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Document Processing Center (TS-790)  
Attention: 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U. S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

Re: TSCA 8(e) Submission 8EHQ-1294-13288

Dear Coordinator:

DuPont had submitted preliminary information under TSCA 9(e) on a cardiac sensitization study that was conducted with HFC-236ea. As a follow up to that submission, you requested a copy of the final report. Please find enclosed that report.

Sincerely,

William J. Brock, Ph.D.  
Staff Toxicologist

WJB/js  
Atch



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**FINAL REPORT**

**STUDY TITLE**

**ACUTE CARDIAC SENSITIZATION STUDY  
OF HFC-236ea IN DOGS BY INHALATION**

**STUDY DIRECTOR**

Dennis J. Naas, B.S.

**STUDY INITIATED ON**

October 11, 1994

**STUDY COMPLETED ON**

May 23, 1996

**PERFORMING LABORATORY**

WIL Research Laboratories, Inc.  
1407 George Road  
Ashland, OH 44805-9281

**LABORATORY STUDY NUMBER**

WIL-189014

**MEDICAL RESEARCH PROJECT NO. 9954**

**SPONSOR**

E. I. du Pont de Nemours & Co.  
Haskell Laboratories  
Elkton Road  
Newark, DE 19714

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Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

COMPLIANCE STATEMENT

This study, designated WIL-189014, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations (40 CFR, Part 792), September 18, 1989, and the Standard Operating Procedures of WIL Research Laboratories, Inc. The study was conducted in accordance with the protocol as approved by the Sponsor.

Study Director:



Dennis J. Neas, B.S.

Assistant Director of Toxicology

5/23/96

Date

Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

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Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

I. SUMMARY:

The cardiac sensitization potential of HFC-236ea by inhalation was evaluated in this study in beagle dogs. Each animal was exposed individually using a nose-only exposure apparatus. There was one group of eight males. The first epinephrine dose (appropriate dose level selected during prestudy) was administered following two minutes of exposure to air (Minute 2). Five minutes later (Minute 7), exposure to the test gas was initiated. The challenge epinephrine dose was administered following five minutes of exposure to HFC-236ea (Minute 12). Exposure to the test gas was terminated after an additional (maximum) five minutes (Minute 17). Electrocardiographic data were recorded continuously throughout the aforementioned procedures (Minutes 0-17). Concentrations tested were 1.25, 2.5, 3.5, 5.0 and 10.0% (12,500, 25,000, 35,000, 50,000 and 100,000 ppm, respectively). At least five dogs were exposed at each concentration. Detailed physical examinations were performed and body weights were recorded for all dogs on the day prior to the first day of exposures and on the completion of all exposures. ECG recordings taken after the challenge epinephrine dose (during test gas exposure) were compared to the ECG recordings taken after the first epinephrine dose (during air exposure) and inspected for responses indicative of cardiac sensitization. Positive responses were considered to consist primarily of multiple PVCs, fibrillation or other serious arrhythmic events. Complete necropsies were performed on animals that died. At study termination, surviving animals were returned to the stock colony.

Two dogs died during exposure to 10.0% (100,000 ppm) HFC-236ea and another dog died during exposure to 5.0% (50,000 ppm) HFC-236ea. There were no gross necropsy findings in the dogs that died which could be ascribed to the test article.

All other animals survived to study termination. No adverse effects on body weights were apparent.

All animals responded positively for cardiac sensitization at 10.0%, the first level tested, and a decreasing incidence of positive responders was observed at the subsequently tested lower concentrations. Based on protocol criteria, there were 0/5,

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0/5, 1/5, 4/6 and 5/5 positive responders at the 1.25, 2.5, 3.5, 5 and 10% concentrations, respectively.

Based on these results, the no observable adverse effect level (NOAEL) was 2.5% (25,000 ppm) with a lowest observable adverse effect level (LOAEL) of 3.5% (35,000 ppm); an EC<sub>50</sub> of approximately 4.4% (44,000 ppm) was calculated by probit analysis.

Acute Cardiac Sensitization Study of  
HFC-236ea in Dogs by Inhalation

II. OBJECTIVE

The objective of this study was to evaluate the cardiac sensitization potential of HFC-236ea during a single acute exposure by inhalation. Cardiac sensitization in this context refers to the potential for the test article to increase the sensitivity of the heart to the pharmacological effects of epinephrine resulting in potentially life-threatening or fatal cardiac arrhythmias. Because of the volatility and potential application of the test article, the route of administration was by inhalation exposure as this is the most likely route of exposure for the general population. The nose-only method of exposure was selected to conserve test article and facilitate epinephrine administration. The beagle dog is recognized as an appropriate model for cardiac function studies.

III. EXPOSURE SCHEDULE AND TREATMENT REGIMEN

<u>Date</u>	<u>Animal Number</u>	<u>Exposure Concentration (ppm)</u>	<u>Epinephrine Dose (<math>\mu</math>g/kg)</u>
12/03/94	2502	100,000	7.5
12/03/94	2264	100,000	12.0
12/03/94	2262	100,000	12.0
12/03/94	2503	100,000	12.0
12/03/94	2475	100,000	12.0
12/10/94	2264	25,000	12.0
12/10/94	2262	25,000	12.0
12/10/94	2503	25,000	12.0
12/10/94	2307	50,000	12.0
12/12/94	2308	25,000	12.0
12/12/94	2263	25,000	12.0
12/12/94	2264	25,000	9.0
12/19/94	2263	12,500	12.0
12/19/94	2262	12,500	12.0
12/19/94	2503	12,500	12.0
12/19/94	2308	12,500	12.0
12/19/94	2264	12,500	9.0
12/21/94	2263	50,000	12.0
12/21/94	2262	50,000	12.0
12/21/94	2503	50,000	12.0
12/21/94	2308	50,000	12.0
12/21/94	2264	50,000	9.0
01/06/95	2308	35,000	12.0
01/06/95	2503	35,000	12.0
01/06/95	2263	35,000	12.0
01/06/95	2264	35,000	9.0
01/06/95	2262	35,000	12.0

IV. EXPERIMENTAL DESIGN - MATERIALS AND METHODSA. INTRODUCTION

The experimental start date, initiating with the first exposure, was December 3, 1994; surviving animals were returned to stock on February 3, 1995.

B. TEST AND CONTROL MATERIALS1. TEST MATERIAL IDENTIFICATION

HFC-236ea was received on October 7, 1994, from E. I. du Pont de Nemours & Co., Wilmington, DE, as follows:

<u>Label Identification</u>	<u>Physical Description</u>	<u>Number of Containers Received</u>
Hexafluoropropane HFC-236ea CAS No. 431-63-0 Lot: FT-1411 Non TSCA For R&D use only	Colorless gas	1 cylinder Gross Weight: 49500.0 g

Stability and purity data for the test substance were the responsibility of the sponsor. The test article was maintained in a sealed container (low pressure cylinder) under ambient conditions. An approximate 2-gram retention sample was collected on October 29, 1994, and stored at WIL Research Laboratories, Inc.

2. EPINEPHRINE SOLUTION IDENTIFICATION

The materials used to prepare the epinephrine challenge doses were received as follows:

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<u>Label Identification</u>	<u>Physical Description</u>	<u>Quantity Received</u>	<u>Supplier</u>	<u>Dates</u>
(-)-EPINEPHRINE ([-]-Adrenalin) 10 g E-4250 Lot 24H0334	White power	1 Bottle Gross Weight: 58.50 g	Sigma Chemical Company, St. Louis, MO	Receipt: 10/3/94 Expiration: 10/3/99
0.9% Sodium Chloride Inj. USP <sup>a</sup>	Clear colorless liquid	24 bags; 1000 ml each	Baxter Healthcare Corporation, Deerfield, IL	Receipt: 9/28/94 Expiration: 2/96
0.9% Sodium Chloride Inj. USP <sup>a</sup>	Clear colorless liquid	36 bags; 1000 ml each	Baxter Healthcare Corporation, Deerfield, IL	Receipt: 10/4/94 Expiration: 2/96

<sup>a</sup> = Lot number and NDC number inadvertently not recorded

### 3. EPINEPHRINE CHALLENGE DOSE PREPARATION

All glassware and utensils were autoclaved prior to use.

All vials were flushed with nitrogen. The 0.9% sterile saline was sparged with argon to eliminate oxygen. The sparged 0.9% sterile saline was transferred to a secondary container and filtered using a sterile Millex-GV 0.22  $\mu\text{m}$  filter unit (millipore). This solution was kept in an ice bath during use.

The 120  $\mu\text{g}/\text{ml}$  epinephrine solution was prepared in the following manner. A small amount of 0.9% sterile saline was added to a precalibrated injection vial. The appropriate amount (-)epinephrine was weighed out and quantitatively transferred to the vial. The weighing vessel was rinsed with 0.9% sterile saline which was added to the injection vial. An adequate amount of 0.9% sterile saline was added to bring the preparation to the proper concentration and volume. A stir bar was added, and the vial was filled with nitrogen and sealed. The solution was kept in an ice bath during dilution procedures.

The 75 and 90  $\mu\text{g}/\text{ml}$  epinephrine solutions were prepared as dilutions of the 120  $\mu\text{g}/\text{ml}$  solution. The appropriate amount of the 120  $\mu\text{g}/\text{ml}$  solution

was transferred to a precalibrated injection vial fitted with a septum cap. A sufficient amount of 0.9% sterile saline was added to bring the solution to the proper concentration and volume. A stir bar was added.

The epinephrine solutions were prepared fresh each day that exposures were performed. All solutions were maintained on ice for transportation and storage between use and were stirred continuously throughout the dosing procedures.

In addition, a portion of each preparation was transferred to a separate vial (with stir bar and capped under inert gas) and maintained in the pharmacy in case of breakage.

#### 4. TEST ATMOSPHERE MONITORING

During the study, exposure concentrations were measured by gas chromatography with a Flame Ionization Detector (FID). Mass air flow, temperature, relative humidity and oxygen content were monitored continuously and were recorded at least every five minutes. Nominal chamber concentrations were determined. The results of these analyses are presented in Appendix A.

#### 5. EXPOSURE METHODS

Each animal was exposed individually using a nose-only exposure apparatus operated under dynamic conditions to sustain air flows of at least 12 to 15 air changes per hour, ensuring a minimum oxygen content of 19 percent and an evenly distributed exposure atmosphere. The protocol specified a temperature range of 20-24°C and a relative humidity range of 40-60% for the test atmospheres. Actual test atmosphere mean temperature ranged from 22.1°-23.8° C and mean relative humidity ranged from 3%-15%. The actual recorded relative humidity values were unavoidably outside the specified range due to the use of dried compressed dilution air for test atmosphere generation; these deviations did not apparently affect the outcome of the study. Exposure methods and conditions are detailed in Appendix A.

6. DETERMINATION OF EPINEPHRINE DOSE

Prior to the first day of test article exposure, an individual response to intravenous epinephrine alone (up to approximately 10 ectopic beats) was established for each individual dog using a range of epinephrine doses. A dose limit of 12  $\mu\text{g}/\text{kg}$  was used. The epinephrine doses were administered via the cephalic vein. All dogs were dosed with epinephrine at 3, 6, 9 and 12  $\mu\text{g}/\text{kg}$  (with the exception of no. 2261 which was eliminated from study after displaying extreme sensitivity to epinephrine doses of 3 and 6  $\mu\text{g}/\text{kg}$ ). On December 1, 1994, the ECG recordings were reviewed by the study director and epinephrine challenge dose levels were selected for most dogs. Additional epinephrine dosings of selected dogs were performed on December 1, 1994, and epinephrine challenge dose levels were selected for these dogs. A dose level of 12  $\mu\text{g}/\text{kg}$  was selected for all dogs with the following exception. An epinephrine challenge dose level of 7.5  $\mu\text{g}/\text{kg}$  was selected for dog no. 2502. In addition, a 12  $\mu\text{g}/\text{kg}$  epinephrine dose level was found to be inappropriate for dog no. 2264, based on the number of ectopic or escape beats occurring during the air-only phase of the exposure regimen, and was subsequently lowered to 9  $\mu\text{g}/\text{kg}$  (see Section IV.B.7.).

During the dose level selection and at the test article exposures, the epinephrine doses were given intravenously into a cephalic vein using a 1.0 ml syringe at a dose volume of 0.1 ml/kg. The injection rate, controlled through the use of a syringe pump, was approximately 0.1 ml/sec.

7. ORGANIZATION AND TREATMENT REGIMEN

The study consisted of one group of eight dogs. Each dog was comfortably restrained using a canvas sling, fitted into the exposure mask and the appropriate ECG limb electrodes were attached. The exposure duration to air was approximately 7 minutes and the exposure duration for the test gas was approximately 10 minutes. (The length of exposure was selected by the sponsor since it was considered to be of sufficient duration to achieve blood levels of HFC-236ea near saturation). Each dog served as its own control in

the following manner. The ECGs recorded following the first epinephrine dose during air exposure were compared to the ECGs recorded following the challenge epinephrine dose during test gas exposure. The treatment regimen was as follows:

<u>Time (minutes)</u>	<u>Event</u>
0	Start ECG recording
2	First epinephrine dose
7	Test gas initiated
12	Challenge epinephrine dose
17	Termination of exposure/ECG

On exposure days, the selected animals were exposed at the appropriate concentration at different times. At the end of each exposure period, the dogs were returned to their home cages. The exposure schedule was as follows:

<u>Date</u>	<u>Animal Number</u>	<u>Exposure Concentration (ppm)</u>	<u>Epinephrine Dose (<math>\mu\text{g}/\text{kg}</math>)</u>
12/03/94	2502	100,000	7.5
12/03/94	2264	100,000	12.0
12/03/94	2262	100,000	12.0
12/03/94	2503	100,000	12.0
12/03/94	2475	100,000	12.0
12/10/94	2264	25,000	12.0
12/10/94	2262	25,000	12.0
12/10/94	2503	25,000	12.0
12/10/94	2307	50,000	12.0
12/12/94	2308	25,000	12.0
12/12/94	2263	25,000	12.0
12/12/94	2264	25,000	9.0

<u>Date</u>	<u>Animal Number</u>	<u>Exposure Concentration (ppm)</u>	<u>Epinephrine Dose (<math>\mu</math>g/kg)</u>
12/19/94	2263	12,500	12.0
12/19/94	2262	12,500	12.0
12/19/94	2503	12,500	12.0
12/19/94	2308	12,500	12.0
12/19/94	2264	12,500	9.0
12/21/94	2263	50,000	12.0
12/21/94	2262	50,000	12.0
12/21/94	2503	50,000	12.0
12/21/94	2308	50,000	12.0
12/21/94	2264	50,000	9.0
01/06/95	2308	35,000	12.0
01/06/95	2503	35,000	12.0
01/06/95	2263	35,000	12.0
01/06/95	2264	35,000	12.0
01/06/95	2262	35,000	9.0

### C. ANIMAL RECEIPT AND ACCLIMATION

On September 21, 1994, nine male outbred beagle dogs (approximately 9-14 months old) were selected from the WIL Research Laboratories, Inc., stock dog colony for possible use on this study. The animals were received from either Ridglan Farms, Inc., Mt. Horeb, Wisconsin, or Marshall Farms, North Rose, New York, and were examined by a qualified technician at that time. Each animal was identified by an ear tattoo. During the acclimation period, the animals were observed twice daily for mortality and changes in general behavior. Body weights were recorded periodically during the acclimation period, including approximately one week prior to the initiation of the exposures and on the day prior to the first day of exposure. Since the animals were selected from available stock, it was not possible to verify that fecal examinations were performed on each dog prior to initiation of exposure. However, it was most likely that all dogs were checked and treated if any parasites were present. This deviation was not expected to have an impact on the outcome of the study.

**D. ANIMAL HOUSING**

The animals were housed individually in clean, stainless steel cages which were cleaned daily during the acclimation period and throughout the study. The animals were given frequent and regular opportunities for exercise and socialization, to the extent possible, on a 0.5 hour/day, seven days per week basis, in accordance with the Final Rules of the Animal Welfare Act Regulations (9 CFR, Part 3) and WIL SOPs. Exercise/socialization may have been suspended, by necessity, on the days of exposure. Animals were maintained in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals"<sup>1</sup>. The animal facilities at WIL Research Laboratories, Inc. are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

**E. DIET, DRINKING WATER AND MAINTENANCE**

Approximately 400 g of the basal ration, Purina® Certified Canine Diet #5007, was offered for approximately 1-2 hours daily. The diet utilized at WIL Research Laboratories, Inc., is a certified feed with appropriate analyses for potential contaminants performed and provided by the manufacturer. The drinking water, delivered by an automatic watering system, was provided *ab libitum* throughout the study period, except during the exposure and exercising periods. Municipal tap water supplying the facility is sampled for contaminants according to Standard Operating Procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, Inc. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study.

**F. ANIMAL ROOM ENVIRONMENTAL CONDITIONS**

Except during exposure, all animals were housed throughout the acclimation period and during the study in environmentally-controlled rooms. Controls were set to maintain a temperature of 72° ± 4°F (20-24°C) and a relative humidity of approximately 30-70%. Room temperature and relative humidity were recorded daily. The actual temperature ranged from 63°F to 75°F (20-22°C) and humidity ranged from 28% to 94% during the study period. Occasional slight variations in

relative humidity during the study period did not apparently affect the outcome of this study. Light timers were set to provide a 12-hour light/12-hour dark photoperiod.

G. IMMUNIZATIONS PRIOR TO ARRIVAL

All animals were immunized prior to arrival at WIL Research Laboratories, Inc. The immunizations included rabies, distemper, hepatitis, leptospirosis, Parvo and parainfluenza.

H. PRETEST PERIOD

Detailed physical examinations and individual body weights were recorded for all animals prior to initiation of test article exposure. Electrocardiographic data were recorded for each dog at least twice prior to epinephrine dose level selection. For acclimation purposes, each dog was placed in the exposure restraint apparatus for 20-40 minutes per day on three days prior to the first exposure.

I. ASSIGNMENT OF ANIMALS TO STUDY

Eight dogs suitable for testing, as judged by the study director, were assigned to the study based on health, body weight, temperament and pretest electrocardiographic data. The dogs weighed 11.4 kg to 15.6 kg prior to the initiation of exposures (day -1).

J. PARAMETERS EVALUATED

1. CLINICAL OBSERVATIONS AND SURVIVAL

The animals were observed twice daily, once in the morning and once in the afternoon for mortality and moribundity. All animals were observed for clinical signs of toxicity prior to initiation of each exposure, continually throughout the exposure period and following each exposure when they were returned to their cages. Detailed physical examinations were conducted on all animals on the day prior to the first day of exposures and on the day following the completion of all exposures. In addition, clinical observations were performed prior to the other exposures (with the exception of the 25,000 ppm re-exposure of dog no. 2264 on December 12, 1994), and on the day that the surviving dogs were returned to stock. Only the results of the examinations

conducted prior to the first exposure and on the day following the last exposure are reported as these were the only examinations required by protocol.

2. BODY WEIGHTS

Individual body weights were recorded on the day prior to the first day of exposures (December 2, 1994) and on the day following the completion of all exposures (January 7, 1995). Additional body weights were recorded for calculation of epinephrine doses; however, they were not required by protocol and will not be reported.

3. ELECTROCARDIOGRAPHIC DATA

Electrocardiographic data were recorded with a Cambridge model 3038/2 three channel electrocardiograph. The ECGs were charted on Cambridge Camco 800 H heat sensitive recording paper. Recordings were made using only leads I, II and III; therefore, only limb clip electrodes were attached to the animals.

ECG data were recorded continuously for approximately two minutes prior to and including the pre-exposure epinephrine dose, exposure to the test gas and for five minutes following the epinephrine challenge dose (total of approximately 17 minutes). The chart speed was 10 mm/sec and response sensitivity was set at 10 mm/mV.

ECG recordings taken after administration of the challenge epinephrine dose during exposure to the test article were compared to the ECG recordings made after administration of the first epinephrine dose that was given in the absence of test article. Individual electrocardiograms were inspected by the study director for abnormal wave-forms and/or arrhythmias which were indicative of cardiac sensitization.

ECG recordings were also reviewed by D. K. Detweiler, V.M.D. (Appendix C).

4. ANATOMIC PATHOLOGY

A complete gross necropsy was conducted on the animals that died. The necropsy included the examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities including viscera. Gross lesions were collected and placed in 10% neutral buffered formalin.

K. STUDY TERMINATION

At study termination, all animals were returned to the WIL Research Laboratories, Inc. stock dog colony (February 3, 1995) as per protocol.

L. DATA RETENTION

The sponsor will have title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product including raw paper data and specimens will be retained in the Archives at WIL Research Laboratories, Inc., as specified in the study protocol.

All raw data in magnetic form, a retention sample of the test article, and the original final report will be retained at WIL Research Laboratories, Inc. in compliance with regulatory requirements.

V. RESULTSA. CLINICAL OBSERVATIONS AND SURVIVAL

Data: Tables 1, 2, 3, 4

At the first concentration tested, 100,000 ppm, two dogs died following epinephrine challenge. During testing at the 50,000 ppm concentration, one dog similarly died. This animal was observed to be struggling during exposure. All other animals survived to study termination.

No test article-related clinical signs were observed. Soft stool was observed for single dogs during exposure at concentrations of 25,000 and 100,000 ppm. Salivation was noted for one dog immediately following exposure at 25,000 ppm. Lacrimation was observed immediately after exposure at 50,000 ppm for a single dog. No other clinical signs were noted.

B. BODY WEIGHTS

Data: Table 5

No adverse effects on body weights were apparent.

C. ELECTROCARDIOGRAPHIC DATA

Data: Table 6

Positive responses were apparent for 0/5, 0/5, 1/5, 4/6 and 5/5 dogs exposed to HFC-236ea at 12,500, 25,000, 35,000, 50,000 and 100,000 ppm, respectively. Positive responses were the occurrence of ventricular fibrillation, ventricular tachycardia, confluent, multifocal repetitive premature ventricular contractions or other similar potential life threatening cardiac arrhythmies. Using these data, the  $EC_{50}$  value calculated by the method of Litchfield and Wilcoxon was found to be 44,434 ppm.

D. ANATOMIC PATHOLOGY

Data: Table 7

Two and one dogs died during exposure to HFC-236ea at concentrations of 100,000 and 50,000 ppm, respectively. No gross findings related to the test article were apparent. Necropsy revealed reddened mucosa or dark red areas in the intestinal tract for all three animals. Splenic capsular scarring and hemorrhagic

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thymus gland were each noted for two of the animals. A hemorrhagic gallbladder was noted for one animal.

**VI. DISCUSSION AND CONCLUSIONS:**

Two dogs died during exposure to 10.0% (100,000 ppm) HFC-236ea and another dog died during exposure to 5.0% (50,000 ppm) HFC-236ea. No test article related gross lesions were apparent.

All other animals survived to study termination. No adverse effects on body weights were apparent.

The design of this study represents a very rigorous assessment of the cardiac sensitization potential of the test article. The epinephrine doses used on this study were higher than those used traditionally on such experiments<sup>2,3</sup> and represented a greater than ten-fold increase over the amount of adrenaline secreted by the human adrenal gland under stress<sup>3</sup>. As two epinephrine administrations given within ten minutes have been shown to cause an increase in the number of abnormal beats following the second dose (previous research<sup>2</sup> and on this study during pre-test epinephrine dose screenings, e.g., dog no. 2263), only a marked increase in the response to the challenge epinephrine dose (during gas exposure) was considered to represent a positive sensitization response.

At the highest concentrations tested, 10% (100,000 ppm), clear positive responses were exhibited for all animals; in fact, two deaths occurred. At the 5% (50,000 ppm) level, a broad range of responses occurred, from death due to severe arrhythmic fibrillation to negative responses for two animals.

At 3.5% (35,000 ppm) only a single positive sensitization response was observed (dog no. 2503). Dog no. 2263, had a weak or equivocal response (few isolated PVCs) to the epinephrine challenge which was not life threatening; thus, this was not considered a positive response.

At 2.5% (25,000 ppm), the results from dog no. 2264 using an epinephrine dose of 12  $\mu$ g/kg were discounted due to the high number of abnormal beats seen during the control epinephrine dosing prior to gas exposure. Even using this inappropriate epinephrine dose, the only response seen for this dog during gas exposure was an increased number of escape beats, the same type of which were seen during the control epinephrine response, over an approximate one minute period. While a slight increase in the number of escape beats was observed for dog no. 2263, these were attributed to

this dog's previously demonstrated increased sensitivity to the second administration of epinephrine and did not occur within a period of time short enough to be considered a positive response. Dog no. 2262 had a weak or equivocal response (few isolated PVCs) to the epinephrine challenge which was not life threatening; thus, this was not considered a positive response.

All responses at the 1.25% (12,500 ppm) level were clearly negative.

The consulting cardiologist who reviewed the ECGs from this study arrived at a somewhat different conclusion (Appendix C). He concluded, based on the results from two dogs (nos. 2503 and 2264), that the LOAEL (lowest observable adverse effect level) was 2.5%.

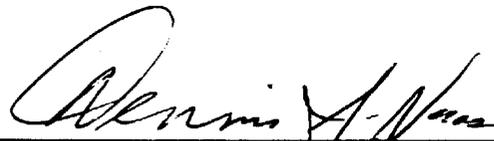
During the epinephrine challenge at 2.5% (25,000 ppm), dog no. 2503 exhibited a short burst of two isolated (nonconfluent) premature ventricular contractions approximately 40 seconds after epinephrine administration. This occurred well outside of the time frame in which such responses might normally be considered positive, was not a life-threatening event and would not be interpreted as a positive response by most investigators familiar with this study design. Further, this animal produced a similar response during the air control exposure prior to testing at 1.25%, indicating that this type of response can occur following epinephrine administration alone and thus is not indicative of sensitization.

Dog no. 2264, during epinephrine challenge at 2.5% (25,000 ppm), produced only escape beats. As would be expected, more escape beats were produced at an epinephrine dose of 12  $\mu\text{g}/\text{kg}$  rather than at 9  $\mu\text{g}/\text{kg}$ . The occurrence of escape beats, even multifocal escape beats, is not life-threatening. There was no evidence of multifocal ventricular extrasystoles or of ventricular tachycardia consistent with the score of five assigned to this animal's response by the consulting cardiologist. Additionally, this animal was negative, even by the consulting cardiologist's criteria, at the 5.0% (50,000 ppm) exposure level. This lack of increased dose responsiveness further suggests that the conclusions reached by the consulting cardiologist at the 2.5% (25,000 ppm) exposure level were not supportable upon detailed examination of all available data.

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Therefore, based on a thorough review of the electrocardiographic data and in accordance with standard methods of interpretation for cardiac sensitization phenomena<sup>2</sup>, there were 5/5, 4/6, 1/5, 0/5 and 0/5 positive cardiac sensitization responses to test atmospheres containing 10%, 5%, 3.5%, 2.5% and 1.25% HFC-236ea, respectively. Based on these results, the lowest observed adverse effect level (LOAEL) was 3.5%, the no observed adverse effect level (NOAEL) was 2.5% and an EC<sub>30</sub> of approximately 4.4% was calculated by probit analysis.



Dennis J. Naas, B.S.  
Study Director

5/23/86

Date

VII. STUDY PERSONNEL AND REPORT SUBMISSION:

Key Personnel:

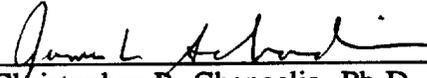
Kerin Clevidence, B.S.	Group Supervisor of Gross Pathology and Developmental Toxicology Laboratory
Sally A. Keets, A.S.	Manager of Vivarium
Gary R. Kiplinger, B.S.	Manager of General Toxicology
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Report Prepared By:

  
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Group Supervisor of Technical Report Writing

5-23-96  
Date

Reviewed By:

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Senior Toxicologist

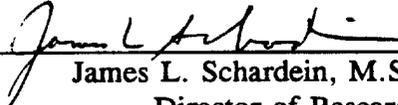
5/23/96  
Date

  
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Robert R. Dahlgren, D.V.M., Ph.D.  
Diplomate A.C.V.P.  
Director of Pathology and Veterinary Medicine

5-22-96  
Date

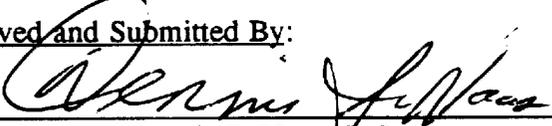
  
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Associate Toxicologist

5/23/96  
Date

  
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James L. Schardein, M.S., A.T.S.  
Director of Research

5/23/96  
Date

Approved and Submitted By:

  
\_\_\_\_\_  
Dennis J. Naas, B.S.  
Assistant Director of Toxicology

5/23/96  
Date

VIII. QUALITY ASSURANCE UNIT STATEMENT:

<u>Date(s) of Inspection(s)</u>	<u>Phase Inspected</u>	<u>Date(s) Findings Reported to Study Director</u>	<u>Date(s) Findings Reported to Management</u>
12/6/94	Animal Care and Equipment	12/6/94	1/25/95
4/17, 18, 25, 30/96	Study Records I-1	4/30/96	5/96
4/17, 18, 19, 25/96	Study Records I-2	4/26/96	5/96
4/22, 25/96	Study Records I-3, I-4	4/25/96	5/96
4/19, 22, 25/96	Study Records I-5, I-8	4/25/96	5/96
4/24, 25/96	Study Records I-6, I-7	4/25/96	5/96
4/24/96	Study Records I-9-I-15	4/24/96	5/96
4/23, 24, 5/2/96	Study Records A-1	5/2/96	6/96
4/23, 24, 25/96	Study Records A-2	4/26/96	5/96
5/1, 2, 6/96	Draft Report (without Appendix A)	5/8/96	6/96
5/2, 6, 8/96	Draft Report (Appendix A)	5/8/96	6/96

This study was conducted and inspected in accordance with the Good Laboratory Practice Regulations, the Standard Operating Procedures of WIL Research Laboratories, Inc., and the sponsor's protocol and protocol amendment(s) with the following exception. Data located in Appendix C were not audited by WIL QA or subject to WIL SOPs. Quality Assurance findings, derived from the inspection during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. A status report is submitted to management monthly.

The raw data, the retention sample(s), if applicable, and the final report will be stored in the Archives at WIL Research Laboratories, Inc., or another location specified by the sponsor.

Deborah L. Little  
 Deborah L. Little  
 Manager of Quality Assurance

5/23/96  
 Date

IX. REFERENCES

1. NIH (1985) Guide for the Care and Use of Laboratory Animals, United States Departments of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23, 83 pages.
2. Reinhardt, C.F., Azar, A., Marfield, M.E., Smith, P.E. and Mullen, L.S. (1971) Cardiac Arrhythmias and Aerosol "Sniffing". Arch Environ Health. 22: 265-279.
3. Mullin, L.S., Reinhardt, C.F. and Hemingway, R.E. (1979) Cardiac arrhythmias and blood level associated with inhalation of Halon 1301. American Industrial Hygiene Association Journal. 40: 653-658.

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Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

Tables 1-7

PROJECT NO.:WIL-189014  
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TABLE 1  
CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
IN-LIFE ANIMAL NUMBERS AND DISPOSITION

PAGE 1

ANIMAL IN-LIFE NUMBER	SEX	DISPOSITION	DATE
2264	M	RETURNED TO STOCK	03-FEB-95
2307	M	FOUND DEAD	10-DEC-94 (50,000 PPM)
2262	M	RETURNED TO STOCK	03-FEB-95
2263	M	RETURNED TO STOCK	03-FEB-95
2503	M	RETURNED TO STOCK	03-FEB-95
2475	M	FOUND DEAD	03-DEC-94 (100,000 PPM)
2308	M	RETURNED TO STOCK	03-FEB-95
2502	M	FOUND DEAD	03-DEC-94 (100,000 PPM)

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TABLE 2 (DETAILED PHYSICAL EXAMINATIONS)  
 CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL CLINICAL OBSERVATIONS  
 TABLE RANGE: 12- 2-94 AND 1- 7-95

ANIMAL	SEX	EXPOSURE LEVEL	CATEGORY	DATE	GRADE	OBSERVATIONS
2264	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
		POST-EXPOSURE	NORMAL	01-07-95	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2307	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2262	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
		POST-EXPOSURE	NORMAL	01-07-95	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2263	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
		POST-EXPOSURE	NORMAL	01-07-95	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2503	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
		POST-EXPOSURE	NORMAL	01-07-95	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2475	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2308	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
		POST-EXPOSURE	NORMAL	01-07-95	P	NO SIGNIFICANT CLINICAL OBSERVATIONS
2502	M	PRE-EXPOSURE	NORMAL	12-02-94	P	NO SIGNIFICANT CLINICAL OBSERVATIONS

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

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CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION

TABLE 3 (DURING EXPOSURE)

INDIVIDUAL CLINICAL OBSERVATIONS

TABLE RANGE: 12- 3-94 TO 1- 6-95

ANIMAL	SEX	EXPOSURE LEVEL	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
2503	M	100,000 PPM	EXCRETA	12-03-94	10:29	P	SOFT STOOL
2307	M	50,000 PPM	BEHAVIOR/CNS	12-10-94	13:47	P	STRUGGLING DURING EXPOSURE
2263	M	25,000 PPM	EXCRETA	12-12-94	14:24	P	SOFT STOOL

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

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TABLE 4 (POST-EXPOSURE)  
 CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL CLINICAL OBSERVATIONS  
 TABLE RANGE: 12- 3-94 TO 1- 6-95

ANIMAL	SEX	EXPOSURE LEVEL	CATEGORY	DATE	TIME	GRADE	OBSERVATIONS
2262	M	25,000 PPM	ORAL/DENTAL	12-10-94	11:41	P	SALIVATION
2264	M	50,000 PPM	EYES/EARS/NOSE	12-21-94	11:43	P	LACRIMATION RIGHT EYE
				12-21-94	11:43	P	LACRIMATION LEFT EYE

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

TABLE 5  
 CARDIAC SENSITIZATION STUDY OF MFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL BODY WEIGHTS AND BODY WEIGHT GAINS (GRAMS)

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ANIMAL	SEX	PRE-EXPOSURE WEIGHT	POST-EXPOSURE WEIGHT	BODY WEIGHT GAIN
2264	M	11350.	11350.	0.
2307	M	13900.	NA	NA
2262	M	14000.	13950.	-50.
2263	M	14800.	16050.	1250.
2503	M	13000.	13400.	400.
2475	M	12600.	NA	NA
2308	M	15600.	12900.	2700.
2502	M	12050.	NA	NA

NA = NOT APPLICABLE; ANIMAL DIED DURING EXPOSURE

TABLE 6  
 CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL CARDIAC SENSITIZATION RESPONSES

EXPOSURE LEVEL	ANIMAL	SEX	RESPONSE	NO. POSITIVE/NO. EXPOSED
100,000 PPM	2262	M	+	5/5
	2264	M	+	
	2475*	M	+	
	2502*	M	+	
	2503	M	+	
50,000 PPM	2262	M	+	4/6
	2263	M	+	
	2264	M	-	
	2307*	M	+	
	2308	M	-	
	2503	M	+	
35,000 PPM	2262	M	-	1/5
	2263	M	-	
	2264	M	-	
	2308	M	-	
	2503	M	+	
25,000 PPM	2262	M	-	0/5
	2263	M	-	
	2264	M	NA	
	2308	M	-	
	2503	M	-	
	2264A	M	-	
12,500 PPM	2262	M	-	0/5
	2263	M	-	
	2264A	M	-	
	2308	M	-	
	2503	M	-	

+ = RESPONSE INDICATIVE OF CARDIAC SENSITIZATION  
 - = NO RESPONSE INDICATIVE OF CARDIAC SENSITIZATION  
 \* = ANIMAL DIED DURING EXPOSURE  
 NA = NOT APPLICABLE DUE TO INAPPROPRIATE EPINEPHRINE DOSE OF 12 UG/KG  
 A = EPINEPHRINE DOSE OF 9 UG/KG

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TABLE 7  
CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
INDIVIDUAL GROSS DESCRIPTION OF ORGANS

PAGE 1

ANIMAL NO. 2307    EXPOSURE LEVEL: 50,000 PPM    MALE    FOUND DEAD    DATE OF DEATH: 12/10/94    GRADE

-----

INTESTINE    GROSS: REDDENED MUCOSA    P  
DUODENUM; JEJUNUM; ILEUM; CECUM; COLON

SPLEEN    GROSS: CAPSULAR SCARRING    P  
STOMACH    GROSS: REDDENED MUCOSA

NO SIGNIFICANT    PYLORIC REGION    P  
CHANGES OBSERVED

GROSS: ADRENAL GLANDS    BRAIN    EPIDIDYMIDES    ESOPHAGUS  
EYES    GALLBLADDER    HEART    KIDNEYS  
LIVER    LYMPH NODE, ME.    LUNGS    MAMMARY GLAND  
PANCREAS    PITUITARY    PROSTATE    SALIVARY GLANDS  
SKIN    TESTES    THYMUS GLAND    THYROID GLANDS  
TRACHEA    URINARY BLADDER

GROSS GRADE CODE: 1-SLIGHT, 2-MODERATE, 3-MARKED, P-PRESENT

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TABLE 7  
 CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL GROSS DESCRIPTION OF ORGANS

PAGE 2

ANIMAL NO.	2475	EXPOSURE LEVEL: 100,000 PPM	MALE	FOUND DEAD	DATE OF DEATH: 12/03/94	GRADE
INTESTINE						P
GROSS: REDDENED MUCOSA						
DUODENUM AND JEJUNUM						
GROSS: CAPSULAR SCARRING						P
GROSS: HEMORRHAGIC						P
NO SIGNIFICANT						
CHANGES OBSERVED						
GROSS: ADRENAL GLANDS						
EYES						
LIVER						
PANCREAS						
SKIN						
TRACHEA						
BRAIN						
GALLBLADDER						
LYMPH NODE, ME.						
PITUITARY						
STOMACH						
URINARY BLADDER						
ESOPHAGUS						
KIDNEYS						
MAMMARY GLAND						
SALIVARY GLANDS						
THYROID GLANDS						
EPIDIDYIMIDES						
HEART						
LUNGS						
PROSTATE						
TESTES						

GROSS GRADE CODE: 1-SLIGHT, 2-MODERATE, 3-MARKED, P-PRESENT

PROJECT NO.: WIL-189014  
 SPONSOR: E. I. DUPONT

TABLE 7  
 CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 INDIVIDUAL GROSS DESCRIPTION OF ORGANS

PAGE 3

ANIMAL NO.	EXPOSURE LEVEL: 100,000 PPM	MALE	FOUND DEAD	DATE OF DEATH: 12/03/94	GRADE
	INTESTINE		GROSS: REDDENED ILEO-CECAL JUNCTION		P
	INTESTINE		GROSS: DARK RED AREAS) MULTIPLE, PINPOINT, DUODENUM; FEW, 5 TO 10 MM IN DIAMETER, JEJUNUM		P
	GALLBLADDER		GROSS: HEMORRHAGIC		P
	THYMUS GLAND		GROSS: HEMORRHAGIC		P
	NO SIGNIFICANT CHANGES OBSERVED		GROSS: ADRENAL GLANDS EYES LYMPH NODE, ME. PITUITARY SPLEEN TRACHEA	BRAIN HEART LUNGS PROSTATE STOMACH URINARY BLADDER	
				EPIDIDYIMIDES KIDNEYS MAMMARY GLAND SALIVARY GLANDS TESTES	ESOPHAGUS LIVER PANCREAS SKIN THYROID GLANDS

GROSS GRADE CODE: 1-SLIGHT, 2-MODERATE, 3-MARKED, P-PRESENT

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Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

**APPENDIX A**

Test Atmosphere Generation and Validation  
and Environmental Conditions During Exposure

Acute Cardiac Sensitization Study of HFC-236ea in Dogs by Inhalation

**APPENDIX A**

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- I. Analytical Methods
  - A. Summary
  - B. Instrumentation and Methods
  - C. Gas Chromatograph Calibration
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    - 2. Results
- II. Exposure Methods
  - A. Inhalation Exposure System Description
  - B. Method of Test Atmosphere Generation
- III. Exposure Period Conditions
  - A. General Conditions of Exposure
    - 1. Exposure Duration
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  - B. Inhalation Test Atmosphere Environmental Data
    - 1. Methods
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    - 1. Analyzed Concentrations
      - a. Methods
      - b. Results
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Acute Cardiac Sensitization Study of HFC-236ea in Dogs by Inhalation

**APPENDIX A**

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Figure 1 - Atmosphere Exposure/Generation System

**Index of Tables**

1. Gas Chromatograph Calibration Data
2. Daily Environmental Conditions Means
3. Test Atmosphere Concentrations (ppm)
4. Nominal Concentrations (ppm)

I. ANALYTICAL METHODS

A. Summary

The test material, HFC-236ea, was analyzed by gas chromatography with a Flame Ionization Detector (FID). The test atmospheres were generated into a 100L Plexiglas inhalation box that had been modified for canine nose-only exposure.

B. Instrumentation and Methods

Gas Chromatography

Instrument:	Hewlett Packard 5890A Series II with a FID and a 3396A or 3393 Integrator
Column:	Nine meter x 1/8 inch O.D. stainless steel packed with 3% SP-1500 on Carbopack B, 80/100 mesh
Carrier:	Helium at approximately 12 ml/min (approximately 35 psig)
Column Temperature:	175°C, Isothermal
Detector:	Flame ionization at 250°C
Injection Port Temperature:	250°C
Injection Volume:	0.25 ml (metered gas sampling valve)
Retention Time:	Approximately 2.1 minutes
Sampling Syringe:	20 cc B-D plastic disposable

C. Gas Chromatograph Calibration

1. Methods

Standards of HFC-236ea in air were prepared using one of two techniques. The first technique involved drawing a known volume of pure gaseous test material out of a Tedlar bag into a plastic disposable syringe of appropriate size. The syringe needle was removed from the bag and the plunger further withdrawn to pull in an additional volume of room air. The total volume held in the syringe is then the vessel volume and the concentration can be calculated. The second technique involved injecting a known volume of HFC-236ea into a vessel of known volume. Vessel volumes were determined in the following manner. Threaded 500 ml Erlenmeyer flasks were tared and then filled with

deionized water. The weight and temperature of the water were then recorded. From Lange's Handbook of Chemistry, the density of water nearest the observed temperature was used to calculate the volume of water and the subsequent volume of the flask. The appropriate volumes of HFC-236ea were withdrawn with a syringe from a Tedlar bag filled with gaseous test material and injected into the appropriate flasks. Three to seven standards, at concentrations encompassing the target exposure levels, were prepared on each day of exposure.

From the area response and concentration of the prepared standards, a calibration curve was constructed (each day that gas chromatography was performed) using the linear analysis option in the HP 3396A integrator. The concentration of each atmosphere sample was calculated from these calibration curves.

2. Results

Mean calibration responses were consistent over the test period. Daily calibration data for the study are presented in Table 1.

## II. EXPOSURE METHODS

### A. Inhalation Exposure System Description

All test atmospheres were generated into a 100L Plexiglas box. The box was operated at approximately 20-30 (10 for initial level) liters per minute (LPM) as measured by a Top-Trak Digital flowmeter. Extending to the approximate center of and exiting the Plexiglas box was a 1/2" diameter pipe section. To this pipe, a series of three-way valves and a non-rebreathing valve were attached. The exposure mask was attached to the appropriate fitting on the non-rebreathing valve during exposure. This fitting was plugged during non-exposure periods. Test atmosphere was continually pulled to the mask during exposure. Chamber exhaust was passed through an uncalibrated Miran 1A as a means of continually monitoring chamber concentrations (in addition to analytical determinations performed on the gas chromatograph). A HyCal Engineering probe and transmitter were used to monitor test atmosphere temperature and relative humidity. Oxygen content was measured by an MSA Remote Sampling Sensor. A diagram of the Generation/Exposure system can be found as Figure 1.

Temperature, relative humidity, oxygen content and negative pressure within the system were continually monitored and recorded every five minutes on exposure days through use of a LabView for Windows Data Acquisition system. Test atmosphere airflows were hand-recorded approximately every five minutes for all exposures except the initial exposures on 12-3-94.

### B. Method of Test Atmosphere Generation

Test atmospheres during animal exposure were generated from a low pressure cylinder of HFC-236ea. The test material was introduced via regulators and flowmeters which were connected to the Plexiglas box with 1/4" O.D. stainless steel tubing. The cylinder and regulator were warmed by various methods to maintain sufficient delivery pressure to produce the desired concentration.

### III. EXPOSURE PERIOD CONDITIONS

#### A. General Conditions of Exposure

##### 1. Exposure Duration

The length of exposure to test material for each dog each time it was exposed was ten minutes from initiation to termination of the supply of HFC-236ea to the nose-only mask, with the only exceptions being deaths, during exposure.

##### 2. Animal Exposure Sequence

During the study, the animals were generally exposed in an arbitrary order.

#### B. Inhalation Test Atmosphere Environmental Data

##### 1. Methods

Temperature, relative humidity, oxygen content and negative pressure within the Plexiglas box were monitored continually during exposure using the LabView for Windows Data Acquisition System. Values for these parameters were recorded every five minutes. Airflows were recorded manually approximately every five minutes.

##### 2. Results

Test atmosphere temperature, relative humidity, oxygen content, and negative pressure (of the Plexiglas box) daily means are presented in Table 2.

It should be noted that some recorded oxygen content readings were in excess of the normal oxygen/air value of 20.9%. This variation was due to the sensitivity of the oxygen detection probe cells to temperature changes. The milliamperage output of the cell increased as the test atmospheres warmed, thereby possibly incurring values above 20.9%.

In addition, due to the requirements of exposure for this study, the relative humidity in the exposure system is acknowledged to have been below the protocol specified range on all exposure days. This condition was unavoidable and is not considered to have adversely affected the outcome of validity of the study.

C. Test Atmosphere Concentration Data

1. Analyzed Concentrations

a. Methods

Atmosphere samples were collected through a valved port located in the test material delivery line between the chamber and the first three-way valve using a 20 cc B-D plastic disposable syringe. The syringe was filled and emptied at least one time before withdrawing a full syringe representative of the test atmosphere being analyzed. The syringe was allowed to fill to atmospheric pressure (1-2 seconds) in order to obtain a sample. The valve was then closed and the syringe removed from the port. The syringe was manually capped and transported to the gas chromatograph. The entire sample was injected into the metered gas sampling valve, which delivered a 0.25 ml of sample to the column/detector.

Samples were taken as required to maintain the chamber concentrations, with at least two samples withdrawn and analyzed for each exposure.

b. Results

Mean concentration per exposure per animal was generally within approximately 5% of target. Daily test atmosphere concentrations are presented in Table 3.

2. Nominal Concentrations

a. Methods

Nominal concentrations were calculated from the weight of test material used and the total chamber air volume for each day of exposure. The total mass air volume includes the volume of fresh air, supplemental oxygen (if applicable) and test material and was calculated by multiplying the mean mass air flow, recorded in standard liters per minute (SLPM), by the length of generation in minutes. Total test material volume was determined by using the formulae listed below.

Relevant Formulae:

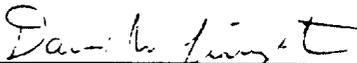
$$\frac{\text{Test Material Volume (L)}}{\text{Total Air Volume (L)}} \times 10^6 = \text{Nominal Concentration (ppm)}$$

$$\text{Weight T.M. (g)} \times \frac{24.45\text{L}}{\text{mol}} \times \frac{\text{mol}}{137.36\text{g}} = \text{Test Material Volume (L)}$$

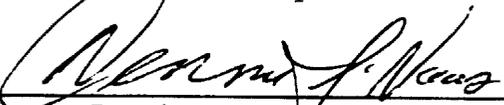
$$\text{Mean SLPM} \times \text{Total Generation Time} = \text{Total Air Volume (L)}$$

b. Results

Nominal concentrations were determined as required by protocol but are of limited utility due to the continual operation of the generation system, including non-animal exposure periods, and (on one day) generation of two different test atmosphere concentrations. Given these limitations, the nominal concentrations appeared reasonable and the results are presented in Table 4.

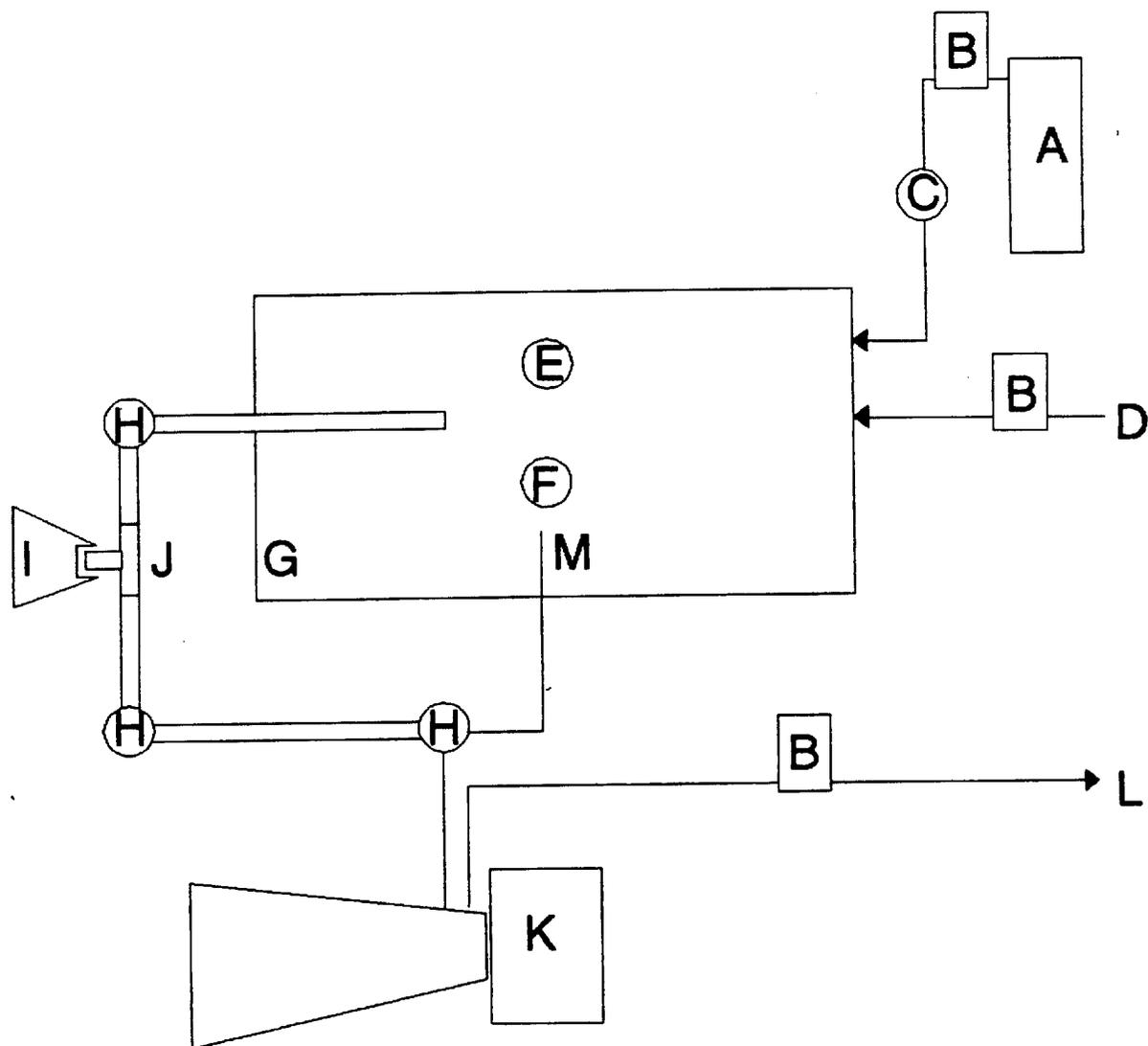
  
\_\_\_\_\_  
David W. Livingston, B.S.  
Acting Section Head, Inhalation

5-23-56  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Dennis J. Naas, B.S.  
Assistant Director of Toxicology

5/23/56  
\_\_\_\_\_  
Date

Figure 1. Atmosphere Exposure/Generation System



- |                                      |                              |
|--------------------------------------|------------------------------|
| A = Test Material Cylinder/Regulator | H = Three-Way Valve          |
| B = Top Trak Digital Flowmeter       | I = Dog Nose-Only Mask       |
| C = Dry Cal DC-1 Flow Calibrator     | J = Non-Rebreathing Valve    |
| D = Laboratory Compressed Air System | K = Miran 1A (monitor only)  |
| E = Negative Pressure Port           | L = Laboratory Vacuum System |
| F = Temperature/Humidity Probe Port  | M = Chamber Monitoring Line  |
| G = 100 L Plexiglas Chamber          |                              |

APPENDIX A (TABLE 1)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236eb IN DOGS BY INHALATION  
 GAS CHROMATOGRAPH CALIBRATION DATA

PROJECT NO.: MIL-189014  
 SPONSOR: DUPONT

DATE	STANDARD (PPH)	RESPONSE	MEAN	STANDARD DEVIATION	C.V. (%)
ANIMAL EXPOSURE:	50,000	8.7358E-04			
12/03/94	100,000	9.2126E-04			
	150,000	8.6741E-04	9.1766E-04	5.1737E-04	5.64
	200,000	9.3056E-04			
	250,000	9.9550E-04			
ANIMAL EXPOSURE:	5,367	1.3391E-03			
12/10/94	10,733	1.4022E-03			
	16,100	1.4446E-03	1.4240E-03	4.2863E-03	3.01
	21,467	1.4345E-03			
	26,834	1.4237E-03			
	32,200	1.4368E-03			
	64,401	1.4371E-03			
ANIMAL EXPOSURE:	21,938	1.2862E-03			
12/12/94	26,834	1.2656E-03	1.2522E-03	4.2266E-03	3.38
	32,432	1.2049E-03			
ANIMAL EXPOSURE:	8,945	1.1925E-03			
12/19/94	12,613	1.2648E-03	1.2253E-03	3.6604E-03	2.99
	18,282	1.2187E-03			
ANIMAL EXPOSURE:	42,934	1.1199E-03			
12/21/94	50,450	1.1644E-03	1.1654E-03	4.6008E-03	3.95
	58,501	1.2119E-03			
ANIMAL EXPOSURE:	26,834	1.7518E-03			
01/06/95	32,432	1.7572E-03	1.7773E-03	3.9525E-03	2.22
	40,219	1.8228E-03			

RESPONSE = AMOUNT/AREA

PROJECT NO.: W1L-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 2)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 DAILY ENVIRONMENTAL CONDITIONS MEANS

PAGE 1

EXPOSURE DATE:	TEMPERATURE (DEGREES CELCIUS)	RELATIVE HUMIDITY (%)	OXYGEN CONTENT (%)	NEGATIVE PRESSURE (IN. H2O)
12/03/94	23.6	10	21.2	0.48
12/10/94	23.8	9	20.0	0.76
12/12/94	23.0	6	20.5	0.80
12/19/94	22.5	14	20.6	0.55
12/21/94	22.5	15	19.7	0.51
01/06/95	22.1	3	19.9	0.77

ANIMAL NO.	DATE: 12/03/94	TIME INTO EXPOSURE(MIN)*	TARGET: 100,000 PPM	SAMPLE NO.	CONCENTRATION
2502		1		1	99,925
		4		2	95,500
		7		3	91,244

MEAN	95,556
S.D.	4,340.77
C.V. (%)	4.54
N	3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 2

DATE: 12/03/94 TARGET: 100,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	101,907
	4	2	95,180
	7	3	90,905
			MEAN 95,997
			S.D. 5,546.35
			C.V. (%) 5.78
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 3

DATE: 12/03/94 TARGET: 100,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2262	1	1	106,774
	4	2	106,735
	7	3	98,486
			MEAN 103,998 S.D. 4,773.86 C.V.(%) 4.59 N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 4

DATE: 12/03/94 TARGET: 100,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2503	1	1	101,507
	4	2	107,719
	7	3	105,912
			MEAN 105,046
			S.D. 3,195.26
			C.V.(%) 3.04
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 5

DATE: 12/03/94

TARGET: 100,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2475	0	1	106,953
	4	2	101,553
			MEAN 104,253
			S.D. 3,818.38
			C.V. (%) 3.66
			N 2

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

PROJECT NO.: MIL-189014      APPENDIX A (TABLE 3)      PAGE 6  
 SPONSOR: DUPONT      ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

DATE: 12/10/94      TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2503	0	1	25,002
	4	2	24,499
	8	3	23,794
			MEAN 24,432
			S.D. 606.81
			C.V. (%) 2.48
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)      \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 7

DATE: 12/10/94 TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2262	0	1	25,166
	4	2	24,510
	8	3	24,819
			MEAN 24,832
			S.D. 328.18
			C.V.(%) 1.32
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 8

DATE: 12/10/94 TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	24,693
	4	2	24,820
	8	3	25,200
			MEAN 24,904
			S.D. 263.81
			C.V.(%) 1.06
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WJL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 9

DATE: 12/10/94

TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2307	0	1	52,737
	4	2	53,633

MEAN 53,185  
S.D. 633.57  
C.V. (%) 1.19  
N 2

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 10

DATE: 12/12/94 TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2263	0	1	25,440
	4	2	25,490
	8	3	25,632
			MEAN 25,521
			S.D. 99.61
			C.V. (%) 0.39
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT  
 APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

DATE: 12/12/94 TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2308	0	1	25,140
	4	2	25,081
	8	3	25,423
			MEAN 25,215 S.D. 182.82 C.V. (%) 0.73 N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 12

DATE: 12/12/94 TARGET: 25,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	24,954
	4	2	25,004
	8	3	25,134
			MEAN 25,031
			S.D. 92.92
			C.V. (%) 0.37
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

DATE: 12/19/94 TARGET: 12,500 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2263	0	1	12,807
	4	2	12,275
	8	3	12,006
			MEAN 12,363 S.D. 407.63 C.V. (%) 3.30 N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPH)

PAGE 14

DATE: 12/19/94

TARGET: 12,500 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2262	0	1	12,479
	4	2	12,441
	8	3	12,511
			MEAN 12,477
			S.D. 35.04
			C.V. (%) 0.28
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 15

DATE: 12/19/94

TARGET: 12,500 PPM

ANIMAL NO.	TIME INTO EXPOSURE (MIN)*	SAMPLE NO.	CONCENTRATION
2503	0	1	12,678
	4	2	12,598
	8	3	12,635
			MEAN 12,637
			S.D. 40.04
			C.V. (%) 0.32
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

DATE: 12/19/94 TARGET: 12,500 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2308	0	1	12,692
	4	2	12,775
	8	3	12,864
			MEAN 12,777
			S.D. 86.02
			C.V.(%) 0.67
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 17

DATE: 12/19/94

TARGET: 12,500 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	12,634
	4	2	12,745
	8	3	12,455
			MEAN 12,611
			S.D. 146.32
			C.V. (%) 1.16
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

DATE: 12/21/94 TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2263	0	1	51,684
	4	2	51,705
	8	3	51,789
			MEAN 51,726
			S.D. 55.56
			C.V.(%) 0.11
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 19

DATE: 12/21/94 TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2262	0	1	51,435
	4	2	51,325
	8	3	49,818
			MEAN 50,859
			S.D. 903.50
			C.V.(%) 1.78
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

DATE: 12/21/94 TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2503	0	1	50,534
	4	2	51,060
	8	3	50,574
			MEAN 50,723
			S.D. 292.82
			C.V.(%) 0.58
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 21

DATE: 12/21/94

TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2308	0	1	51,162
	4	2	50,328
	8	3	51,217
			MEAN 50,902
			S.D. 498.15
			C.V.(%) 0.98
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 22

DATE: 12/21/94 TARGET: 50,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	50,490
	4	2	49,887
	8	3	49,456
			MEAN 49,944
			S.D. 519.38
			C.V. (%) 1.04
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014      APPENDIX A (TABLE 3)      PAGE 23  
 SPONSOR: DUPONT      ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

DATE: 01/06/95      TARGET: 35,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2263	0	1	35,033
	4	2	36,882
	8	3	36,688
			MEAN    36,201
			S.D.    1016.16
			C.V.(%)    2.81
			N    3

CONCENTRATION = PARTS PER MILLION (PPM)      \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 24

DATE: 01/06/95 TARGET: 35,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2262	0	1	35,591
	4	2	35,516
	8	3	35,747
			MEAN 35,618
			S.D. 117.84
			C.V.(%) 0.33
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 25

DATE: 01/06/95 TARGET: 35,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2503	0	1	35,216
	4	2	35,879
	8	3	35,619
			MEAN 35,571
			S.D. 334.06
			C.V. (%) 0.94
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 26

DATE: 01/06/95 TARGET: 35,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2308	0	1	36,473
	4	2	36,512
	8	3	36,596
			MEAN 36,527
			S.D. 62.86
			C.V.(%) 0.17
			N 3

CONCENTRATION = PARTS PER MILLION (PPM) \* = AFTER TEST GAS INITIATED

PROJECT NO.: MIL-189014  
 SPONSOR: DUPONT

APPENDIX A (TABLE 3)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 TEST ATMOSPHERE CONCENTRATIONS (PPM)

PAGE 27

DATE: 01/06/95 TARGET: 35,000 PPM

ANIMAL NO.	TIME INTO EXPOSURE(MIN)*	SAMPLE NO.	CONCENTRATION
2264	0	1	35,736
	4	2	34,936
	8	3	35,502
			MEAN 35,391
			S.D. 411.32
			C.V.(%) 1.16
			N 3

CONCENTRATION = PARTS PER MILLION (PPM)

\* = AFTER TEST GAS INITIATED

APPENDIX A (TABLE 4)  
 ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION  
 PROJECT NO.: WIL-189014  
 SPONSOR: DUPONT

EXPOSURE DATE:	MEAN AIRFLOW (LPM)	T.M. USED (GRAMS)	GENERATION LENGTH (MINUTES)	NOMINAL CONCENTRATION (PPM)
12/03/94	10.9	1350	196	112,479
12/10/94	24.815	900	215	30,045
12/12/94	25.924	550	124	30,455
12/19/94	23.316	400	203	15,043
12/21/94	22.047	1550	200	62,571
01/06/95	22.961	800	215	28,846

PPM = PARTS PER MILLION      LPM = LITERS PER MINUTE

WIL-189014

E. I. DuPont de Nemours & Co.

Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

**APPENDIX B**

Study Protocol



Study Number: WIL-189014

**PROTOCOL AMENDMENT I**

Sponsor: E. I. DuPont de Nemours & Co.

A. Title of Study:

Acute Cardiac Sensitization Study of HFC-236ea in Dogs by Inhalation

B. Protocol Modification:

1) VI. TEST SYSTEM

The dogs weighed approximately 11-16 kg at initiation of exposures.

C. Reason for Protocol Modification:

1) Information added to protocol for GLP compliance.

Approved By:

E.I. du Pont de Nemours & Co.  
Haskell Laboratories  
Elkton Road  
Newark, DE 19714

William J. Brock, Ph.D.  
Project Monitor

5/14/96

Date

Prepared By:

WIL Research Laboratories, Inc.  
Ashland, OH 44805-9281

Dennis J. Naas, B.S.  
Study Director

5/9/96

Date



**PROTOCOL**

**Acute Cardiac Sensitization Study of HFC-236ea in Dogs by Inhalation**

WIL Study No.: WIL-189014

For

E.I. du Pont de Nemours & Co.  
Haskell Laboratories  
Elkton Road  
Newark, Delaware 19714

By

WIL Research Laboratories, Inc.  
Ashland, Ohio 44805-9281

October 11, 1994



**PROTOCOL**

**Acute Cardiac Sensitization Study of HFC-236ea in Dogs by Inhalation**

WIL Study No.: WIL-189014

For

E.I. du Pont de Nemours & Co.  
Haskell Laboratories  
Elkton Road  
Newark, Delaware 19714

By

WIL Research Laboratories, Inc.  
Ashland, Ohio 44805-9281

October 11, 1994

# ACUTE CARDIAC SENSITIZATION STUDY OF HFC-236ea IN DOGS BY INHALATION

WIL Study No.: WIL-189014

## I. OBJECTIVE OF STUDY

The objective of this study is to evaluate the cardiac sensitization potential of the test article during a single acute exposure by inhalation. Cardiac sensitization in this context refers to the potential for the test article to increase the sensitivity of the heart to the pharmacological effects of epinephrine resulting in potentially life-threatening or fatal cardiac arrhythmias.

The study will be conducted in compliance with the EPA (Environmental Protection Agency) Good Laboratory Practice Standards, 40 CFR Part 792.

## II. PERSONNEL INVOLVED IN THE STUDY

### A. Sponsor Representative

William J. Brock, Ph.D.  
Toxicology Consultant

### B. WIL Study Director

Dennis J. Naas, B.S.  
Assistant Director of Toxicology

### C. WIL Toxicology Department Responsibilities

1. E. Crosby Tompkins, Ph.D., D.A.B.T.  
Vice President, Director of Toxicology
2. Christopher P. Chengelis, Ph.D., D.A.B.T.  
Associate Director of Toxicology
3. Jerry E. Bennick  
Manager, Inhalation Toxicology
4. Lisa Simon, B.S., M.T.(ASCP)  
Supervisor of Clinical Pathology
5. Stanley E. Kopp  
Systems Manager
6. Sally A. Keets, A.S.  
Manager of In-Life Facilities
7. Deborah L. Little  
Manager of Quality Assurance

II. PERSONNEL INVOLVED IN THE STUDY (continued)

8. Kerin Clevidence, B.S.  
Section Head II - Pathology and  
Developmental Toxicology Laboratory
9. Gary R. Kiplinger, B.S.  
Manager of General Toxicology
10. Charlene M. Lindsey, M.S.  
Manager of Technical Report Writing
11. Robert R. Dahlgren, D.V.M., Ph.D.  
Diplomate A.C.V.P.  
Director of Pathology
12. Chris Nelson  
Acting Section Head I - Pharmacy
13. Robert Hamlin, D.V.M.  
Consulting Veterinary Cardiologist  
Ohio State University  
School of Veterinary Medicine

III. STUDY SCHEDULE DATA

- A. Proposed Experimental Start Date: October, 1994
- B. Proposed Experimental Termination Date: November, 1994
- C. Proposed Draft Report Date: January, 1994

IV. TEST ARTICLE DATA

- A. Identification: HFC-236ea
- B. Lot Number: FT-1411
- C. Purity: > 99 %
- D. Stability: Test article stability data are the responsibility of the Sponsor.
- E. Physical Description: HFC-236ea is a nonflammable, colorless liquified gas at room temperature.

IV. TEST ARTICLE DATA (continued)

- F. Storage Conditions: Sealed container (low pressure cylinder) under ambient conditions.
- G. Reserve Samples: A reserve sample will be collected in a small stainless steel cylinder and stored in the Archives at WIL Research Laboratories, Inc.
- H. Personnel Safety Data: Long sleeves (lab coat), eye protection, latex or nitrile gloves and organic respirator required when handling material or when potential high exposure may occur. Refer to attached Material Safety Data Sheet for complete available precautions.

V. POSITIVE CONTROL ARTICLE DATA

- A. Identification: CFC-11 (CAS No. 75-69-4)
- B. Container Number: 20405
- C. Purity: 99.98 %
- D. Stability: Stability data are the responsibility of the Sponsor.
- E. Physical Description: Clear, colorless liquid under pressure, colorless gas under ambient (atmospheric) conditions.
- F. Storage Conditions: Sealed container (low pressure cylinder) under ambient conditions.
- G. Reserve Samples: A reserve sample will be collected in a small stainless steel cylinder and stored in the Archives at WIL Research Laboratories, Inc.
- H. Personnel Safety Data: Long sleeves (lab coat), eye protection, latex or nitrile gloves and organic respirator required when handling material or when potential high exposure may occur. Refer to attached Material Safety Data Sheet for complete available precautions.

VI. TEST SYSTEM

- A. Species: Dog
- B. Strain: Beagle
- C. Source: Standard U.S.D.A. - approved supplier.

VI. TEST SYSTEM (continued)

- D. Number of Animals: Eight males will be placed on study.
- E. Approximate Age: Animals will be approximately 10 to 14 months of age when placed on study.
- F. Identification System: Animals will be uniquely identified by an ear tattoo. Individual cage cards will be affixed to each cage and will display the animal number and study number.
- G. Justification for Selection: This species and breed of animal is recognized as appropriate model for cardiac function studies.

VII. SPECIFIC (NONEXPOSURE PERIOD) MAINTENANCE SCHEDULEA. Animal Housing

All animals will be housed individually in clean suspended stainless steel cages in an environmentally controlled room. The cages will be elevated above stainless steel flush pans which will be cleaned daily. Dogs will be given frequent and regular opportunities for exercise and socialization, to the extent possible on a 0.5 hour/day, seven days per week basis, in accordance with the Animal Welfare Act. Exercise/socialization may be suspended, by necessity, during the exposure phase of the study.

B. Environmental Conditions

Controls will be set to maintain temperature at  $72^{\circ} \pm 4^{\circ}\text{F}$  and relative humidity at approximately 30-70%. Fluorescent lighting will provide illumination for 12 hours per day. Temperature and relative humidity will be recorded once daily.

C. Drinking Water

Tap water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures. Municipal water supplying the laboratory will be analyzed periodically for contaminants according to Standard Operating Procedures to ascertain that none are present at concentrations that would be expected to affect the outcome of the study.

D. Basal Diet

Approximately 400 g of Purina® Certified Canine Diet #5007 (pellet) will be offered daily for approximately 1-2 hours. Analyses of the certified feed for the presence of contaminants will be provided by the manufacturer to ensure that none are present at concentrations that would be expected to affect the outcome of the study.

VIII. EXPERIMENTAL DESIGN

A. Animal Receipt and Acclimation

The dogs used on this study will be selected from available stock based upon age, health and acceptable pretest ECG.

Each animal will be inspected by qualified personnel upon receipt. Dogs judged to be in good health and suitable as test animals will be acclimated for at least 14 days. Dogs will be weighed periodically during acclimation until study start, including approximately one week prior to initiation of the exposures and on the day prior to the first day of exposures. During the acclimation period each dog will be observed twice daily for changes in general appearance and behavior. Immunizations prior to arrival of the dogs will include: distemper, hepatitis, leptospirosis (DHL), Parvo and parainfluenza. Stool samples will be collected during the acclimation period and checked for parasites. Each dog will be given a thorough pretest ECG evaluation.

During the week prior to the start of the study, each dog will be placed in the exposure restraint apparatus for several 20 to 40 minute periods in order to acclimate them to test article exposure conditions.

B. Randomization

Animals judged to be suitable for testing will be assigned to the study from available stock based on health, body weight, temperament and pretest ECG results.

C. Route and Rationale of Test Article Administration

Because of the volatility and potential application of the test article, the route of administration will be by inhalation exposure as this is the most likely route of exposure for the general population. The nose-only method of exposure was selected to conserve test article and facilitate epinephrine administration.

D. Exposure Levels

Initially, three test article exposure concentrations (10, 15 and 20%, i.e., 100,000, 150,000 and 200,000 ppm, HFC-236ea) and a positive control atmosphere containing 2% (v/v), i.e., 20,000 ppm, CFC-11 will be studied. Additional test article exposure concentrations or replicates of previous exposure concentrations may be evaluated as necessary to define a dose-response and/or clarify previous responses (by amendment at additional cost).

VII. EXPERIMENTAL DESIGN (continued)

E. Determination of Epinephrine Dose

An individual response to intravenous epinephrine alone (up to approximately 10 ectopic beats) will be established for each individual dog using a range of epinephrine doses; a dose limit of 12  $\mu\text{g}/\text{kg}$  will be used. The epinephrine doses will be administered via the cephalic vein. A starting dose of 3  $\mu\text{g}/\text{kg}$  will be used. If no ectopic beats are observed for a particular dog the epinephrine dose will be increased until a response is observed. Doses of epinephrine to be used are 3, 6, 9 or 12  $\mu\text{g}/\text{kg}$ ; others may be used at the discretion of the Study Director. If too many ectopic beats are observed, the epinephrine dose will be reduced until an acceptable response is found. The dog will be rested for at least 10 minutes between these determinative epinephrine doses. During this phase of the study, the dogs will be exposed to air only. The nose-only apparatus may be removed during the resting period but will be in place during epinephrine dosing and the subsequent ECG recording.

If necessary, the Study Director will apply judgement in the selection of an appropriate epinephrine dose, in the best interests of the dog and the study integrity. When an appropriate dose is found, that dose will be used for exposure to the test material.

F. Organization and Treatment Regimen

There will be one group of eight dogs. Each dog will be exposed to the same concentrations of test material, but at different times. At the end of each exposure period the dogs will be returned to their home cages. There will be a minimum of approximately 24 hours between exposure periods for each animal.

Each dog will be comfortably restrained using a canvas sling and the appropriate ECG leads attached.

The exposure duration to air will be approximately 7 minutes and the exposure duration for the test gas will be approximately 10 minutes. (This exposure duration selected by the Sponsor should be of sufficient duration to achieve blood levels near saturation of HFC-236ea based on cardiac sensitization results obtained with HFC-236fa and a blood:air partition coefficient for HFC-236ea of 0.56). In this regimen, a dog will serve as its own control based pre-study determination of the epinephrine dose. The treatment regimen will be:

<u>TIME (minutes)</u>	<u>Event</u>
0	Start ECG recording
2	First epinephrine dose
7	Test gas initiated
12	Challenge dose of epinephrine
17	Termination of exposure/ECG

VII. EXPERIMENTAL DESIGN (continued)

G. Exposure Methods

All animals will be exposed to the test article using a nose-only exposure apparatus operated under dynamic conditions to sustain air flows of at least 12 to 15 air changes per hour, ensuring an adequate oxygen content of approximately 19 percent or above and an evenly distributed exposure atmosphere. Oxygen will be supplemented as necessary to maintain a level of approximately 19% or above. The method of exposure will be documented in the study records and described in detail in the final report. Feed and water will be withheld during the exposure period. Controls will be set to maintain test atmosphere exposure temperature at approximately 22°C ( $\pm 2^\circ$ ) and humidity between approximately 40 to 60 percent.

H. Test Atmosphere Monitoring

Exposure concentrations will be measured by gas chromatography or other suitable method (to be documented in the study records).

Mass air flow, oxygen content, temperature and humidity shall be monitored continuously and recorded approximately every 5 minutes during exposures.

I. Epinephrine Challenge

The epinephrine challenge doses will be given intravenously into a cephalic vein. The dose volume will be 0.1 ml/kg and will be administered using a 1.0 ml syringe. The injection rate, controlled through use of a syringe pump, will be approximately 0.1 ml/sec. Epinephrine solutions will be prepared fresh daily and will be kept on ice. The free base ([-] adrenalin, Sigma catalog # E4250) will be used. Equimolar amounts of HCl will be used to assure dissolution. The vehicle will be sterile 0.9% saline (U.S.P.) for injection. Catheters may be used, if necessary, to accommodate the epinephrine dosings.

IX. PARAMETERS TO BE EVALUATED

A. Viability Checks

All animals will be checked for mortality and moribundity each morning and afternoon.

B. Clinical Observations

The dogs will be observed for clinical signs of toxicity prior to initiation of each exposure, continually during the exposure period, and following each exposure when they are returned to their cages.

**IX. PARAMETERS TO BE EVALUATED (continued)**

Detailed physical examinations will be conducted on all animals on the day prior to the first day of exposures and after completion of all exposures.

**C. Individual Body Weights**

Individual body weights will be recorded on the day prior to the first day of exposures and after completion of all exposures.

**D. Electrocardiographic Examinations**

ECG's will be recorded with a Cambridge model 3038/2 three channel electrocardiograph or equivalent. Only modified lead 1, 2, 3 electrocardiograms will be collected. Alligator clip electrodes will be placed on each limb. Chart speed will be 10 mm/sec and response sensitivity will be set at 5 mm/mV. ECG's will be recorded continuously throughout the pre-exposure epinephrine dosing, exposure to the test gas and for five minutes following administration of the challenge epinephrine dose (total of approximately 17 minutes). The points at which epinephrine was given and when exposure to the test article was initiated will be clearly marked on each ECG recording.

During the pretreatment periods different chart speeds may be used to more fully evaluate the cardiovascular condition of the animals.

**E. Anatomic Pathology**

Gross necropsies will be done on the animals that die. Tissues will be collected at the discretion of the pathologist and/or study director. Microscopic examination may be performed at the discretion of the pathologist and study director. Animals that survive the treatment regimen will not be subjected to necropsy and will be returned to stock.

**X. EVALUATION OF RESULTS**

ECG recordings taken following exposure to the test article and after administration of the challenge epinephrine dose will be compared to the ECG recordings made after administration of the first epinephrine dose that was given in the absence of test article. Reactions or changes indicative of cardiac sensitization will be interpreted in accordance with the criteria described by Reinhardt, *et al*.

Copies of the ECG recordings will be supplied to the Sponsor so that they may review/confirm positive and negative findings and assist in the interpretation of equivocal (unclear) results.

**XI. QUALITY ASSURANCE**

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with Good Laboratory Practice regulations, adherence to the protocol and to WIL Standard Operating Procedures. The final report will be audited by the WIL Quality Assurance Unit prior to submission to the sponsor to assure that the final report accurately describes the conduct and the findings of the study.

This study is an EPA regulated study and shall be included on the master schedule.

**XII. RECORDS TO BE MAINTAINED**

All original raw data records will be stored in the Archives at WIL Research Laboratories, Inc. Records to be retained will include, but are not limited to, the following:

- A. Protocol and protocol amendments
- B. Master protocol computer printout
- C. WIL study personnel involved in the conduct of the study
- D. Study schedule
- E. Purina® Certified feed lot records
- F. Animal receipt and identification records including purchase orders and shipping records
- G. Laboratory animal inventory
- H. Acclimation period body weights and observation records
- I. Documentation of animal selection for study
- J. Test material preparation records with balance accuracy records
- K. Computer randomization records
- L. Body weight computer archive reports
- M. Observation computer archive reports
- N. Room temperature and humidity records
- O. Animal room cleaning records
- P. Mortality/Moribundity records
- Q. Computer raw data edit records
- R. Original electrocardiograms
- S. Unscheduled deaths/euthanization records
- T. Gross pathological and histopathological raw data computer records
- U. Documentation of exposure system temperature, relative humidity and oxygen content
- V. Documentation of diluent air and exhaust flow
- W. Documentation of calibration for:
  - 1. Analytical instrument
  - 2. Diluent air and exhaust flow
  - 3. Temperature and relative humidity probes
- X. Method of atmosphere generation
- Y. Method of atmosphere concentration verification
- Z. Verification of atmosphere concentration

**XIII. WORK PRODUCT**

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, magnetically encoded records and specimens will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, Inc. Thereafter, WIL Research Laboratories will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

**XIV. REPORTS**

The final report will contain a summary, test material data, methods and procedures, and an interpretation and discussion of the study results. The final report will be comprehensive and shall attempt to define level(s) inducing toxic effects as well as "no-effect" level(s) under the condition of this investigation. The report will contain all information necessary to conform with current EPA-TSCA specifications.

The contents of the report will be as follows:

**A. Text**

1. Summary
2. Introduction
3. Objective
4. Experimental Design
5. Results of Clinical Observations
6. Results of Body Weights
7. Results of ECG Examinations
8. Results of Macroscopic Examination (if conducted)
9. Results of Microscopic Examination (if conducted)
10. Discussion and Conclusion

**B. Tables**

1. Summary of Observations
2. Summary of Mean Body Weights and Changes
3. Summary of ECG Findings
4. Summary of Macroscopic and Microscopic Findings (if conducted)
5. Individual Body Weights
6. Individual ECG Findings
7. Individual Macroscopic and Microscopic Findings (if conducted)

**C. Appendices**

1. Atmosphere generation methods and exposure data (including environmental conditions)
2. Methods and results of atmosphere concentration analyses

XIV. REPORTS (continued)

Two copies of the final report will be supplied

XV. PROTOCOL MODIFICATION

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director.

XVI. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

1. The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.
2. Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.
3. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.
4. Methods of euthanasia used during this study are in conformance with the above referenced regulation.

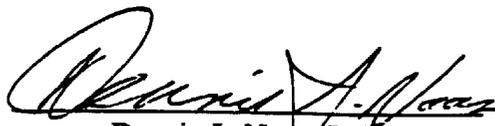
XVII. PROTOCOL APPROVAL

E.I. DuPont de Nemours & Co.  
Haskell Laboratories  
Elkton Road  
Newark, Delaware 19714

  
\_\_\_\_\_  
William J. Brock, Ph.D.  
Project Monitor

10/20/94  
Date

WIL Research Laboratories, Inc.  
Ashland, Ohio 44805-9281

  
\_\_\_\_\_  
Dennis J. Naas, B.S.  
Study Director

10/11/94  
Date

References

1. Mullin, L., Reinhardt, C. and Hemingway R. (1979) Cardiac Arrhythmias and Blood Levels Associated with Inhalation of Halon 1301, Am. Ind. Hygiene Assoc. Jour., 40, 653-658.
2. Reinhardt, C., Azar A., Marfield, M., Smith, P., and Mullin, L., (1971) Cardiac Arrhythmias and Aerosol "Sniffing", Arch. Envir. Health, 22, 265-279.



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DúPont  
Material Safety Data Sheet

Page 2

## (HAZARDS IDENTIFICATION - Continued)

Dizziness, deep breathing, possible nausea.

Skin: Discoloration of skin to gray or white.

## Carcinogenicity Information

None of the components present in this material at concentrations equal to or greater than 0.1% are listed by IARC, NTP, OSHA or ACGIH as a carcinogen.

-----  
FIRST AID MEASURES  
-----

## First Aid

## INHALATION

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

## SKIN CONTACT

Flush skin with water after contact. Wash contaminated clothing before reuse.

## EYE CONTACT

In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

## INGESTION

Get medical attention.

-----  
FIRE FIGHTING MEASURES  
-----

## Flammable Properties

## Fire and Explosion Hazards:

Toxic fumes or mists of fluoride may be produced on thermal decomposition.

## Extinguishing Media

Dry Chemical, CO<sub>2</sub>.

## Fire Fighting Instructions

Wear self-contained breathing apparatus. Wear full protective equipment.

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DuPont  
Material Safety Data Sheet

Page 3

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 ACCIDENTAL RELEASE MEASURES
 

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## Safeguards (Personnel)

NOTE: Review FIRE FIGHTING MEASURES and HANDLING (PERSONNEL) sections before proceeding with clean-up. Use appropriate PERSONAL PROTECTIVE EQUIPMENT during clean-up.

## Spill Clean Up

Soak up with sawdust, sand, oil dry or other absorbent material. Shovel or sweep up.

---

 HANDLING AND STORAGE
 

---

## Handling (Personnel)

Avoid breathing vapors or mist.

## Storage

Store in a well ventilated place. Store in a cool place. Keep container tightly closed.

Store in dry area.

---

 EXPOSURE CONTROLS/PERSONAL PROTECTION
 

---

## Engineering Controls

Use only with adequate ventilation.

## Personal Protective Equipment

Eye/Face	: Safety Glasses. Coverall chemical splash goggles.
Respirator	: NIOSH/MSHA - Approved respirator if exposure limits exceeded.
Protective Gloves	: Rubber or neoprene

## Exposure Guidelines

## Exposure Limits

1,1,1,2,3,3-HEXAFLUOROPROPANE		
PEL	(OSHA)	: None Established
TLV	(ACGIH)	: None Established

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73210255

DuPont  
Material Safety Data Sheet

Page 4

-----  
PHYSICAL AND CHEMICAL PROPERTIES  
-----

## Physical Data

Boiling Point : 6 C (43 F)  
Vapor Density : >1 (Air = 1)  
Solubility in Water : Insoluble

-----  
STABILITY AND REACTIVITY  
-----

## Chemical Stability

Stable at normal temperatures and storage conditions.

## Incompatibility with Other Materials

Incompatible with strong oxidizer, and alkali metals.

## Decomposition

Hazardous gases/vapors produced are HF, CO, and CO2.

## Polymerization

Polymerization will not occur.

-----  
DISPOSAL CONSIDERATIONS  
-----

## Waste Disposal

Treatment, storage, transportation, and disposal must be in accordance with applicable Federal, State/Provincial, and Local regulations.

-----  
OTHER INFORMATION  
-----

## Additional Information

The above information is based on an MSDS issued by PCR Inc. dated 8/31/90.

The data in this Material Safety Data Sheet relates only to the specific material designated herein and does not relate to use in combination with any other material or in any process.

Responsibility for MSDS : MSDS Coordinator  
Address : Du Pont Chemicals  
Corpus Christi  
Telephone : 512-776-6614

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271CA

-----  
"FREON" 11  
2090FR Revised 23-SEP-1991 Printed 31-AUG-1994  
-----

-----  
CHEMICAL PRODUCT/COMPANY IDENTIFICATION  
-----

\* Material Identification

"FREON" is a registered trademark of DuPont.

Corporate MSDS Number : DU000026  
CAS Number : 75-69-4  
Formula : CCl3F  
Molecular Weight : 137.36

Tradenames and Synonyms

F-11  
CC0119

Company Identification

MANUFACTURER/DISTRIBUTOR

Du Pont  
1007 Market Street  
Wilmington, DE 19898

PHONE NUMBERS

Product Information : 1-800-441-9442  
Transport Emergency : CHEMTREC: 1-800-424-9300  
Medical Emergency : 1-800-441-3637

-----  
COMPOSITION/INFORMATION ON INGREDIENTS  
-----

Components

Material	CAS Number	x
-METHANE, TRICHLOROFLUORO- ("FREON" 11)	75-69-4	100

\* Regulated as a Toxic Chemical under Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 and 40 CFR part 372.

-----  
HAZARDS IDENTIFICATION  
-----

\* Potential Health Effects

Inhalation of high concentrations of vapor is harmful and may cause heart irregularities, unconsciousness, or death. Intentional misuse or deliberate inhalation may cause death without warning. Vapor reduces oxygen available for breathing and is heavier than air. Causes skin and eye irritation.

## (HAZARDS IDENTIFICATION - Continued)

## HUMAN HEALTH EFFECTS:

Human health effects of overexposure by eye contact may include eye irritation with discomfort, tearing, or blurring of vision. Skin contact with the liquid may cause drying of the skin with repeated contact resulting in mild skin irritation with discomfort or rash. Overexposure by inhalation may cause temporary nervous system depression with anesthetic effects such as dizziness, headache, confusion, incoordination, and loss of consciousness; temporary alteration of the heart's electrical activity with irregular pulse, palpitations, or inadequate circulation, or the effects of exclusion of oxygen with grossly excessive exposures. Ingestion may include nonspecific discomfort, such as nausea, headache, or weakness.

Individuals with preexisting diseases of the central nervous or cardiovascular system may have increased susceptibility to the toxicity of excessive exposures.

## Carcinogenicity Information

None of the components present in this material at concentrations equal to or greater than 0.1% are listed by IARC, NTP, OSHA or ACGIH as a carcinogen.

-----  
FIRST AID MEASURES  
-----

## First Aid

## INHALATION

If high concentrations are inhaled, immediately remove to fresh air. Keep person calm. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

## SKIN CONTACT

In case of skin contact, flush skin with plenty of water for 15 minutes. Get medical attention if irritation is present.

## EYE CONTACT

In case of eye contact, immediately flush eyes with plenty of water for 15 minutes. Call a physician.

## INGESTION

If swallowed, no specific intervention is indicated as the compound is not likely to be hazardous by ingestion. However, consult a physician if necessary.

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## (FIRST AID MEASURES - Continued)

## Notes to Physicians

Because of possible disturbances of cardiac rhythm, catecholamine drugs, such as epinephrine, should be used with special caution in situations of emergency life support.

-----  
FIRE FIGHTING MEASURES  
-----

## Flammable Properties

Flash Point : Will not burn  
Method : TOC  
Flammable limits in Air, % by Volume  
LEL : Not applicable  
UEL : Not applicable  
Autoignition : Not determined  
Autodecomposition : >593 C (>1099 F)

## Fire and Explosion Hazards:

Drums may rupture under fire conditions. Decomposition may occur.

## Extinguishing Media

As appropriate for combustibles in area.

## Fire Fighting Instructions

Self-contained breathing apparatus (SCBA) is required if containers rupture and contents are spilled under fire conditions.

-----  
ACCIDENTAL RELEASE MEASURES  
-----

## Safeguards (Personnel)

NOTE: Review FIRE FIGHTING MEASURES and HANDLING (PERSONNEL) sections before proceeding with clean-up. Use appropriate PERSONAL PROTECTIVE EQUIPMENT during clean-up.

## Accidental Release Measures

Ventilate area. Do not flush into sewers. Dike spill. Collect on absorbent material and transfer to steel drums for recovery or disposal. Use self-contained breathing apparatus (SCBA) for large spills. Comply with Federal, State and local regulations on reporting releases.

-----  
HANDLING AND STORAGE  
-----

## Handling (Personnel)

Use with sufficient ventilation to keep employee exposure below recommended limits.

## Storage

Clean, dry area. Do not store above 125 deg F (52 deg C).

-----  
EXPOSURE CONTROLS/PERSONAL PROTECTION  
-----

## Engineering Controls

Normal ventilation for standard manufacturing procedures is generally adequate. Local exhaust should be used when large amounts are released. Mechanical ventilation should be used in low or enclosed places.

## Personal Protective Equipment

Impervious gloves should be used to avoid prolonged or repeated exposure. Chemical splash goggles should be available for use as needed to prevent eye contact. Under normal manufacturing conditions, no respiratory protection is required when using this product. Self-contained breathing apparatus (SCBA) is required if a spill occurs.

## Exposure Guidelines

## Exposure Limits

"FREON" 11	
PEL (OSHA)	: 1,000 ppm, 5,600 mg/m <sup>3</sup> , 8 Hr. TWA
TLV (ACGIH)	: 1,000 ppm, 5,620 mg/m <sup>3</sup> , Ceiling
AEL - (Du Pont)	: None Established

\* AEL is Du Pont's Acceptable Exposure Limit. Where governmentally imposed occupational exposure limits which are lower than the AEL are in effect, such limits shall take precedence.

-----  
PHYSICAL AND CHEMICAL PROPERTIES  
-----

## \* Physical Data

Boiling Point	: 23.9 C (75 F)
Vapor Pressure	: 14.7 psia at 25 deg C (77 deg F)
Vapor Density	: 4.9 (Air = 1)
% Volatiles	: 100 WT%
Evaporation Rate	: (CCl <sub>4</sub> = 1) Greater than 1

## (PHYSICAL AND CHEMICAL PROPERTIES - Continued)

Solubility in Water : 0.1 WT% @ 25 C (77 F)  
pH : Neutral  
Odor : Slight ethereal  
Form : Liquid  
Color : Colorless  
Density : 1.48 g/cc at 25 deg C (77 deg F)

Appearance : Clear

-----  
STABILITY AND REACTIVITY  
-----

## Chemical Stability

Stable.

However, avoid open flames and high temperatures.

## Incompatibility with Other Materials

Incompatible with alkali or alkaline earth metals- powdered Al, Zn, Be, etc.

## Polymerization

Polymerization will not occur.

## Other Hazards

Decomposition : Decomposition products are hazardous. "FREON" 11 can be decomposed by high temperatures (open flames, glowing metal surfaces, etc.) forming hydrochloric and hydrofluoric acids and possibly carbonyl halides.

-----  
TOXICOLOGICAL INFORMATION  
-----

## Animal Data

Inhalation 4-hour LC50: 26,200 ppm in rats  
Oral ALD : 3725 mg/kg in rats

The compound is not a skin irritant but is a mild eye irritant. Toxic effects in rats exposed by inhalation include central nervous system and anesthetic effects at high concentrations. Concentrations of 0.35% and higher caused cardiac sensitization in dogs. Various cardiovascular and circulatory abnormalities have also been reported in other animals. Changes in the lungs, liver, brain and spleen were observed in a study of rats exposed by inhalation to 12 times the TLV. In another study at 25 times the TLV, rats, guinea pigs, and cats exhibited no

(TOXICOLOGICAL INFORMATION - Continued)

microscopic evidence of damage to the heart, lungs, kidney, liver or spleen. Exposures by ingestion or skin resulted in no evidence of toxicity in rats, dogs or rabbits.

-----  
ECOLOGICAL INFORMATION  
-----

Ecotoxicological Information

Aquatic Toxicity

"FREON" 11: 96-hour LC50, rainbow trout: 190 mg/L

-----  
DISPOSAL CONSIDERATIONS  
-----

Waste Disposal

Reclaim by distillation or remove to a permitted waste disposal facility. Comply with Federal, State, and local regulations.

-----  
TRANSPORTATION INFORMATION  
-----

Shipping Information

DOT

DOT/IMO

Proper Shipping Name : RQ ENVIRONMENTALLY HAZARDOUS SUBSTANCE  
LIQUID, N.O.S.  
(TRICHLOROFLUOROMETHANE)

Hazard Class : 9

UN No. : 3082

DOT/IMO Label : CLASS 9

Packing Group : III

Shipping Containers

Tank Cars.

Tank Trucks.

Drums

Reportable Quantity : 5000 lbs/2270 kg

"FREON" 11 IS NOT REGULATED AS A HAZARDOUS MATERIAL BY DOT, IMO OR ICAO IN CONTAINERS LESS THAN 5000 LBS.

BEST COPY AVAILABLE

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REGULATORY INFORMATION  
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## U.S. Federal Regulations

TSCA Inventory Status : Reported/Included.

TITLE III HAZARD CLASSIFICATIONS SECTIONS 311, 312

Acute : Yes  
Chronic : No  
Fire : No  
Reactivity : No  
Pressure : No

## LISTS:

Extremely Hazardous Substance -No  
CERCLA Hazard Substance -Yes  
Toxic Chemicals -Yes-----  
OTHER INFORMATION  
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## NFPA, NPCA-HMIS

NPCA-HMIS Rating  
Health : 1  
Flammability : 0  
Reactivity : 1

Personal Protection rating to be supplied by user depending on use conditions.

## Additional Information

"FREON" 11 contains very low levels of carbon tetrachloride and chloroform, chemicals known to the State of California to cause cancer.

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The data in this Material Safety Data Sheet relates only to the specific material designated herein and does not relate to use in combination with any other material or in any process.Responsibility for MSDS : W. J. Brock  
Address : Du Pont Chemicals  
P. O. Box 80709, Chestnut Run  
Wilmington, DE 19880-0709  
Telephone : 302-999-5072

\* Indicates updated section.

End of MSDS

WIL-189014

E. I. DuPont de Nemours & Co.

Acute Cardiac Sensitization Study  
of HFC-236ea in Dogs by Inhalation

**APPENDIX C**

Electrocardiographic Examination Report (D.K. Detweiler, V.M.D.)

M E M O R A N D U M

TO: Dennis J. Naas  
WIL Research Laboratories, Inc.  
Ashland, OH 44805-9281

FROM: Dr. D.K. Detweiler  
Suite A212, 1400 Waverly Road  
Gladwyne, PA 19035

DATE: March 14, 1995

RE: Study Number WIL-189014

Enclosed is the electrocardiographic report and/or data sheets on the above captioned study.

Sincerely,

*DK Detweiler*

D.K. Detweiler, V.M.D., M.S., D.Sc.  
Diplomate, A.C.V.I.M, Cardiology

DKD/dl

Enclosures

P.S. ( ) There were no significant changes in the electrocardiograms in this study at the \_\_\_\_\_ interval.

( ) The following electrocardiographic changes were present in this study at the \_\_\_\_\_ interval.

D. K. DETWEILER, V.M.D.  
Waverly Heights, Suite A 212  
1400 Waverly Road  
Gladwyne, PA 19035

WIL Research Laboratories, Inc.  
Study No.: 189014

ACUTE CARDIAC SENSITIZATION STUDY OF  
HFC-236ea IN DOGS BY INHALATION

METHOD

Three lead (I, II, III) electrocardiograms were taken twice pretest at 25 mm/sec. paper speed and continuously during drug trials at 10 mm/sec. paper speed.

RESULTS

All pretest electrocardiograms were within normal limits. Following epinephrine administration both in the absence and in the presence of the test gas, various electrocardiographic manifestations of epinephrine effect occurred as follows:

Increase in nomotopic heart rate.

Decrease in nomotopic heart rate.

Sinus bradycardia.

Atrioventricular (AV) nodal escape beats.

Ventricular escape beats.

Abnormal cardiac arrhythmias varying in their degree of negative effect on cardiac performance from slow ventricular or AV nodal rhythms, ventricular extrasystoles, ventricular tachycardia, multiform ventricular tachycardia (torsades de pointes) to fatal ventricular fibrillation.

ANALYSIS

The criteria described by Reinhardt, *et al.* (see Protocol page 9) were not used in this analysis because they do not distinguish between vagal escape beats, which are essentially life-sustaining, from other ectopic rhythms which can be life-threatening. The so-called escape beats serve to maintain the blood pressure when the sinoatrial nodal pacemaker activity is excessively slowed or interrupted owing to the increase in vagal tone caused by the baroreceptor reflex.

Instead the various electrocardiographic effects described in the foregoing were organized into an arbitrary scoring system to depict the relative severity of the changes with respect to cardiac function.

ARBITRARY SCORING SYSTEM

<u>SCORE</u>	<u>ELECTROCARDIOGRAPHIC CHANGE</u>
0.0	None.
0.2	Increase in heart rate accompanied by increase in positive T wave amplitude in lead II (T peaking).
0.5	Heart rate slowing with or without T peaking.
1.0	T peaking and sinus bradycardia (heart rates below 61 bpm.).
2.0	T peaking, sinus bradycardia with ventricular or AV nodal escape beats.
3.0	T peaking, sinus bradycardia, ventricular escape beats, slow AV nodal or ventricular rhythm no more than a single ventricular extrasystole.
4.0	As in 3.0 plus more than one ventricular extrasystole.
5.0	Multifocal ventricular extrasystoles and/or ventricular tachycardia.
6.0	Multiform ventricular tachycardia (torsades de pointes) and/or ventricular fibrillation.

EXPLANATION OF SCORING SYSTEM

The first scores from 0.2 through 3.0 are considered to be caused by the pharmacological action of epinephrine related to its direct stimulation of cardiac pacemaker cells and reflex vagal effects on rate and rhythm (sinus bradycardia and resultant vagal escape beats). The vagal effects result from action of the increased blood pressure on the baroreceptor reflex.

The next scores from 4 through 6 represent increasingly dangerous cardiac arrhythmias. Number 6 represents the fatal arrhythmias caused by epinephrine.

The fractional numbers 0.2 and 0.5 were used because these changes do not represent serious changes in cardiac function and therefore the scores were purposely kept low.

Table 1 lists the scores found with the various doses used in the epinephrine only trials. The smallest dose 3 microgram/kg. resulted in scores of 1 or less in seven of eight dogs. At a dose of 6 microgram/kg. the scores were 1 or less in six of eight dogs. The 9 microgram/kg. dose resulted in scores of 1 or less in five of eight dogs. The highest dose, 12 microgram/kg., caused scores of 2 or 3 in six of eight dogs. In dog 2263 two 12 microgram/kg. doses were given resulting in scores of 1 and 3 respectively. Two dogs, 2262 and 2502, had higher scores than the other dogs and dog 2308 had not scores exceeding 1. Thus, it can be seen from this table that doses of epinephrine of 12 microgram/kg. and below cause only expected pharmacological effects none of which are life-threatening. It is also obvious that sensitivity to the pharmacological action varies between dogs and in the same dog at different times, e.g., dog 2263 that received two sequential doses of 12 microgram/kg. Note, however, that the scores never exceeded 3 in this series and scores exceeding this value in the same group of dogs can be considered to represent an increase in sensitivity to the actions of epinephrine on cardiac rhythm and function. Note further that the mere occurrence of escape beats is not considered a toxic or life-threatening sign.

Tables 2 and 3 list the scores reached in response to epinephrine challenge before and during exposure to the test gas. The asterisks in each table mark the trials in which a 9 microgram/kg. rather than the 12 microgram/kg. dose of epinephrine was used. The results of responses at the lower dose will not be used in making comparisons of the responses at different gas concentrations.

In Table 2 the scores are grouped in decreasing order of the gas concentrations used and the gain or loss in score during gas exposure over that reached before gas exposure is represented as a + or - change. At a gas concentration of 100,000 PPM the response to epinephrine was markedly enhanced in all dogs and two dogs died owing to fatal cardiac arrhythmias. At the 50,000 PPM concentration the scores of four of the dogs receiving a dose of 12 microgram/kg. epinephrine during gas exposure researched 5 or 6 and one dog died. One dog (2308) did not have a toxic reaction to the 12 microgram/kg. challenge. At 35,000 PPM concentration only two of the four animals receiving the 12 microgram/kg. dose of epinephrine had dangerous reaction scores of 5. The result was approximately the same at a concentration of 25,000 PPM during which two of the five dogs had dangerous responses of 4

and 5 respectively. There was no evidence of sensitization to epinephrine at the concentration of 12,500 PPM. Curiously, at this low level two dogs had higher response scores before exposure to gas and more modest responses during gas exposure.

In Table 3 it can be seen that dog 2308 was not sensitized to epinephrine challenge at gas concentrations up to and including 50,000 PPM. Dog 2503, on the other hand, had dangerous responses (scores of 4 and 5) at all but the lowest gas concentrations.

The mean response scores pre-exposure and during exposure are summarized in Table 4. Note that mean response scores exceeded 3 at all concentrations except the lowest.

#### INTERPRETATION

In this analysis response scores of 3 or below are considered evidence of the expected pharmacological effect of epinephrine on the heart rate and rhythm caused by its direct action on cardiac pacemaker cells and its indirect effect on vagal tone caused by the baroreceptor response to epinephrine induced hypertension. Scores greater than 3 are considered evidence of dangerous effects that can or do lead to serious or fatal arrhythmias. It is evident from the results that these responses can vary between different dogs and in the same dog at different times. For example, dog 2308 had no dangerous arrhythmias to any epinephrine doses before exposure and at exposure levels up to 50,000 PPM whereas dogs 2262 and 2503 had test scores of 4 before gas exposure at the 12,500 PPM level.

Despite this variability, when the scoring results are tabulated for the group of dogs as a whole it is evident that the test article produces a concentration related sensitization to the dangerous arrhythmogenic properties of 12 microgram/kg. doses of epinephrine at all concentration levels except 12,500 PPM as summarized in Table 4.

*D.K. Detweiler*

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D.K. Detweiler, V.M.D., M.S., D.Sc.  
Diplomate, A.C.V.I.M., Cardiology

March 14, 1995

DKD/dl

TABLE 1. RESPONSES TO EPINEPHRINE ALONE

<u>DOG NO.</u>	<u>DOSE microgram/kg</u>	<u>SCORE</u>
2262	3	2
	6	2
	9	3
	12	3
2263	3	1
	6	1
	9	1
	10.5	1
	12	1 and 3
2264	3	0.5
	6	1
	9	2
	12	3
2307	3	0.5
	6	0.5
	9	1.0
	12	0.5
2308	3	1
	6	1
	9	1
	12	1
2475	3	0.5
	6	1
	9	1
	12	2
2502	3	1
	6	2
	9	3
	12	3
2503	3	0.5
	6	0.5
	9	1.
	12	2

TABLE 2. RESPONSES TO EPINEPHRINE BEFORE AND DURING EXPOSURE TO GAS AT VARIOUS CONCENTRATIONS

<u>GAS CONCENTRATION</u>	<u>DOG</u>	<u>PRE</u>	<u>DURING</u>	<u>CHANGE</u>	<u>COMMENT</u>
100,000	2262	2	5	+3	
	2264	2	5	+3	
	2475	3	6	+3	Died^
	2502	3	6	+3	Died^
	2503	2	5	+3	
50,000	2262	3	5	+2	
	2263	1	5	+4	
	2264*	3	2	-1	
	2307	1	6	+5	Died^
	2308	3	1	-2	
	2503	1	5	+4	
35,000	2262	3	3	0	
	2263	1	5	+4	
	2264*	1	1	0	
	2308	3	1	-2	
	2503	0.5	5	+4.5	
25,000	2262	3	3	0	
	2263	3	3	0	
	2264	2	5	+3	
	2308	3	3	0	
	2503	0.2	4	+3.8	
12,500	2262	3	3	0	
	2263	1	1	0	
	2264*	2	2	0	
	2308	3	3	0	
	2503	4	1	-3	

^ Died following epinephrine challenge during exposure to gas.

\* The dose of epinephrine was reduced to 9 microgram/kg. in these trials; all other doses were 12 microgram/kg.

TABLE 3. RESPONSES TO EPINEPHRINE BEFORE AND DURING EXPOSURE TO GAS AT VARIOUS CONCENTRATIONS

<u>DOG</u>	<u>GAS CONCENTRATION</u>	<u>PRE</u>	<u>DURING</u>	<u>COMMENT</u>
2262	100,000	2	5	
	50,000	3	5	
	35,000	3	3	
	25,000	3	3	
	12,500	3	3	
2263	50,000	1	5	
	35,000	1	5	
	25,000	3	3	
	12,500	1	1	
2264	100,000	2	5	
	50,000*	3	2	
	35,000*	1	1	
	25,000	2	5	
	25,000*	3	3	
	12,500*	2	2	
2307	50,000	1	6	Died^
2308	50,000	3	1	No increased sensitivity
	35,000	3	1	
	25,000	3	3	
	12,500	3	3	
2475	100,000	3	6	Died^
2502	100,000	3	6	Died^
2503	100,000	2	5	
	50,000	1	5	
	35,000	0.5	5	
	25,000	0.2	4	
	12,500	4	1	

^ Died following epinephrine challenge during exposure to gas.

\* The dose of epinephrine was reduced to 9 microgram/kg. in these trials; all other doses were 12 microgram/kg.

TABLE 4. MEAN SCORES BY GAS CONCENTRATION

<u>GAS CONCENTRATION</u>	<u>NO. DOGS</u>	<u>MEAN SCORES</u>		<u>DEATHS</u>
		<u>PRE</u>	<u>DURING</u>	
100,000	5	2.5	5.4	2
50,000	5	1.8	4.4	1
35,000	4	1.9	3.5	0
25,000	5	2.2	3.6	0
12,500	4	2.8	2.0	0