

bp

FYI-1002-01435

62696


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October 2nd, 2002

Attn: FYI
Room G99 East Tower
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460-0001

2002 OCT -9 AM 10:46

RECEIVED
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For your information, enclosed is a copy of the report of the results of an inhalation study to investigate the cardiac sensitization potential of a prospective halon replacement in the Beagle Dog. This study was a follow on from other toxicology studies on three agents that were being evaluated as prospective halon replacements by the Advanced Agent Working Group (AAWG) that you have already been advised of. Information on the test agent, test protocol, and test results are contained in the enclosed report. The No Adverse Effect Level was determined to be 0.49% v/v and the Low Adverse Effect Level was determined to be 1.00% v/v.

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Sincerely,



David V. Catchpole
On Behalf of the AAWG

Enclosures



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Huntingdon

AGENT 873

**AN INHALATION STUDY TO INVESTIGATE THE CARDIAC SENSITISATION
POTENTIAL IN THE BEAGLE DOG**



AGENT 873

**AN INHALATION STUDY TO INVESTIGATE THE CARDIAC SENSITISATION
POTENTIAL IN THE BEAGLE DOG**

Sponsor

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Report issued: 16th September 2002

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- HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2001 33

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

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

The United Kingdom Good Laboratory Practice Regulations, 1999, (Statutory Instrument No. 3106).

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

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

Information regarding the purity and expiry date of the test material, were not available to Huntingdon Life Sciences for compliance with the Good Laboratory Practice Regulations given above.



.....
Paul J. Horrell, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

16 SEPTEMBER 2002

.....
Date

QUALITY ASSURANCE STATEMENT

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

Study Phases Inspected	Date of Inspection	Date of Reporting
Protocol Review	09 July 2001	12 July 2001
Study Based Inspections		
Study preparation)		
Test item control and disposition)		
Exposure and generation of test atmospheres)	23 August 2001	28 August 2001
Atmosphere sampling)		
Clinical signs examination)		
Report audit	04 September 2002	05 September 2002

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

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

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

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

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

A. Thompson

Amanda Thompson, M.R.Q.A.,
Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

16 September 2002

Date

CONTRIBUTING SCIENTISTS

STUDY MANAGEMENT

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Section Head, Aerosol Technology and Analysis.

SUMMARY

This study was designed to assess the cardiac sensitisation potential of Agent 873 in beagle dogs.

Adrenaline was administered by intravenous injection before and during inhalation of test substance. The effect of the adrenaline on the ECG pattern was examined. Positive evidence of cardiac sensitisation would be observed as the presence of multiple multifocal ectopic beats or ventricular fibrillation following adrenaline administration during inhalation of the test substance. No such effect should be observed in the absence of the test substance.

Agent 873 was administered at concentrations of 0.49, 1.00 , and 1.50 % v/v in air.

At 0.49% v/v Agent 873 there was no evidence of cardiac sensitisation.

At 1.00% v/v Agent 873, 1 out of 6 dogs exposed gave a positive cardiac sensitisation response.

At 1.50% v/v Agent 873, the single dog exposed gave a positive response.

It is concluded from the results of the study that for Agent 873 the No Adverse Effect Level is 0.49% v/v and the Low Adverse Effect Level is 1.00% v/v.

INTRODUCTION

The potential of Agent 873 to cause cardiac sensitisation by inhalation in the beagle dog was determined.

Six dogs from an available batch of 9 were used on this study. All of the six dogs had been used for previous studies of the same type.

Cardiac sensitisation to adrenaline is a phenomenon associated with the inhalation of a number of unsubstituted and halogenated hydrocarbons (Beck *et al*, 1973; Clark, 1973, 1982; Hardy, 1994). After inhalation of the sensitising agent, challenge with adrenaline causes cardiac arrhythmias. This type of response was first demonstrated by Levy (Levy, 1913) when he induced ventricular fibrillation in cats during inhalation of chloroform, by injecting adrenaline.

There are no regulatory guidelines for this type of study, therefore design was in accordance with accepted pharmacological principles and methods (Reinhardt, 1971, 1973).

The technique used involved the intravenous injection of adrenaline before and during the inhalation of the test gas. The effect of adrenaline on the electrocardiogram was examined in both cases and compared to assess any positive response to the test gas. It should be noted that the mammalian response to exogenous adrenaline can be dependant upon a number of biological factors alone or in combination for example plasma hormone levels and temperament. This stresses the importance of acclimatisation and training.

The beagle dog was chosen for three reasons. Firstly available cardiac sensitisation data pertains to beagles and therefore provides comparative data. Secondly, a docile and accommodating species is required to accept training and venepuncture. Thirdly, a non-rodent species provides more suitable electrocardiographic data when extrapolating the data to man.

The protocol was approved by the Study Director and Huntingdon Life Sciences Management on 28 June 2001 and by the Sponsor on 20 July 2001.

On completion of the study all data pertaining to the study, including a copy of this final report, were transferred to Huntingdon Life Sciences Ltd Archives. The data will be retained in Archives for at least 5 years from the completion date for the study.

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

The experimental start and finish dates for this study were 30 July and 26 November 2001 respectively.

The Stage II exposures were carried out during the period 13 August to 12 September 2001.

TEST SUBSTANCES

Common name:	Agent 873
Chemical name:	2-bromo-3, 3, 3-triflouoropropene
Purity:	Not given
Presentation:	liquid
Storage conditions:	Ambient temperature and humidity in the dark
Batch (lot):	#83-70
Expiry date:	Not given. Assumed stable for the duration of the study
Date received:	15 May 2001
Supplier:	Sponsor

The complete description of the chemical and physical properties of the test substance, together with information regarding its purity, composition and stability are the responsibility of the Sponsor.

MATERIALS AND METHODS

TEST ANIMALS

Nine pure-bred male beagle dogs, approximately 6 - 16 months old, were available for the study.

All dogs were obtained from Interfauna UK Ltd, Abbots Ripton Road, Wyton, Huntingdon, England.

At the start of the Stage II procedures (Air only) the dogs weighed between 9.5 and 19.5 kg.

The dogs were each identified by a unique number tattooed on the ear pinna by the supplier. Permanent study numbers were assigned to all the dogs. The individual permanent study number was tattooed on the inside aspect of the left hindleg.

ACCLIMATISATION

The dogs were acclimatised to laboratory conditions and handling procedures. Each dog was further trained in order to acclimatise it to the exposure apparatus. The training consisted of at least 5 sessions during which the dogs were restrained in a canvas sling whilst breathing through a face mask and non-return valve connected to a reservoir gas bag containing air only. Details of training are stored with the raw data for this study but are not commented on further in this report.

ACCOMMODATION AND HUSBANDRY

The dogs were housed in pens within a purpose designed dog holding facility (Building J24, Unit 25). The pens were designed in accordance with the requirements of the United Kingdom Home Office Code of Practice for Housing and Care of Animals used in Scientific Procedures. The pens had a floor area of 4.5 square metres and could accommodate up to 2 dogs of the same sex and group. The pens could be subdivided into equal areas by means of a moveable partition so that each day the dogs could be segregated for assessment of individual clinical signs and food consumption. Both parts of the pen were fitted with an automatic valve for the supply of drinking water. During week days the dogs were segregated from approximately 0800 to 1500 hours. At weekends dogs were paired up at approximately 1200 hours.

Animal room temperature controls were set to maintain, as far as possible, a temperature range of 15 - 25°C. Relative humidity was recorded continuously but was not controlled. Fluorescent lighting was controlled to give 12 hours of light and 12 hours of dark in each 24-hour period. Air extraction, *via* a balanced system, provided approximately 10 - 12 air changes per hour. Actual minimum and maximum temperature and relative humidity values were 17/23°C and 40/90% respectively.

The exposure sessions were carried out in the Department of Dog Toxicology (Building J24 Room 1005). The dogs were transported from the holding unit to the experimental unit as required.

The dogs were provided with 500 - 600 g of dry diet daily (Diet A, Special Diets Services Ltd) and fresh tap water daily. There was no information available to indicate to the Study Director the presence, in food or water, of any non-nutrient substance likely to influence the outcome of this study. The results of chemical analysis during the study are lodged in the Huntingdon Life Sciences Archives.

As part of the routine husbandry, food consumption records and weekly bodyweights were recorded and veterinary records were maintained. These have been archived and are not commented on further in this report.

On completion of the study the surviving dogs were retained for possible use in further studies of the same type.

EXPOSURE SYSTEM (Figure 1)

The dogs were exposed to the test gas by a snout-only system.

A Halls face mask (Phoenix Medical Ltd) was used. The mask consisted of a rubber cone, one end of which was connected to the air supply line, while the other end was placed over the dog's snout. To ensure a reasonably airtight fit around the dog's snout, a latex sheet with a hole was placed over the snout end of the mask and the dog's snout protruded through the sheet into the mask. The mask was held in place by a leather muzzle. The dog was restrained in a canvas sling through which the limbs extended, the dog could thus support itself standing or be supported by the sling.

The dog face mask was connected *via* a non-return valve to a reservoir gas bag (Tedlar® SKC Inc, USA) of 200 litres capacity. The dogs inhaled from the gas bag through the non-return valve and exhaled to waste. During the first experimental session the gas bag was filled with room air only.

For gas exposures, the reservoir gas bag contained known concentrations of the test material. The test atmosphere was produced by injecting volumes of the test material through a port into a gas bag containing a known volume of air which had been metered into the bag through a wet-type gas meter (Alexander Wright and Co (Westminster) Ltd). The liquid test substance was then allowed to vaporise completely.

During the gas exposure period the dog was permitted to breathe through the non-return valve from the test gas bag and exhale to waste. In this way the concentration in the gas bag remained constant throughout the exposure period.

The test atmosphere in the reservoir gas bag was sampled before and after exposure for each dog. Samples were analysed using infra-red spectrophotometry. The procedures are detailed in Appendix 4.

MEASUREMENT OF THE ELECTROCARDIOGRAM (ECG)

The standard lead II electrocardiograph was used throughout the study.

Appropriate areas on the dog's limbs were shaved and electrode gel applied. Standard ECG limb leads were then connected to the prepared areas on the dog with blunt clips. The electrocardiograph (Electromed multi-trace II) was calibrated with 1mV peaks. The electrocardiogram was then checked for clarity before dosing commenced.

EXPERIMENTAL PROCEDURE

The experimental procedure was based on published techniques (Reinhardt, 1971, 1973).

The gas bags were prepared before each exposure session and the target concentration was established before any dogs were exposed. Once the level was established, the dog was placed in the sling and fitted with the mask. At this stage the dog was breathing room air.

Adrenaline solutions were prepared immediately prior to each exposure session from a stock solution (Adrenaline 1: 1000, Batch 026007, Phoenix Pharmaceuticals Ltd., Gloucester, England and Batch 184575, Martindale Pharmaceuticals, Romford, Essex, England) and sterile pyrogen-free water (Formulation Department). Injections were given at a rate of 0.1 ml/second. Appropriate dilutions were used such that the volume of adrenaline solution used was consistently 0.1 ml/kg bodyweight.

The ECG was established (as described previously) and the timing commenced as shown below:

Time (minutes)	Event
0	Start ECG recording
2	First adrenaline (D1) challenge administered
7	Gas reservoir connected to air supply line
12	Second adrenaline (D2) challenge administered
17	Gas reservoir disconnected Stop ECG recording

The test animals were exposed to various test gas concentrations as detailed in the **EXPERIMENTAL DESIGN** section of this report.

EXPERIMENTAL DESIGN

The study was performed in 2 stages:

STAGE 1

In this stage an individual response to adrenaline alone was selected for each of the 9 dogs using a range of adrenaline doses.

The response to administration of adrenaline is dependant on the dose given and the responsiveness of each individual dog. Typically the response consists of a transient increase in heart rate followed by a reflex slowing of the heart rate and an increase in the height of the T-wave, occasionally multiple unifocal ventricular tachycardia may occur.

The purpose of Stage 1 of the study was to establish an adrenaline dose at which there was a clear but minimal effect on the ECG ideally with a few ectopic beats. The actual dose level at which the various responses occurred was dependent on the individual dog.

A suitable adrenaline dose had been selected for each of the previously used dogs and was established for each of the naive dogs in the following manner.

The initial dose adrenaline for each dog was 4 $\mu\text{g}/\text{kg}$. If the response to the first adrenaline challenge was too large, as defined by an excessive number of ectopic beats (more than approximately 10) or following both injections there were no ectopic beats, the experimental session did not proceed further and the dog was allowed to rest for at least 20 minutes. The procedure was then repeated with an adrenaline dose half or double that previously given (except that the highest dose was 12 $\mu\text{g}/\text{kg}$, *ie*: if there was no response at 8 $\mu\text{g}/\text{kg}$, this was followed by 12 $\mu\text{g}/\text{kg}$).

It was recognised that there were occasions when a judgement needed to be applied, for example, if there were 15 - 20 ectopic beats at 8 $\mu\text{g}/\text{kg}$ but at 4 $\mu\text{g}/\text{kg}$ there were none, then 8 $\mu\text{g}/\text{kg}$ was likely to be acceptable as a suitable dose.

Each previously used dog was re-tested at the established dose and where necessary a new adrenaline dose was established. If no ectopic beats were observed for a particular dog the adrenaline dose was increased until a response was observed. Conversely, if too many ectopic beats were observed the dog was allowed to recover and the adrenaline dose was reduced until an acceptable dose was found. A maximum dose of 12 $\mu\text{g}/\text{kg}$ was used.

If necessary, the Study Director and monitoring toxicologist applied a judgement in the selection of an appropriate adrenaline dose in the best interests of the dog and the study integrity.

When an appropriate dose had been selected, that dose was used for the exposures in Stage 2.

Each of the dogs allocated to the study was tested as indicated in the **EXPERIMENTAL PROCEDURE**, except that no test gas was used and the dogs received air only for the 17 minutes.

On completion of Stage 1, 6 of the dogs were allocated for use in Stage 2. Details of the results of Stage 1 of the study and the rationale for the selection of the dogs for Stage 2 of the study are given in the **RESULTS** section of this report.

STAGE 2

Each of the dogs selected for Stage 2 was exposed to the test gas according to the **EXPERIMENTAL PROCEDURE**. The dogs were scheduled to be exposed according to the following schedule,

Exposure session	Agent 873 concentration (% v/v in air)
1	Air only
2	1
3	3
4	5
5	7
6	10
7	15

At least one calendar day was planned between each exposure session to allow the dogs to recover.

INTERPRETATION OF RESULTS

The study was designed to provide information as to any dose level that gave rise to clear signs of test gas related cardiac sensitisation. The criterion for a positive effect was the appearance of a burst of multifocal ventricular ectopic activity (MVEA) or ventricular fibrillation (VF) at any time during exposure to the test gas.

Interpretation of the results was made for each dog, taking into account the response of each dog during Stage 1 of this study.

DEVIATIONS FROM PROTOCOL

The correct name of the dog breeder is Harlan UK Ltd, not Harlan UK U.K. Ltd as stated in section 4.1.1 of the Protocol and Protocol Amendment 1.

The date of arrival, at Huntingdon Life Sciences, for Dogs 1061, 1063, 1065 and 1067 was incorrectly stated, in Protocol Amendment 1, as 10/07/00. The correct date of arrival was 10/07/01.

Dog 1061 was removed from the study, prior to the start of Stage 1 procedures due to ill health, and was replaced with Dog 1315.

These deviations from Protocol have had no impact on the integrity of the study.

RESULTS

STAGE 1 - CHALLENGES WITH ADRENALINE ALONE

The results of the experimental sessions in which the dogs were challenged with adrenaline in the absence of any test gas are summarised in Appendix 1.

On the basis of these results the dose of adrenaline selected for each dog was:

1 µg/kg:	Dog:	1065
2 µg/kg:	Dogs:	1315, 1063, 1067 and 1133
8 µg/kg:	Dog:	1003
12 µg/kg:	Dogs:	1001 and 1131

Dog 1307 would not tolerate the challenge process.

STAGE 2 - EXPOSURE TO TEST ATMOSPHERE

Six dogs were selected from the 9 available.

The dogs selected represented a range of responses to adrenaline. The following dogs were selected:

Dog number	Adrenaline dose (µg/kg)
1001	12
1003	8
1063	2
1065	1
1131	12
1133	2

The results are presented as follows:

- Table 1 - Summary of cardiac responses to adrenaline administration during Agent 873 exposure.
- Appendix 2 - Results of Stage 2 - Cardiac responses to adrenaline administration during exposure to air only.
- Appendix 3 - Results of Stage 2 - Cardiac responses to adrenaline administration during exposure to Agent 873.

The achieved exposure concentrations for each dog are presented as follows:

- Table 2 - Mean analysed concentrations of 2-bromo-3,3,3-trifluoropropene
- Appendix 5 - Gas bag concentrations of 2-bromo-3,3,3-trifluoropropene - individual sample values

The original exposure regimen required exposure at an initial concentration of 1% v/v in air progressing to 15% v/v in air. However, due to a positive response seen during the first exposure the regimen was revised. Following the positive response at 1.00% v/v the sensitisation potential was confirmed by exposing a single animal to 1.5% v/v. Subsequently an exposure at half the concentration used in the first exposure was conducted in order to either further refine the Low Adverse Effect Level or establish a No Adverse Effect Level. If the latter occurred no further exposures would be required. The revised target concentrations and actual gas bag concentrations achieved for 2-bromo-3,3,3-trifluoropropene are summarised below:

Target concentration (% v/v)	Mean achieved concentration (% v/v)
1.0	1.00
1.5	1.50
0.5	0.49

There was good agreement between achieved and target concentrations of test material.

The results of exposure are presented below:

At the 1.00% level 6 dogs were exposed. Clinical signs included agitation, deep slow breathing, muscle tremors and struggling. One animal responded positively with an episode of multifocal ventricular ectopic activity (MVEA) approximately 6 seconds after administration of the second adrenaline challenge.

At the 1.50% level 1 dog was exposed. Clinical signs included deep slow breathing, agitation and limb tremors. The animal responded positively with an episode of MVEA approximately 13 seconds after administration of the second adrenaline challenge.

At the 0.49% level 6 dogs were exposed. Clinical signs included agitation, deep slow breathing and muscle tremors. There were no positive cardiac responses.

DISCUSSION AND CONCLUSION

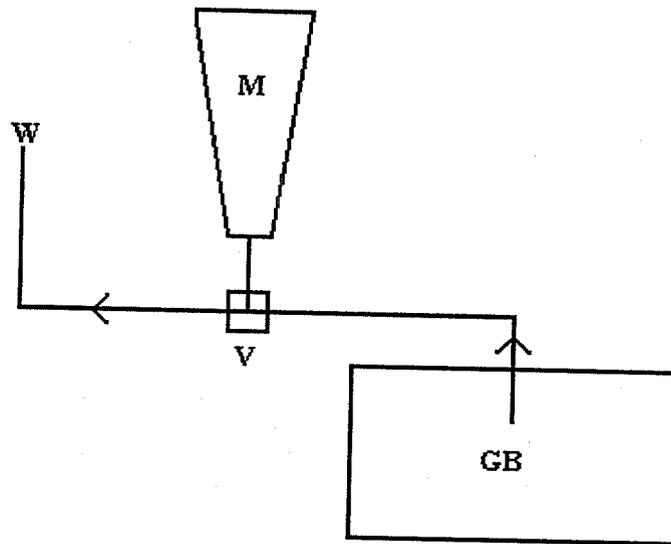
Agent 873 is being developed by the Sponsor for fire suppressant use.

In this study the Low Adverse Effect Level was 1.00% v/v and the No Adverse Effect Level was 0.49% v/v Agent 873 in air.

REFERENCES

- BECK PHILLIPPA, S., CLARK, D.G. AND TINSTON, D.J. (1973). The pharmacological actions of bromochlorodifluormethane (BCF) *Tox. and App. Pharmacol.*, **24**, 1 - 10.
- CLARK, D.G. and TINSTON, D.J. (1973). Correlation of the cardiac sensitisation potential of some halogenated and non-halogenated hydrocarbons. *Brit. j. Pharmacol.*, **49**, 355 - 357.
- CLARK, D.G. and TINSTON, D.J. (1982). Acute inhalation toxicity of some halogenated and non-halogenated hydrocarbons *Human Toxicol.*, **1**, 239 - 247.
- HARDY, C.J., KIERAN, P.C., SHARMAN, I.J. (1994). Assessment of the cardiac sensitisation potential of a range of halogenated materials. Poster Presentation at Society of Toxicology 1994 Annual Meeting, Dallas, Texas.
- LEVY, A.G. (1913), The exciting causes of ventricular fibrillation in animals under chloroform anaesthesia *Heart*, **4**, 319 - 378.
- REINHARDT *et al* (1971). Cardiac arrhythmias and aerosol sniffing *Arch. Env. Hlth.*, **22**, 265 - 279.
- REINHARDT *et al* (1973). Epinephrine induced cardiac arrhythmia potential of some common industrial solvents *J. Occup. Med.*, **15**, (12) 953 - 955.

FIGURE 1
Exposure system



GB Gas bag reservoir
M Mask
V Non-rebreathing valve
W Waste

To breathe the air V is open to ambient
To dose GB is connected to V

TABLE 1

**Summary of cardiac responses to adrenaline administration
during Agent 873 exposure**

Dog number	Adrenaline dose ($\mu\text{g}/\text{kg}$)	Concentration in air (% v/v)		
		0.49	1.00	1.50
1001	12	N	N	NP
1003	8	N	N	P (MVEA)
1063	2	N	N	NP
1065	1	UA	N	NP
1131	12	N	P (MVEA)	AP
1133	2	N	N	NP
Number positive		0/6	1/6	2/2

- N Negative response
P Positive response
UA Unable to assess due to struggling disturbing ECG recording
NP Not performed
AP Assumed positive due to positive response at lower concentration
MVEA Multifocal ventricular ectopic activity

TABLE 2

Mean analysed concentrations of 2-bromo-3,3,3-trifluoropropene

Target 0.5% v/v

Dog identification	Mean exposure concentration	
	(ppm)	(% v/v)
1131	4932	0.493
1001	4911	0.491
1003	4945	0.494
1063	4928	0.493
1133	4866	0.487
1065	4866	0.487
Mean	4908	0.491

Target 1.0% v/v

Dog identification	Mean exposure concentration	
	(ppm)	(% v/v)
1001	10200	1.02
1131	10180	1.02
1003	9946	0.99
1063	9876	0.99
1133	9929	0.99
1065	9950	0.99
Mean	10014	1.00

Target 1.5% v/v

Dog identification	Mean exposure concentration	
	(ppm)	(% v/v)
1003	14988	1.50

APPENDIX 1

Results of Stage 1 - individual responses to adrenaline alone

Dog number	Adrenaline dose ($\mu\text{g}/\text{kg}$)	Description of cardiac response following adrenaline challenge*		
		Number of ectopic beats		Comment (response)
		First challenge	Second challenge	
1001	4	0	0	L
	8	0	0	L
	12	1	11	A
1003	4	0	0	L
	8	0	2	A
1315	4	23	NP	H
	2	0	0	A
	4	19	29	H
1063	4	0	15	H
	2	0	8	A
1065	4	20	NP	H
	2	14	16	H
	1	13	9	A
1067	4	11	40	H
	2	0	0	A
	4	28	NP	H
1131	12	0	1	A
1133	2	0	18	A
	1	0	0	L

* In this appendix, mention is made only of ectopic beats or unexpected responses. The expected response to intravenous injection of adrenaline (initial tachycardia followed by bradycardia and increase in height of T-wave) is not mentioned in the "Description of cardiac response"

L Adrenaline dose considered too low
H Adrenaline dose considered too high
A Adrenaline dose considered acceptable
NP Not performed due to excessive response after first challenge

APPENDIX 2

**Results of Stage 2 - Cardiac responses to adrenaline administration
during exposure to air only**

Dog number	Adrenaline dose ($\mu\text{g}/\text{kg}$)	Description of cardiac response following adrenaline challenge*		
		Number of ectopic beats		Comment (response)
		First challenge	Second challenge	
1001	12	0	13	(N)
1003	8	1	4	(N)
1131	12	0	0	(N)
1133	2	0	3	(N)
1063	2	0	0	(N)
1065	1	0	0	(N)

* In this appendix, mention is made only of ectopic beats or unexpected responses. The expected response to intravenous injection of adrenaline (initial tachycardia followed by bradycardia and increase in height of T-wave) is not mentioned in the "Description of cardiac response".

(N) Negative response

APPENDIX 3

**Results of Stage 2 - cardiac responses to adrenaline administration
during exposure to Agent 873**

Gas concentration (% v/v)	Dog number	Adrenaline dose (µg/kg)	Description of cardiac response following adrenaline challenge*		
			Number of ectopic beats		Comment (response)
			First challenge	Second challenge	
1.00	1001	12	10	1	Agitation, arched back, pawing at mask, vocalising, deep slow breathing, struggling (N)
	1131	12	0	MVEA (VEA)	Agitation, arched back, deep slow breathing, struggling, pawing at mask, back legs tense (P)
	1003	8	8	8	Agitation, arched back, muscle tremors, deep slow breathing (N)
	1063	2	0	3	Arched back, deep slow breathing, agitation, struggling (N)
	1133	2	2	8	Agitation, arched back, deep slow breathing (N)
	1065	1	1	0	Agitation, pawing at mask, muscle tremors, struggling (N)
1.50	1003	8	1	MVEA	Deep slow breathing, arched back, agitation, limb tremors (P)
0.49	1001	12	0	1	Agitation, deep slow breathing, arched back, muscle tremors, shallow breathing (N)
	1131	12	1	1	Agitation, deep slow breathing, salivation, muscle tremors (N)
	1003	8	0	2	Agitation, deep slow breathing, muscle tremors, shallow breathing (N)
	1063	2	0	0	Agitation, arched back, muscle tremors, shallow breathing, struggling (N)
	1133	2	0	3	Arched back, deep slow breathing, agitation (N)
	1065	1	0	UA	Violent struggling (UA)

* In this appendix, mention is made only of ectopic beats or unexpected responses. The expected response to intravenous injection of adrenaline (initial tachycardia followed by bradycardia and increase in height of T-wave) is not mentioned in the "Description of cardiac response"

(N) Negative response

(P) Positive response

(UA) Unable to assess due to struggling disturbing ECG recording.

VEA Ventricular ectopic activity

MVEA Multifocal ventricular ectopic activity

APPENDIX 4**Methods of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene****SAMPLE COLLECTION****Chamber concentration**

Samples of the test atmosphere were drawn directly from the 200 litre gas-sampling bag containing the test atmosphere into the cell of the infra-red (IR) spectrometer to exhaust using a Metal Bellows sample pump. The absorbance at 8.6 μm was measured relative to room air and compared to that of standards prepared in gas-sampling bags. The method of sample analysis is detailed, together with a summary of the method validation, in the Inhalation Analytical Procedure at the end of this Appendix.

Preparation of the test atmospheres

The test atmospheres were prepared in gas sampling bags (*ca.* 200 litre capacity). A known volume of air was metered into the bag and the required volume of 2-bromo-3,3,3-trifluoropropene vapour added. The contents of the bag were mixed thoroughly prior to use.

CALCULATIONS

The method for calculating the approximate 2-bromo-3,3,3-trifluoropropene concentration from the volumes used to prepare each bag is as follows:

$$\text{Concentration (ppm)} = \frac{V}{V_a + V} \times 10^6$$

where V = volume of 2-bromo-3,3,3-trifluoropropene vapour added to the bag (litres);
 V_a = volume of air added to the bag (litres).

The exact concentration of 2-bromo-3,3,3-trifluoropropene present in each test atmosphere was determined analytically by IR analysis.

In order to minimise the cumulative errors that 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 and recalculation may lead to small apparent discrepancies with other data in the report.

APPENDIX 4

(Method of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene - continued)**Preparation of calibration standards**

Draw the required amount of 2-Bromo-3,3,3-trifluoropropene into a syringe (of appropriate volume) fitted with a mininert syringe valve, taking care to remove any air bubbles from the syringe. Close the valve and accurately weigh (to 0.1 mg) the syringe and contents. After weighing, dispense the contents of the syringe into a Tedlar gas sampling bag (containing a known volume of air) via the injection port. Close the syringe valve and reweigh the syringe. From the weight of 2-Bromo-3,3,3-trifluoropropene dispensed the standard concentration is calculated using the following equations:

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

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

where

V	=	gaseous volume of 2-Bromo-3,3,3-trifluoropropene (ml)
W	=	mass of 2-Bromo-3,3,3-trifluoropropene (mg)
M	=	molecular weight of 2-Bromo-3,3,3-trifluoropropene (174.95 g/mol)
Gas constant	=	0.08205 L atm./mol.K
Temperature (K)	=	temperature (°C) + 273
Atm. Pressure	=	barometric pressure (mm Hg)
V _a	=	volume of air added to the calibration bag

The range of calibration concentrations used in the measurement of 2-Bromo-3,3,3-trifluoropropene is detailed in the study specific supplement.

Storage of standards

Type	Storage conditions	Maximum storage period
Standard gas bags (5,000 – 20,000 ppm)	ambient	1 Day

APPENDIX 4

(Method of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene - continued)

Calibration and quantification

The instrument is allowed to warm-up for at least 15 minutes, then zeroed on room air. The wavelength of the instrument is fixed and room air is pumped through the flowcell immediately prior to measurement. Relative absorbance measurements are made by starting the integrator, switching the flow of sample through the chamber for up to 10 seconds, then switching back to room air. The integrator measures peak height which is proportional to absorbance of the sample.

Calibrate by measuring replicates of each calibration standard, as detailed in the study specific supplement. Measure the absorbance (peak height) for each calibration standard and derive the line of best fit using an unweighted least squares linear method.

For each sample, measure the absorbance at 8.6 μm and determine the concentration of 2-Bromo-3,3,3-trifluoropropene present using the equation below:

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

Where A = Absorbance of the sample
 S = Slope of calibration line derived from calibration data
 I = Intercept of calibration line derived from calibration data

Infra-red spectrophotometer conditions

IR Spectrophotometer	Wilkes Miran 1A-CVF
Cell type	1 cm path length cell fitted with zinc selenide windows
Operating wavelength	8.6 μm
Slit width	1 mm
Detector range	0.25 A for concentrations between 5,000 and 20,000 ppm
Meter response settings	1 second

Quality assurance measures

Calibration of the instrument will be performed with the appropriate standards on each day of use.

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

A quality check standard must follow every 6 test atmosphere samples for the analysis to be regarded as valid. The results of the quality check standards must lie within 5% of the nominal value.

Samples must lie within the range of acceptable quality check standards for the result to be regarded as valid. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

APPENDIX 4**(Method of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene - continued)****Summary of method validation**

The raw data for the method validation is located in study AAS/001.

Test blanks (room air, drawn through the flow cell) produced no quantifiable peaks at the measurement wavelength.

Precision data for repeatability (peak height) showed coefficients of variation for 2-Bromo-3,3,3-trifluoropropene of less than 5% using standard concentrations of 20,000 to 6,000 ppm.

Least squares regression analysis with no weighting of the peak height response against concentration of standard (20,000 to 6,000 ppm) produced a correlation coefficient of 0.9995 and relative errors less than 3%. The Limit of Quantification (LOQ) for 2-Bromo-3,3,3-trifluoropropene will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 2,943 and 971 ppm respectively (calculated statistically using the standard deviation obtained for a standard of concentration 5,963 ppm).

Standards of 2-Bromo-3,3,3-trifluoropropene in air of 20,000 to 6,000 ppm stored at ambient temperature for 1 day and subsequently analysed against fresh standards showed concentrations within 5% of their nominal concentrations.

APPENDIX 4

(Method of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene - continued)

AAS/001 - STUDY SPECIFIC SUPPLEMENT to the Inhalation analytical procedure for 2-Bromo-3,3,3-trifluoropropene

This supplement details additions and amendments to the procedure to be used for the IR assay of 2-Bromo-3,3,3-trifluoropropene obtained from air samples collected on the above study.

The assay, incorporating the additions and amendments, is suitable for the analysis of 2-Bromo-3,3,3-trifluoropropene, in air, at concentrations within the range of 5,000 to 20,000 ppm.

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

EFFECTIVE DATE :	16 August 2001
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Analytical standard

Name	2-Bromo-3,3,3-trifluoropropene
Batch number	83-70
Purity	98%
Expiry date	08/04
Supplier	BP Exploration

Preparation of calibration standards

Prepare calibration standards in the nominal range 5,000 to 20,000 ppm.

Instrument conditions

Detector range	0.25 A
Attenuation	1024

EFFECTIVE DATE :	23 August 2001
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Instrument conditions

Attenuation	2048
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EFFECTIVE DATE :	28 August 2001
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Instrument conditions

Detector range	1 A
Attenuation	512

APPENDIX 4

(Method of sample collection and analysis for 2-bromo-3,3,3-trifluoropropene - continued)

EFFECTIVE DATE :	11 September 2001
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Instrument conditions

Detector range	0.25 A
Attenuation	1024

Additional Method Validation

The raw data for this method validation is located in study AAS/001.

Precision data for repeatability (peak height) showed coefficients of variation for 2-Bromo-3,3,3-trifluoropropene of less than 1% using standard concentrations of 9,000 to 3,000 ppm.

Least squares regression analysis with no weighting of the peak height response against concentration of standard (9,000 to 3,000 ppm) produced a correlation coefficient of 0.997 and relative errors less than 4.5%. The Limit of Quantification (LOQ) for 2-Bromo-3,3,3-trifluoropropene will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 275 and 91 ppm respectively (calculated statistically using the standard deviation obtained for a standard of concentration 3,172 ppm).

Standards of 2-Bromo-3,3,3-trifluoropropene in air of 9,000 to 3,000 ppm are stable on the day of preparation (when analysed against alternate standards they showed concentrations within 5% of their nominal concentrations).

APPENDIX 5

Gas bag concentrations of 2-bromo-3,3,3-trifluoropropene - individual sample values

Target concentration 0.5% v/v

Sample ID	Gas bag No.	Measured concentration	
		(ppm)	(% v/v)
Pre dog 1131	1	4943	0.494
Post dog 1131, Pre dog 1001	1	4921	0.492
Post dog 1001	1	4900	0.490
Pre dog 1003	2	4948	0.495
Post dog 1003, Pre dog 1063	2	4941	0.494
Post dog 1063	2	4914	0.491
Pre dog 1133	3	4873	0.487
Post dog 1065	3	4859	0.486

Target concentration 1.0% v/v

Sample ID	Gas bag No.	Measured concentration	
		(ppm)	(% v/v)
Pre dog 1001	1	10194	1.02
Post dog 1001, Pre dog 1131	1	10206	1.02
Post dog 1131	1	10154	1.02
Pre dog 1003	2	9983	1.00
Post dog 1003, Pre dog 1063	2	9910	0.99
Post dog 1063	2	9842	0.98
Pre dog 1133	3	9929	0.99
Post dog 1133, Pre dog 1065	3	9930	0.99
Post dog 1065	3	9969	1.00

Target concentration 1.5% v/v

Sample ID	Gas bag No.	Measured concentration	
		(ppm)	(% v/v)
Pre dog 1003	1	14950	1.49
Post dog 1003	1	15026	1.50

HUNTINGDON RESEARCH CENTRE GLP COMPLIANCE STATEMENT 2001



THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
Huntingdon Life Sciences Huntingdon Research Centre Wooley Road Alconbury Huntingdon Cambs. PE28 4HS	Analytical Chemistry Clinical Chemistry Ecosystems Environmental Fate Environmental Toxicity Phys/Chem Testing Toxicology

DATE OF INSPECTION
15th January 2001

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

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



3/4/01

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Head, UK GLP Monitoring Authority

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