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VOLUME II

ARMSTRONG LABORATORY

GENETIC TOXICITY EVALUATION OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE VOLUME II OF III: RESULTS OF *IN VIVO* MOUSE BONE MARROW ERYTHROCYTE MICRONUCLEUS TESTING

A. D. Mitchell

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January 1995

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FINAL REPORT FOR THE PERIOD MARCH THROUGH DECEMBER 1994

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TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1995-0008
VOLUME II

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

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

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

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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12a. DISTRIBUTION/AVAILABILITY STATEMENT

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12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words) Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated, examined the potential of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce structural chromosome aberrations in erythropoietic cells of the bone marrow. Genesys used the mouse micronucleus test which measures the clastogenic (chromosome breaking) action of chemicals by the induction of micronuclei in bone marrow cells, as observed in erythrocytes from the peripheral blood of male and female mice obtained approximately 24 hours after steady-state dosing.

Based on preliminary toxicity information obtained by ManTech, a mouse bone marrow micronucleus test of HFC-227ea was conducted using 2.6%, 5.3%, and 10.5% HFC-227ea, administered to male and female Swiss Webster mice by inhalation for six hours on each of three consecutive days. Bone marrow cells were obtained from the mice which were sacrificed 24 hours after the third exposure. Cells from mice exposed to the test material, and to the negative and positive controls, were evaluated for toxicity and the presence of micronuclei. The positive control, 0.4 mg triethylenemelamine (TEM)/kg (administered intraperitoneally) significantly (p,0.001) elevated the number of micronuclei in newly-formed erythrocytes (PCEs, polychromatic erythrocytes) from male and female mice.

Neither criterion for a positive response-a dose-related increase in micronuclei and the induction by one or more of the doses of a statistically significant ($p<0.05$) increase in micronuclei induction-were observed in male and female mice. Therefore, HFC-227ea was evaluated as negative in the mouse bone marrow micronucleus test. Hence, HFC-227ea was not clastogenic *in vivo*.

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PREFACE

The U.S. Air Force is investigating chemical replacements for the fire suppressant/extinguishant Halon 1301. 1,1,1,2,3,3,3-Heptafluoropropane (HFC-227ea) has excellent solvent properties and may serve as a "drop in" extinguishant replacement. Results from laboratory animal *in vivo* studies indicate that HFC-227ea has a low order of acute toxicity. A comprehensive literature search indicated that no information was available on the mutagenic potential of HFC-227ea. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of HFC-227ea. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing Guidelines.

This document, Volume II of III, serves as a final report detailing the results of the *in vivo* mouse bone marrow erythrocyte micronucleus testing in the genetic toxicity evaluation of HFC-227ea. Volumes I and III will describe, respectively, the results of the *salmonella typhimurium* histidine reversion assay (Ames assay) and the results of the forward mutation assay using L5178Y mouse lymphoma cells.

The research described herein began in March 1994 and was completed in December 1994 by Genesys Research, Inc., Research Triangle Park, NC under a subcontract to ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F30). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

SUMMARY

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated examined the potential of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce structural chromosome aberrations in erythropoietic cells of the bone marrow. Genesys used the mouse micronucleus test which measures the clastogenic (chromosome breaking) action of chemicals by the induction of micronuclei in bone marrow cells, as observed in erythrocytes from the peripheral blood of male and female mice obtained approximately 24 hours after steady-state dosing.

Based on preliminary toxicity information obtained by ManTech, a mouse bone marrow micronucleus test of HFC-227ea was conducted using 2.6%, 5.3%, and 10.5% HFC-227ea, administered to male and female Swiss Webster mice by inhalation for six hours on each of three consecutive days. Bone marrow cells were obtained from the mice which were sacrificed 24 hours after the third exposure. Cells from mice exposed to the test material, and to the negative and positive controls, were evaluated for toxicity and the presence of micronuclei. The positive control, 0.4 mg triethylenemelamine (TEM)/kg (administered intraperitoneally) significantly ($p < 0.001$) elevated the number of micronuclei in newly-formed erythrocytes (PCEs, polychromatic erythrocytes) from male and female mice.

Neither criterion for a positive response—a dose-related increase in micronuclei and the induction by one or more of the doses of a statistically significant ($p < 0.05$) increase in micronuclei induction—were observed in male and female mice. Therefore, HFC-227ea was evaluated as negative in the mouse bone marrow micronucleus test. Hence, HFC-227ea was not clastogenic *in vivo*.

GENESYS RESEARCH INCORPORATED
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

IN VIVO MOUSE BONE MARROW ERYTHROCYTE MICRONUCLEUS TESTING OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea)

Genesys Research Incorporated's portion of the above titled study was reviewed for compliance with Quality Assurance (QA) regulations and with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The practices used in the study were found to be in compliance with these regulations.

Ann D. Mitchell 12/17/94

Ann D. Mitchell, Ph.D. Date
Study Director

GENESYS RESEARCH INCORPORATED
QUALITY ASSURANCE STATEMENT

With the exception of animal husbandry and exposure of the mice to the test material and the handling, storage, dilution (for exposure of the mice) and analytical chemistry of the test material, which were the responsibility of ManTech Environmental Technology, Incorporated, the data and the report for the following study carried out at Genesys Research, Incorporated has been reviewed and approved for compliance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The final report accurately describes the methods that were used and accurately reflects the raw data of the study.

ManTech Environmental Technology, Incorporated Study Number: 1093-F30

Genesys Research, Incorporated Study Number: 93037

Type of Study: Mouse Bone Marrow Erythrocyte Micronucleus Test

Protocol Signed by Study Director: March 19, 1994

Date Testing Started at Genesys: May 5, 1994

Critical Phase Audit(s): May 9 and 30, 1994

Date Testing Completed: June 19, 1994

Date Draft Report Audited: September 6 and 8, 1994

Date Audit Findings Reported to Management: May 9 and 30 and September 9, 1994

Approved: Helen M. King
Helen M. King, B.S.
Quality Assurance Officer for Genesys

Date: 12/17/94

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

Study Director: Allen Ledbetter

ManTech Environmental Technology's portion of this study was conducted in accordance with EPA Good Laboratory Practice Regulations (GLP) as set forth in the Code of Federal Regulations (40 CFR 792). There were no significant deviations, in the work conducted by ManTech, from the aforementioned GLP regulations that would have affected the integrity of the study or the interpretation of the test results. The ManTech generated raw data have been reviewed by the Study Director, who certifies that the information contained in this report represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria. Deviations are listed below:

1. The sponsor was responsible for the test substance characterization, stability and homogeneity analysis.

All original ManTech generated raw data are retained in the ManTech Environmental Technology's Archives, at 5 Triangle Drive, Research Triangle Park, NC 27709, with a copy of the final study report.

SUBMITTED BY:

Study Director:

Allen Ledbetter 12/16/94

Allen Ledbetter Date

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED QUALITY ASSURANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

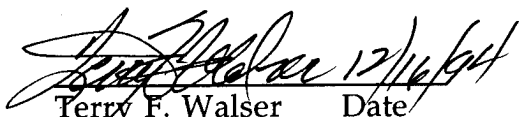
Study Director: Allen Ledbetter

Report Audit Dates:

This study has been subjected to inspections and the report has been audited by ManTech Environmental Technology's Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect ManTech's raw data. ManTech's raw data and a copy of the final report will be stored in room 210 in the MET building at Research Triangle Park, NC. The sponsor was responsible for the Iodotrifluoromethane characterization, stability and homogeneity analyses.

The following are the inspection dates, and the dates inspection reports were submitted:

Phase(s)	Date(s) of Inspection	Report Submitted to Study Director	Report Submitted to Management
Exposure	5/11/94	5/11/94	5/11/94
Protocol	8/2/94	8/2/94	8/2/94
Data Review	12/15/94	12/16/94	12/16/94



Terry F. Walser Date
Quality Assurance Officer

IN VIVO MOUSE BONE MARROW ERYTHROCYTE MICRONUCLEUS TESTING OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea)

1. INTRODUCTION

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated examined the potential of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce structural chromosome aberrations in erythropoietic cells of the bone marrow. Genesys used the mouse micronucleus test which measures the clastogenic (chromosome breaking) action of chemicals by the induction of micronuclei in bone marrow cells, as observed in erythrocytes from the peripheral blood of male and female mice obtained approximately 24 hours after dosing. Allen Ledbetter, ManTech Environmental Technology, Incorporated, Research Triangle Park, North Carolina (ManTech/RTP), was responsible for handling, storage, dilution (for exposure of the mice), and analytical chemistry of the test material. ManTech/RTP was also responsible for animal husbandry and for dosing of the mice with the test material.

Testing at Genesys consisted of all procedures not performed by ManTech/RTP and was conducted under the direction of Ann D. Mitchell, Ph.D., Study Director, by J. Thom Deahl, M.S., and Diane M. Brecha, B.S., Genetic Toxicologists, in accordance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97). Testing at Genesys was initiated on May 5, 1994 and concluded on June 19, 1994 with the final microscopic evaluations of erythrocytes. The protocol, protocol amendments, raw data obtained by Genesys, and a copy of this report will be retained in Genesys' archives located at 2300 Englert Drive, Durham, NC 27713.

2. BACKGROUND

The mouse bone marrow micronucleus test is a rapid, *in vivo* cytogenetic assay based on the observation that cells with broken chromosomes or impairment of the spindle apparatus often have disturbances in the distribution of chromatin during cell division. Micronuclei arise when chromosomes or their fragments lag at anaphase and fail to be incorporated within the daughter cell nucleus. Acentric chromosomal fragments, bridged chromosomes, and chromosomes lost due to spindle abnormalities are the major types of genetic damage which result in micronucleus formation.

The treated cell population consists of erythroblasts undergoing their final chromosome replication and mitosis before expulsion of the nucleus to form erythrocytes. After division, the daughter cells contain the displaced chromatin as distinct micronuclei in the cytoplasm (Schmid, 1976). Because RNA is present in erythrocytes for at least 24 hr after expulsion of the nucleus, newly-formed erythrocytes (that were exposed to

the test material or its metabolites when they were erythroblasts in the bone marrow) can be distinguished from the pre-existing population of erythrocytes (that were not exposed to the test material or its metabolites when they were erythroblasts) by differential staining. Although micronucleated erythrocytes are selectively removed from peripheral blood by the spleen in certain species, there is little or no selective removal in the mouse. Therefore, measurement of the frequency of micronucleated erythrocytes in the circulating blood of this species provides a convenient index of clastogenic damage that occurred in the bone marrow.

Micronuclei are visualized in erythrocytes from the bone marrow or peripheral blood stained with Giemsa or a fluorescent stain such as acridine orange. Micronuclei are detected more rapidly in fluorescent-stained preparations, but Giemsa stained preparations do not require fluorescence microscopy and are considered by many to be to more appropriate for permanent archiving. With Giemsa staining, polychromatic erythrocytes (PCEs, which contain RNA) are readily differentiated from normochromatic erythrocytes (NCEs, which have lost RNA) because the PCEs are a light blue-violet whereas the NCEs are light salmon red, and nuclear material (e.g., micronuclei) is a darker violet to reddish purple.

Because some chemicals are known to retard the progression of cells to mitosis, clastogenic effects may not be detected within the first 24 hours following a single dose. Two approaches can be used to overcome this problem: the mice can be dosed with the test material on each of two or three consecutive days to achieve steady state of the test material or its metabolites, with samples obtained within approximately 24 hr of the last dose, or single doses can be used, followed by multiple harvest times. Each approach presents advantages and disadvantages. While there is some evidence that the clastogenic effects of some chemicals may be missed if the times of multiple harvests are incorrect, the use of multiple dosing to achieve steady state may preclude testing to sufficiently high doses of toxic materials. For this study, to preclude the first problem, a multiple dosing of the test material, by inhalation, was used, followed by one sacrifice time.

It has been found that during continuous exposure *via* diet or drinking water, or during repeated daily exposures to test agents by i.p. injection, gavage, or inhalation, the frequencies of micronucleated cells in peripheral blood approach steady-state within two to three days in RNA-positive PCEs and within five to six weeks in RNA-negative NCEs (MacGregor, *et al.*, 1990). Therefore, efficiency of the micronucleus test is markedly improved by using a repeated dose schedule with a single sample taken at steady-state.

The percentage of PCEs in the erythrocyte populations of blood or bone marrow cells (PCEs plus NCEs), which is determined based on a statistical sampling of erythrocytes, provides a measure of potential cytotoxic effects of the test material. Because peripheral blood normally contains much lower PCE ratios ($\leq$ 5%) than the bone marrow (~ 50%), much larger sample sizes are required to detect a significant reduction in PCE ratios in peripheral blood; therefore, peripheral blood preparations provide a less informative

measure of toxicity. The highest dosage selected for micronucleus scoring is one in which the percentage of PCEs is depressed. However, if fewer than 0.5% PCEs are observed, that dosage is not scored. Alternately, if no depression the percentage of PCEs is observed, the highest dosage selected for scoring is the highest testable dosage.

3. METHODS

3.1. Identification, Storage, and Dilution of the Test Material

The test material, 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea, or F_7C_3H; molecular weight 170; CAS Number 431-89-0), a colorless gas, was received in a steel gas container from ManTech/Dayton on April 4, 1994 then transferred to Allen Ledbetter, ManTech/RTP, who was responsible for handling, storage, and dilution of the test material. The HFC-227ea was stored at ManTech/RTP at room temperature (approximately 72°F). ManTech/Dayton documented the strength, purity, and composition of the test material and provided a Material Safety and Data Sheet (MSDS) from Great Lakes Chemical Corporation for HFC-227ea. Upon acceptance of the final report, the remaining test material will be returned to the Sponsor. No reserve sample will be retained by ManTech/RTP.

3.2. Administration of the Test Material

ManTech/RTP exposed four groups of Swiss Webster mice six hours per day for three consecutive days at concentrations of either 0, 2.6%, 5.3% or 10.5% HFC-227ea via nose-only inhalation. An additional group served as a positive control and was dosed on exposure day 3 via i.p. injection. The HFC-227ea mice were exposed in Cannon 52-port nose only chambers (Lab Products, Maywood, NJ) and the negative control mice were exposed in a nose-only chamber made by IN-TOX Products (Albuquerque, NM). due to a shortage of the Cannon Chambers. Forty of the 52 ports of the Cannon chambers were sealed to allow for reduced air flow in an attempt to conserve the test material.

3.3. Test Atmosphere Generation

For each exposure group, the test atmosphere was generated by metering the HFC-227ea gas from the cylinder into either a 2000 or 4000 ml Erlenmeyer flask that served as a mixing plenum. Air was metered into the flask to provide the desired exposure concentration. Each flowmeter used to deliver the gases to the generation system was calibrated with the gas for which it was to be used. The air/HFC-227ea mixture exited the flask and entered the top of the exposure chambers.

The test mixture was distributed to each animal port at a rate of approximately 52 ml/min (total chamber flow was 621 ml/min).

To maintain proper chamber airflow, the exhaust line controller was adjusted so the static pressure inside the chamber was approximately zero, indicating that the amount

of flow entering the chamber matched the amount of flow exiting the chamber. This approach was required because it was not possible to accurately measure the exhaust flow using a rotameter since the flow properties change as the percentage of HFC-227ea changes (HFC-227ea is heavier than air).

3.4. Test Atmosphere Monitoring

The nominal concentration for each exposure was determined by dividing the total amount of the test material consumed (weight of the gas cylinder determined before and after each exposure) by the total exposure chamber airflow.

Actual chamber concentrations were determined by infrared analyses (IR) (Miran 1A, Foxboro Analytical, South Norwalk, CT). The IR analyzer was calibrated using a closed-loop calibration method with HFC-227ea gas either just prior to the exposure or, if an existing calibration was used, the calibration was checked, via the closed loop method, just prior to exposure, and the IR calibration was rechecked after each exposure.

The IR absorbance response, expressed in recorder chart lines, was determined for each known quantity of HFC-227ea injected. A least-squares regression was determined using a Texas TI-60 calculator. Samples for injection into the IR analyzer were collected using a gas-tight syringe from an unused animal port. The IR settings were: pathlength, 15.75 meters; wavelength, 9.05 microns; absorbance, 0.25; response, x 1; slit, 1; and meter response, 4. Oxygen levels from the chamber exhausts were determined using an O_2/Explosion meter (MSA Model 421).

3.5. Animal Source, Environment, Husbandry, and Evaluation

Thirty male and 30 female Swiss Webster mice, approximately 42 days old at receipt, were purchased, by ManTech/RTP, from Charles River Laboratories in Raleigh, NC. The mice were held in quarantine for approximately 1 week and examined carefully to ensure their health and suitability as test subjects. All work involving animals was reviewed and approved by the ManTech/RTP's Animal Care and Use Committee prior to the initiation of the work.

During all non-exposure periods, the mice were individually housed in suspended stainless steel wire-mesh cages with dimensions of 7" x 4" x 5" (L x W x H, 28 in^2 floor space). The animal rooms were maintained at approximately 71°F (range 65-78°F) and 57% (range 46-64%) relative humidity. Fluorescent lighting was provided automatically on a 12 hours light: 12 hours dark regimen.

Certified rodent feed (Purina® Certified Rat Chow 5002, St. Louis MO) and water, via automatic watering, were available *ad libitum*, except during the actual inhalation exposure periods. The mice were identified by cage number. All mice were weighed at arrival, at randomization and just prior to sacrifice. At the end of the quarantine period, the mice were given a physical examination, weighed and assigned to study groups based on a weight stratified method. Each study group for the negative control,

the three concentrations of HFC-227ea, and the positive control consisted 5 male and 5 female mice. Thus, a total of 50 mice were used for the assay. Mice that were not used for the assay were sacrificed by ManTech. Necropsy was not done on any of the mice.

All mice were observed during the exposure period and immediately upon removal from the exposure chamber and daily during the post-exposure observation period. Necropsy was not done on any of the mice. All cage changes and dosing were performed by ManTech. Sacrifice of the mice and preparation and analysis of the slides were performed by Genesys.

3.6. Positive Control

Genesys was responsible for administering the positive control, and approximately 24 hours prior to sacrifice each positive control animal for the micronucleus test received one i.p. dose of 0.4 mg/kg triethylenemelamine (TEM, CAS No. 51-18-3), which gives a reproducible increase in the frequency of micronucleated PCEs in Swiss Webster mice in our laboratory.

3.7. Sacrifice and Slide Preparation

Slides of peripheral blood smears were made for all animals at 24 ± 3 hours after the last exposure by the following procedure. Bovine calf serum, 2-3 µl, was placed on a slide pre-cleaned with methanol. Each mouse was sacrificed by cervical dislocation and 2-3 µl of blood per slide was obtained from the mid-ventral tail vein of a mouse and placed on top of the serum. The blood was mixed with the serum and spread on the slide to produce a thin, even film, then the slide was allowed to air-dry. Three slides were prepared per mouse, and, after the slides were dry, the erythrocytes were fixed by placing the slides in absolute methanol for two minutes; then they were allowed to air-dry vertically. ManTech/RTP was responsible for disposal of the carcasses.

3.8. Staining of Slides

The slides were stained for 20 minutes in 5% Giemsa stain in phosphate buffer containing 3% methanol and 3% 0.1M citric acid, rinsed by dipping them in deionized water until clear, and allowed to air dry vertically. Coverslips were attached with Permount before the erythrocytes were analyzed at 100X, oil immersion, magnification.

3.9. Scoring of Slides

Micronuclei were scored in slides from male and female mice from each HFC-227ea exposure group, and in slides from the positive and negative control animals. The treated and control slides were divided into three identical groups. Two groups of slides were coded by an individual not involved in the scoring or analysis, and the third group of slides was held in reserve, uncoded. Two observers were utilized, one for each set of coded slides.

Each bone marrow smear was inspected under low power to observe the distribution of cells and to select an area with good cell morphology and thin, even density (without overlapping cells) for scoring. Each slide was then scored for micronuclei using oil immersion objectives. The criteria which distinguish micronuclei from artifacts have been described by Schmid (1976). Micronuclei are identified as round or oval shaped bodies found in the cytoplasm of erythrocytes. Bodies which are refractile, improperly shaped or stained, or which are not in the focal plane of the cell are not scored as micronuclei. Cells containing more than one micronucleus are scored as a single micronucleated cell.

3.10. Raw Data Collection

All procedures used and the results were recorded on standard forms which were bound together with the protocol at the end of the study. After the microscopic analysis was completed, the slides were decoded, and the slide numbers, the ratios of PCEs per 1000 erythrocytes, and the number of micronuclei per observed PCEs and NCEs were recorded for each experimental and control animal. As five mice per sex were exposed per treatment group, the ratios for each group were then expressed per 5000 erythrocytes.

3.11. Analysis and Interpretation of Results

a. Data Analyzed

When the slides from the micronucleus assay had been decoded and tabulated, the ratios (‰) of PCEs per 5000 erythrocytes were calculated for all groups of mice. Treatment groups in which the ‰ micronuclei ratios were elevated above the untreated mice were then compared with the concurrent, untreated negative control group for that sex using Student's *t* test, as described in Hayes (1989).

b. Criteria for Interpretation

• **Positive.** A test material is considered to have elicited a positive response in the mouse erythrocyte micronucleus test if there is a dose-related increase in micronuclei and if one or more of the doses induces a statistically significant ($p < 0.05$) increase in micronuclei induction.

• **Negative.** A test material is considered to have elicited a negative response if the criteria for a positive response are not met.

Both biological and statistical significance were considered together in the evaluation of the results; the final interpretation of the results was the responsibility of the Study Director.

4. RESULTS AND DISCUSSION

Actual IR chamber concentrations during exposure in ppm (10,000 ppm = 1%) were:

Group	Mean of Three Daily Exposures	Standard Deviation	Relative Standard Deviation
Negative Control	0	0	0
2.62%	25,873	3,301	12.8
5.25%	53,864	2,409	4.5
10.50%	105,196	6,703	6.4

No mice died during the study, and all mice appeared normal throughout the study. When weighed before exposure to HFC-227ea in the micronucleus assay, male mice weighed 23.5 to 32.5 g and female mice weighed 21.9 to 27.2 g. When weighed after exposure and immediately before sacrifice, male mice weighed 27.1 to 33.4 g and female mice weighed 21.2 to 29.0 g. The majority of the mice in all exposure groups, including the negative control group, lost weight from day -1 (randomization) to day 4 (sacrifice). Weight loss during exposure was not treatment related and averaged 0.4 g for the male mice and 0.9 g for the female mice. Otherwise, all mice appeared to be normal from the time of dosing until the times of sacrifice; no toxic symptoms were noted by ManTech. Slides of bone marrow cells were prepared as described above and stained by Genesys.

Summaries of the results obtained in the micronucleus assay of HFC-227ea in male and female mice are presented in Tables 1 and 2. Negative control values were within Genesys' historical ranges, and each positive control group yielded a positive response that was significant at $p < 0.001$. Although not required by the protocol, MN/NCE (‰) were noted and are included in the tables of results. However, as NCEs persist for up to 60 days in the peripheral blood of mice, ‰ MN/NCE is considered to be, at most, only an indicator of micronuclei that may have been present before treatment; the key indicators for this assay are the number of newly formed PCEs per 1000 erythrocytes, which is a measure of toxicity, and ‰ MN/PCE, which is an index of potential chromosome breakage.

In the negative control mice, PCE ratios were 11.55‰ for male mice and 10.76‰ for female mice, values which were low but within historical ranges for the laboratory. Appropriately low numbers of MN/PCE were observed in the negative control mice: 1.0‰ MN/PCE in male mice and 2.0‰ MN/PCE in female mice, and, although PCE/erythrocyte ratios were not depressed in the positive control mice, MN/PCE ratios were elevated. In response to 0.4 mg/kg TEM, the PCE ratios were 12.96‰ in male mice and 9.36‰ in female mice, and 21.0‰ MN/PCE were observed in cells from male mice and 18.0‰ MN/PCE in cells from female mice.

In comparison to the negative controls, PCE/erythrocyte ratios were not significantly depressed in male or female mice exposed to HFC-227ea, and neither statistically significant increases in MN (at $p < 0.05$) nor dose-related increases in MN were observed in

male or female mice. Therefore, since neither criterion for a positive response was met in mice of either sex, HFC-227ea was clearly negative for the induction of micronuclei, which indicates that HFC-227ea is not capable of inducing structural chromosomal aberrations *in vivo*.

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Table 1

MICRONUCLEUS ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) IN MALE MICE

Chemical	Average Dose	Animal #	PCE Ratio*	MN/PCE (‰)	MN/NCE (‰)	Notes
Air	N/A	1	8.96	0.00	0.00	
		2	14.91	0.00	0.00	
		3	14.93	0.00	0.00	
		4	12.94	5.00	3.02	
		5	5.99	0.00	2.01	
		Average/Dose:	11.55	1.00	1.01	
HFC-227ea	2.6%	1	4.00	0.00	1.00	
		2	9.96	10.00	1.01	
		3	11.92	5.00	3.02	
		4	4.00	5.00	2.01	
		5	17.82	0.00	0.00	
		Average/Dose:	9.56	4.00	1.41	
HFC-227ea	5.3%	1	9.96	0.00	0.00	
		2	12.91	5.00	3.02	
		3	6.99	5.00	0.00	
		4	7.97	0.00	0.00	
		5	6.99	0.00	1.01	
		Average/Dose:	8.97	2.00	0.80	
HFC-227ea	10.5%	1	12.96	5.00	0.00	
		2	15.97	10.00	3.09	
		3	12.94	0.00	0.00	
		4	14.93	0.00	0.00	
		5	16.93	0.00	0.00	
		Average/Dose:	14.74	3.00	0.61	
TEM	0.4 mg/kg	1	2.00	30.00	0.00	
		2	9.94	10.00	0.00	
		3	11.00	10.00	0.00	
		4	28.52	40.00	4.05	
		5	13.09	15.00	5.10	
		Average/Dose:	12.96	21.00	1.82	◆◆

* = ‰ Erythrocytes
MN= Micronuclei
PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes
TEM = Triethylenemelamine
◆◆ = Positive Response, $p < 0.001$, for MN/PCE (‰)

Table 2

MICRONUCLEUS ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) IN FEMALE MICE

Chemical	Average Dose	Animal #	PCE Ratio*	MN/PCE (‰)	MN/NCE (‰)	Notes
Air	N/A	1	7.98	0.00	0.00	
		2	10.95	0.00	0.00	
		3	13.97	5.00	0.00	
		4	13.89	0.00	0.00	
		5	6.98	5.00	1.00	
		Average/Dose:	10.76	2.00	0.20	
HFC-227ea	2.6%	1	5.00	0.00	1.01	
		2	6.99	0.00	2.01	
		3	7.98	0.00	0.00	
		4	24.63	0.00	0.00	
		5	6.98	0.00	1.00	
		Average/Dose:	10.35	0.00	0.80	
HFC-227ea	5.3%	1	6.97	5.00	1.00	
		2	8.00	0.00	0.00	
		3	5.99	0.00	0.00	
		4	4.99	0.00	2.00	
		5	14.91	0.00	1.01	
		Average/Dose:	8.18	1.00	0.80	
HFC-227ea	10.5%	1	12.92	0.00	0.00	
		2	10.97	0.00	0.00	
		3	5.99	0.00	0.00	
		4	13.92	5.00	1.01	
		5	10.99	0.00	0.00	
		Average/Dose:	10.96	1.00	0.20	
TEM	0.4 mg/kg	1	1.00	5.00	0.00	
		2	22.64	35.00	0.00	
		3	5.00	10.00	2.01	
		4	3.00	30.00	3.01	
		5	14.90	10.00	0.00	
		Average/Dose:	9.36	18.00	1.00	◆◆

* = ‰ Erythrocytes
MN= Micronuclei
PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes
TEM = Triethylenemelamine
◆◆ = Positive Response, $p < 0.001$, for MN/PCE (‰)

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