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TOXICITY OF HALON 2402

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The acute toxicity of Halon 2402 was assessed and compared to that of Halon 1211. Separate lots of Fischer-344 rats were exposed for 4 hours by inhalation to various concentrations of either Halon 2402 or 1211. The Maximum Tolerated Concentration, Approximate Lethal Concentration, and No Observable Effect Level were determined for each agent. Test animals were clinically observed daily for 14 days after exposure and euthanized. Tissues were examined for histopathological changes. This study indicates that Halon 2402 is more toxic than Halon 1211. It was difficult, however, to conclude that any pathological findings were a direct result of the test agents.

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EXECUTIVE SUMMARY

A. OBJECTIVE: The purpose of this effort was to determine the acute toxicity of Halon 2402 in laboratory animals and to compare this toxicity to that of Halon 1211.

B. BACKGROUND: Because of the interest in Halon 2402 as a potential firefighting agent, it was necessary to evaluate the toxicity of this agent and compare it to that of Halon 1211, the standard USAF agent. Therefore, the New Mexico Engineering Research Institute (NMERI), in collaboration with the University of New Mexico College of Pharmacy, evaluated the acute toxicity of Halons 1211 and 2402 in rats.

C. METHODOLOGY: Because of the high vapor pressure of halons, the route of exposure posing the highest risk is inhalation. Therefore, determining the toxic effects of acute inhalation provides an important step in understanding the health risks of the agent.

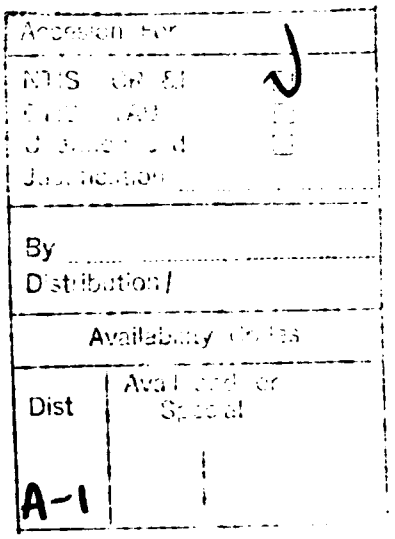
D. TEST DESCRIPTION: Separate lots of Fischer-344 rats were exposed for 4 hours by inhalation to various concentrations of Halons 1211 and 2402. The Maximum Tolerated Concentration (MTC), Approximate Lethal Concentration (ALC), and No Observable Effect Level (NOEL) were determined for each agent. The MTC is the highest concentration of agent tolerated by animals without death during the exposure period. The MTC is an essential parameter for designing a subchronic study. The ALC is the approximate concentration that produces death during exposure. The NOEL is the highest concentration at which no behavioral or clinical abnormalities are apparent during the exposure. Test animals were clinically observed daily for 14 days after the exposure period. Following the observation period, the rats were euthanized and tissues were examined for histopathological changes.

F. RESULTS: The MTC of Halon 2402 was 7.5 percent for Halon 2402 and 18 percent for Halon 1211. The ALC was 8.75 percent for Halon 2402 and

20 percent for Halon 1211. The NOEL was less than 2.5 percent for Halon 2402 and 6.4 percent for Halon 1211. The toxic effects of Halon 2402 occurred at a markedly lower concentration than did the effects of Halon 1211. The toxic effects were similar for both halons; the test animals experienced respiratory distress, hypothermia, agitation, and, at relatively high concentrations, death. Histological investigation revealed lung petechial hemorrhages in some test animals from each group examined.

G. CONCLUSIONS: It was difficult to conclude that any pathological findings were a direct result of the test agents; however, this study indicates that Halon 2402 is more toxic than Halon 1211.

H. RECOMMENDATIONS: In order to assess fully the toxicity of Halon 2402, longer exposures using repeated doses, measurements of different endpoints, and examinations of the toxic effects of dermal exposure are needed.



PREFACE

This report was prepared by the New Mexico Engineering Research Institute (NMERI), University of New Mexico, Albuquerque, New Mexico 87131, under Contract F29601-84-C-0080 (Subtask 3.18 "Toxicity of Halon 2402"), for the Headquarters Engineering and Services Center, Air Force Engineering and Services Laboratory, Tyndall Air Force Base Florida 32403.

The HQ AFESC/RDCF project officers were Mr. Bryce Mason and Captain John R. Floden. This report summarizes work done between 16 August 1985 and 30 September 1986.

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

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

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LIST OF ABBREVIATIONS

ALC	Approximate Lethal Concentration
ALD	Approximate Lethal Dosage
ARF	Animal Resource Facility
BCM	Bromochloromethane
CNS	Central Nervous System
DBM	Dibromomethane
GC-FID	Gas Chromatography - Flame Ionization Detector
MTC	Maximum Tolerated Concentration
NFPA	National Fire Protection Association
NOEL	No Observable Effects Level
OECD	Organization for Economic Cooperation and Development
SCFH	Standard Cubic Feet Per Hour
SOP	Standard Operating Procedure
TCD	Thermal Conductivity Detector

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SECTION I
INTRODUCTION

A. OBJECTIVE

The objective of this effort was to evaluate the toxicological properties of 1,2-dibromotetrafluoroethane (Halon 2402) and compare these properties to those of the fire extinguishing agent Halon 1211, which has permanent National Fire Protection Association (NFPA) standards. This study involved inhalation exposure of test animals to various concentrations of Halons 2402 and 1211 and examination of the animals for behavioral and pathological changes.

B. SCOPE/APPROACH

The scope of this effort was to determine the airborne concentration of Halon 2402 and a halocarbon of known toxicity, Halon 1211, necessary to produce acute toxic responses in laboratory animals. The Maximum Tolerated Concentration (MTC), the highest concentration of halocarbon tolerated by the animals without death, was assessed for both halocarbons. In addition, the approximate lethal concentration (ALC) and no observable effects level (NOEL) were determined for each agent. The exposure levels used here were determined during the testing period and were based initially on previous studies on these agents. A 14-day observation period with daily clinical examination of test animals followed the exposures. Histological examinations followed the observation period in control animals as well as in those exposed to the highest and lowest concentrations.

This effort was divided into two phases: Phase I included a technology review, test plan development, and procurement of equipment; Phase II involved the actual acute testing program for Halons 1211 and 2402. This report documents each phase, details the protocol for each agent, and presents the results and conclusions.

C. BACKGROUND

Halon fire suppressants are clean halogenated hydrocarbon agents in general use throughout the world. The three agents most widely available are Halon 1301 (bromotrifluoromethane), Halon 1211 (bromochlorodifluoromethane), and Halon 2402 (1,2-dibromotetrafluoroethane). The properties of these three agents are compared in Table 1.

TABLE 1. PROPERTIES OF HALON FIRE EXTINGUISHANTS.

Property	Halon 1301	Halon 1211	Halon 2402
Formula	CF_3Br	CF_2ClBr	CF_2BrCF_2Br
Molecular Weight	148.9	165.4	259.9
Boiling Point at 1 atm, ^oC	-57.8	-3.9	46.7
Freezing Point at 1 atm, ^oC	-161	-168	-111.5
Density of Liquid at 25 ^oC, g/mL	1.54	1.80	2.16
Vapor Pressure at 25 ^oC, atm	15.9	2.64	0.46

A number of studies have shown that Halon 2402 is more effective than either Halon 1301 or Halon 1211 in extinguishment of most types of fire. The higher effectiveness of Halon 2402 is due to both physical and chemical properties. Its higher boiling point, lower vapor pressure, and higher density permit a longer throw range, less dispersion during delivery, lower buoyant losses owing to fire updrafting, and improved securing ability. These characteristics make Halon 2402 particularly effective against deep-seated fires, outdoor fires in unfavorable ambient conditions, large buoyant

fires, and Class B fires, where there is danger of burnback. The larger bromine content per molecule (compared to Halons 1301 and 1211) gives Halon 2402 an improved extinguishment capability since hydrogen bromide, formed during agent use, is the primary free radical terminator effecting suppression.

Although Halon 2402 is a superior fire extinguishing agent, concerns about its potential toxicity have been raised. In an attempt to address these concerns, the present effort has been undertaken to compare the acute inhalation responses to Halon 2402 and Halon 1211.

SECTION II
PHASE I

Phase I provides a summary of known toxicity information for Halons 2402 and 1211 and the testing protocol for the determination of acute toxicity for these materials.

A. TOXICITY REVIEW

Halogenated hydrocarbons exhibit two major health effects. First, these chemicals can cause Central Nervous System (CNS) responses such as dizziness, impaired coordination, and anesthesia. Second, inhalation of halogenated hydrocarbons can cause cardiac arrhythmia (irregular heartbeat) and cardiac sensitization to epinephrine (adrenalin) (Reference 1). The possibility of serious arrhythmias is particularly noted when exposure to both halon and epinephrine occurs. Cardiac arrhythmias and CNS effects appear to be readily reversible upon termination of exposure; however, both effects can lead to death under certain conditions. The following section describes the effects of Halons 1211 and 2402.

1. Halon 1211

The Department of Environmental Medicine, Medical College of Wisconsin, conducted a study to evaluate human performance when exposed to low concentrations of Halon 1211 (Reference 2). Nineteen human volunteers were exposed to concentrations ranging from 500 to 2000 ppm for periods of 15 minutes to 1 hour. At these concentrations and exposure times, none of the subjects exhibited any problems on the performance tests or showed any adverse health effects. At 1000 ppm, some of the subjects noted the odor of the material. At 2000 ppm for 15 minutes or longer, all subjects noted the odor. Although blood and breath analyses were performed on the subjects during and after exposure, no definite toxic effects were seen from these exposure concentrations.

Beck and coworkers (Reference 3) conducted an extensive study of the effects of Halon 1211 exposure on conscious and anesthetized rats, mice, guinea pigs, dogs, and a monkey. Short-term exposures of a few minutes utilized concentrations of 5 to 30 percent Halon 1211. Long-term exposures of several hours used exposure levels of 3300 and 10,000 ppm Halon 1211. Results showed that the inhalation of Halon 1211 in the test animals had three toxicological actions. First, the CNS was affected, producing responses ranging from tremors to convulsions, depression, and, finally, death. Second, the contraction force of the heart was decreased, leading to alteration of the pulse rate. Third, Halon 1211 sensitized the heart to epinephrine, producing arrhythmia. These reactions are similar to those produced by other halogenated hydrocarbons.

Van Stee (Reference 4) compared toxic hazards of halon fire extinguishing agents based on previous published studies and original investigations. Halons 1211, 1301, and 1011 were investigated. Anesthetized, open-chested mongrel dogs, weighing over 25 kilograms each, were used for the inhalation study. Concentrations of 27 to 75 percent of Halon 1301, 4 to 12 percent Halon 1211, and 0.3 to 1.0 percent Halon 1011 were used. While not claiming to be comprehensive, this report does offer an overview of the relative toxic hazards of short-term inhalation exposure to halons. The author proposed "Exposure Criteria" for Halon 1301, Halon 1211, and Halon 1011. Criteria for the first two chemicals are given in Table 2.

The authors define little or no effect as "a slightly perceptible feeling of light-headedness with the possibility of occasional slight tingling sensations in the extremities; no cardiovascular effects, with the possible exception of a slight increase in heart rate." A moderate effect is defined as "a definite feeling of light-headedness that might be perceived by some individuals as a symptom of impending unconsciousness; tingling sensations (paresthesia) would be felt by some; heart rate would be

TABLE 2. EXPOSURE CRITERIA FOR HALONS 1301 AND 1211.[a]

	Halon 1301 % by volume	Halon 1211 % by volume
Little or no effect, 3-5 minutes	7	1.2
Little or no effect, 20 minutes	5	0.8
Moderate effect, 20 minutes	10	1.7

[a]Reference 4.

moderately accelerated and a few individuals would develop serious electrocardiagraphic abnormalities" (Reference 4). The onset of symptoms indicating a moderate effect should alert the subject to discontinue further exposure.

Orlowski et al. (References 5 and 6) studied the effect of Halon 1211 on hemotological parameters. Four groups of male rats, each group consisting of 10 animals, were used in these studies. Three groups were exposed to Halon 1211 in a dynamic chamber for 4 hours a day, 6 days a week, for periods of either 2, 4, or 12 weeks. The fourth group of animals was used as a control. At the conclusion of the exposure, the animals were euthanized, and blood from the heart ventricles was collected for analyses. Results showed that total protein and lipoprotein fractions changed with respect to the control group. No changes were found in the glycoprotein or protein fractions compared to the control group. Results showed an increase in cholesterol and phospholipid concentrations during the entire exposure times. The concentration of glyceride glycol decreased after 2 weeks, stabilized after 4 weeks, and then increased after 12 weeks. Decreases in the blood serum sodium, potassium, and calcium were reported. The chloride concentration increased at the end of a 2-week exposure, then stablized and

did not change any further relative to the control group. The significance of these findings were not discussed by the authors.

2. Halon 2402

Rainaldi, from Montecatini Edison, S.p.A, reported on a Halon 2402 toxicity study conducted by the University of Milan, Italy (Reference 7). Male Sprague Dawley rats were used to determine the Approximate Lethal Dosages (ALD) for 0, 50, and 100 percent mortality when the halon was administered by inhalation. An ALD_0 of 131,600 ppm, an ALD_{50} of 173,900 ppm, and an ALD_{100} of 216,200 ppm were reported for a 4-hour inhalation exposure. A 4-hour exposure to 47,000 ppm of Halon 2402 produced trembling and involuntary muscular contractions in the test animals but did not produce narcosis. This test program followed that proposed by the National Academy of Science.

Stewart et al. (Reference 8) studied the effects of low Halon 2402 concentrations (250 to 2000 ppm). Several physical and perceptual tests were used to quantify the performance response of eighteen male volunteers. Complete physical examinations were performed on the individuals before exposure, and all were in good health. For concentrations below 1000 ppm and up to 4-hours duration, no harmful effects were observed. Subjects initially noted a strong odor, which diminished with time. Dizziness was observed after 1-hour exposure while performing the eyes-closed modified Romberg test. Disorientation was noted after 2 minutes of exposure at 2200 ppm. Dizziness was noted first, followed by loss of equilibrium and an inability to maintain normal balance. Based on results from these limited studies, the authors recommended that exposure to Halon 2402 of 2000 ppm for more than a few seconds should be avoided. Exposure to 1000 ppm for short durations may be acceptable; for longer exposure times, a concentration of 500 ppm may be acceptable.

Zielinska-Psuja et al. (Reference 9) performed an oral chronic toxicity study on rats. Halon 2402 was orally administered for 4 days at

two different dosage levels: 2 grams and 20 grams of Halon 2402 per kilogram of body weight. The amount of halon was increased by a factor of 1.5 after 4 days with the studies lasting 10 or 33 days depending on the initial dose. The animals exposed to the lower dosage experienced a decrease in body weight after 25 days. The animals receiving the larger dose experienced a decrease in body weight throughout the entire experiment. A cumulation coefficient was calculated and indicated a weak accumulation of metatoxic effects of Halon 2402 in the experimental animals.

3. Dermal Absorption Studies of Halomethanes

Inhalation studies have been the focus of most research dealing with halons; little attention has been paid to the possibility of dermal absorption of these compounds. Personnel involved in firefighting operations may be provided with respirators, but not full-body protection, and may be dermally exposed to toxic materials in certain circumstances.

McDougal and coworkers (References 10 and 11) constructed a body-only chamber that allowed rats to be exposed to halomethanes via dermal absorption without pulmonary uptake of the toxic materials. Because of these high lipid solubility and small molecular size, dibromomethane (DBM) and bromochloromethane (BCM) were used for these studies. Fischer-344 male rats, weighing between 180 and 260 grams each, were used as test animals. Exposure concentrations of 500, 1000, 5000, and 10,000 ppm (Reference 10) and 500 to 40,000 ppm (Reference 11) were used for these test materials. Results showed that DBM was rapidly absorbed through the skin even at the lowest concentration level. The absorption increased over 100-fold between the 500 ppm and 10,000 ppm concentration levels. BCM was also absorbed very rapidly, with a 40-fold increase between the lowest and highest concentration levels. Based on these findings, the authors suggested that due to the large surface area available for absorption, the skin can offer a viable route for metabolic toxic processes. The possibility of dermal absorption has, however, not been examined using halon fire extinguishing agents now in general use.

B. ACUTE INHALATION TESTING PROTOCOL

Guidelines from the Organization for Economic Cooperation and Development (OECD) were used to develop the protocol necessary to conduct the acute inhalation testing. The animals were obtained through the University of New Mexico Animal Resource Facility (ARF). Before testing was initiated, the protocol was submitted to ARF for approval (Appendix A, Animal Use Protocol). The testing methods and materials used are discussed below. Except for the mechanisms of halon and fresh air introduction into the exposure chamber, the protocols for Halon 1211 and Halon 2402, were nearly identical. Where differences in methods occurred, they are discussed separately. In each case, the test animals were exposed to either Halon 1211 or Halon 2402 for 4 hours and were then clinically observed for 14 days. After the observation period, animals were euthanized and necropsy and histological examinations performed.

1. Experimental Animals

Young, healthy, adult male Fischer-344 rats weighing 200-225 grams and adult female rats weighing 160-185 grams were exposed to Halon 2402. Only healthy, young male (150-200 grams) Fischer-344 rats were used for exposure to Halon 1211. The animals were acclimated to the laboratory environment for at least 5 days prior to the exposure. Two rats were randomly housed in each plastic cage. Water, food, and aspen bedding were replaced daily. The temperature in the animal housing area was maintained at 22 ^{o}C and the relative humidity was maintained at 40 to 60 percent. Each animal was weighed, marked with an identification number, and examined for general health status before being placed in the inhalation chamber. Five male and five female rats were exposed at each concentration level of Halon 2402. Five male rats were exposed at each concentration of Halon 1211. Prior to and following the exposure, the animals for each exposure level were kept as a group (two or three rats per cage). The males and females were exposed separately. The rats had free access to water and conventional rat food except during the exposure period.

2. Exposure Material

a. Halon 2402

Halon 2402 was analyzed for contamination using a Hewlett Packard Model 5780 Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID). A contaminant identified as diisooctylphthalate, a plasticizer, was found in the Halon 2402 obtained directly from the shipping barrels. Therefore, Halon 2402 was distilled using a 30-inch vigraux fractionation column located between the distillation flask and the condenser to remove any plasticizer (Figure 1). The diisooctylphthalate concentration was monitored using a GC-FID, and a standard curve was developed by serial injections of known concentrations. If the concentration of the phthalate reached 50 ppm or above, the halon was redistilled or new distilled halon was introduced.

b. Halon 1211

Halon 1211 was used as received from the manufacturer without further purification.

3. Exposure Method

a. Halon 2402

Each exposure was 4-hours long, commencing at the time the desired concentration was reached. Purity, temperature, and humidity of the air stream were controlled by a Compucool air filtration system. Fresh air was drawn into the Compucool and purified, and the temperature and humidity were adjusted. The air stream was divided at this point. Approximately two-thirds of the stream was directed to a mixing chamber, and the rest was directed to the halon introduction mechanism. The temperature inside the

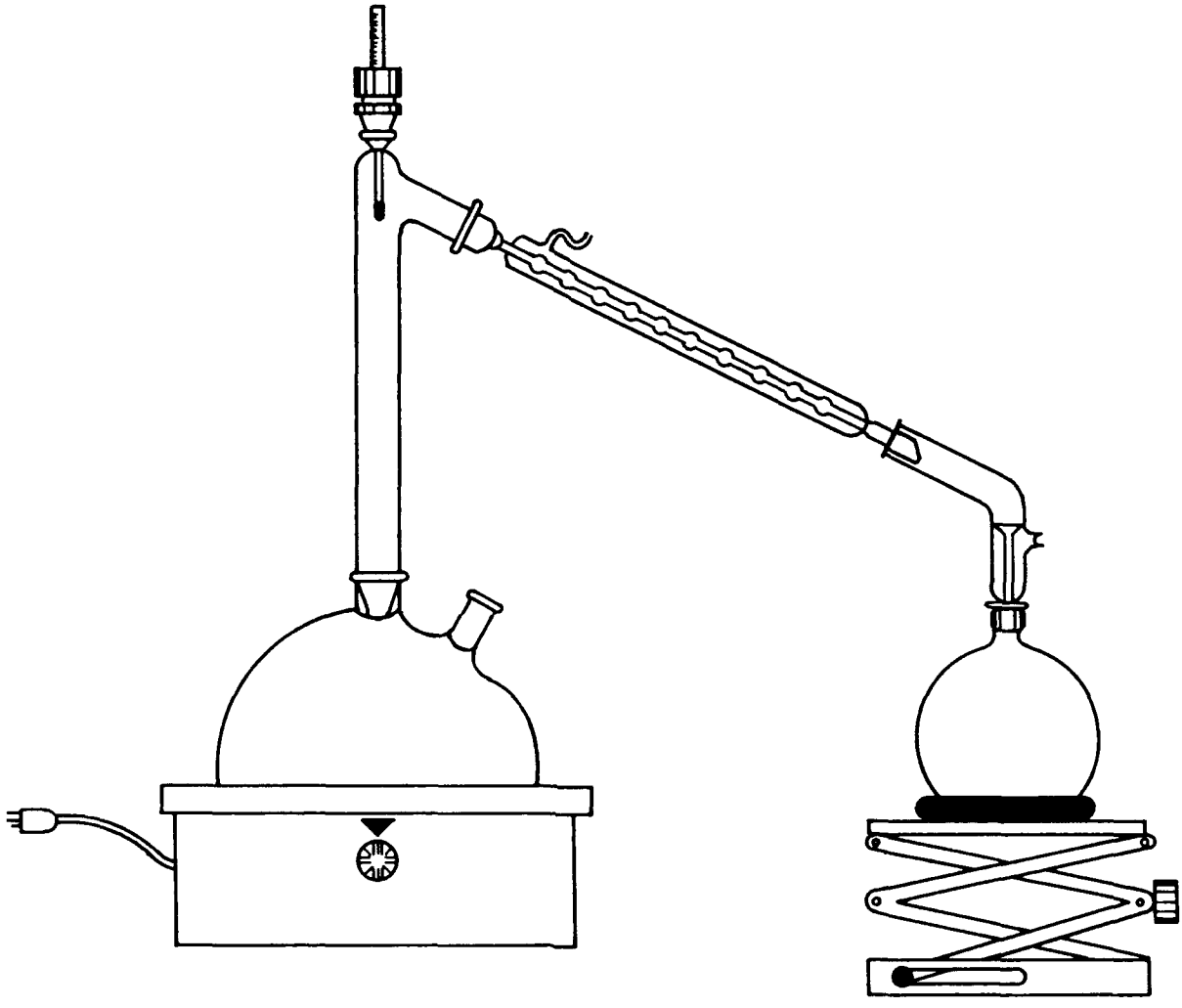

Figure 1. Halon 2402 Distillation Apparatus.

exposure chamber was maintained at 22 ± 2 °C. Since animal body heat was sufficient to increase the chamber temperature, ice water was pumped through a coil placed within the chamber to maintain the desired temperature. The relative humidity was maintained between 30 and 70 percent. The desired Halon 2402 concentration in the chambers was produced by mixing purified air with halon saturated air. The air was saturated with halon by passing it through a series of three constant-temperature halon evaporators. The exposure system is diagrammed in Figure 2.

The total rate of air flow through the chamber (16 to 20 liters per minute) was sufficient to produce 12 to 15 complete exchanges per hour. The air flow rate was monitored every 15 minutes at the beginning of each exposure and every hour once the system was stablized. Before the inflow of air was adjusted, the air supply to the evaporators was set and maintained at 3 pounds per square inch. The halon-saturated air was supplied to the gas-mixing prechamber and was mixed with a sufficient amount of purified air to produce the desired final halon concentration. A Dwyer 0.5-5 SCFH (Standard Cubic Feet per Hour) or 1-10 SCFH flowmeter, depending on the desired halon concentration, was used to monitor the halon-saturated air that was supplied to the mixing prechamber. The purified air supplied to the mixing chamber was monitored using a Dwyer 10 to 100 SCFH flowmeter. All flowmeters were carefully calibrated and corrected daily for variances in temperature and atmospheric pressure.

Frequent monitoring of the final halon concentration within the chamber ensured that the exposure concentration was that desired. This was accomplished by withdrawing an 8- to 10-mL sample from a septum port located at the top center of the exposure chamber into a gas-tight syringe and injecting this material into a GC equipped with a Thermal Conductivity Detector (TCD). The area under the curve was determined and compared with a standard curve of peak area versus concentration. The standard curve, generated by analysis of known air-halon mixtures, was calibrated daily

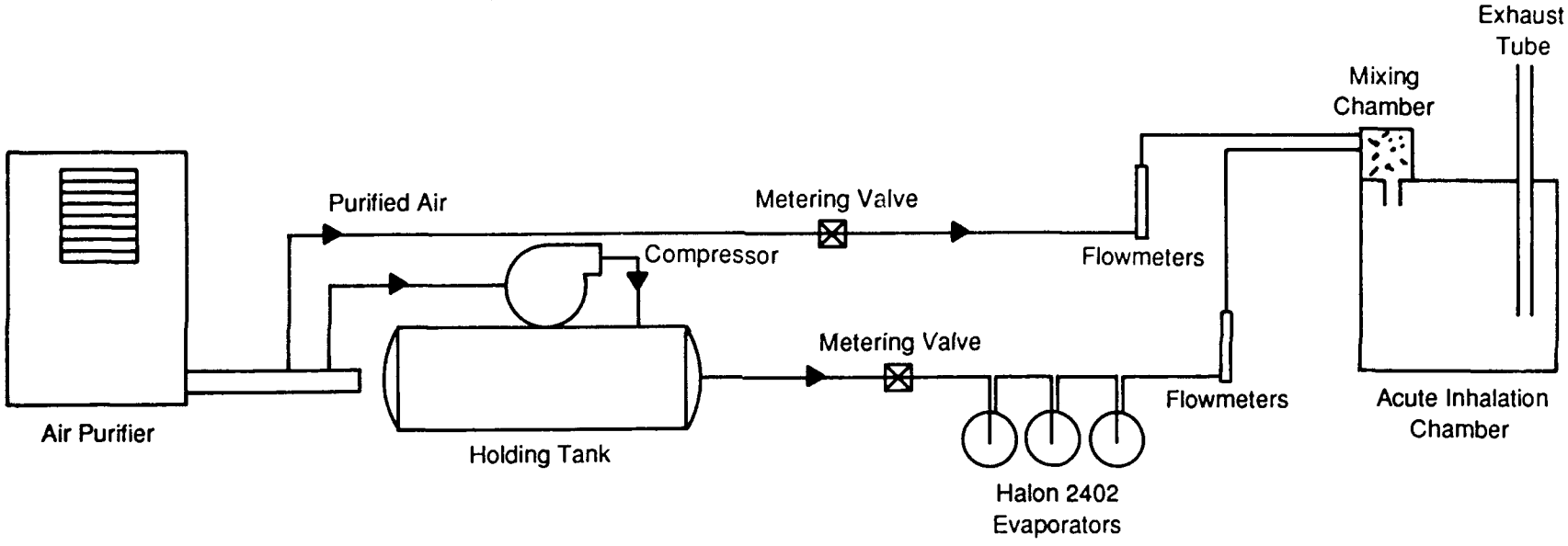

Figure 2. Halon 2402 Exposure System.

using air-halon mixtures corresponding to the halon concentration in the exposure chamber. A deviation from the desired concentration of up to ± 0.5 percent was considered acceptable. If the concentration fell outside the specified range, the flow rate of halon-saturated air was corrected.

b. Halon 1211

The exposure time for Halon 1211 was the same as that for Halon 2402. The temperature and humidity inside the chamber were again maintained at 22 ± 2 ^{o}C and between 30 and 70 percent, respectively. Air and oxygen from pressurized tanks were used as the clean air source. Oxygen was added to maintain 19 percent O_2 when large concentrations of halon were used. Halon 1211 was contained in a cylinder fitted with a regulator. Vaporization of the halon was achieved by wrapping the cylinder with heating tape. Halon 1211, breathing air, and oxygen were combined in a mixing chamber to give the required concentrations before introduction into the acute inhalation chamber. Figure 3 depicts the exposure system for Halon 1211.

The Halon 1211 concentration was monitored in the same manner, with the same frequency, and with the same equipment as used for Halon 2402. Standard curves were also developed for this material and were used to determine the concentration inside the chamber.

4. Assessment of Toxicity

The objective of the acute phase of the study was to establish the MTC, ALC, and NOEL of Halon 2402 and 1211 in Fischer-344 rats. The MTC was that which was tolerated for the 4-hour exposure period without lethality during exposure or within 14 days after exposure. The ALC was the estimated concentration that produced lethality during exposure. The NOEL was the

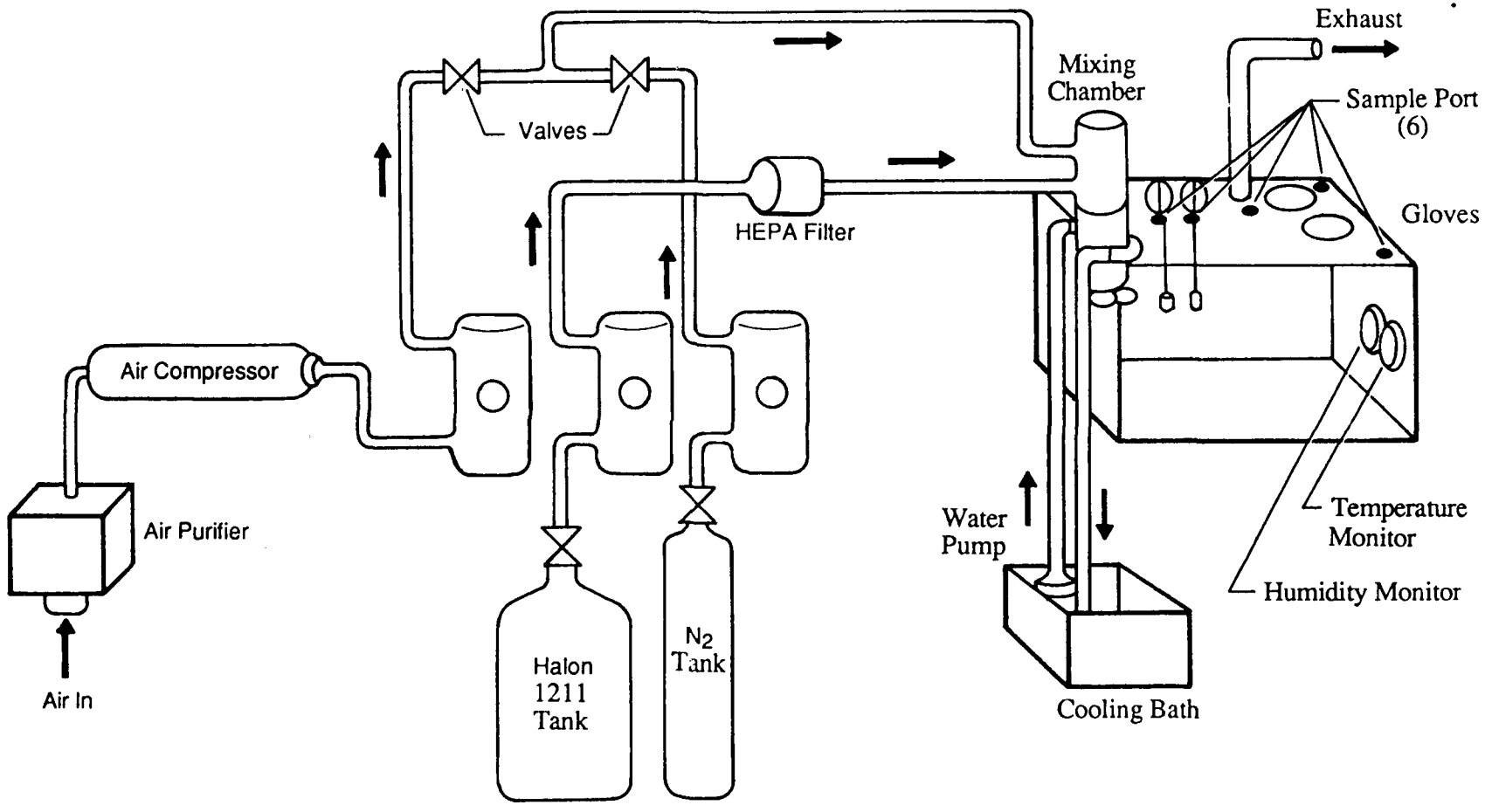

Figure 3. Halon 1211 Exposure System.

level of halon at which no notable alterations in behavior or clinical findings were observed in the test animals during exposure. The appearance and behavior of the animals during segments of the exposure period were recorded on video tape for reference. The animals were clinically observed during the exposure period and daily thereafter for 14 days. These clinical observations were carried out in compliance with the recommendations of a certified Veterinary Pathologist.

At the termination of the 14-day post-exposure observation period, the animals exposed to the highest, lowest, and control concentrations of Halon 2402 and the MTC and control levels of Halon 1211 were euthanized and a necropsy conducted under the direction and supervision of a certified Veterinary Pathologist according to standard procedures (Appendix B). An example of a gross necropsy results form is presented in Appendix C.

SECTION III
PHASE II

Phase II reports the results and conclusions obtained during the acute testing of Halon 2402 and Halon 1211 in Fischer-344 rats.

A. RESULTS

1. Halon 2402

Groups of five males and five females were exposed at concentration levels of 0, 2.5, 5.0, 7.5, or 10 percent. The maximum level of Halon 2402 tolerated for the entire 4-hour exposure without lethality was 7.5 percent (Table 3). At a 10-percent concentration, body temperature depression and respiratory depression necessitated termination of the exposure in the male rats after 37 minutes and the female rats after only 10 minutes. Based on these data, the ALC of Halon 2402 to Fischer-344 rats is estimated to be 8.75 percent.

Although lethality was not produced at 7.5 percent Halon 2402, the animals were severely stressed and would not have been likely to survive a longer exposure at that concentration. At 5 percent, the animals were stressed to a similar degree, while at a concentration of 2.5 percent, the animals were only partially incapacitated, their respiration was adequate, and their body temperatures were only slightly depressed. Therefore, the NOEL is less than 2.5 percent.

Sex differences in the acute toxic effects of Halon 2402 were observed. Following a 4-hour exposure to 5 or 7.5 percent, and after the 14-day observation period, female, but not male, rats showed significant weight loss compared to the control rats. The mean weight changes are summarized in Table 4. Since the exposure to 10-percent Halon 2402 was

TABLE 3. HALON 2402 CONCENTRATIONS AND CORRESPONDING CLINICAL EFFECTS.

Halon concentration, % by volume	Effects observed
2.5	Slight hypothermia
5.0	Stressed/respiratory depression
7.5	Stressed/respiratory depression/hypothermia
10.0[a]	Severe respiratory depression/severe hypothermia

[a]Animals exposed to this concentration were unable to withstand a 4-hour exposure period; therefore, the test was ended prematurely.

TABLE 4. MEAN BODY WEIGHT GAIN OR LOSS FOLLOWING EXPOSURE OF RATS TO HALON 2402.[a]

Sex	Control	2.5 Percent	5 Percent	7.5 Percent	10 Percent
Male	+26.0 ± 8.6	+12.3 ± 3.4	+ 4.8 ± 5.6	+18.9 ± 2.4	[b]
Female	+ 5.7 ± 18.5	+12.1 ± 2.8	-13.9 ± 13.9	-13.5 ± 0.9	[b]

[a]Numerical values denote mean weight changes plus or minus the standard deviation for each exposure group (5 rats per group). Positive values are indicative of weight gain. The numbers were calculated by subtracting the body weights immediately prior to exposure from the weights 14 days after exposure.

[b]These animals were exposed to the Halon 2402 for a much shorter time period than were the animals in the other treatment groups (37 and 10 minutes, respectively, for males and females). Therefore, comparison of these data with other treatment groups is not meaningful.

terminated at less than 4 hours (37 minutes for male rats, 10 minutes for females), these exposure levels were apparently not of sufficient duration to produce weight loss in either males or females.

The animals showed behavioral signs of stress, with the females again being the most profoundly affected. The female rats exposed to 5 or 7.5 percent Halon 2402 were withdrawn and lethargic, with oversensitive reactions to physical stimulation (prodding) for one to two days following exposure. Male rats showed a similar reaction, but only after the 7.5 percent exposure, and the effect lasted for only one day.

Necropsies were performed on the animals exposed to the lowest and highest concentrations of Halon 2402 and on the control animals. The only marked pathological finding was petechial hemorrhages in the animals exposed to 7.5 percent 2402 for 4 hours (Table 5). The pathology was most severe in the female rats. However, it cannot be concluded that this pathology is the result of exposure to the test material, since petechial hemmorhages were found in two of the control males, one of the control females, and three males exposed to 2.5 percent Halon 2402. Although the hemorrhages occurred more frequently in the treated animals, more animals would be needed for conclusive data.

2. Halon 1211

Under conditions nearly identical to those used for exposure to Halon 2402, the MTC, ALC, and NOEL for Halon 1211 were determined in order to compare the toxicity of the two halons. Groups of five male rats were exposed to concentrations levels of 0, 3.2, 6.4, 12.9, 18, and 24.4 percent.

At concentrations of 3.2 percent and 6.4 percent, no clinical signs or syptoms were observed following exposure to Halon 1211 (Table 6). At concentrations higher than 12.9 percent, tremors, respiratory depression,

TABLE 5. INCIDENCE OF HISTOPATHOLOGICAL CHANGES IN RATS EXPOSED TO HALON 2402.

Halon concentration, % by volume	Finding	Incidence
0	Lung petechial hemorrhage	3
0	Eye redness enlargement	1
2.5	Lung petechial hemorrhage	3
7.5	Lung petechial hemorrhage	6
7.5	Kidney abnormality	1

and hypothermia were observed in all the animals exposed. Since two rats died during the first hour of the 4-hour exposure to 24.4 percent, the exposure period was terminated early in the remaining animals. Therefore, the ALC is 20 percent for Halon 1211. The MTC and the NOEL were determined to be 18 percent and 6.4 percent, respectively.

Following a 4-hour exposure to Halon 1211, no significant decrease in body weight gain was observed in exposure groups until concentrations exceeding 18 percent were reached (Table 7). At 18 percent and 24.4 percent Halon 1211 concentrations, significant decreases in body weight gain were observed.

The incidence of histopathological changes was determined after the 14-day observation period in control animals and in those exposed to 18 percent Halon 1211. Two out of ten rats in the 18-percent Halon 1211 exposure group had mild centrilobular liver necrosis and mononuclear cell infiltration (Table 8). Four experimental animals in the 18 percent Halon 1211 exposure group developed lung petechial hemorrhages, whereas only two control animals developed similar types of lesions. It is unclear whether

these findings were directly related to the test substance exposure since consistent results were not noted for either group of animals on which a necropsy was conducted.

TABLE 6. HALON 1211 CONCENTRATIONS AND CORRESPONDING CLINICAL EFFECTS.

Halon concentration, % by volume	Effects observed
3.2	None
6.4	None
12.9	Tremor/respiratory depression
18.0[a]	Tremor/respiratory depression
24.4[b]	Hypothermia/death

[a]Body temperature: 34 ^{o} C.
[b]Body temperature: 32 ^{o} C.

TABLE 7. BODY WEIGHT GAIN OR LOSS FOLLOWING EXPOSURE OF MALE RATS TO HALON 1211.

Halon concentration, % by volume	Weight loss or gain[a]
0.0	+25.9 ± 2.05
3.2	+21.6 ± 1.30
6.4	+27.5 ± 0.68
12.9	+28.1 ± 1.60
18.0	+1.60 ± 0.68[b]
24.2	c

[a]Numerical values denote mean weight changes plus or minus the standard deviation for each exposure group (5 rats per group). Positive values are indicative of weight gain. The numbers were calculated by subtracting the body weights immediately prior to exposure from the weights 14 days after exposure.

[b]Significantly different from control group at $P < 0.05$ using Analysis of Variance.

[c]These animals were exposed to Halon 1211 for a much shorter period. Therefore, a meaningful comparison with the other groups is not possible.

TABLE 8. INCIDENCE OF HISTOPATHOLOGICAL CHANGES IN MALE RATS EXPOSED TO HALON 1211.

Halon concentration, % by volume	Finding	Incidence
0	Lung petechial hemorrhages	2
18.0	Lung petechial hemorrhages	4
18.0	Liver necrosis and infection	2

B. CONCLUSIONS

Toxic effects of Halon 2402 occur at a markedly lower concentration than do the effects of Halon 1211. The MTC of Halon 2402 is 7.5 percent, whereas the MTC of Halon 1211 is 18 percent. The toxic effects are similar for both halons; the animals experience respiratory dysfunction, hypothermia, agitation, and death at relatively high concentrations. Significant weight loss was noted in female rats exposed to high concentrations of Halon 2402 (7.5 percent). A lower weight gain was found in males exposed to high concentrations of Halon 1211 (7.5 percent) and Halon 2402 (18 percent) compared to control animals. Histological examination, showed similar pathological findings in animals exposed to both halons. Animals exposed to either Halon 2402 or Halon 1211 had lung petechial hemorrhages; however, some control animals also showed signs of such lesions. Petechial hemorrhages occurred at much lower concentrations in the animals exposed to Halon 2402 than in those exposed to Halon 1211.

In order to assess fully the toxicity of Halon 2402, longer exposures using repeated doses, measurements of different end points, and examinations of the toxic effects of dermal exposure are needed. A test plan for a subchronic toxicity evaluation is presented in Appendix D.

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APPENDIX A

ANIMAL USE PROTOCOL

PROJECT TITLE: TOXICITY STUDIES OF HALON 2402 (DIBROMOTETRAFLUOROETHANE) IN RATS.

1. Research Objectives. Due to recent deaths among firefighting personnel using halon fire extinguishing agents, it has become necessary to re-evaluate the toxic properties of these agents. The U. S. Air Force has contracted the New Mexico Engineering Research Institute to carry out a two-phase study to evaluate the toxicity of Halons 1211 and 2402 in rats resulting from acute (4-hour) inhalation exposure. This study is to be carried out in collaboration with University of New Mexico College of Pharmacy.

2. Animal Type and Number. Both phases of the study will be carried out using male and female Fischer-344 rats. In the acute studies, as many as 10 rats will be used for each exposure level, and it is expected that as many a 5 exposure levels may be utilized. In order to evaluate the two agents, the acute studies may require 100 rats, and possible as many as 140.

3. Animal Need. Studies carried out to determine the systemic effects of inhaled agents must be done in live animals. The Fischer-344 rat was chosen as the animal model because it is relatively inexpensive, and because this particular strain has been widely used in similar studies. We had also considered the guinea pig as a animal model, but the rat was selected as the animal of choice.

4. Procedures and Methodology.

A. Acute Studies. Groups of up to 10 animals will be exposed to test materials at the highest initial concentration that will not be expected to produce toxic effects. The concentration will then be doubled with each subsequent exposure until potential lethality is seen. The concentration

below this level will be designated as the Maximum Tolerated Concentration (MTC). Animals will be monitored closely during the exposures, and the animals will be removed from the chambers if they appear to be suffering in any detectable way. The literature indicates that the maximum tolerated levels of the test materials will range between 1 and 7 percent in the air, due to the CNS depressant actions of these agents. At the end of the exposure period, all surviving rats will be euthanized, a necropsy will be performed, and histopathology will be done on selected organs.

5. Methods of Euthanasia. The rats will be anesthetized with CO_2 and sacrificed by exsanguination through the auxillary artery.

APPENDIX B

STANDARD OPERATING PROCEDURES

1.0 GROSS NECROPSY

1.1 Purpose--This Standard Operating Procedure (SOP) describes the procedures for gross necropsy examination of rats, the method of recording the gross findings, and the tissues to be sampled for histopathology.

1.2 Equipment and supplies--

Dissection instruments (forceps, scalpels, scissors)
Latex necropsy gloves, disposable
Formalin containers (300-500 mL vol.)
10 percent buffered formalin solution
Tape and felt-tipped pens for labeling containers
"Gross Necropsy Results" forms
2 cork dissecting boards and pins
Suture material for ligation of trachea
Lung perfussion apparatus
Clip boards
Aprons, disposable
Euthanasia apparatus
Balance for recording body weights

1.3 Body weights--Rats are weighed and the terminal body weight is recorded prior to necropsy examination.

1.4 Euthanasia--Live rats to be examined are killed by CO_2 anesthesia and exsanguination. Necropsy is begun immediately after euthanasia.

1.5 Preservation of rats that die naturally--Rats that die naturally are refrigerated (not frozen) as soon as possible after death. Necropsy is performed within 12 hours after being found.

1.6 Dissection and specimen collection--The skin, mammary glands, eyes and eyelids, oral cavity, and anus are examined visually.

A mid-ventral skin incision is made from the pubis to the mandible and the skin is reflected to expose the body wall. The abdominal wall is opened longitudinally and the ventral half of the rib cage is removed. The skeletal musculature, subcutis, pleural cavity, and peritoneal cavity are examined visually.

The trachea and esophagus are cut in the mid-cervical region and the distal trachea, distal esophagus, heart, aorta, and thymus are removed as a unit and examined visually. The lungs and distal trachea are freed from the other organs, and the lungs are perfused with formalin solution via the trachea. The perfusion pressure is 30 cm water. The trachea is ligated; and the lungs are placed into the formalin container.

The proximal trachea and larynx are opened longitudinally and the mucosa is examined visually. The thyroids, salivary glands, lachrymal glands (exorbital) and submandibular lymph nodes are examined visually.

The liver is examined visually and samples approximately 5 mm thick are taken for histopathology from the right and left lobes.

The spleen is examined visually.

Both kidneys and both adrenals are examined visually. A transverse sample 5 mm thick is taken for histopathology from the center of the right kidney.

The stomach is opened and the mucosa is examined visually. The small intestine, cecum, and rectum, are examined visually. They are not opened. The pancreas is examined visually.

The gonads and uterus/accessory genital organs are examined visually.

The unopened urinary bladder is examined visually.

The dorsal aspect of the cranium is removed to expose the brain. The brain is examined visually in situ.

Samples for histopathology are taken from all gross lesions observed.

1.7 Records--A "Gross Necropsy Results" form is filled out for each rat (Appendix C). Any abnormalities found are described on the back of this form. At the end of the necropsy examination this form is initialed and dated by the pathologist and the prosector.

2.0 HISTOPATHOLOGHY SOP

2.1 Purpose--The purpose of this SOP is to describe the method of preparation of tissue sections, to list the tissues to be examined microscopically, and to describe the slide reading procedure.

2.2 Equipment and supplies--Supplies, reagents, and equipment are provided by the histology laboratory that is to prepare the slides.

2.3 Fixation--Tissues are fixed for a minimum of 48 hours in 10 percent buffered formalin solution. A minimum of 10 volumes of solution are used to fix the tissues from each rat.

2.4 Tissue cutdown--Tissues are trimmed to a thickness of approximately 3 mm and are placed into embedding cassettes. The following tissues are trimmed in from each rat:

1. Lung--Sections through the right cranial, right middle, and right caudal lobes along the axes of major airways. A single sagital section through the left lobe.

2. Liver--Single section from right lobe.

3. Kidney--Single transverse section from right kidney.

4. Gross lesions--Section through any gross lesion not included in the above sections.

2.5 Preparation of stained tissue sections--The paraffin infiltration, paraffin embedding, sectioning, and staining of tissue specimens are done according to normal procedures. The manual of standard operating procedure for tissue processing is available in that laboratory. Tissue sections are routinely stained with hematoxylin and eosin (H & E). Special stains are done as required by the pathologist.

2.6 Microscopic evaluation of tissue sections (histopathology)--All tissue sections are examined microscopically by an A.C.V.P. certified Veterinary Pathologist. All microscopic lesions found are described, and a list of microscopic lesions is prepared for each rat.

APPENDIX C

ACUTE INHALATION TOXICITY STUDY
GROSS NECROPSY RESULTS

Pathologist VT 9/2/86
Prosector

Rat number 5
Group 75% , 4 hr & 8/14 ♀
Time & date of death 9:17 9/2/86
Time & date of necropsy 9:17 9/2/86
Manner of death CO2 exsang.
Body weight 136.2 gm

*	Organ/Tissue	*	Organ/Tissue
✓	Skin	✓	Trachea
✓	Mammary glands	✓	Liver
✓	Subcutaneous tissue	✓	Kidneys
✓	Eyes and eyelids	✓	Adrenals
✓	Skeletal muscle	✓	Accessory genital organs
✓	Submandibular lymph node	✓	Urinary bladder
✓	Salivary gland	✓	Gonads
✓	Lachrymal gland	✓	Spleen
✓	Esophagus	✓	Stomach
✓	Lungs	✓	Pancreas
✓	Heart	✓	Small intestine
✓	Aorta	✓	Cecum
✓	Thymus	✓	Colon
✓	Thyroids	✓	Rectum
✓	Pleural cavity	✓	Anus
✓	Peritoneal cavity	✓	Brain
✓	Larynx	✓	Uterus
✓	Oral cavity		

EXAMPLE

* Normal = ✓, Abnormal = A. All gross lesions described on back.

NOTE:
Lung, liver, kidney and organs with gross lesions are taken for histo.

APPENDIX D
TEST PLAN FOR SUBCHRONIC TOXICITY EVALUATION (PHASE III)

Objective:

To evaluate the subchronic toxicity of Halon 2402 in Fischer-344 white rats.

Experiments:

The concentrations to be used for the subchronic 90-day study are determined from the acute Maximum Tolerated Concentration (MTC) for a 4-hour exposure in Fischer-344 rats. The MTC is the maximum dose tolerated by the animals without death. Based on a MTC of 7.5 percent determined in the acute toxicity study, subchronic exposure concentrations must be below this level.

Healthy, young adult Fischer-344 rats will be used in the subchronic study. The rats will be housed in the University of New Mexico's Animal Resource Facility prior to exposure, then acclimated to the laboratory exposure environment before the subchronic exposures begin. The rats will be separated by gender and housed two or three to a cage. Food and water will be provide ad libitum during nonexposure times.

Animals will be randomly divided into five exposure groups consisting of 24 animals per group (12 females and 12 males). Three of the five groups will be exposed to Halon 2402 at concentrations of 0.5 percent, 1 percent, and 2 percent for 8 hours per day, 5 days per week, for 90 days. Another group will be used as an exposure control group, and the last group will be a necropsy and histopathology control group. During the exposure period, the animals will be allowed free access to water but food will be withheld.

The rats will be individually weighed weekly; clinical observations will be made at the beginning of each exposure day.

The Halon 2402 will be purified by distillation through a 30-inch Vigreax column. The concentration of contaminants will be monitored by GC-FID. The temperature and relative humidity will be monitored hourly during the exposure period. The temperature inside the exposure chamber will be maintained at 22 $\pm$ 3 ^{o}C, and the relative humidity will remain between 30 and 70 percent.

The total air flow rate through the chambers will be sufficient to produce 12 to 15 air exchanges per hour. This requires 16 to 20 liters per minute of total air flow as determined by summing the fresh air supply and the halon-saturated air flow to each chamber.

At the end of the 90-day exposure period, the rats will be euthanized, blood samples will be taken for clinical biochemistry, a necropsy examination will be performed, and vital organs will be submitted for tissue histopathology.

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