

APPENDIX 2 - continued

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
1M	4	Coat			
		Wet fur, Moderate		2-4, 8-12, 17-19, 23-24	
		Staining			
		Red, around both eyes		4-5, 8, 10, 17, 22, 25	
	5	Coat			
		Wet fur, Moderate		3, 5, 9-10, 12, 15-16, 19, 24	
		Wet fur, Marked		11	
		Staining			
		Red, around both eyes		4, 10, 16, 18-19, 22, 25	

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
2M	7	Coat			
		Wet fur, Moderate		16-17, 23	
		Wet fur, Marked		22	
		Staining			
		Red, around both eyes		2, 5, 22, 26	
	8	Coat			
		Wet fur, Moderate		5, 10	
		Wet fur, Marked		23	
		Staining			
		Red, around both eyes		5, 9, 15, 18-19, 22, 26	
	9	Coat			
		Wet fur, Moderate		1-3, 5, 9, 11-12, 15	
		Wet fur, Marked		16	
		Staining			
		Red, around both eyes		4-5, 8, 10-12, 15, 17-19, 22	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
2M	10	Coat			
		Wet fur, Moderate		3, 5, 11-12, 15, 25	
		Wet fur, Marked		2, 4	
		Staining			
		Red, around both eyes		3, 5, 9-12, 15-17, 19, 22, 25	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
3M	11	Coat			
		Wet fur, Moderate		1-2, 5, 8, 10-12, 19	
		Wet fur, Marked		9	
		Staining			
		Red, around both eyes		3, 8, 11-12, 18-19	
	12	Coat			
		Wet fur, Moderate		1-4, 10, 12, 15, 23	
		Staining			
		Red, around both eyes		8-9, 11-12	
	13	Coat			
		Wet fur, Moderate		3-5, 10, 22	
		Wet fur, Marked		11	
		Staining			
		Red, around both eyes		2, 10, 19	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days Pre exposure	On return to home cage	Late check
3M	14	Coat			
		Wet fur, Moderate		3, 8, 19, 22	
		Wet fur, Marked		15	
		Staining			
		Red, around both eyes		2, 9-12, 17, 19	
	15	Coat			
		Wet fur, Moderate		8, 10, 12, 16, 22, 25	
		Wet fur, Marked		17-18	
		Staining			
		Red, around both eyes		2-3, 8-11, 24-26	

Huntingdon Life Sciences
ATS0031

APPENDIX 2 - continued

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
4M	16	Coat			
		Wet fur, Moderate		1, 3-4	
		Staining			
		Red, around both eyes		3, 5, 10, 22	
	17	Coat			
		Wet fur, Moderate		1, 10, 12, 16, 24	
		Staining			
		Red, around both eyes		2, 5, 12	
	18	Coat			
		Wet fur, Moderate		1-2, 4	
		Staining			
		Red, around both eyes		3, 8, 10-12, 16, 24	
	19	Coat			
		Wet fur, Moderate		1-2, 8	
		Staining			
		Red, around both eyes		2, 9-10, 12, 19, 22, 26	

Huntingdon Life Sciences
ATS0031

APPENDIX 2 - continued

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Pre exposure	Days On return to home cage	Late check
4M	20	Coat			
		Wet fur, Moderate		1, 5, 8-9, 16, 24	

APPENDIX 2 - continued

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
1F	21	Coat			
		Wet fur, Moderate		1, 3-4, 8-12, 15, 22, 25-26	
		Wet fur, Marked		2, 5	
		Staining			
		Red, around both eyes		2, 8-10, 12, 23, 25	
	22	Coat			
		Wet fur, Moderate		4-5, 8, 11-12	
		Staining			
		Red, around both eyes		3-4, 8, 10-11, 15, 17-19	
	23	Coat			
		Wet fur, Moderate		4, 9, 17, 22-26	
		Wet fur, Marked		1-3, 5, 8, 10-12, 15-16, 18-19	
		Staining			
		Red, around both eyes		4, 8, 12, 18	

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days Pre exposure	On return to home cage	Late check
1F	24	Coat			
		Wet fur, Moderate		2-3, 9, 15-17	
		Wet fur, Marked		5	
		Staining			
		Red, around both eyes		22-23	
	25	Coat			
		Wet fur, Moderate		2-5, 9, 11, 24	
		Staining			
		Red, around both eyes		3-4, 11, 23	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
2F	26	Coat			
		Wet fur, Moderate		1-5, 8-12, 15, 17, 19, 22, 25	
		Staining			
		Red, around both eyes		2, 12, 25-26	
	27	Coat			
		Wet fur, Moderate		1-2, 8-12, 15-16, 18, 24	
		Wet fur, Marked		4-5, 17, 19	
		Staining			
		Red, around both eyes		2-3, 10, 12, 19	
	28	Coat			
		Wet fur, Slight		3	
		Wet fur, Moderate		1, 4, 8-10, 17-18	
		Staining			
		Red, around both eyes		3, 9, 12	

Huntingdon Life Sciences
ATS0031

APPENDIX 2 - continued

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
2F	29	Coat			
		Wet fur, Moderate		2, 4, 9, 11-12, 19, 22, 24	
		Wet fur, Marked		16	
		Staining			
		Red, around both eyes		2-4, 8-9, 11, 17, 19, 22, 25	
	30	Coat			
		Wet fur, Moderate		1, 4, 8-9, 15, 19, 23-24, 26	
		Wet fur, Marked		2, 5, 10-12, 16-18, 22, 25	
		Staining			
		Red, around both eyes		8-10, 12, 19	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
3F	31	Coat			
		Wet fur, Moderate		4, 9-12, 16, 19, 24	
		Wet fur, Marked		1-3, 5, 18	
		Staining			
		Red, around both eyes		9-10, 15, 18-19	
	32	Coat			
		Wet fur, Moderate		1, 4, 8-9, 11-12, 15-19, 22, 25	
		Wet fur, Marked		2, 10	
		Staining			
		Red, around both eyes		9, 11, 15	
	33	Coat			
		Wet fur, Moderate		1-4, 8, 16-17, 22-23, 26	
		Wet fur, Marked		5, 9, 11-12, 15, 18	
		Staining			
		Red, around both eyes		2, 4, 8, 10, 12, 15, 18-19, 26	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Pre exposure	Days On return to home cage	Late check
3F	34	Coat			
		Wet fur, Moderate		4, 8, 11-12, 16, 24	
		Wet fur, Marked		3	
		Staining			
		Red, around both eyes		10-12	
	35	Coat			
		Wet fur, Moderate		2, 4, 8-12, 15-16, 23-24	
		Wet fur, Marked		5	
		Staining			
		Red, around both eyes		2-5, 10-12, 15-16, 19, 24-25	

Huntingdon Life Sciences
ATS0031

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
4F	36	Coat			
		Wet fur, Moderate		1-5, 9, 17, 22	
		Staining			
		Red, around both eyes		2, 4, 8	
	37	Coat			
		Wet fur, Moderate		2-5, 8-10, 15-16, 18, 22	
		Wet fur, Marked		1, 11-12, 17	
		Staining			
		Red, around both eyes		10-11	
	38	Coat			
		Wet fur, Moderate		1-3, 5, 15, 18	
		Staining			
		Red, around both eyes		3-4	
	39	Coat			
		Wet fur, Moderate		1-2, 5, 8, 12, 15	
		Staining			
		Red, around both eyes		3, 12, 19	

Huntingdon Life Sciences
ATS0031

APPENDIX 2 - continued

Request ID: 167472

Dose observations - individual signs

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Sign	Days: Pre exposure	On return to home cage	Late check
4F	40	Coat			
		Wet fur, Moderate		3-5, 8-10, 12, 24	
		Wet fur, Marked		1-2	
		Staining			
		Red, around both eyes		3	

Huntingdon Life Sciences
ATS0031

APPENDIX 3

Request ID: 167322

Bodyweights - individual values (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Week -1	Week 0	Week 1	Week 2	Week 3	Week 4
1M	1	198	241	282	314	338	339
	2	200	241	285	315	340	344
	3	183	223	262	299	331	341
	4	203	243	289	322	337	340
	5	193	235	271	291	312	313
2M	6	204	252	303	334	367	377
	7	194	235	269	287	313	309
	8	208	254	306	353	393	398
	9	203	233	276	303	333	327
	10	189	227	260	295	326	328
3M	11	191	230	274	294	306	313
	12	215	265	317	357	398	406
	13	193	228	276	316	342	341
	14	198	235	271	303	335	342
	15	209	249	291	316	339	342
4M	16	184	226	271	305	336	335
	17	215	256	304	333	361	358
	18	211	259	304	330	369	362
	19	204	243	287	325	357	354
	20	195	233	275	310	336	331

Huntingdon Life Sciences
ATS0031

APPENDIX 3 - continued

Request ID: 167322

Bodyweights - individual values (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Week -1	Week 0	Week 1	Week 2	Week 3	Week 4
1F	21	198	204	218	234	250	249
	22	173	195	200	216	227	225
	23	186	200	218	221	225	233
	24	175	188	208	225	237	238
	25	195	197	219	230	236	238
2F	26	187	202	210	225	238	235
	27	179	200	221	229	234	235
	28	192	210	224	238	248	240
	29	178	190	207	220	229	220
	30	164	179	190	196	207	204
3F	31	189	203	213	236	247	236
	32	177	191	208	204	224	225
	33	181	199	215	224	233	227
	34	186	200	222	229	226	238
	35	165	182	194	205	214	207
4F	36	174	192	211	205	231	207
	37	176	186	206	213	224	200
	38	177	190	199	210	223	204
	39	166	181	194	197	215	190
	40	175	190	211	215	231	206

APPENDIX 4

Request ID: 167323

Food consumption - individual values (g/animal/week)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Cage Number	Week -1	Week 1	Week 2	Week 3	Week 4
1M	1	178	170	183	187	170
2M	2	185	178	193	199	166
3M	3	186	183	194	202	173
4M	4	186	181	194	204	176

Huntingdon Life Sciences
ATS0031

APPENDIX 4 - continued

Request ID: 167323

Food consumption - individual values (g/animal/week)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Cage Number	Week -1	Week 1	Week 2	Week 3	Week 4
1F	5	130	130	135	143	123
2F	6	130	130	137	141	117
3F	7	130	130	133	142	119
4F	8	128	133	133	143	112

APPENDIX 5

Ophthalmic examination - individual observations - pretreatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

		Group and sex/Animal number							
Structure	Observation	1M	2M	3M	4M	1F	2F	3F	4F
Lens	Persistent hyaloid artery	2L	6						38R
Cornea	Superficial opacity, faint		7			22L			
	Superficial opacity, slight			11L					
	Superficial opacity, slight, medial				16L				
Iris	Haemorrhage, slight, dorsomedial				20L				
Number of animals examined :		5	5	5	5	5	5	5	5

Huntingdon Life Sciences
ATS0031

APPENDIX 5 - continued

Ophthalmic examination - individual observations - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

		Group and sex/Animal number							
Structure	Observation	1M	2M	3M	4M	1F	2F	3F	4F
Lens	Persistent hyaloid artery	2L	6						38R
Cornea	Superficial opacity, faint		7			22L			
	Superficial opacity, faint, linear					22R			
	Superficial opacity, slight			11L					
	Superficial opacity, slight, medial				16L				
Number of animals examined :		5	5	5	5	5	5	5	5

Huntingdon Life Sciences
ATS0031

APPENDIX 6

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Hct L/L	Hb g/dL	RBC $x10^{12}/L$	Retic %	MCH pg	MCHC g/dL	MCV fL
1M	1	0.476	16.5	8.48	1.95	19.5	34.7	56.2
	2	0.458	15.8	8.72	1.66	18.1	34.5	52.5
	3	0.465	15.8	8.21	2.35	19.3	34.1	56.6
	4	0.472	16.2	8.91	1.59	18.1	34.3	52.9
	5	0.451	15.7	8.56	1.49	18.3	34.8	52.7
2M	6	0.439	15.3	8.27	2.29	18.5	34.9	53.0
	7	0.466	16.2	8.87	1.45	18.3	34.8	52.5
	8	0.448	15.5	8.05	2.73	19.2	34.5	55.7
	9	0.477	16.4	9.02	1.43	18.2	34.4	52.9
	10	0.444	15.5	7.99	2.18	19.3	34.8	55.5
3M	11	0.487	17.1	9.37	0.55	18.2	35.1	52.0
	12	0.495	17.3	9.50	1.96	18.2	34.9	52.1
	13	0.493	17.1	9.23	1.57	18.5	34.6	53.4
	14	0.464	16.0	8.52	1.70	18.8	34.5	54.5
	15	0.437	15.1	8.28	1.38	18.2	34.5	52.8
4M	16	0.469	16.4	8.78	1.75	18.6	34.9	53.4
	17	0.468	16.2	8.88	1.06	18.2	34.6	52.7
	18	0.490	16.9	8.80	1.47	19.1	34.4	55.7
	19	0.496	17.1	8.96	1.48	19.0	34.4	55.4
	20	0.498	17.1	9.13	1.53	18.8	34.4	54.5

Huntingdon Life Sciences
ATS0031

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	WBC $x10^9/L$	N $x10^9/L$	L $x10^9/L$	E $x10^9/L$	B $x10^9/L$	M $x10^9/L$	LUC $x10^9/L$	Plt $x10^9/L$
1M	1	14.97	2.89	11.53	0.15	0.03	0.25	0.11	1128
	2	13.50	1.07	11.84	0.06	0.07	0.27	0.17	1372
	3	13.38	0.86	11.93	0.11	0.06	0.31	0.11	1294
	4	20.41	2.95	16.38	0.12	0.25	0.55	0.17	1349
	5	15.35	1.70	13.05	0.08	0.11	0.34	0.07	1196
2M	6	13.64	3.21	9.80	0.22	0.09	0.25	0.07	1310
	7	16.76	4.67	11.28	0.11	0.08	0.52	0.10	1463
	8	13.92	0.95	12.50	0.07	0.09	0.24	0.07	1143
	9	15.90	1.02	14.32	0.09	0.15	0.20	0.12	1345
	10	12.55	1.58	10.44	0.09	0.08	0.31	0.07	1427
3M	11	10.74	1.18	9.18	0.08	0.08	0.18	0.03	1315
	12	15.59	1.43	13.23	0.13	0.10	0.57	0.13	1284
	13	12.73	1.40	10.73	0.14	0.10	0.27	0.09	1353
	14	14.88	1.12	13.14	0.12	0.08	0.20	0.21	1401
	15	14.54	2.44	11.46	0.08	0.19	0.27	0.09	1216
4M	16	14.15	3.68	9.73	0.14	0.06	0.39	0.15	1359
	17	13.87	0.84	12.37	0.15	0.11	0.29	0.11	1294
	18	9.78	2.52	6.73	0.14	0.04	0.32	0.04	1421
	19	14.23	1.02	12.58	0.17	0.14	0.19	0.13	1310
	20	15.69	0.99	14.20	0.09	0.10	0.22	0.08	1194

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Aniso-cytosis	Micro-cytosis	Macro-cytosis	Hypo-chromasia	Hyper-chromasia
1M	1	-	-	-	-	-
	2	-	-	-	-	-
	3	-	-	-	-	-
	4	-	-	-	-	-
	5	-	-	-	-	-
2M	6	-	-	-	-	-
	7	-	-	-	-	-
	8	-	-	-	-	-
	9	-	-	-	-	-
	10	-	-	-	-	-
3M	11	-	-	-	-	-
	12	-	-	-	-	-
	13	-	-	-	-	-
	14	-	-	-	-	-
	15	-	-	-	-	-
4M	16	-	-	-	-	-
	17	-	-	-	-	-
	18	-	-	-	-	-
	19	-	-	-	-	-
	20	-	-	-	-	-

Huntingdon Life Sciences
ATS0031

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	PT sec	APTT sec
1M	1	14.1	17.7
	2	13.4	14.7
	3	14.9	19.7
	4	14.8	14.5
	5	15.7	20.3
2M	6	14.2	20.6
	7	13.4	23.0
	8	14.2	18.1
	9	14.2	17.4
	10	14.4	20.0
3M	11	15.4	17.0
	12	14.9	17.3
	13	14.9	19.8
	14	14.7	20.2
	15	15.3	21.5
4M	16	14.4	19.2
	17	14.6	21.5
	18	14.9	17.8
	19	14.4	17.1
	20	14.8	20.8

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Hct L/L	Hb g/dL	RBC $x10^{12}/L$	Retic %	MCH pg	MCHC g/dL	MCV fL
1F	21	0.428	15.2	7.84	2.26	19.4	35.4	54.6
	22	0.426	15.2	8.13	1.10	18.6	35.5	52.4
	23	0.422	15.1	8.13	2.13	18.5	35.7	51.9
	24	0.435	15.7	8.80	1.53	17.8	36.0	49.4
	25	0.406	14.4	7.83	1.93	18.4	35.4	51.9
2F	26	0.451	15.7	8.94	0.70	17.5	34.8	50.4
	27	0.432	15.7	8.50	1.41	18.4	36.2	50.9
	28	0.426	15.3	8.39	0.76	18.3	36.0	50.7
	29	0.416	15.0	8.03	0.96	18.6	35.9	51.8
	30	0.422	14.9	8.61	1.24	17.4	35.4	49.0
3F	31	0.436	15.8	8.14	0.99	19.4	36.1	53.6
	32	0.434	15.7	8.48	1.08	18.6	36.2	51.2
	33	0.461	16.6	9.21	0.87	18.0	36.0	50.0
	34	0.438	15.5	8.06	1.82	19.2	35.3	54.4
	35	0.444	15.8	9.10	1.09	17.4	35.6	48.9
4F	36	0.451	15.7	8.19	1.17	19.2	34.9	55.1
	37	0.432	15.7	8.44	0.80	18.6	36.3	51.2
	38	0.424	15.2	8.21	1.37	18.5	35.8	51.7
	39	0.430	15.2	8.40	1.26	18.1	35.4	51.2
	40	0.433	15.4	8.62	1.11	17.9	35.7	50.2

Huntingdon Life Sciences
ATS0031

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	WBC $x10^9/L$	N $x10^9/L$	L $x10^9/L$	E $x10^9/L$	B $x10^9/L$	M $x10^9/L$	LUC $x10^9/L$	Plt $x10^9/L$
1F	21	9.21	1.15	7.70	0.07	0.02	0.23	0.03	1235
	22	9.03	1.09	7.37	0.11	0.05	0.33	0.07	1325
	23	13.74	0.79	12.37	0.09	0.08	0.27	0.15	1576
	24	12.97	0.93	11.45	0.19	0.05	0.25	0.10	1374
	25	11.18	1.25	9.49	0.16	0.06	0.15	0.06	1292
2F	26	9.39	0.43	8.62	0.14	0.04	0.10	0.06	1354
	27	9.24	0.57	8.25	0.09	0.05	0.17	0.11	1194
	28	9.28	0.45	8.44	0.11	0.02	0.17	0.08	1175
	29	9.39	1.08	8.03	0.07	0.04	0.12	0.04	1441
	30	8.24	1.78	6.13	0.12	0.02	0.14	0.05	1292
3F	31	9.04	1.07	7.72	0.06	0.03	0.10	0.05	1295
	32	7.77	0.44	7.04	0.06	0.05	0.13	0.06	1323
	33	6.89	0.29	6.37	0.10	0.03	0.07	0.03	1219
	34	8.26	2.72	5.19	0.14	0.04	0.15	0.04	NVR
	35	8.09	0.87	6.80	0.11	0.02	0.20	0.09	1333
4F	36	9.74	0.79	8.52	0.09	0.04	0.23	0.07	1037
	37	7.26	0.39	6.55	0.08	0.04	0.18	0.03	1375
	38	8.65	0.87	7.28	0.13	0.01	0.26	0.09	1284
	39	9.06	1.11	7.56	0.11	0.05	0.16	0.07	1273
	40	7.97	0.57	7.20	0.07	0.02	0.08	0.03	1461

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Aniso-cytosis	Micro-cytosis	Macro-cytosis	Hypo-chromasia	Hyper-chromasia
1F	21	-	-	-	-	-
	22	-	-	-	-	-
	23	-	-	-	-	-
	24	-	-	-	-	-
	25	-	-	-	-	-
2F	26	-	-	-	-	-
	27	-	-	-	-	-
	28	-	-	-	-	-
	29	-	-	-	-	-
	30	-	-	-	-	-
3F	31	-	-	-	-	-
	32	-	-	-	-	-
	33	-	-	-	-	-
	34	-	-	-	-	-
	35	-	-	-	-	-
4F	36	-	-	-	-	-
	37	-	-	-	-	-
	38	-	-	-	-	-
	39	-	-	-	-	-
	40	-	-	-	-	-

Huntingdon Life Sciences
ATS0031

APPENDIX 6 - continued

Haematology - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	PT sec	APTT sec
1F	21	15.3	18.5
	22	15.2	19.7
	23	14.7	19.0
	24	15.0	14.5
	25	12.9	17.2
2F	26	15.2	16.1
	27	15.3	18.3
	28	15.4	18.9
	29	15.3	20.8
	30	15.2	19.4
3F	31	15.2	19.5
	32	15.6	17.9
	33	15.9	17.4
	34	15.6	16.8
	35	15.2	18.3
4F	36	15.9	16.4
	37	15.9	16.8
	38	15.6	17.0
	39	16.1	17.0
	40	14.7	15.8

Huntingdon Life Sciences
ATS0031

APPENDIX 7

Blood chemistry - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	ALP U/L	ALT U/L	AST U/L	gGT U/L	Bili µmol/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	Trig mmol/L
1M	1	158	48	89	0	2	4.81	30	6.50	1.78	0.46
	2	159	53	97	0	2	5.08	29	7.51	2.25	0.84
	3	187	62	96	14	2	4.52	26	5.83	1.39	0.65
	4	247	65	118	0	2	5.92	31	8.08	1.59	0.71
	5	161	49	99	0	2	5.13	30	8.93	1.27	0.40
2M	6	175	50	84	0	1	4.94	26	6.61	1.95	0.82
	7	200	59	89	0	2	4.92	31	8.50	2.33	0.54
	8	139	51	88	0	2	4.48	33	6.21	1.56	0.50
	9	137	43	159	16	2	5.61	30	7.49	1.16	0.40
	10	157	47	79	0	2	4.15	29	9.13	1.75	0.88
3M	11	148	48	90	0	1	5.23	28	6.39	1.19	0.70
	12	251	63	156	1	2	4.91	26	8.04	1.97	0.64
	13	185	49	75	0	2	5.09	24	7.87	1.67	0.40
	14	147	51	79	0	2	7.16	32	9.11	1.97	0.48
	15	175	44	96	0	1	4.56	28	7.90	1.48	0.30
4M	16	175	50	142	11	2	4.45	27	7.69	1.41	1.03
	17	121	42	90	2	1	4.97	27	8.07	1.30	0.79
	18	158	51	124	0	2	4.83	33	5.19	1.35	0.81
	19	162	62	132	0	1	6.59	32	9.33	1.41	0.98
	20	153	48	151	0	2	6.23	31	10.73	1.17	0.44

Huntingdon Life Sciences
ATS0031

APPENDIX 7 - continued

Blood chemistry - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Na mmol/L	K mmol/L	Cl mmol/L	Ca mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	A/G Ratio
1M	1	145	3.7	102	2.76	2.42	69	36	1.09
	2	145	3.9	100	2.69	2.51	66	37	1.28
	3	143	4.1	102	2.77	2.51	61	34	1.26
	4	143	4.4	101	2.72	2.37	67	37	1.23
	5	142	3.9	99	2.62	2.31	64	34	1.13
2M	6	143	3.8	101	2.78	2.84	69	36	1.09
	7	142	3.9	101	2.73	2.47	78	36	0.86
	8	143	4.2	102	2.71	2.66	64	35	1.21
	9	143	4.0	101	2.70	2.62	65	37	1.32
	10	144	4.0	102	2.63	2.60	63	36	1.33
3M	11	142	5.4	102	2.74	2.41	62	35	1.30
	12	142	5.1	101	2.70	2.62	63	35	1.25
	13	144	3.7	102	2.67	2.26	64	35	1.21
	14	143	4.2	99	2.70	2.33	68	36	1.13
	15	143	4.0	104	2.56	2.44	61	32	1.10
4M	16	143	4.4	102	2.65	2.48	66	36	1.20
	17	142	4.1	102	2.66	2.34	64	33	1.06
	18	144	3.8	101	2.73	2.34	73	37	1.03
	19	142	5.1	102	2.66	2.29	67	37	1.23
	20	144	4.2	102	2.66	2.29	62	35	1.30

Huntingdon Life Sciences
ATS0031

APPENDIX 7 - continued

Blood chemistry - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	ALP U/L	ALT U/L	AST U/L	gGT U/L	Bili µmol/L	Urea mmol/L	Creat µmol/L	Gluc mmol/L	Chol mmol/L	Trig mmol/L
1F	21	77	37	84	0	1	6.05	35	5.92	1.83	0.60
	22	85	38	79	0	2	4.66	35	7.16	1.32	0.88
	23	81	44	105	0	2	6.41	37	8.05	1.53	0.80
	24	116	50	92	0	2	9.24	42	6.69	1.63	1.32
	25	96	29	68	0	2	4.83	35	5.79	1.49	0.55
2F	26	118	45	81	1	1	5.60	31	5.98	2.29	1.11
	27	97	43	83	0	2	5.81	43	6.73	1.90	0.55
	28	106	32	68	0	1	6.08	33	6.24	1.59	1.02
	29	124	38	73	1	2	5.11	33	7.52	1.23	0.36
	30	96	41	109	0	2	5.75	36	6.93	1.28	0.34
3F	31	71	37	69	0	2	7.49	35	6.67	1.23	0.69
	32	100	40	119	0	1	5.05	32	7.18	1.55	0.94
	33	81	30	96	0	3	6.27	35	5.92	1.76	0.99
	34	97	35	82	0	1	5.89	37	4.74	1.28	0.48
	35	144	33	74	2	2	7.28	33	7.18	1.42	0.74
4F	36	107	43	131	0	3	6.08	36	7.24	1.55	0.74
	37	90	35	132	0	2	5.69	35	5.71	1.25	0.38
	38	82	47	129	0	2	5.46	35	6.00	1.60	0.39
	39	91	30	80	0	2	6.63	29	6.88	1.55	0.79
	40	92	36	93	0	1	5.45	31	5.87	1.12	0.52

Huntingdon Life Sciences
ATS0031

APPENDIX 7 - continued

Blood chemistry - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Na mmol/L	K mmol/L	Cl mmol/L	Ca mmol/L	Phos mmol/L	Total Prot g/L	Alb g/L	A/G Ratio
1F	21	144	3.5	103	2.65	1.95	64	34	1.13
	22	144	3.6	102	2.68	1.73	68	37	1.19
	23	142	3.3	99	2.61	1.73	66	37	1.28
	24	143	3.5	101	2.77	2.04	69	38	1.23
	25	142	3.9	101	2.71	1.86	72	40	1.25
2F	26	145	4.2	103	2.70	2.15	68	36	1.13
	27	142	3.1	101	2.62	1.73	69	37	1.16
	28	143	3.9	101	2.74	1.88	69	37	1.16
	29	143	3.4	103	2.62	1.94	66	37	1.28
	30	144	3.7	104	2.52	1.89	67	34	1.03
3F	31	142	3.8	101	2.65	1.98	71	37	1.09
	32	145	3.5	103	2.69	1.92	65	37	1.32
	33	143	3.7	102	2.62	1.68	67	37	1.23
	34	144	3.4	104	2.61	1.77	73	36	0.97
	35	142	4.1	101	2.64	2.07	64	36	1.29
4F	36	144	3.8	100	2.73	2.11	65	36	1.24
	37	142	4.0	101	2.55	1.97	64	35	1.21
	38	143	3.7	102	2.65	1.99	67	37	1.23
	39	143	3.9	102	2.64	2.06	63	34	1.17
	40	144	3.8	104	2.59	1.82	62	35	1.30

Huntingdon Life Sciences
ATS0031

APPENDIX 8

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	App	Vol mL	pH	SG g/L	Gluc	Keto	Bili	UBld
1M	1	PY	3.4	6.6	1037	NEG	1+	NEG	NEG
	2	PY	7.0	7.7	1028	NEG	1+	NEG	NEG
	3	PY	3.6	6.8	1039	NEG	1+	NEG	NEG
	4	PY	4.5	6.7	1039	NEG	1+	NEG	NEG
	5	PY	4.5	7.4	1033	NEG	1+	NEG	NEG
2M	6	PY	6.7	6.9	1033	NEG	1+	NEG	NEG
	7	PY	8.3	7.3	1028	NEG	2+	NEG	NEG
	8	PY	8.5	7.0	1030	NEG	2+	NEG	NEG
	9	PY	11.5	7.2	1026	NEG	2+	NEG	NEG
	10	PY	3.8	7.1	1035	NEG	2+	NEG	NEG
3M	11	CPY	4.4	7.0	1036	NEG	2+	NEG	NEG
	12	PY	10.0	6.8	1030	NEG	2+	NEG	NEG
	13	PY	5.8	6.5	1033	NEG	1+	NEG	NEG
	14	PY	9.3	6.9	1027	NEG	1+	NEG	NEG
	15	PY	4.1	6.4	1033	NEG	2+	NEG	NEG
4M	16	PY	6.2	7.0	1026	NEG	3+	NEG	NEG
	17	PY	10.3	6.5	1024	NEG	2+	NEG	NEG
	18	MY	6.8	6.0	1036	NEG	2+	NEG	NEG
	19	PY	8.2	6.8	1029	NEG	2+	NEG	NEG
	20	PY	13.0	7.3	1024	NEG	3+	NEG	NEG

Huntingdon Life Sciences
ATS0031

APPENDIX 8 - continued

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Casts	A	CLEU /HPF	CURB /HPF	CCRY /HPF	CSP /HPF	CEPI /HPF
1M	1	0	0	0	0	0	6	0
	2	0	0	0	0	1	0	0
	3	0	0	0	0	0	0	0
	4	0	0	0	0	2	7	0
	5	0	0	0	0	2	20	0
2M	6	0	0	0	0	0	6	0
	7	0	0	0	0	2	0	0
	8	0	0	0	0	0	0	0
	9	0	0	0	0	2	0	0
	10	0	0	0	0	1	4	0
3M	11	0	0	0	0	4	0	0
	12	0	0	0	0	13	7	0
	13	0	0	0	0	2	8	0
	14	0	0	0	0	4	8	0
	15	0	0	0	0	0	0	0
4M	16	0	0	0	0	0	0	0
	17	0	0	0	0	0	0	0
	18	0	0	0	0	0	3	0
	19	0	0	0	0	0	4	0
	20	0	0	0	0	0	0	0

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APPENDIX 8 - continued

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Prot g/L	U-Na mmol/L	U-K mmol/L	U-Cl mmol/L	T-Na mmol	T-K mmol	T-Cl mmol
1M	1	1.28	18.0	227.5	33.0	0.061	0.774	0.112
	2	0.94	102.0	164.5	100.0	0.714	1.152	0.700
	3	0.73	81.0	228.0	82.0	0.292	0.821	0.295
	4	1.33	144.0	199.5	121.0	0.648	0.898	0.545
	5	1.32	87.0	162.5	68.0	0.392	0.731	0.306
2M	6	0.72	85.0	198.0	84.0	0.570	1.327	0.563
	7	0.70	93.0	160.0	80.0	0.772	1.328	0.664
	8	0.86	79.0	182.0	81.0	0.672	1.547	0.689
	9	0.80	105.0	130.0	76.0	1.208	1.495	0.874
	10	0.95	104.0	180.0	113.0	0.395	0.684	0.429
3M	11	0.72	89.0	223.5	71.0	0.392	0.983	0.312
	12	0.70	109.0	140.5	73.0	1.090	1.405	0.730
	13	0.70	97.0	154.5	81.0	0.563	0.896	0.470
	14	0.54	78.0	156.0	86.0	0.725	1.451	0.800
	15	0.76	83.0	189.0	66.0	0.340	0.775	0.271
4M	16	0.62	108.0	133.5	63.0	0.670	0.828	0.391
	17	0.52	97.0	103.0	100.0	0.999	1.061	1.030
	18	0.98	84.0	139.5	66.0	0.571	0.949	0.449
	19	0.75	88.0	151.5	78.0	0.722	1.242	0.640
	20	0.36	116.0	107.0	73.0	1.508	1.391	0.949

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APPENDIX 8 - continued

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	App	Vol mL	pH	SG g/L	Gluc	Keto	Bili	UBld
1F	21	PY	0.9	6.4	1039	INS	INS	INS	INS
	22	PY	1.9	6.3	1030	NEG	NEG	NEG	NEG
	23	PY	3.7	6.3	1031	NEG	TRA	NEG	NEG
	24	PY	7.4	7.3	1023	NEG	NEG	NEG	NEG
	25	PY	4.6	6.6	1029	NEG	TRA	NEG	NEG
2F	26	PY	3.0	7.3	1030	NEG	1+	NEG	NEG
	27	PY	7.8	6.3	1029	NEG	1+	NEG	NEG
	28	PY	5.7	7.0	1024	NEG	1+	NEG	NEG
	29	PY	2.9	6.0	1038	NEG	TRA	NEG	NEG
	30	MY	1.8	5.5	1066	NEG	1+	NEG	NEG
3F	31	MY	3.0	5.7	1060	NEG	TRA	NEG	NEG
	32	PY	3.6	6.0	1033	NEG	1+	NEG	NEG
	33	PY	3.0	5.9	1036	NEG	1+	NEG	NEG
	34	PY	7.0	5.8	1032	NEG	2+	NEG	NEG
	35	PY	5.4	6.3	1032	NEG	1+	NEG	NEG
4F	36	PY	4.4	5.8	1036	NEG	2+	NEG	NEG
	37	PY	8.5	6.6	1026	NEG	3+	NEG	NEG
	38	PY	3.7	6.2	1032	NEG	1+	NEG	NEG
	39	PY	4.0	6.0	1037	NEG	1+	NEG	NEG
	40	PY	4.0	5.9	1044	NEG	2+	NEG	NEG

APPENDIX 8 - continued

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Casts	A	CLEU /HPF	CURB /HPF	CCRY /HPF	CSP /HPF	CEPI /HPF
1F	21	INS	INS	INS	INS	INS	INS	INS
	22	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0
2F	26	0	0	0	0	0	0	0
	27	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0
	29	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0
3F	31	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0
	33	0	0	0	0	0	0	0
	34	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0
4F	36	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0
	38	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0

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APPENDIX 8 - continued

Urinalysis - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Prot g/L	U-Na mmol/L	U-K mmol/L	U-Cl mmol/L	T-Na mmol	T-K mmol	T-Cl mmol
1F	21	0.18	63.0	196.0	58.0	0.057	0.176	0.052
	22	0.20	82.0	125.5	50.0	0.156	0.238	0.095
	23	0.18	64.0	156.0	68.0	0.237	0.577	0.252
	24	0.11	42.0	130.0	38.0	0.311	0.962	0.281
	25	0.18	106.0	106.5	79.0	0.488	0.490	0.363
2F	26	0.24	82.0	146.5	57.0	0.246	0.440	0.171
	27	0.10	69.0	101.0	37.0	0.538	0.788	0.289
	28	0.16	69.0	117.0	33.0	0.393	0.667	0.188
	29	0.22	112.0	142.0	56.0	0.325	0.412	0.162
	30	0.29	76.0	303.5	56.0	0.137	0.546	0.101
3F	31	0.25	115.0	231.0	97.0	0.345	0.693	0.291
	32	0.18	95.0	108.5	72.0	0.342	0.391	0.259
	33	0.20	60.0	140.5	68.0	0.180	0.422	0.204
	34	0.13	95.0	99.0	51.0	0.665	0.693	0.357
	35	0.13	93.0	146.0	76.0	0.502	0.788	0.410
4F	36	0.19	100.0	139.5	68.0	0.440	0.614	0.299
	37	0.14	94.0	84.5	51.0	0.799	0.718	0.434
	38	0.15	114.0	146.0	72.0	0.422	0.540	0.266
	39	0.16	103.0	128.5	85.0	0.412	0.514	0.340
	40	0.17	86.0	173.0	73.0	0.344	0.692	0.292

APPENDIX 9

Urinary fluoride - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Fluoride expressed as F (mg/L)
1M	1	1.3
	2	1.1
	3	1.4
	4	1.0
	5	1.1
2M	6	8.4
	7	8.6
	8	6.0
	9	5.1
	10	8.7
3M	11	9.7
	12	4.7
	13	8.9
	14	5.5
	15	8.5
4M	16	7.4
	17	8.5
	18	7.6
	19	8.3
	20	6.7

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APPENDIX 9 - continued

Urinary fluoride - individual values - during Week 4 of treatment

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Fluoride expressed as F (mg/L)
1F	21	1.2
	22	<0.7
	23	0.7
	24	0.5
	25	0.9
2F	26	3.0
	27	2.3
	28	3.3
	29	7.3
	30	8.5
3F	31	7.4
	32	4.5
	33	4.9
	34	4.4
	35	4.4
4F	36	7.3
	37	9.4
	38	8.0
	39	7.1
	40	8.7

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APPENDIX 10

Request ID: 167473

Organ weights - individual values - absolute (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Terminal bodyweight	Adrenals	Heart	Kidneys	Liver	Lungs + Bronchi	Spleen	Testes
1M	1	339	0.050	1.022	2.15	14.26	1.246	0.814	3.63
	2	343	0.055	1.157	2.29	15.34	1.288	0.618	3.25
	3	343	0.051	1.320	2.13	13.56	1.219	0.596	3.41
	4	342	0.054	1.407	2.18	15.29	1.385	0.575	3.35
	5	311	0.053	1.549	2.15	11.98	1.360	0.744	3.21
2M	6	377	0.067	1.512	2.71	17.06	1.525	0.869	3.46
	7	309	0.043	1.505	2.16	12.12	1.121	0.778	3.33
	8	398	0.053	1.306	2.63	17.54	1.438	0.707	3.43
	9	330	0.068	1.225	2.30	14.12	1.394	0.722	3.16
	10	328	0.057	1.185	2.44	13.96	1.176	0.514	3.36
3M	11	313	0.051	1.133	2.04	12.91	1.079	0.536	2.56
	12	406	0.052	1.390	2.43	17.51	1.595	0.903	3.26
	13	341	0.053	1.334	2.37	14.97	1.367	0.682	3.15
	14	342	0.066	1.311	2.02	14.57	1.301	0.552	3.28
	15	342	0.078	1.083	2.19	13.94	1.373	0.751	3.52
4M	16	335	0.055	1.152	2.16	12.68	1.133	0.544	2.97
	17	358	0.051	1.208	2.27	14.05	1.287	0.717	2.82
	18	362	0.060	1.284	2.23	17.35	1.500	0.709	3.04
	19	354	0.049	1.344	2.33	16.63	1.266	0.613	3.37
	20	331	0.055	1.182	2.01	11.95	1.209	0.756	2.66

APPENDIX 10 - continued

Request ID: 167473

Organ weights - individual values - absolute (g)

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Group /Sex	Animal Number	Terminal bodyweight	Adrenals	Heart	Kidneys	Liver	Lungs + Bronchi	Spleen
1F	21	249	0.061	1.013	1.45	11.04	1.147	0.638
	22	226	0.051	0.830	1.38	8.57	1.033	0.445
	23	233	0.072	0.808	1.41	8.53	1.028	0.667
	24	238	0.058	0.892	1.27	8.79	1.038	0.580
	25	238	0.052	0.845	1.42	9.02	1.020	0.505
2F	26	235	0.058	1.042	1.37	8.47	1.082	0.489
	27	235	0.064	0.903	1.41	7.48	1.125	0.461
	28	240	0.065	0.934	1.51	8.57	1.113	0.501
	29	219	0.068	0.790	1.55	7.32	1.138	0.401
	30	204	0.052	0.858	1.35	7.10	0.937	0.466
3F	31	237	0.072	0.937	1.57	8.91	1.242	0.477
	32	225	0.057	0.781	1.42	8.56	1.075	0.465
	33	228	0.064	0.832	1.43	8.32	1.105	0.506
	34	238	0.064	1.058	1.60	10.45	1.153	0.511
	35	205	0.055	0.742	1.37	7.47	0.907	0.379
4F	36	209	0.060	0.906	1.48	7.77	1.014	0.525
	37	200	0.076	0.841	1.50	7.64	1.053	0.488
	38	204	0.050	1.039	1.45	6.51	1.084	0.526
	39	190	0.053	0.857	1.34	7.48	1.037	0.506
	40	206	0.052	0.899	1.47	7.10	1.048	0.425

APPENDIX 11

Request ID: 174455

Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 1 Sex: Male Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 338.8 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
LN Mandibular Enlarged, one on each side, 10mm. Congested, Right	LN Mandibular Sinus Erythrocytosis/Erythrophagocytosis, Slight Plasmacytosis, Slight
Liver Median cleft pale area(s), One, subcapsular, 2mm.	Liver No significant lesions
	Larynx Inflammation, Slight
	Testes Seminiferous Tubular Atrophy, Focal, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 2 Sex: Male Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 343.1 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Lungs + Bronchi Arterial Mural Mineralisation, Minimal Larynx Inflammation, Minimal Testes Seminiferous Tubular Atrophy, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 3 Sex: Male Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 342.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
LN Mandibular Congested, Right	LN Mandibular Sinus Erythrocytosis/Erythrophagocytosis, Slight Plasmacytosis, Slight
	Lungs + Bronchi Alveolar Osseous Metaplasia, Present
	Larynx Inflammation, Minimal
Stomach Antrum white nodule(s), Mucosa, Punctate, One, near to limiting ridge.	

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 4 Sex: Male Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 341.8 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Liver Inflammation, Focal, Minimal Lungs + Bronchi Aggregations of Alveolar Macrophages, Minimal Larynx Inflammation, Minimal Testes Seminiferous Tubular Vacuolation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 5 Sex: Male Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 310.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
LN Mandibular Enlarged, one on each side, 10mm.	LN Mandibular Plasmacytosis, Moderate
	Liver Inflammation, Focal, Minimal
	Larynx Inflammation, Minimal
	Testes Seminiferous Tubular Vacuolation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 6 Sex: Male Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 376.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight
LN Mandibular Enlarged, one on each side, 10mm.	LN Mandibular Plasmacytosis, Moderate Lymphoid Hyperplasia, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 7 Sex: Male Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 309.1 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight
LN Mandibular Enlarged, one on each side, 10mm.	LN Mandibular Plasmacytosis, Moderate Lymphoid Hyperplasia, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 8 Sex: Male Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 398.0 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Myocardial Fibrosis, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 9 Sex: Male Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 329.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Congestion, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 10 Sex: Male Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 327.8 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Congestion, Minimal

Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 11 Sex: Male Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 312.8 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	***ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED***

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 12 Sex: Male Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 405.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Moderate Myocardial Fibrosis, Minimal
LN Bronchial Not visible, tissue taken from region.	LN Bronchial Tissue missing

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 13 Sex: Male Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 340.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Congestion, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 14 Sex: Male Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 342.2 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal Congestion, Minimal
Stomach Antrum white nodule(s), Mucosa, One, near to limiting ridge, 1mm.	

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 15 Sex: Male Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 341.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Myocardial Fibrosis, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 16 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 334.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight
LN Mandibular Enlarged, one on each side, 10mm.	LN Mandibular Plasmacytosis, Moderate
	Larynx Inflammation, Minimal
	Testes Seminiferous Tubular Vacuolation, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 17 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 357.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Myocardial Fibrosis, Minimal Lungs + Bronchi Aggregations of Alveolar Macrophages, Minimal Larynx Inflammation, Moderate Testes Seminiferous Tubular Vacuolation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 18 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 362.3 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Myocardial Fibrosis, Minimal
LN Mandibular Enlarged, one on each side, up to 12mm.	LN Mandibular Plasmacytosis, Marked
	Liver Inflammation, Focal, Slight
	Lungs + Bronchi Aggregations of Alveolar Macrophages, Slight Perivascular Inflammatory Cells, Slight
	Larynx Inflammation, Slight
	Tracheal Bifurc. Subepithelial Inflammatory Cell Aggregates, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 18 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 362.3 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
continued from previous page...	
	Testes
	Seminiferous Tubular Atrophy, Minimal
	Seminiferous Tubular Vacuolation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 19 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 353.8 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY

ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED

HISTOPATHOLOGY

Heart
Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight

Lungs + Bronchi
Perivascular Inflammatory Cells, Minimal

Nasal turbinates
Olfactory Epithelium - Rosette Formation, Minimal

Testes
Seminiferous Tubular Vacuolation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 20 Sex: Male Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 331.4 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Liver Inflammation, Focal, Slight Larynx Inflammation, Minimal Nasal turbinates Respiratory Epithelium - Pseudogland Formation, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 21 Sex: Female Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 248.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
Kidneys Pelvic dilatation, Right	Kidneys Cortical Cyst(s), Minimal Mineralisation, Corticomedullary, Minimal
LN Mandibular Enlarged, Right, One, 10mm.	LN Mandibular No significant lesions
	Liver Inflammation, Focal, Minimal
	Larynx Inflammation, Minimal
	Nasal turbinates Respiratory Epithelium - Pseudogland Formation, Minimal Olfactory Epithelium - Rosette Formation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 22 Sex: Female Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 225.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Minimal Liver Inflammation, Focal, Minimal Larynx Inflammation, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 23 Sex: Female Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 233.3 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
	Liver Single Cell Necrosis, Slight
	Spleen Haemosiderosis, Slight
Uterus Fluid distension	Uterus Luminal Dilatation, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 24 Sex: Female Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 237.9 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Minimal
	Lungs + Bronchi Aggregations of Alveolar Macrophages, Minimal
	Larynx Inflammation, Minimal
Uterus Fluid distension	Uterus Luminal Dilatation, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 25 Sex: Female Dose Group: 1 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 237.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Larynx Inflammation, Slight
	Nasal turbinates Olfactory Epithelium - Degeneration, Focal, Slight Olfactory Epithelium Mineralisation, Focal, Minimal
Uterus Fluid distension	Uterus Luminal Dilatation, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 26 Sex: Female Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 234.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	***ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED***

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 27 Sex: Female Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 234.9 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal
Uterus Fluid distension	Uterus Luminal Dilatation, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 28 Sex: Female Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 239.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 29 Sex: Female Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 219.2 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
Uterus	Uterus
Fluid distension	Luminal Dilatation, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 30 Sex: Female Dose Group: 2 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 204.4 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 31 Sex: Female Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 236.6 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal
LN Mandibular Enlarged, one on each side, 10mm.	LN Mandibular Plasmacytosis, Moderate Lymphoid Hyperplasia, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 32 Sex: Female Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 224.9 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal
Stomach Antrum white nodule(s), Mucosa, One, near to limiting ridge, 1mm.	

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 33 Sex: Female Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 227.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal Myocardial Fibrosis, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 34 Sex: Female Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 238.0 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal
LN Mandibular Enlarged, Right, up to 10mm.	LN Mandibular Plasmacytosis, Moderate Lymphoid Hyperplasia, Moderate
Stomach Forestomach depression(s), Epithelial aspect, One, crater-like, 2mm.	Stomach Epithelial Hyperplasia - Nonglandular Region, Moderate Hyperkeratosis - Nonglandular Region, Slight Submucosal Inflammation - Nonglandular Region, Slight
Uterus Fluid distension	Uterus Luminal Dilatation, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 35 Sex: Female Dose Group: 3 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 204.9 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	***ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED***

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 36 Sex: Female Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 208.5 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY

ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED

HISTOPATHOLOGY

Heart
Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight

Liver
Single Cell Necrosis, Slight

Larynx
Inflammation, Minimal

Nasal turbinates
Respiratory Epithelium Mineralisation, Moderate

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 37 Sex: Female Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 200.0 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Minimal
	Larynx Inflammation, Minimal
	Nasal turbinates Olfactory Epithelium Cyst, Minimal
Uterus Fluid distension	Uterus No significant lesions

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 38 Sex: Female Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 203.7 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Adrenals One of pair missing Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Liver Inflammation, Focal, Minimal Lungs + Bronchi Aggregations of Alveolar Macrophages, Slight Larynx Inflammation, Slight

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 39 Sex: Female Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 190.2 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
ANIMAL HAS NO MACROSCOPIC FINDINGS RECORDED	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Myocardial Fibrosis, Slight Liver Single Cell Necrosis, Slight Larynx Inflammation, Slight Nasal turbinates Olfactory Epithelium Mineralisation, Focal, Minimal

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Macropathology and histopathology - individual findings

Group	:	1	2	3	4
Compound	:	Control	HFO 1243zf	HFO 1243zf	HFO 1243zf
Dose (ppm)	:	0	4967	14953	49686

Animal Number: 40 Sex: Female Dose Group: 4 Phase: Treatment Sacrifice Status: Final phase sacrifice
Date of Death: 18-May-09 Day of Death: 29 Week of Death: 5 Subgroup: 1 Terminal Bodyweight: 206.1 g

PATHOLOGY OBSERVATIONS

MACROPATHOLOGY	HISTOPATHOLOGY
	Heart Focal/Multifocal Inflammation, Vacuolation, Myofibre Degeneration, Slight Congestion, Moderate
Kidneys Pelvic dilatation, Right	Kidneys No significant lesions
	Liver Single Cell Necrosis, Slight
	Lungs + Bronchi Arterial Mural Mineralisation, Minimal Aggregations of Alveolar Macrophages, Minimal Perivascular Inflammatory Cells, Minimal
	Larynx Inflammation, Minimal
	Tracheal Bifurc. Subepithelial Inflammatory Cell Aggregates, Slight

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Annex 1 Administration of HFO 1243zf to rats

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Administration of HFO 1243zf by Inhalation to Rats

HLS study number:	ATS0031
Author:	Andrew Allen

1. Administration

1.1 Administration

Rats were exposed to air or an atmosphere containing the test gas by snout-only exposure for 6 hours a day, 5 consecutive days/week for 4 weeks.

Different doses were achieved by varying the concentration of the test gas in the exposure chamber air, whilst keeping the duration of exposure constant.

1.2 Exposure system

The inhalation system comprised of a snout only inhalation exposure chamber and an atmosphere generation system. Accessories included air supply and extract lines, attached to the top and bottom of the chamber, respectively. A filtration system was incorporated in the extract line.

Each inhalation chamber was connected to an atmosphere generation system supplying a flow of test atmosphere or air. An air flow differential was maintained between the inlet and outlet to provide a small negative pressure within the exposure system. Separate exposure systems were used for the test and control groups.

A schematic of the exposure system is presented in Figure A. The exposure system operating conditions are presented in Table A. The component parts of the system are described in further detail as follows:

1.2.1 Test atmosphere generation

The test gas was dispensed directly from the test cylinder supplied. The test cylinder was fitted with a cylinder regulator to allow isolation of the test gas supply. The flow of test gas to each dose group was regulated with a Porter flowmeter, needle and toggle valves, prior to dilution in the exposure chamber.

1.2.2 Inhalation chamber

The ADG snout-only inhalation chambers were of modular apparatus of aluminium alloy construction comprising a base unit, a variable number of animal exposure sections and a top section incorporating a central aerosol inlet surrounded by a tangential air inlet. A single exposure level was used for this study.

1.2.3 Rat restraining tubes

The restraining tubes were moulded polycarbonate tubes tapered at one end to allow only the snout to project from the tapered end. The other end was closed by insertion of an expanded plastic bung. A push rod passed through the centre of the bung and was adjustable to maintain the position of the rat during restraint. Tubes were attached to the chamber by means of push fit "O" ring seals located in the exposure ports of the animal exposure sections.

1.2.4 Air supply and extract

The compressed air supply was filtered to remove any residual particulate and dried. This was used to operate the supplementary tangential air inlet introduced above the top of the inhalation chamber.

Air extract, attached to the base of the inhalation exposure system, was provided by vacuum pumps and incorporated a filtration system to remove water vapour. Downstream of the filtration system were charcoal drums to remove the test gas from the airstream.

Air supply and extract were monitored using in-line flowmeters and recorded manually. The in-line flowmeters were calibrated prior to each exposure against high quality tapered tube rotameters and were used to measure the free flow of air at the points of attachment of the supply and extract lines to the exposure system.

1.3 Exposure conditions

1.3.1 Gas concentration

The samples were collected directly from the single chamber level of the exposure chambers using gas tight syringes. The gas concentration of the HFO 1243zf within the exposure chambers was measured by Formulation and Inhalation Analysis and details are given in Appendix A.

1.3.2 Nominal concentration

A mean daily nominal concentration was calculated for all test groups from the mass of test gas used over the exposure period and the airflow through the exposure chamber. The ideal gas equation was used with the molecular weight of the test gas and the measured chamber conditions to compute the volume of gas produced from the mass of test gas used. The calculation is detailed in Table C.

1.3.3 Temperature

This parameter was recorded manually, every 30 minutes during exposure.

2. Results

2.1 Gas concentration

The data are presented as follows and are summarised below:

Mean gas concentrations - Table B
Individual gas concentrations - Appendix B

Group	Chamber concentration (ppm)	
	Target	Analysed
2	5000	4967
3	15000	14953
4	50000	49686

The mean analysed concentrations were all very close to the target concentrations.

The coefficients of variations for all test groups were less than 5%. This confirms consistent generation throughout the duration of the study.

2.2 Nominal concentration

The nominal concentrations are presented in Table C.

The mean unrounded analysed/nominal ratio for the test groups were fractionally lower than 100%, which was well within the tolerance limits in the measurement of test gas and air flows.

2.3 Chamber temperature

The daily mean chamber temperatures are presented in Table D.

The chamber temperatures were similar for each group on each day of the study. The observed temperatures for all groups remained within the normally accepted range for inhalation exposure of rats.

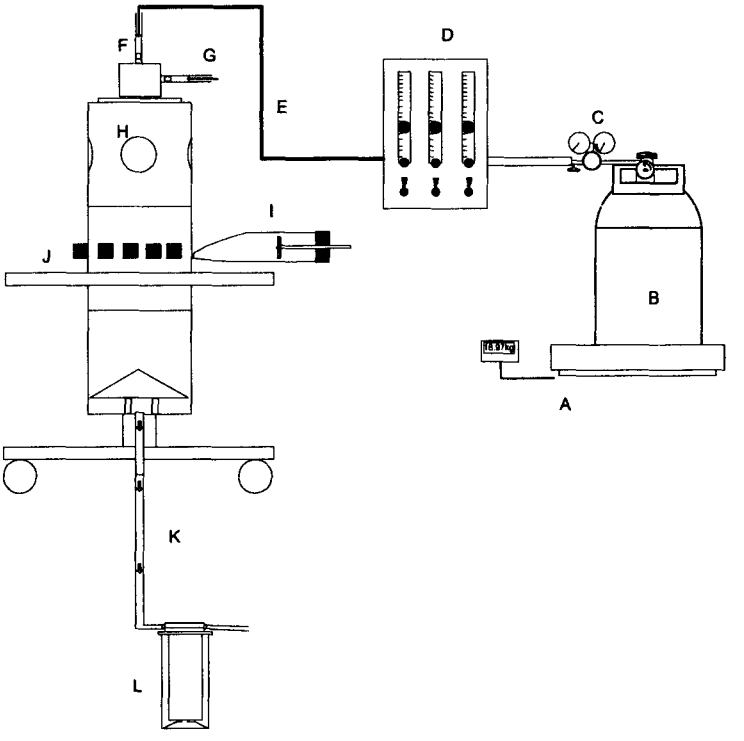
3. Discussion

Control of the delivery of HFO 1243zf gas to the exposure chambers was very good for all test groups.

4. Calculations

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

Figure A Schematic of a rodent inhalation dosing system



Key

A	Balance	G	Diluent line
B	Test material cylinder	H	Exposure chamber
C	Regulator / Gauges	I	Rat restraint tube
D	Porter flow meter panel	J	Blanking bung
E	Feed line	K	Extract pipe
F	Test material connection to chamber	L	Extract filtration

Table A Operating conditions for the inhalation exposure system

Parameter	Group 1	2	3	4
Chamber airflows (L/minute)				
Inlet	9	9	9	9
Extract	10	10	10	10
Chamber level of the rats	1	1	1	1

Table B Chamber concentrations of HFO 1243zf - mean exposure values

Exposure number	Atmosphere concentration (ppm) Group 1	Group 2	Group 3	Group 4
1	ND	5119	15679	52349
2	ND	4923	15426	51891
3	ND	5089	14404	49420
4	ND	5039	16083	47221
5	ND	5132	14833	49289
6	ND	5176	14971	49737
7	ND	5054	14894	50093
8	ND	4715	14123	48185
9	ND	4816	14889	51027
10	ND	4886	15010	49561
11	ND	4919	15182	49475
12	ND	5054	15405	50697
13	ND	5057	15735	50967
14	ND	5050	14918	50857
15	ND	5035	14058	48670
16	ND	4754	13886	47404
17	ND	4913	15470	49607
18	ND	5036	15306	49954
19	ND	4659	14337	47685
20	ND	4915	14450	49630
Mean		4967	14953	49686
sd		144.4	601.6	1393.5
CV (%)		2.9	4.0	2.8

ND Not detected
sd Standard deviation
CV (%) Coefficient of variation (sd × 100/mean)

Table C Nominal concentrations of HFO 1243zf - individual and mean exposure values

Exposure number	Mean temperature of dose groups (°C)	Barometric pressure (mmHg)	Test gas usage (kg)	Analysed conc. (ppm)	Nominal conc. (ppm) [1]	A/N (%)
1	20.4	771	1.144	73147	72837	100
2	20.4	771	1.115	72241	71122	102
3	20.5	771	1.047	68913	67104	103
4	20.4	767	1.040	68343	66989	102
5	20.5	763	1.056	69254	68288	101
6	20.2	746	1.037	69884	68517	102
7	20.3	751	1.040	70040	68297	103
8	20.4	755	1.120	67022	72829	92
9	20.4	759	1.117	70733	72278	98
10	20.4	768	1.131	69457	72331	96
11	20.3	770	1.108	69575	70770	98
12	20.5	765	1.102	71157	70887	100
13	20.4	762	1.095	71758	70703	101
14	20.5	758	1.068	70824	69442	102
15	20.4	753	1.069	67763	69917	97
16	20.4	766	1.052	66044	67777	97
17	20.4	766	1.084	69990	69695	100
18	20.2	761	1.059	70296	68579	103
19	20.3	755	1.047	66681	68386	98
20	20.2	749	1.063	68995	69838	99
			Mean	69606	69829	100
			sd	1836.3	1845.6	2.8
			CV (%)	2.6	2.6	2.8

[1] Nominal concentrations were calculated from the following equations:

$$\text{Concentration (ppm)} = \frac{V}{V_a + V} \times 1000000 \qquad V = \frac{W \times R \times T}{M} \times \frac{760\ \text{mm Hg}}{\text{Atm}}$$

where
- V = gaseous volume of HFO 1243zf
- W = mass of HFO 1243zf
- M = molecular weight HFO 1243zf (96.05 g/mole)
- R = gas constant (0.08206 l atm mol^{-1} K^{-1})
- T = temperature (K), = temperature (°C, see Table D) + 273
- Atm = atmospheric pressure (mmHg)
- V_a = volume of air (litres) passing through the chamber during the Exposure (airflow (see Table A) x duration)

A/N (%) Analysed/nominal concentration ratio expressed as a percentage
sd Standard deviation
CV (%) Coefficient of variation (sd × 100/mean)

Table D Chamber temperature (°C) - exposure mean values

Exposure number	Group 1	2	3	4
1	21.6	20.7	20.5	20.0
2	21.7	20.6	20.5	20.1
3	21.8	20.7	20.7	20.2
4	22.0	20.6	20.5	20.2
5	21.9	20.6	20.5	20.3
6	21.3	20.3	20.4	20.0
7	21.5	20.5	20.5	20.0
8	21.5	20.6	20.5	20.2
9	21.5	20.6	20.4	20.1
10	21.9	20.5	20.6	20.1
11	21.4	20.4	20.4	20.1
12	21.9	20.6	20.7	20.2
13	21.3	20.6	20.4	20.2
14	21.8	20.6	20.6	20.3
15	21.5	20.6	20.6	20.1
16	21.4	20.6	20.3	20.2
17	21.3	20.5	20.5	20.1
18	20.8	20.3	20.4	19.9
19	21.4	20.4	20.5	20.1
20	21.0	20.3	20.2	20.0
Mean	21.5	20.5	20.5	20.1
sd	0.31	0.13	0.12	0.11
CV (%)	1.4	0.6	0.6	0.5

sd Standard deviation
CV (%) Coefficient of variation (sd × 100/mean)

Appendix A Method of analysis for HFO 1243zf

Flow diagram of the analytical method for the analysis of HFO 1243zf in aerosol samples

Sample Assay

A 20 mL syringe containing HFO 1243zf, extracted directly from animal dose chamber.

↓

Inject onto the GC in duplicate.

Calibration

Dispense at least 500 mL of HFO 1243zf into a gas bag that has been purged with test item.

↓

Using gas tight syringes add appropriate volumes of air to clean gas bags, followed by appropriate volumes of HFO 1243zf from the primary standard gas bag. Prepare standards at 0.3, 0.5, 1.5, 3.0, 5.0 and 7.0% v/v HFO 1243zf.

↓

Inject the six standards onto the GC prior to sample assay, to ensure GC system is operating correctly, and an appropriate bracketing standard during sample assay, for calibration.

GC conditions

Column:	SPB-5, 30 m × 0.32 mm ID, 0.25 μm film thickness
Injection mode:	Manual injection in split mode
Injector temperature:	100 °C
Injection volume:	Load 5 mL into injector system
Injection loop volume:	250 μL
Detector type:	Flame Ionisation
Detector temperature:	250 °C
Range:	5
Oven temperature programme:	Isothermal at 30 °C
Run time:	2 min
Gases:	
Carrier:	Helium at 7.5 mL/min
Split vent:	Helium at 150 mL/min (split ratio 1:20)
Make up:	Helium at 30 mL/min
Oxidant:	Air at 400 mL/min
Fuel:	Hydrogen at 35 mL/min

These conditions were established using an Agilent 5890 Gas Chromatograph

Appendix B Chamber concentrations of HFO 1243zf - individual exposure values (ppm)

Group 1

Exposure number	Sample number 1	2	3	4	5	6
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND
11	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND
13	ND	ND	ND	ND	ND	ND
14	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND
16	ND	ND	ND	ND	ND	ND
17	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	ND
19	ND	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND	ND

ND Not detected

Group 2

Exposure number	Sample number 1	2	3	4	5	6
1	5872	5948	4549	4716	4831	4799
2	4981	4789	4939	4985	4906	4939
3	5277	5422	5031	4842	4948	5011
4	5130	4767	5081	4980	5084	5191
5	5092	5186	5221	5149	4996	5147
6	5370	5258	5007	5262	5087	5074
7	5435	5010	5035	5027	4914	4901
8	3978	4486	4666	5040	5148	4969
9	4738	4769	4712	4639	4959	5081
10	4909	4974	4733	4844	4986	4869
11	4808	5178	5192	4759	4733	4841
12	4984	4992	5077	5046	5161	5063
13	5127	5242	5046	4832	4969	5124
14	5004	5098	5028	5058	4965	5144
15	5090	5129	4973	5038	5140	4837
16	3964	4532	4922	5126	4826	5155
17	4858	5222	4914	4766	4842	4877
18	5309	5066	4946	4863	5086	4947
19	4606	4733	4678	4574	4768	4595
20	4707	4697	5246	4955	4726	5157

Appendix B Chamber concentrations of HFO 1243zf - individual exposure values (ppm)(continued)

Group 3

Exposure number	Sample number 1	2	3	4	5	6
1	15802	15705	15743	15994	14957	15871
2	15639	16227	15535	16165	14864	14127
3	14838	14452	14252	14358	14213	14313
4	17870	18056	16883	13870	14646	15174
5	14635	14608	14798	14941	15045	14972
6	15320	14843	15299	14608	14860	14893
7	14839	14251	14991	15248	14818	15216
8	11989	13894	13550	14240	15801	15262
9	14518	14554	15216	15694	14380	14974
10	14701	14991	15022	15498	15009	14838
11	14992	15678	14933	15028	15289	15170
12	15746	15171	15437	15415	15444	15219
13	15787	14828	15692	16721	16035	15344
14	14881	15218	15108	15010	14588	14701
15	14224	14254	13696	13699	14156	14317
16	11961	12335	14163	14311	15009	15537
17	14741	16557	15651	15261	15465	15144
18	15229	15518	15610	15022	15270	15187
19	14759	14163	14353	13743	14553	14449
20	13901	15224	13749	14910	14196	14719

Group 4

Exposure number	Sample number 1	2	3	4	5	6
1	52071	52200	52165	52196	52865	52595
2	52837	52192	51204	52872	51090	51152
3	49737	51701	48192	49651	48455	48785
4	47595	49334	48849	44666	46378	46502
5	47957	50377	49511	49872	49087	48927
6	48247	49315	48329	51592	49955	50984
7	49930	50053	51620	49645	50600	48707
8	43712	46104	46638	49926	50703	52026
9	50257	49872	50396	56197	49522	49918
10	50313	49330	48131	48524	50763	50307
11	47724	47590	50124	50374	51202	49837
12	47635	50199	51212	50335	53115	51688
13	50434	49767	50530	51481	51665	51923
14	48565	50926	51678	52257	49886	51830
15	46154	49985	47168	50612	49533	48570
16	43827	46802	46499	50280	48508	48507
17	49400	49189	50842	48776	49846	49589
18	49294	50244	51168	50072	49343	49601
19	49478	52586	45036	46399	46951	45662
20	47142	47120	47066	49397	52488	54568

Annex 2 Urinary fluoride report

BUTTERWORTH
LABORATORIES

Contract Analytical Chemistry

Butterworth Laboratories Ltd

GLP Study Report

Delegated Phase

Study Title:
3,3,3-Trifluoroprop-1-ene (HFO1243zf) Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks

Study ID:
ATS0031

Objective of the Delegated Phase:
Determination of Fluoride in Urine

BLL Schedule No:
0904-0054

Study Director:
Jeanette Blair

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Registered in England & Wales
Company registration No 1185121

Contents **Page No**

Cover Page	1
Contents Page	2
Summary of Results	3-5
Sample/test item types	3
Description of test items	3
Date(s) of receipt of sample(s)	3
Date(s) of analysis	3
Identification of Reference Items	3
Test Results including the Identification of Test Items	3-5
Methodology	5
Contributing Scientists	5
Conclusions and Discussion of results	5
Archiving Requirements	6
GLP Compliance Statement	6
QA Report	7-8

Butterworth Laboratories Ltd

BLL Schedule No: 0904-0054
Study ID: ATS/0031

Summary of Results

Samples of: Rat urine

Description of Samples: Yellow liquids with a small amount of suspended matter

Dateof receipt: 18 May 2009

Date of analysis: 17 June 2009

Reference Item(s): 1000ppm fluoride standard solution purchased from Romil, product E3F4, lot S537418

Test Results:

BLL Reference	Sample Reference	Fluoride expressed as F (mg/l)
	Group 1	
BL 05/0270-09	Male 1	1.3
BL 05/0271-09	Male 2	1 1
BL 05/0272-09	Male 3	1.4
BL 05/0273-09	Male 4	1.0
BL 05/0274-09	Male 5	1.1
BL 05/0275-09	Female 21	1.2
BL 05/0276-09	Female 22	<0.7
BL 05/0277-09	Female 23	0.7
BL 05/0278-09	Female 24	0.5
BL 05/0279-09	Female 25	0.9
	Group 2	
BL 05/0280-09	Male 6	8.4
BL 05/0281-09	Male 7	8.6
BL 05/0282-09	Male 8	6.0
BL 05/0283-09	Male 9	5.1
BL 05/0284-09	Male 10	8.7

BLL Reference	Sample Reference	Fluoride expressed as F (mg/l)
	Group 2	
BL 05/0285-09	Female 26	3.0
BL 05/0286-09	Female 27	2.3
BL 05/0287-09	Female 28	3.3
BL 05/0288-09	Female 29	7.3
BL 05/0289-09	Female 30	8.5
	Group 3	
BL 05/0290-09	Male 11	9.7
BL 05/0291-09	Male 12	4.7
BL 05/0292-09	Male 13	8.9
BL 05/0293-09	Male 14	5.5
BL 05/0294-09	Male 15	8.5
BL 05/0295-09	Female 31	7.4
BL 05/0296-09	Female 32	4.5
BL 05/0297-09	Female 33	4.9
BL 05/0298-09	Female 34	4.4
BL 05/0299-09	Female 35	4.4
	Group 4	
BL 05/0300-09	Male 16	7.4
BL 05/0301-09	Male 17	8.5
BL 05/0302-09	Male 18	7.6
BL 05/0303-09	Male 19	8.3
BL 05/0304-09	Male 20	6.7
BL 05/0305-09	Female 36	7.3
BL 05/0306-09	Female 37	9.4

BLL Reference	Sample Reference	Fluoride expressed as F (mg/l)
	Group 4	
BL 05/0307-09	Female 38	8.0
BL 05/0308-09	Female 39	7.1
BL 05/0309-09	Female 40	8.7

Results in mg/l relate to samples as received.

Methodology: BLM 147 issue 5

This method was known to be due for review before commencement of the analysis of samples. The method has been assessed for it validity and appropriateness for this study and it has been deemed to be fit for purpose.

Contributing Scientists:

Tim Goddard
David Riches

Discussion of Results:

It was not possible to perform any duplicate tests due to insufficient sample available.

A single preparation was spiked with fluoride. The recovery was 104%.

Archiving Requirements

An authorised copy of this report together with copies of raw data, original electronic data, instrument calibration records, documented in-house methods, standard operating procedures and any other associated documentation will be held in the Archives of Butterworth Laboratories Limited for a period of not less than six years. Original documentation including Raw Data and copies of the QA inspection form is to be archived by Huntingdon Life Sciences.

GLP Compliance: Statement

I, the undersigned, accept responsibility for the conduct of the delegated phase of the Study and for validity of the data produced. I confirm that this was conducted at a facility that is part of the UK GLP Compliance Programme. The work has been conducted in compliance with the Principles of Good Laboratory Practice (GLP) as set forth in "The Good Laboratory Practice Regulations 1999" - Statutory Instrument 1999 No 3106 and the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 No 994.

Signed by: _____ Date 26 June 2003

David Riches BSc CChem MRSC
Principal Investigator – Butterworth Laboratories Ltd

QA Report:

The QA unit has audited this work for the aspects listed below. Details of observations and findings made are located in the QA inspection form which is included in the Raw Data pack provided to the Study Director.

Aspect Monitored	Date Performed	Date Reported*
Review of Study Plan	23 April 2009	24 April 2009
Analysis	17 June 2009	17 June 2009
Analytical Results	19 June 2009	22 June 2009
Draft Report	19 June 2009	22 June 2009
Report	26 June 2009	26 June 2009

Findings

1. The Study Plan requested that the Principal Investigator submitted a signed pdf copy of his report.
2. The Study Plan identifies the method of analysis as BLM147 (Issue 5). This method became due for review before the analysis was carried out.
3. During the inspection of the analytical phase it was noted that a stirrer was used which did not have an internal ID.

Corrective Actions

1. Study Plan Amendments address reporting, archiving and test facility QA inspection details.
2. The Principal Investigator has assessed the validity of the method as it is and has approved its used for this study. It was not deemed practical to review and re-issue the method during the course of the study.
3. The stirrer was new and has now been correctly labelled.

*All findings reported to the Principal Investigator, the Study Director, Lead QA at Huntingdon Life Sciences and Butterworth Laboratories Ltd. Management. Lead QA at Huntingdon Life Sciences report findings to Management.

In addition the above study specific inspections, a number of scheduled non-study specific process based inspections are performed by Butterworth QA throughout the calendar year. The most recent process based inspection was carried out on: 16 June 2009. Facility based inspections are also performed on an annual schedule covering all areas of analytical operations.

Butterworth Laboratories Ltd

BLL Schedule No: 0904-0054
Study ID: ATS/0031

This report complies with the requirements of the Study Plan and any corrective actions required have been completed to the satisfaction of the QA department of Butterworth Laboratories Ltd. This report accurately reflects the raw data generated by Butterworth Laboratories Ltd.

Signed by _____ Date 26/6/09

David J Hawkins BSc CBiol MIBiol
QA Manager – Butterworth Laboratories Ltd

Annex 3 Pathology report

Pathology Report

3,3,3-Trifluoroprop-1-ene (HFO 1243zf)

Toxicity Study by Inhalation Administration to CD Rats for 4 Weeks

Laszlo Nemet
DVM MSc
Huntingdon Life Sciences

Date: 13 August 2009

1. Introduction

1.1 Objective

Assessment of systemic toxic potential of the test substance (refrigerant) in a 4 week inhalation study in CD rats.

Group	Treatment	Exposure level ppm	Number of animals	
			Males	Females
1	Air Control	0	5	5
2	HFO 1243zf	4967	5	5
3	HFO 1243zf	14953	5	5
4	HFO 1243zf	49686	5	5

A restricted number of organs were processed for histology from the control and high dose groups: nasal turbinates, larynx, trachea, tracheal bifurcation, lungs, liver, kidney, spleen, adrenals, testes and any abnormalities. Hearts were examined in all groups.

2. Results

2.1 Decedents

There were no unscheduled deaths amongst the animals in this study.

2.2 Macroscopic Pathology

2.2.1 Animals sacrificed after 4 weeks of treatment

The macroscopic examination performed after 4 weeks of treatment revealed no intergroup differences of note.

The nature and incidence of all the findings were consistent with the commonly seen background of macroscopic changes.

2.3 Microscopic Pathology

2.3.1 Animals sacrificed after 4 weeks of treatment

Treatment related findings

Changes considered related to treatment with HFO 1243zf were seen in the hearts.

Heart

Focal or multifocal myocardial inflammation, vacuolation and myofibre degeneration was seen in rats from all treated groups. In some of the rats there was also focal myocardial fibrosis and/or congestion. Control rats showed only presence of minimal inflammatory cell foci which was considered an incidental background finding.

Summary of treatment related findings in the heart for animals sacrificed after 4 weeks of treatment

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Exposure level ppm	0	4967	14953	49686	0	4967	14953	49686
Focal / Multifocal Inflammation Vacuolation, Myofibre Degeneration								
Minimal	0	0	1	0	0	2	4	1
Slight	0	4	2	5	0	1	0	4
Moderate	0	0	1	0	0	0	0	0
Total	0	4	4	5	0	3	4	5
Myocardial Fibrosis								
Minimal	0	0	2	2	0	0	1	0
Slight	0	1	0	0	0	0	0	1
Total	0	1	2	2	0	0	1	1
Congestion								
Minimal	0	2	2	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
Total	0	2	2	0	0	0	0	1
Number of animals examined	5	5	5	5	5	5	5	5

Incidental findings

All other findings were considered incidental and unrelated to the test substance.

3. Discussion

Focal/multifocal inflammation was present in the heart of control and treated animals. However, in all groups of treated animals the inflammation was accompanied by degenerative changes of myofibres of a minimal to moderate degree. Myofibre degeneration presented as fine vacuolation of the peripheral (subsarcolemmal) region of cardiac muscle cells.

No predilection site was seen regarding the distribution of the change.

A clear dose-relationship could not be observed histologically, but heart changes were more pronounced in male animals than in females.

It is supposed that the slight heart toxicity found in this study correlated with the increased adjusted heart weights in all treated females and with the increased serum AST levels in the high dose treated females and males respectively.

No treatment-related change was evident in the heart weight of male animals, but this may have been masked by the decreased body weights in the treated groups.

4. Conclusion

The 4 week inhalation treatment with HFO 1243zf caused heart toxicity at all applied exposure levels (4967, 14953 and 49686 ppm).

No dose-relationship was found by histological observation. Male animals were more susceptible than females.

Annex 4 Certificates of Analysis

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100493	**Cylinder Number**	160573
Batch No:	Pures1	**Ineos R&T Reference**	IF20090305
Date of Manufacture:	05-Mar-2009	**Expiry Date**	05-Mar-2010
Date/Time of Sampling :	09/03/2009 08:03:00	**Date/Time Logged :**	09/03/2009 08:23:17
Quality:	PASS	**Specification Ref :**	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	5.3	ppm w/w		AWEIR
134a	3.4	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	6.0	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	246	ppm w/w		AWEIR
12	155	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	31	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.955	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 09-Mar-2009 10:29 a

Date Report Printed : 09-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100602	Cylinder Number	160496
Batch No:	Pures2	Ineos R&T Reference	20090310_1
Date of Manufacture:	10-Mar-2009	Expiry Date	10-Mar-2010
Date/Time of Sampling :	10/03/2009 13:28:00	Date/Time Logged :	12/03/2009 13:29:08
Quality:	PASS	Specification Ref :	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	2.8	ppm w/w		AWEIR
134a	2.1	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	3 4	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	194	ppm w/w		AWEIR
12	106	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	16	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.968	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 2.00 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	**100603**	**Cylinder Number**	160551
Batch No:	Pures2	**Ineos R&T Reference**	20090310_1
Date of Manufacture:	10-Mar-2009	**Expiry Date**	10-Mar-2010
Date/Time of Sampling :	10/03/2009 09:33:00	**Date/Time Logged :**	12/03/2009 13:29:57
Quality:	PASS	**Specification Ref :**	**1243ZF_PRODUCT**

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	2.8	ppm w/w		AWEIR
134a	2.1	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	3.4	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	194	ppm w/w		AWEIR
12	106	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	16	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.968	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1:59 pm

Date Report Printed : **12-Mar-2009**

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100604	Cylinder Number	160493
Batch No:	Pures3	Ineos R&T Reference	20090310_3
Date of Manufacture:	10-Mar-2009	Expiry Date	10-Mar-2010
Date/Time of Sampling :	10/03/2009 13 52 00	Date/Time Logged :	12/03/2009 13.32.32
Quality:	PASS	Specification Ref :	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	6.8	ppm w/w		AWEIR
134a	5.0	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	7.6	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	241	ppm w/w		AWEIR
12	260	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	43	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.944	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1:58 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100605	**Cylinder Number**	160557
Batch No:	Pures3	**Ineos R&T Reference**	20090310_3
Date of Manufacture:	10-Mar-2009	**Expiry Date**	10-Mar-2010
Date/Time of Sampling :	10/03/2009 13 52 00	**Date/Time Logged :**	12/03/2009 13:33:00
Quality:	PASS	**Specification Ref :**	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	6.8	ppm w/w		AWEIR
134a	5.0	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	7.6	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	241	ppm w/w		AWEIR
12	260	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	43	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99 944	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1 58 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100606	Cylinder Number	160476
Batch No:	Pures4	Ineos R&T Reference	20090310_2
Date of Manufacture:	10-Mar-2009	Expiry Date	10-Mar-2010
Date/Time of Sampling :	10/03/2009 11:03:00	Date/Time Logged :	12/03/2009 13:33:52
Quality:	PASS	Specification Ref :	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	1.4	ppm w/w		AWEIR
134a	1.3	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	1.8	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	142	ppm w/w		AWEIR
12	54	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	10	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.979	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1.57 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Refereuce No:	**100607**	**Cylinder Number**	160571
Batch No:	Pures4	**Ineos R&T Reference**	20090310_2
Date of Manufacture:	10-Mar-2009	**Expiry Date**	10-Mar-2010
Date/Time of Sampling :	10/03/2009 11:03:00	**Date/Time Logged :**	12/03/2009 13:34:14
Quality:	**PASS**	**Specification Ref :**	**1243ZF_PRODUCT**

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	1.4	ppm w/w		AWEIR
134a	1.3	ppm w/w		AWEIR
1225zc	N/D	ppm w/w		AWEIR
1234yf	1.8	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	142	ppm w/w		AWEIR
12	54	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	10	ppm w/w		AWEIR
1122	N/D	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.979	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1.57 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

INEOS Fluor

INEOS FLUOR R&TLABORATORY
Name:3,3,3-Trifluoroprop-1-ene

Synonyms:HFO1243zf

Sample Status: Authorized

LIMS Reference No:	100608	**Cylinder Number**	160517
Batch No:	Pures5	**Ineos R&T Reference**	20090310_4
Date of Manufacture:	10-Mar-2009	**Expiry Date**	10-Mar-2010
Date/Time of Sampling :	10/03/2009 14:43:00	**Date/Time Logged :**	12/03/2009 13:35:11
Quality:	PASS	**Specification Ref :**	1243ZF_PRODUCT

Issue No :

Analyte	Result	Units	Spec. Status	Analyst
125	N/D	ppm w/w		AWEIR
143a	N/D	ppm w/w		AWEIR
134a	N/D	ppm w/w		AWEIR
1225zc	<1	ppm w/w		AWEIR
1234yf	N/D	ppm w/w		AWEIR
134	N/D	ppm w/w		AWEIR
1225ye-Z	N/D	ppm w/w		AWEIR
152a	65	ppm w/w		AWEIR
12	20	ppm w/w		AWEIR
263fb	N/D	ppm w/w		AWEIR
Methyl Chloride	<1	ppm w/w		AWEIR
1122	<1	ppm w/w		AWEIR
Vinyl Chloride	N/D	ppm w/w		AWEIR
31	N/D	ppm w/w		AWEIR
1233xf	N/D	ppm w/w		AWEIR
1233zd	N/D	ppm w/w		AWEIR
Unknown1	N/D	ppm w/w		AWEIR
Unknown2	N/D	ppm w/w		AWEIR
Unknown3	N/D	ppm w/w		AWEIR
Unknown4	N/D	ppm w/w		AWEIR
Unknown5	N/D	ppm w/w		AWEIR
Total Unknowns	N/D	ppm w/w		AWEIR
1243zf Purity	99.991	% wt		LIMS Calc.

Notes:

Reviewed By : AWEIR
Date Reviewed : 12-Mar-2009 1:57 pm

Date Report Printed : 12-Mar-2009

Page -1 of 1

Annex 5 GLP Compliance Statements

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2008

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Huntingdon Life Sciences Huntingdon Research Centre Woolley Road Alconbury Cambs. PE28 4HS	Analytical/Clinical Chemistry Ecosystems Environmental Fate Environmental Toxicity (terrestrial only) Flow Cytometry Histology/pathology (slide reading only) Microbiology Toxicology

DATE OF INSPECTION

23rd – 25th September 2008

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

8/1/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2009

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Huntingdon Life Sciences Ltd. **Huntingdon Research Centre** **Woolley Road** **Alconbury** **Cambridgeshire** **PE28 4HS**	**Analytical Chemistry** **Clinical Chemistry** **Ecosystems** **Environmental Fate** **Environmental Toxicity** **Toxicology**

DATE OF INSPECTION

2nd November 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

11/01/10

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

EYE RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2009

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Huntingdon Life Sciences **Eye Research Centre** **Occold** **Eye** **Suffolk** **IP23 7PX**	**Analytical/Clinical Chemistry** **Ecosystems** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Phys/Chem Testing** **Toxicology**

DATE OF INSPECTION

17-19 February 2009

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

1/5/09

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

EYE RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2010

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

Huntingdon Life Sciences
Eye Research Centre
Occold
Eye
Suffolk
IP23 7PX

TEST TYPE

Analytical/Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Physico-chemical Testing
Residue Studies
Toxicology

DATE OF INSPECTION

26 January 2010

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

22/3/10

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

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