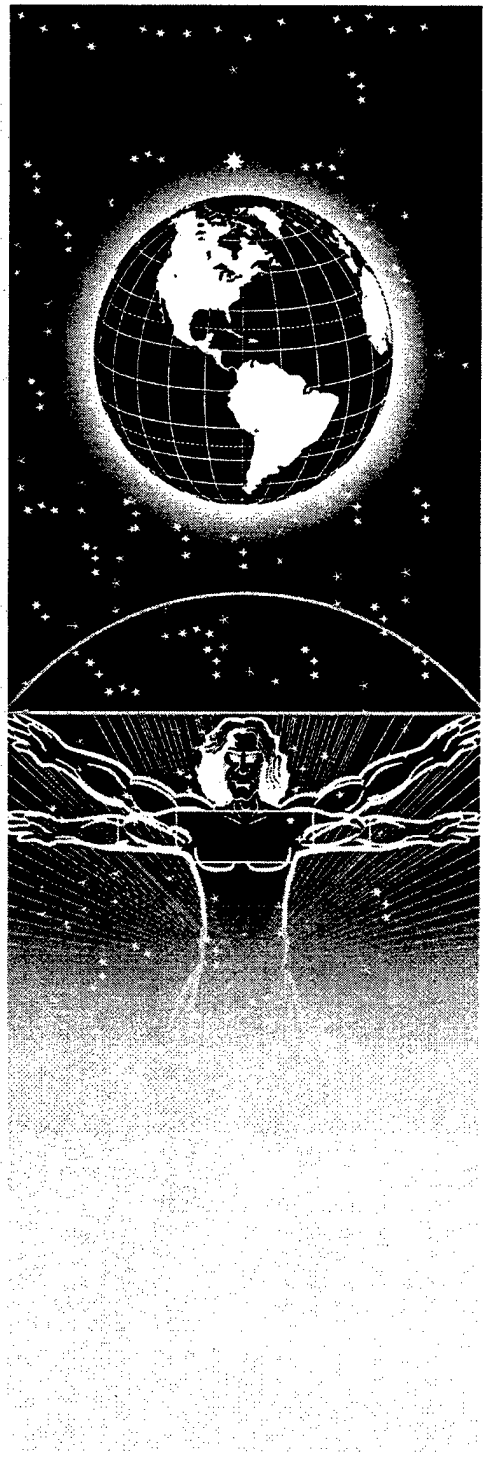
AL/OE-TR-1995-0031



UNITED STATES AIR FORCE ARMSTRONG LABORATORY

Iodotrifluoromethane And Iodoheptafluoropropane Assessment Of Cardiac Sensitisation Potential In Dogs

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February 1995

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TECHNICAL REVIEW AND APPROVAL

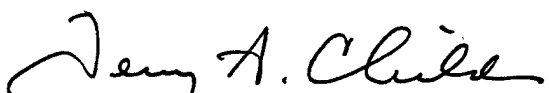
AL/OE-TR-1995-0031

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	21 February 1995	Final -November 1993 - February 1995

4. TITLE AND SUBTITLE
Iodotrifluoromethane and Iodoheptafluoropropane Assessment of Cardiac Sensitisation Potential in Dogs

5. FUNDING NUMBERS
Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630200
WU 63021410

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Huntingdon Research Centre Ltd.
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Huntingdon, Cambridgeshire
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8. PERFORMING ORGANIZATION REPORT NUMBER
MAH 1/943246

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING;MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1995-0031

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

This study was designed to assess the cardiac sensitisation potential of CF_3I and C_3F_7I in beagle dogs. Adrenaline was administered by intravenous injection before and during inhalation of test substances. The effect of the adrenaline of the ECG pattern was examined. Positive evidence of cardiac sensitisation would be observed as the presence of multifocal ventricular ectopic activity or ventricular fibrillation following adrenaline administration during inhalation of the test substance. CF_3I was administered to dogs at concentration in air of 0.1, 0.2, 0.4, and 1%v/v. At 0.1 and 0.2% there was no positive result. At each of 0.4 and 1.0% the first dog exposed responded positively with fatal ventricular fibrillation and consequently no other dogs were exposed at these levels.

C_3F_7I was administered to 3 dogs at concentrations in air of 0.1, 0.2, and 0.4%v/v. At each of 0.1 and 0.4% one dog responded positively with multifocal ventricular ectopic bets. The dog responding positively at 1.0% was not subsequently exposed to any higher concentrations. At 0.2% there was no positive result.

It is concluded that CF_3I has cardiac sensitisation potential at concentrations of 0.4%v/v in air and that C_3F_7I has cardiac sensitisation potential at concentrations of 0.1%v/v in air.

14. SUBJECT TERMS		15. NUMBER OF PAGES
Iodotrifluoromethane Cardiac Sensitisation	Iodoheptafluoropropane	38
		16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

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PREFACE

This document serves as a final report summarizing the work performed to assess the cardiac sensitisation potentialof CF_3I and C_3F_7I in beagle dogs. The research described herein was performed under subcontract to Huntingdon Research Centre, Ltd, Cambridgeshire, England for ManTech Environmental Technology, Inc. under the direction of Darol E. Dodd, Ph.D., Director of the Toxic Hazards Research Unit, located at Wright-Patterson Air Force Base, OH.

This research began on 1 November 1993 and was completed in February 1995 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F34). Lt Col Terry Childress served as the Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was cosponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, Army Medical Research Detachment, Walter Reed Army Institute of Research.

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IODOTRIFLUOROMETHANE AND IODOHEPTAFLUOROPROPANE

ASSESSMENT OF CARDIAC SENSITISATION POTENTIAL IN DOGS

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Report issued 21 February 1995

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APPENDICES

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.

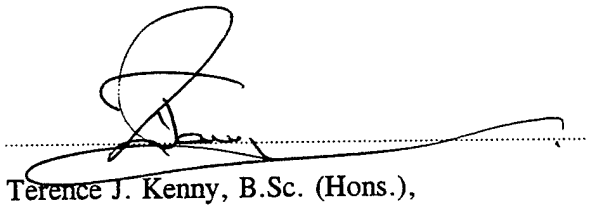
Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

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

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).



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Study Director,
Huntingdon Research Centre Ltd.

21 February 1995

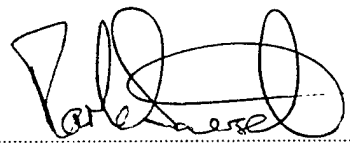
Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at HRC and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	13 May 1994
Pre-experimental Period	1 June 1994	2 June 1994
Experimental Period	13 June 1994	14 June 1994
	22 June 1994	24 June 1994
	1 - 8 July 1994	11 July 1994
	6 September 1994	7 September 1994
Date of reporting audit findings to the Study Director and HRC Management		26 January 1995



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13 February 95

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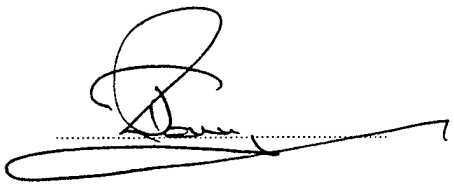

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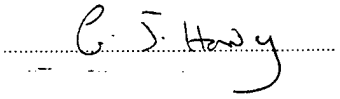

SUMMARY

This study was designed to assess the cardiac sensitisation potential of CF_3I and C_3F_7I 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 multifocal ventricular ectopic activity 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.

CFC 11 was administered to 1 dog as a positive control, the dog responded positively with fatal ventricular fibrillation.

CF_3I was administered to dogs at concentrations in air of 0.1, 0.2, 0.4 and 1% v/v. At 0.1 and 0.2% there was no positive result. At each of 0.4 and 1.0% the first dog exposed responded positively with fatal ventricular fibrillation and consequently no other dogs were exposed at these levels.

C_3F_7I was administered to 3 dogs at concentrations in air of 0.1, 0.2 and 0.4% v/v. At each of 0.1 and 0.4% one dog responded positively with multifocal ventricular ectopic beats. The dog responding positively at 0.1% was not subsequently exposed to any higher concentration. At 0.2% there was no positive result.

It is concluded that CF_3I has cardiac sensitisation potential at concentrations of 0.4% v/v in air and that C_3F_7I has cardiac sensitisation potential at concentrations of 0.1% v/v in air.

INTRODUCTION

The potential of CF_3I and C_3F_7I to cause cardiac sensitisation by inhalation in the beagle dog was determined.

A batch of 9 dogs were obtained for use on this study. One of these dogs gave a positive response to CFC 11 (a known cardiac sensitiser) at a concentration of 2% and died. These data confirm that the test system is capable of detecting cardiac sensitisers.

Cardiac sensitisation to adrenaline is a phenomenon associated with the inhalation of a number of unsubstituted and halogenated hydrocarbons (Beck *et al* 1973; Clark and Tinston 1973, 1982; Hardy *et al* 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 *et al* 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 dependent upon a number of biological factors alone or in combination *eg* 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 venipuncture. 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 HRC Management on 29 March 1994 and by the Sponsor on 4 April 1994. Four amendments were issued and approved by the Study Director on 12 May, 1 July, 22 July and 5 October 1994 respectively. These amendments were approved by the Sponsor on 23 May, 14 July, 29 July and 14 October 1994 respectively.

On completion of the study all data pertaining to the study, including a copy of this final report, were transferred to HRC Archives.

The exposures to the test gases were carried out during the period of 29 June to 13 September 1994.

MATERIALS

Test substances:

Iodotrifluoromethane

Chemical name:	Iodotrifluoromethane
Chemical formula:	CF_3I
Presentation:	Pressurised gas in cylinders
Received from:	Sponsor
Date received	14 March 1994
Batch no.:	Cylinder 1 labelled: Iodotrifluoromethane gas ARC G-2-112 Cylinder 2 labelled: Trifluoromethyl Iodide (no lot number)
Storage conditions:	Room temperature

Purity and stability of the test substances are the responsibility of the Sponsor.

Iodoheptafluoropropane

Chemical name:	Iodoheptafluoropropane
Chemical formula:	C_3F_7I
Presentation:	Liquid in amber glass bottle
Received from:	Sponsor
Date received:	5 August 1994
Batch no.:	None given
Storage conditions:	Room temperature in a fume hood or 4°C in the dark

Purity and stability of the test substances are the responsibility of the Sponsor.

CFC 11

Common name:	CFC 11
Chemical formula:	CCl_3F
Presentation:	Liquid
Received from:	Fisons Scientific Equipment, Loughborough, England.
Date received:	24 February 1994
Batch no.:	30490332
Purity:	99.5%
Stability:	Adequate
Storage conditions:	4°C in the dark

TEST ANIMALS

Nine pure-bred male beagle dogs, approximately 6 - 7 months old, were available for the study.

The dogs used on the study were obtained from Interfauna UK Ltd, Abbots Ripton Road, Wyton, Huntingdon, England. The dogs arrived at HRC on 13 April 1994.

At the start of the experimental procedures (Stage 1) the dogs weighed between 12.7 kg and 15.7 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 of the dogs. The individual permanent study number was tattooed on the inside aspect of the left hind leg.

ACCLIMATISATION

The dogs were acclimatised to laboratory conditions and handling procedures for approximately 4 weeks before experimental work was commenced. During the following 4 weeks the dogs were restrained in canvas slings and fitted with face masks with gradually increasing duration to accustom them to the restraint procedures to be applied during the experimental sessions. Details of the periods of restraint 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 (HRC Building no. J24, Unit 32). 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 2 equal areas by means of a movable partition so that each day, from approximately 0800 to 1700 hours, 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.

The exposure sessions (Stages 1, 2 and 3) were carried out in the Department of Inhalation Toxicology (HRC Building no. M12, Room 014). The dogs were transported, in a ventilated box, from the holding unit to the experimental unit as required, usually in groups of 3 dogs.

The dogs were provided with 400 g of dry diet daily (Diet A, Special Diets Services Ltd) and fresh tap water daily. Quality control and analytical data on food and water are stored in the HRC Archives.

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

On completion of the study the surviving dogs were retained for use in further studies by the Sponsor.

EXPOSURE SYSTEM

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

A Halls face mask (Veterinary Drug Co.) 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 air-tight fit around the dog's snout a latex sheet with a hole was placed over the snout end of the face 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.

CF_3I (Figure 1)

The apparatus used to produce the required test gas flow into the dog breathing line is shown in Figure 1. The test gas was metered from the pressurised cylinder through a flow-meter and added to a supply of air passing into the dog's face mask. A pair of solenoid valves, acting in tandem, could be switched so as to expose the dog to either clean air or to the test gas. The relative flows of test gas and diluent air were adjusted individually to result in a total air flow of approximately 40 litres/minute at the mask. The mask was exhausted to waste through a 4 m × 1.5 m tube which acted as a ballast if the inspiratory flow rate of the dog exceeded the supply air flow rate.

The test atmosphere was sampled continuously using a metal bellows pump and analysed using a Miran 1A CVF infrared gas analyser. The output of the analyser was recorded continuously on a Philips PM 8252 chart recorder. The measuring wavelength for CF_3I was 8.55 μm for the concentration range of 0.05 - 50% v/v in air. The analyser was calibrated using gas sampling bags (SKC Inc. U.S.A.) containing known concentrations of the test gas.

Calibration was carried out prior to and after each experimental session. During the exposure the test gas flow and air flow were monitored and adjusted as necessary to maintain the required target concentration at the dog mask.

C_3F_7I (Figure 2)

The air line connected to the dog face mask was in turn connected via a non-return valve to a reservoir gas bag (Tedlar® SKC Inc, USA) of either 100 litres or 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 substance. The test atmospheres were produced by injecting known volumes of the test liquid from a gas-tight syringe through a self sealing septum into the 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 was allowed to evaporate and the contents of the gas bag were mixed thoroughly by agitation. 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 using a sample line connected to a Miran 1A CVF infra-red analyser (see Figure 2). The output of the analyser was recorded using a Philips PM8252 chart recorder. The measuring wavelength for C_3F_7I was 8.9 μm for the concentration range 0.05 - 0.5% v/v in air. The analyser was calibrated using gas bags similar to the reservoir but of smaller volumes containing known concentrations of the test substance vapour.

Calibration was carried out prior to, and after each exposure session. During each exposure session, the reservoir gas bag atmosphere was renewed and/or maintained between dog exposures as necessary.

CFC 11

The exposure system was similar to that used for CF_3I. However in place of the pressurised gas cylinder a copper coil vapouriser was used. Liquid CFC 11 was metered into a copper tube immersed in a water bath maintained at approximately 80°C. Heated air was passed through the coil and the vapour/air mixture passed into the exposure system. The analytical wavelength for CFC 11 was 9.3 μm.

MEASUREMENT OF THE ELECTROCARDIOGRAM (ECG)

The standard lead II electrocardiogram was used throughout the study.

Appropriate areas on the dog's limbs were shaved and a conduction medium applied. Standard ECG limb leads were then connected to the prepared areas on the dog with blunt clips. The electrocardiograph (Devices 3442 ECG amplifier with a two-channel chart recorder) was calibrated with 1 mV peaks. The electrocardiogram was then checked for clarity before dosing commenced.

EXPERIMENTAL PROCEDURE

The experimental procedure was based on published techniques (Reinhardt *et al* 1971).

The exposure system was calibrated before each exposure session and the target level established before any dogs were exposed. When the system settings had been determined 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 310002, Phoenix Pharmaceuticals, Gloucester, England) and sterile pyrogen-free water (Formulation Department, HRC). 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	Test gas supplied to dog
12	Second adrenaline (D2) challenge administered
17	Test gas supply discontinued Stop ECG recording

The test animals were exposed to various test gas concentrations as detailed below.

EXPERIMENTAL DESIGN

The study was carried out in 3 stages.

Stage 1

Each of the 9 dogs on the study was tested according to the procedure given previously except that fresh air only was supplied to the face mask. Various concentrations of adrenaline solution were used in order to establish the response of each individual dog to adrenaline alone. On completion of Stage 1 the dogs were assigned to treatments for use in Stages 2 and 3 of the study. Details of the results of Stage 1 of the study are given in the **RESULTS** section of this report.

An appropriate initial adrenaline dose was selected for each dog following review of the results of Stage 1 of the study.

Stage 2

Two dogs were selected for exposure to CFC 11 according to the procedure given previously. The dogs selected were 1151 and 1157. The purpose of these exposures was to show that the experimental model used gave a positive response with CFC 11, a known cardiac sensitiser. Details of the results of Stage 2 of the study are given in the **RESULTS** section of this report.

Stage 3

Each of the 6 dogs selected for exposures 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. At this point the adrenaline response of each dog was assessed and compared to previously reported responses.

Details of the results of air only exposures are given in the **RESULTS** section of this report.

Each of the 6 dogs selected was exposed to the gas according to the **EXPERIMENTAL PROCEDURE**. The dogs were exposed according to the following schedule:

Exposure session	Test gas	Concentration in air % v/v
1	Air only	-
2	CF_3I	0.1
3	CF_3I	1.0
4	CF_3I	0.2
5	CF_3I	0.4
6	C_3F_7I	0.1
7	C_3F_7I	0.2
8	C_3F_7I	0.4

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

There was a gap of approximately 8 weeks between the end of exposures with CF_3I and the start of exposures with C_3F_7I. Only the surviving 4 dogs from the CF_3I exposures were used in the exposures with C_3F_7I.

When a clear positive response was seen in any dog no further exposures were undertaken on that dog with that test gas. It is considered that a positive response would be seen for that dog at higher exposure levels, this was reported as 'assumed positive' (AP).

If a dog died as a result of exposure to the test gas no further exposures at that concentration or higher concentrations were performed.

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). Ventricular tachycardia alone was not necessarily considered definitive evidence of a positive response.

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. The response of a dog to administration of adrenaline was dependent on the dose given and the responsiveness of each individual dog. Typically the response consisted of a transient increase in heart rate followed by a slowing of heart rate and an increase in the height of the T-wave (see Figure 3). In some dogs multiple unifocal ventricular tachycardia occurred (see Figure 4).

Examination of the results provided in Appendix 1 indicates that 7 of 9 dogs tested could be divided into 3 types of responder.

Dog 1145 was rejected during stage 1 due to excessive struggling.

Dog 1161 was rejected during stage 1 due to cardiac anomalies (probably bundle branch block) visible on the ECG trace and was subsequently sacrificed.

Dog 1155 was very responsive to adrenaline and had a large number of ectopic beats even at low levels of adrenaline administration.

Dog 1151 showed a minimal response at levels of adrenaline up to 12 μg/kg.

The remaining dogs (except 1145 and 1161) showed a predictable and minimal response to adrenaline given at levels of 4 or 8 μg/kg.

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

1 μg/kg:	Dog 1155, 1159
4 μg/kg:	Dogs 1157
8 μg/kg:	Dogs 1147, 1149, 1153
12 μg/kg:	Dog 1151

STAGE 2 - EXPOSURE TO CFC 11

The two dogs selected for Stage 2 of the study were 1151 and 1157.

The results are shown in Appendix 2.

Dog 1151 produced a positive response at 2% in air with fatal ventricular fibrillation (Figure 5). Dog 1157 was not exposed due to humane considerations. These data are consistent with previously published data (Reinhardt *et al*, 1971) and shows that the test is capable of detecting substances that are cardiac sensitisers.

STAGE 3 - EXPOSURE TO CF_3I

The results are presented as follows:

Table 1 Summary of cardiac responses to adrenaline administration during CF_3I exposure

Appendix 3 Cardiac responses to adrenaline administration during exposures with CF_3I

All dogs were tested at 0.1% CF_3I in air. All dogs were negative and nothing abnormal was observed.

One dog (dog 1147) was tested at 1.0% CF_3I in air. The dog responded positively and died (Figure 6). Exposures were terminated at this concentration. All remaining dogs were tested at 0.2% CF_3I in air. All dogs were negative and nothing abnormal was observed.

One dog (dog 1149) was tested at 0.4% CF_3I in air. The dog responded positively and died (Figure 7). Exposures were terminated with CF_3I.

EXPOSURE TO C_3F_7I

The results are presented as follows:

Table 2 Summary of cardiac responses to adrenaline administration during C_3F_7I exposure

Appendix 4 Cardiac responses to adrenaline administration during exposures with C_3F_7I

The 4 surviving dogs were tested at 0.1% C_3F_7I in air. One dog (dog 1153) responded positively and recovered (Figure 8). All remaining dogs were negative and nothing abnormal was observed.

All remaining dogs were tested at 0.2% C_3F_7I in air. All dogs were negative and nothing abnormal was observed.

All remaining dogs were tested at 0.4% C_3F_7I in air. One dog (dog 1157) responded positively and recovered (Figure 9). All remaining dogs were negative and nothing abnormal was observed.

DISCUSSION AND CONCLUSION

The results of exposure show that CF_3I has potential to cause cardiac sensitisation in dogs at a concentration of 0.4% v/v in air. The results also show that C_3F_7I has potential to cause cardiac sensitisation in dogs at a concentration of 0.1% v/v in air.

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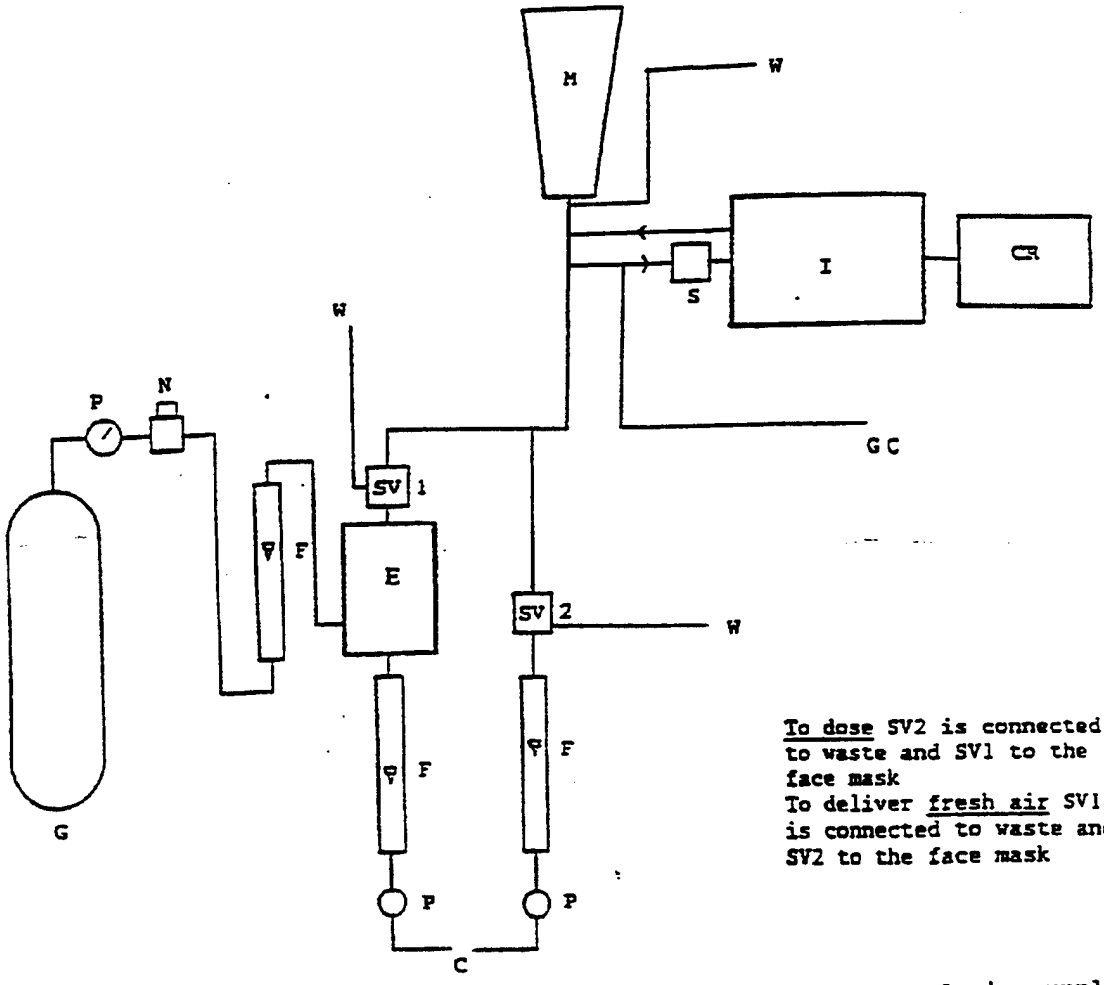
FIGURE 1

Exposure system - CF_3I

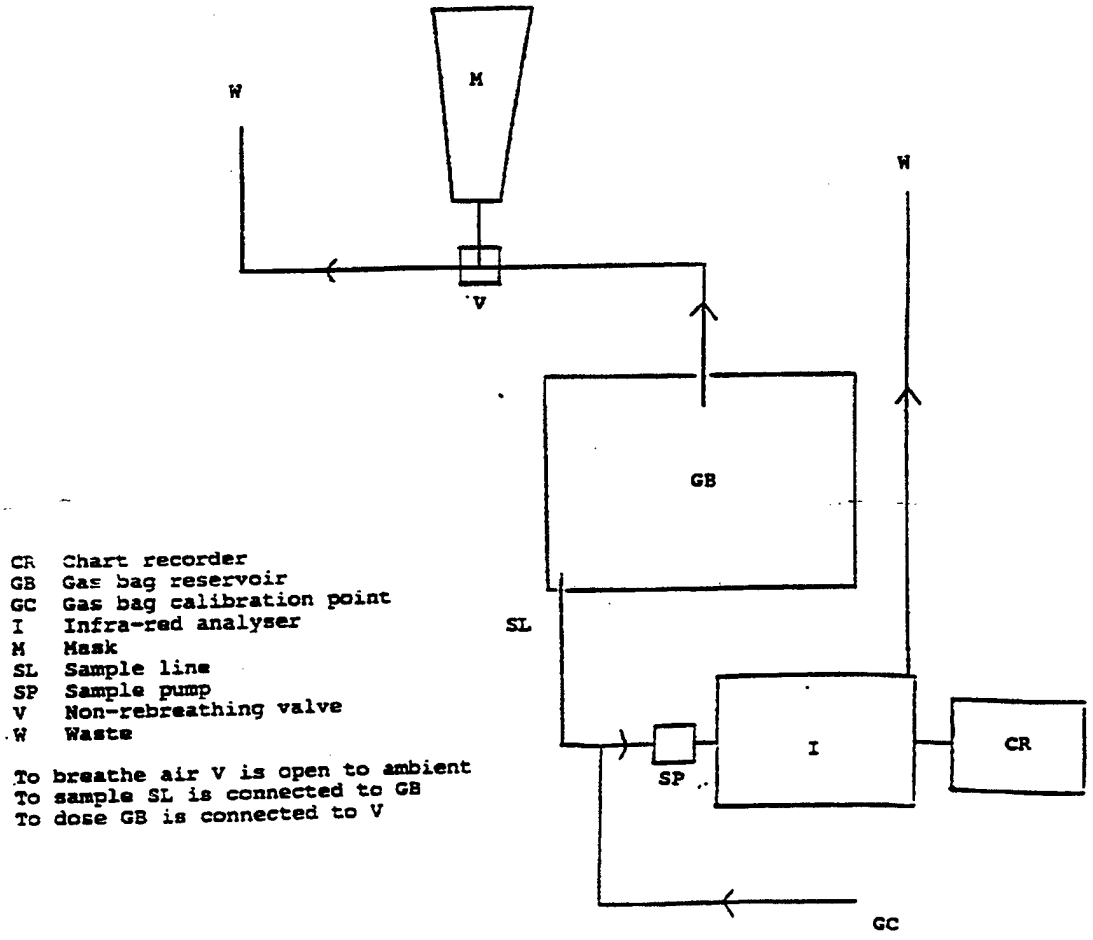


To dose SV2 is connected to waste and SV1 to the face mask
To deliver fresh air SV1 is connected to waste and SV2 to the face mask

C Compressed air supply
CR Chart recorder
E Expansion vessel
F Flowmeter
G Gas cylinder
GC Gas calibration point
I Infra-red analyser
M Mask
N Needle valve
P Pressure regulator
S Sample pump
SV Solenoid valve
W Waste

FIGURE 2

Exposure system - C_3F_7I



CR Chart recorder
GB Gas bag reservoir
GC Gas bag calibration point
I Infra-red analyser
M Mask
SL Sample line
SP Sample pump
V Non-rebreathing valve
W Waste

To breathe air V is open to ambient
To sample SL is connected to GB
To dose GB is connected to V

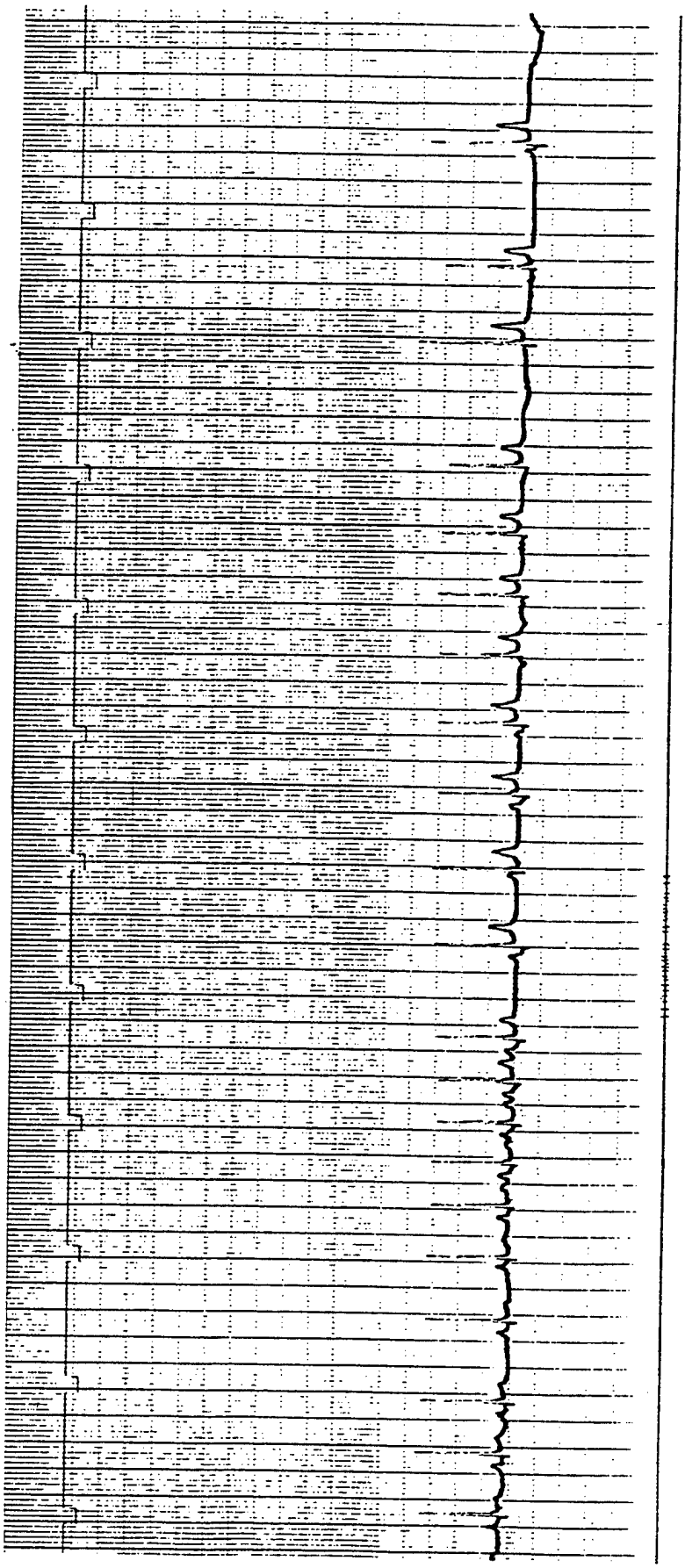


FIGURE 3

Example of a typical response to administration of adrenaline alone (Dog 1153: 8 µg/kg adrenaline)

FIGURE 4

Example of a typical response to administration of adrenaline alone (Dog 1157: 4 μg/kg adrenaline)

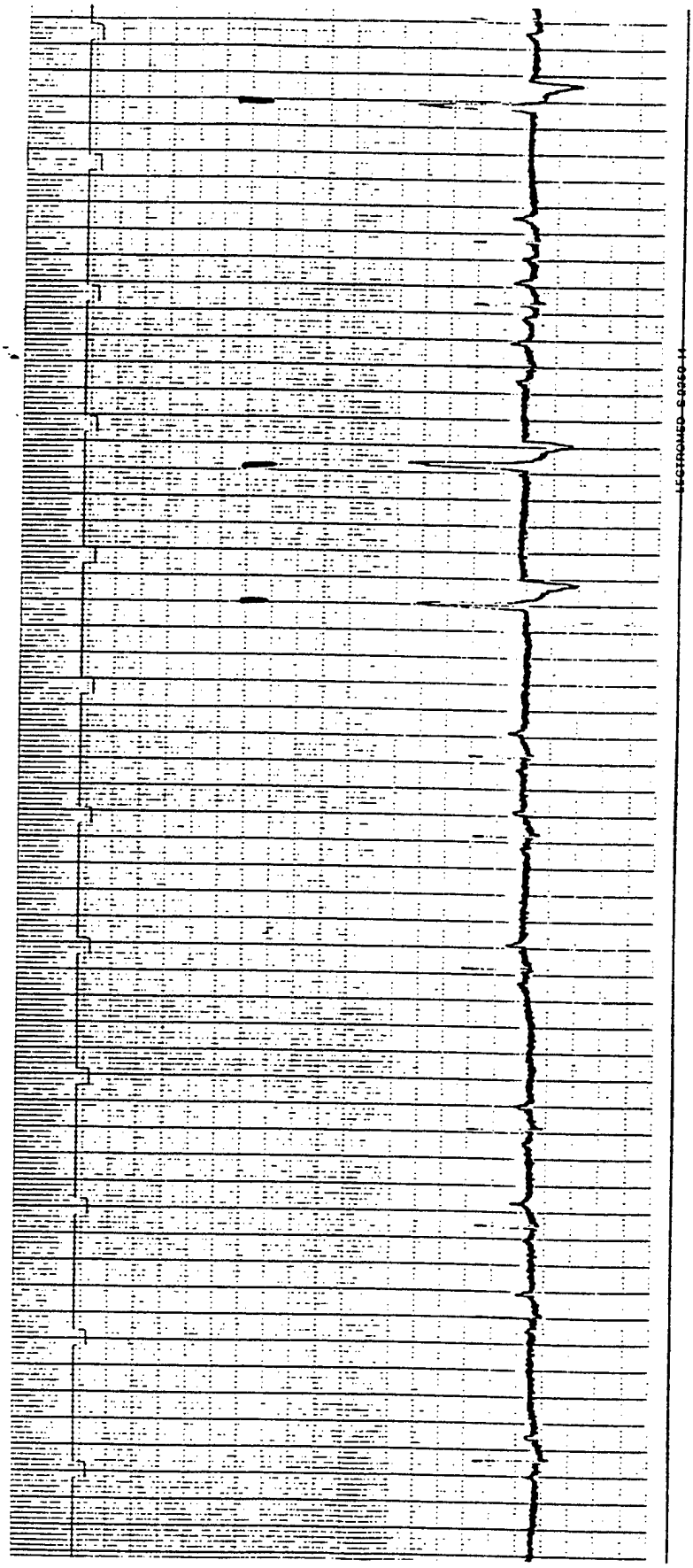


FIGURE 5

Response of Dog 1151 to administration of adrenaline during exposure to 2.0% v/v CFC 11 (response classified as positive)

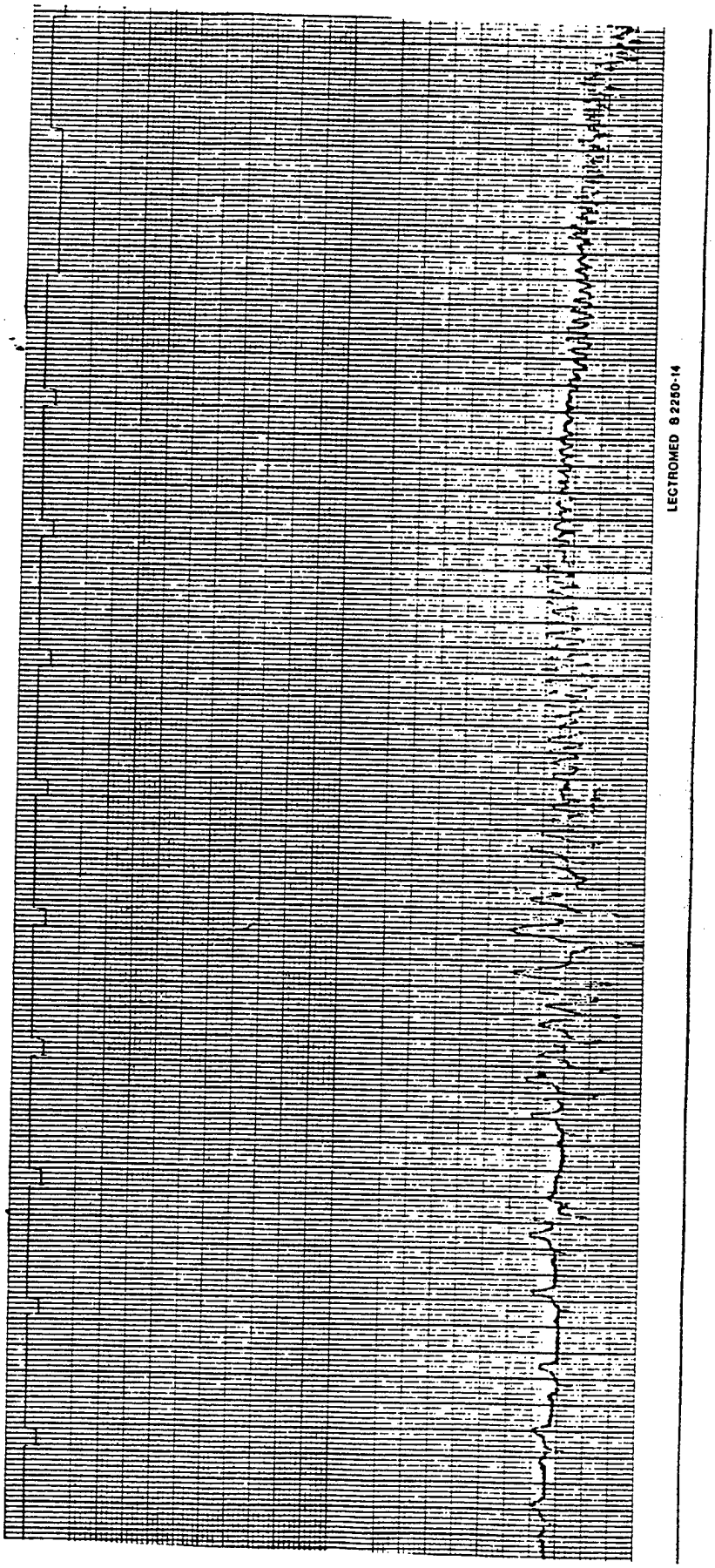


FIGURE 6

Response of Dog 1147 to administration of adrenaline during exposure to 1.0% v/v CF_3I (response classified as positive)

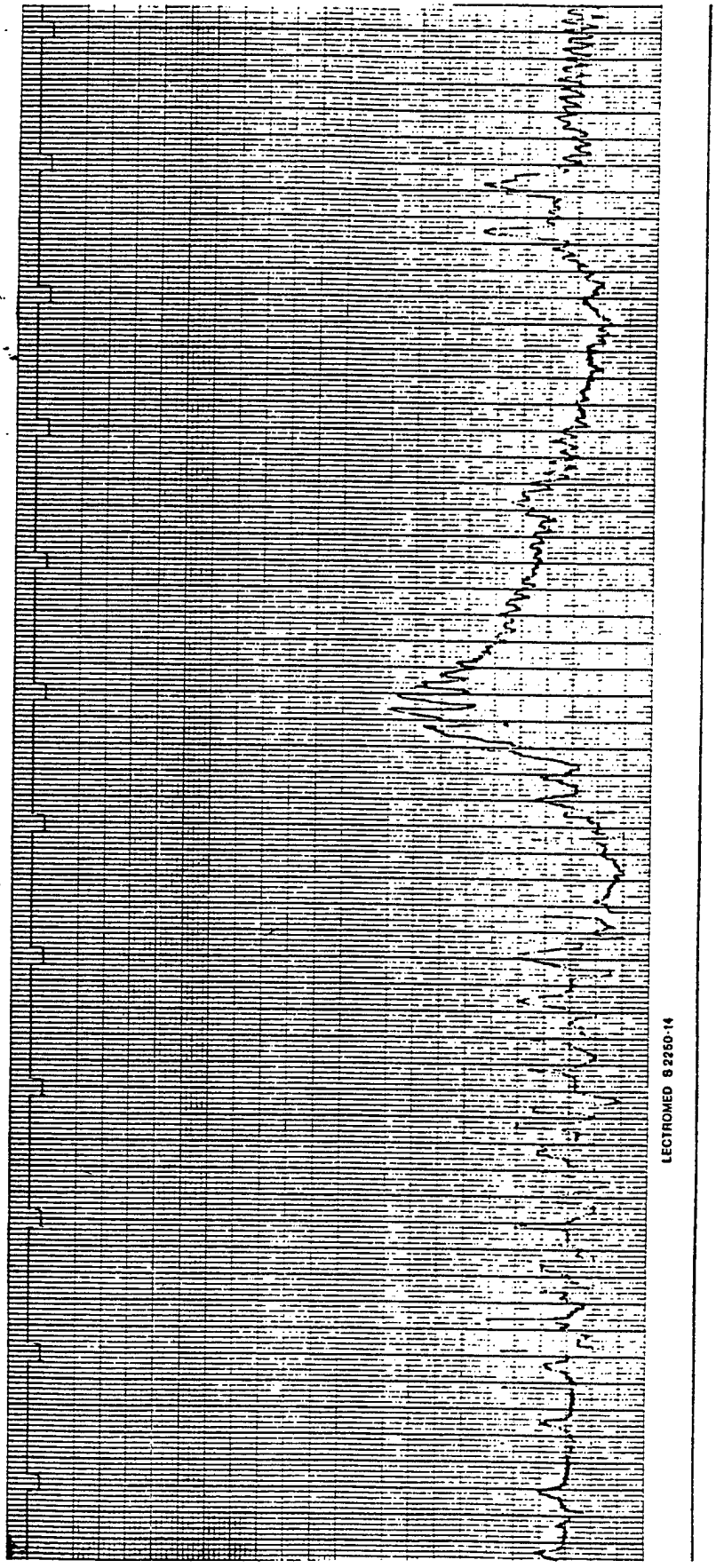


FIGURE 7

Response of Dog 1149 to administration of adrenaline during exposure to 0.4% v/v CF_3I (response classified as positive)

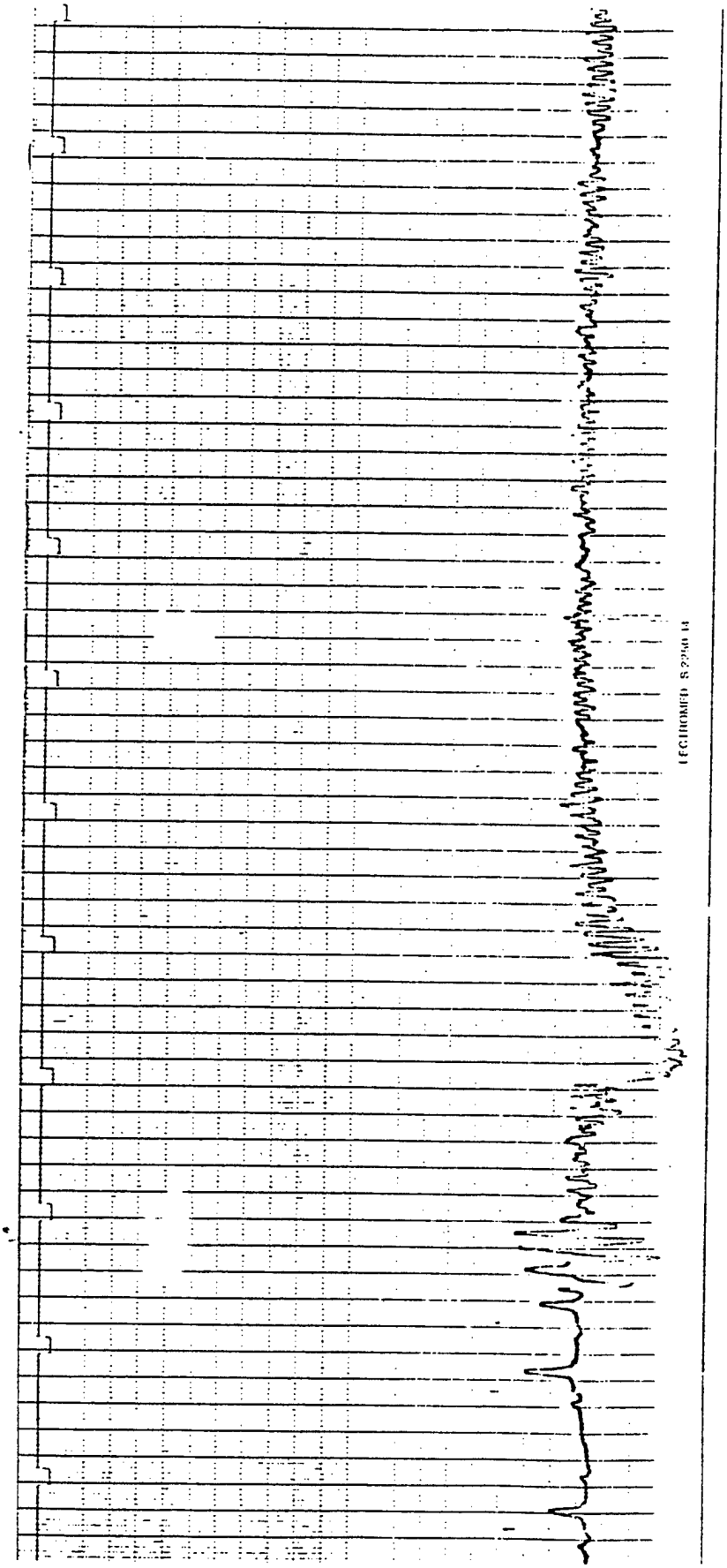


FIGURE 8

Response of Dog 1153 to administration of adrenaline during exposure to 0.1% v/v C_3F_7I (response classified as positive)

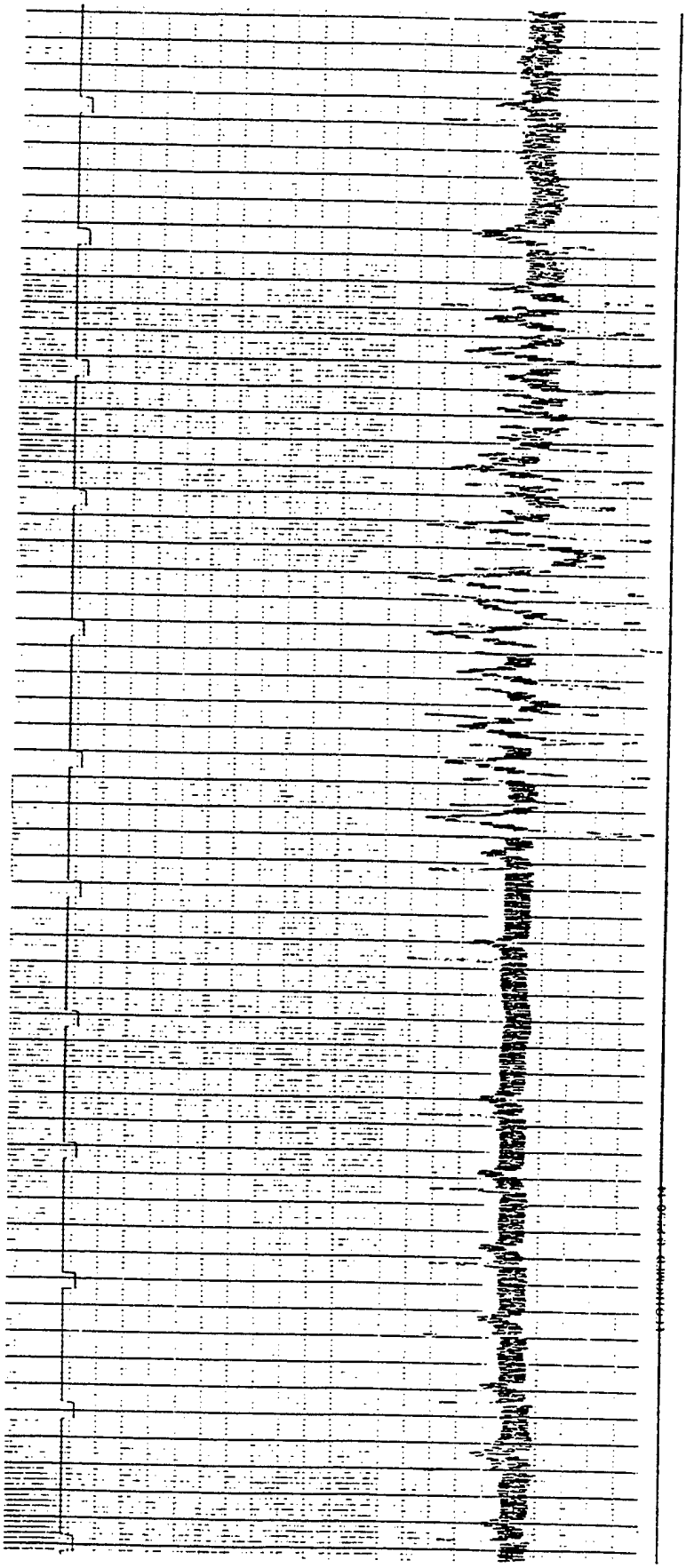


FIGURE 9

Response of Dog 1157 to administration of adrenaline during exposure to 0.4% v/v C_3F_7I (response classified as positive)

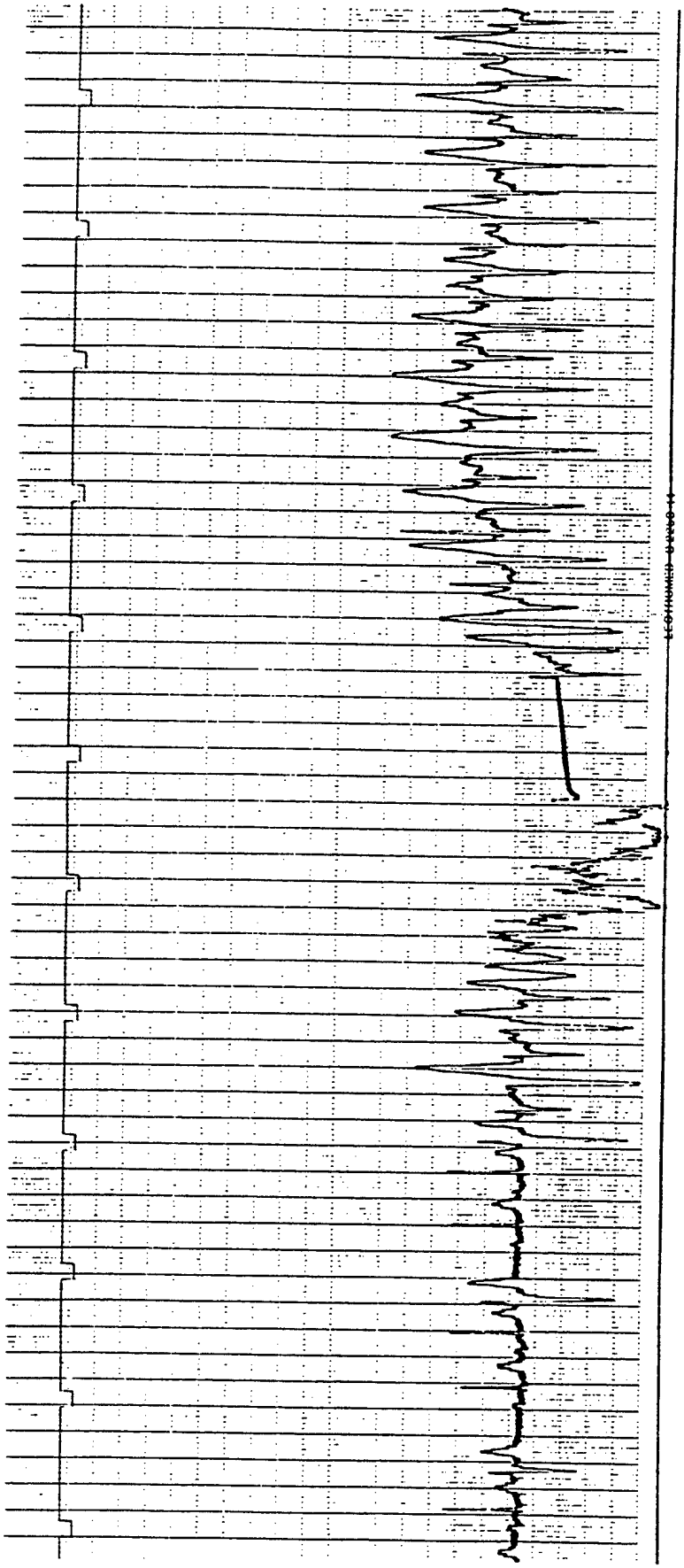


TABLE 1

Summary of cardiac responses to adrenaline administration during CF_3I exposure

Dog number	Adrenaline dose (μg/kg)	Concentration in air			
		0.1%	0.2%	0.4%	1.0%
1147	8	(N)	NP	NP	FVF (P)
1149	8	(N)	(N)	FVF (P)	NP (AP)
1153	8	(N)	(N)	NP	NP
1155	1	(N)	(N)	NP	NP
1157	4	(N)	(N)	NP	NP
1159	1	(N)	(N)	NP	NP
Incidence of positive response		0/6	0/5	1/1	2/2
Cumulative % positive responses		0	0	100	100

FVF Fatal ventricular fibrillation
NP Not performed
(N) Negative response
(P) Positive response
(AP) Assumed positive

TABLE 2

Summary of cardiac responses to adrenaline administration during C_3F_7I exposure

Dog number	Adrenaline dose (μg/kg)	Concentration in air		
		0.1%	0.2%	0.4%
1153	8	MVEA (P)	NP (AP)	NP (AP)
1155	1	(N)	(N)	(N)
1157	4	(N)	(N)	MVEA (P)
1159**	1	NP	NP	NP
Incidence of positive response		1/3	1/3	2/3
Cumulative % positive responses		33	33	67

** Dog 1159 not successfully dosed due to severe struggling
MVEA Multifocal ventricular ectopic activity
NP Not performed
(N) Negative response
(P) Positive response
(AP) Assumed positive

APPENDIX 1

Results of Stage 1 of the study - challenges with adrenaline alone

Date	Dog number	Adrenaline dose (μg/kg)	Summary of cardiac response* following adrenaline challenges		
			Number of ectopic beats		Comment
			Following first challenge	Following second challenge	
1994					
13 June	1145	4	-	-	Failed injection
	1147	4	0	0	
	1149	4	0	0	
	1151	4	0	0	
	1153	4	0	0	
	1155	4	50	-	No second challenge
	1157	4	13(6)	-	No second challenge
	1159	4	15	-	Failed second injection
	1161	4	0	0	Numerous abnormal beats
14 June	1147	8	2	4	
	1149	8	2	1	
	1151	8	0	0	
	1153	8	1	3	
	1155	1	2	(11)	
	1157	2	0	0	
	1161	2	0	0	Numerous abnormal beats
15 June	1145	4	0	0	
	1159	2	11	-	No second challenge
16 June	1145	8	-	-	Failed injection
	1151	12	0	3	
	1157	4	0	0	
	1157	4	1	11	
	1159	1	1	2	

* 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, increase in height of T wave) is not mentioned in the "summary of cardiac response"

( ) Values in parentheses indicate abnormal beats of uncertain origin typically escape beats

APPENDIX 2

Results of Stage 2 of the study - cardiac responses to administration of adrenaline during exposure to CFC 11

Dog number	Gas concentration (%)	Adrenaline dose (μg/kg)	Summary of cardiac response*		
			Number of ectopic beats		Comments (clinical signs)
			First challenge	Second challenge	
1151	2.0	12	0	MVEA (3S) FVF	Positive

* The cardiac response typically consisted of an initial rate increase and increase in T wave amplitude followed by a rate decrease and a number of unifocal ventricular ectopic beats

MVEA Multifocal ventricular ectopic activity

FVF Fatal ventricular fibrillation

(S) Duration in seconds of event observed

APPENDIX 3

Cardiac responses to adrenaline administration during exposure to CF_3I

Dog number	Gas concentration (%)	Adrenaline dose (μg/kg)	Summary of cardiac response*		
			Number of ectopic beats		Comments (clinical signs)
			First challenge	Second challenge	
1147	0	8	2 (+8)	9	NAD
	0.1	8	12	14	NAD
	1.0	8	3	MVEA (5S), FVF	Positive
1149	0	8	6 (+1)	7	NAD
	0.1	8	0	14	NAD
	0.2	8	3	12	NAD
	0.4	8	8	MVEA (0.5S), FVF	Positive
1153	0	8	0	0	NAD
	0.1	8	0	0	NAD
	0.2	8	0	0	NAD
1155	0	1	10	22 (+13)	NAD
	0.1	1	0 (+1)	22 (+6)	NAD
	0.2	1	12	22	NAD
1157	0	4	3 (+1)	3 (+1)	NAD
	0.1	4	1	0	NAD
	0.2	4	7	24	NAD
1159	0	1	1	0	Struggling
	0.1	1	0	0	Struggling
	0.2	1	NP	NP	Struggling (severe)

* The cardiac response typically consisted of an initial rate increase and increase in T wave amplitude followed by a rate decrease and a number of unifocal ventricular ectopic beats

NAD Nothing abnormal detected

( ) Values in parentheses indicate abnormal beats of uncertain origin typically escape beats

NP Not performed

MVEA Multifocal ventricular ectopic activity

FVF Fatal ventricular fibrillation

(S) Duration in seconds of event observed

APPENDIX 4

Cardiac responses to adrenaline administration during exposure to C_3F_7I

Dog number	Gas concentration (%)	Adrenaline dose (μg/kg)	Summary of cardiac response*		
			Number of ectopic beats		Comments (clinical signs)
			First challenge	Second challenge	
1153	0.1	8	1	MVEA (5S), 3	Positive
1155	0.1	1	0	0	(Rapid breathing)
	0.2	1	0	0	NAD
	0.4	1	0	0 (+9)	NAD
1157	0.1	4	2	6	NAD
	0.2	4	24	50	NAD
	0.4	4	10	3, MVEA (19S) VT (9S)	Positive
1159	0.1	1	NP	NP	Struggling (severe)
	0.2	1	NP	NP	Struggling (severe)
	0.4	1	NP	NP	Struggling (severe)

* The cardiac response typically consisted of an initial rate increase and increase in T wave amplitude followed by a rate decrease and a number of unifocal ventricular ectopic beats

NAD Nothing abnormal detected

( ) Values in parentheses indicate abnormal beats of uncertain origin typically escape beats

NP Not performed

MVEA Multifocal ventricular ectopic activity

FVF Fatal ventricular fibrillation

VT Ventricular tachycardia

(S) Duration in seconds of event observed