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DATA EVALUATION REPORT

Study Type: Subchronic Oral Toxicity Study Guideline 82-1

Test Animal: CDBR Sprague-Dawley Rats

ID #: MRID No.: 417994-05 DP Barcode No.: D164205
Caswell No.: 454E HED Project No.: 1-1237

Test Material: Sulfluramid (96.6% linear and 3.4% branched isomers) - MRD-89-472 (Batch #I)

Synonym: N-ethyl perfluoro-octanesulfonamide

Dosages: 0, 10, 50, and 150 ppm

Sponsors: Griffin Corporation, Rocky Ford Rd., Valdosia, GA 31603

Study Number: 247254

Study Period: January 31, 1990 to May 4, 1990 (In Life Study)

Testing Facility: Exxon Biomedical Sciences, Inc. Toxicology Laboratory, Mettlers Rd., CN 2350

Title of Report: 90-Day Subchronic Dietary Toxicity Study in Rats Sulfluramid (MRD-89-472)

Author: Gary W. Trimmer

Report Issued: October 10, 1990

Conclusions:

Sulfluramid (96.6% linear and 3.4% branched isomeric mixture - MRD-89-472) was fed in the diet of Sprague-Dawley rats at dose levels of 0, 10, 50, and 150 ppm for a period of 90 days.

Ten rats (6♂ and 4♀) of the 150 ppm dose group died prior to study termination.

Treatment-related clinical signs (agitation, convulsions, hyperactivity, and emaciation), and reduced body weight and food consumption were noted in the high dose group (150 ppm).

Based on the results of the mean absolute organ weight and organ weight/body weight ratio data, the increase of brain/body weight, kidney/body weight, and testis/body weight ratios in the 150 ppm dose rats are attributed to treatment-related inhibition of body weight gain. The increase of the absolute liver weight and the liver/body weight ratio in the 50 ppm and 150 ppm dose rats are related to treatment.

The reduction of the red blood cell, hematocrit, and hemoglobin and the elevation of alkaline phosphatase, and bilirubin in the 150 ppm rats of both sexes, and the elevation of alanine transferase in the 150 ppm females are judged to be related to treatment.

Treatment-related gross abnormalities in the 150 ppm dose group included emaciation, fur staining, thickened liver, liver discoloration, abnormal GI tract contents, dark areas on the stomach, and undescended testes in the males.

Centrilobular hepatocellular hypertrophy and an associated hepatocellular vacuolation in the 50 ppm and 150 ppm rats of both sexes are considered to be related to treatment.

The NOEL for Sulfluramid 96.6% linear and 3.4% branched isomeric mixture (MRD-89-472) was determined to be 10 ppm, when fed in the diet for period of 90 days. The LOEL is 50 ppm based on body weight, food consumption, hematology, clinical chemistry, organ weight, and histo-pathological data.

CLASSIFICATION: Core-Minimum

Study Title: 90-Day Subchronic Dietary Toxicity Study in Rats Sulfluramid (MRD-89-472)

Author: Gary W. Trimmer

Testing Facility: Exxon Biomedical Sciences, Inc. Toxicology Lab. Mettlers Rd., CN 2350

Report Issued: October 10, 1990 Study No.: 247254

Testing Facility: Exxon Biomedical Sciences, Inc. Toxicology Lab. Mettlers Rd., CN 2350

Test Material: Sulfluramid (96.6% linear and 3.4% branched isomers) MRD-89-472 (Batch #I)

Test Animal: CDBR Sprague-Dawley Rats

1. OBJECTIVE

The objective of this study was to evaluate the toxicity of Sulfluramid (MRD-89-472) when administered in the diet to Sprague-Dawley rats for a period of 90 days.

2. MATERIALS AND METHODS

The in-life and necropsy phases, and the diet mixture analysis of this study were conducted at the Exxon Biomedical Sciences Inc., East Millstone, NJ and the histopathologic phase was conducted at the Research Pathology Services, Inc., New Britain, PA 18901. The sperm count and motility assay were conducted at the Argus Research Laboratories, Inc., Horsham, PA 19044.

Test Material

- Physical Description: A white powder with 96.6% linear and 3.4% branched isomers. MRD-89-472 (Batch no. I)
- Source: Griffin Corporation, Valdosia, GA 31603
- Storage: Room temperature (pure material) and in the freezer for mixtures.

Test Animals

- Species: CDBR Sprague-Dawley Rat
- Source: Charles River Breeding Laboratory, Inc., Charles River Kingston, Stoneridge, N.Y. 12484
- Total Number: 50 males and 50 females (125 rats ordered)
- Age: Approximately 7 weeks old at start of study
- Body Weight: ♂ = 218.9-254.5 g; ♀ = 164.0-198.4 g on day 0
- Caging: In individual suspended stainless steel cages
- Acclimation period: 15 days

Feed and Water

Purina Certified Rodent Chow mash (from Ralston Purina Co., St. Louis MO.) and water (Elizabethtown Water Company, Elizabeth NJ) were provided ad libitum. Feed was withheld one day prior to blood collection and prior to scheduled necropsy.

Environmental Parameter: Air temperature = 68-76°F; Relative Humidity= 40% to 70%; 12 hours dark/light cycle; fresh air exchanges not provided in the study report.

Experimental Design

Study Duration

Surviving animals in all other groups were terminated during week 13.

Group Arrangement

Animals were assigned to the study using a computer-generated randomization as follows:

Dose Group	Dosage (ppm)	# Males	# Females
Control	0	15	15
Low Dose	10	10	10
Mid Dose	50	10	10
High Dose	150	15	15

Diet Preparation

Sulfluramid was mixed in the basal diet to the appropriate dose level and fed to the animals for a period of at least 13 weeks. Fresh diet were given to the animals weekly.

Diet Analyses

Samples of the diets and the pure test article were collected at the start and at termination of the study for further analysis.

Clinical Observations

The rats were checked twice daily and once on weekends and holidays for mortality, moribundity and signs of toxicity. Detailed physical examinations were also conducted on the day of scheduled sacrificed.

Body Weights

Individual body weights were taken during the week prior to study initiation, on day 0, and weekly thereafter to termination. Body weights were also taken at the scheduled sacrifice and at death for moribund sacrificed rats.

Food Consumption

Individual food consumption was taken weekly.

Individual Compound Intake

Individual compound intake was not calculated.

Clinical Pathology Evaluation

Blood samples were collected from the abdominal aorta of the rats while under methoxyflurane anesthesia from each group at terminal sacrifice.

a. Hematology

The following hematological parameters were evaluated:

- o Hemoglobin
- o Hematocrit
- o Erythrocyte count
- o Leukocyte count (total & differential)
- o Platelet count
- o Reticulocyte count (only if other RBC parameters are abnormal)

b. Clinical Chemistry

The following clinical chemistry parameters were evaluated:

- o Alkaline phosphatase
- o Blood urea nitrogen (BUN)
- o Lactic dehydrogenase
- o Alanine aminotransferase
- o Aspartate aminotransferase
- o Glucose
- o Total protein
- o Cholesterol
- o Albumin
- o Total bilirubin
- o Creatinine
- o Calcium
- o Phosphorus
- o Sodium and Potassium
- o Chloride
- o Globulin (not taken)

c. Urinalysis

Urinalysis was not determined in this study.

Ophthalmologic Examination

Ophthalmologic examination by a veterinary ophthalmologist was conducted prior to the start of the study and prior to termination.

Gross Macroscopic Examinations

All rats which died or were sacrificed in extremis and all rats sacrificed at the scheduled sacrifice were subjected to gross macroscopic examination. Surviving rats sacrificed at terminal necropsy, were fasted overnight, anesthetized with methoxyflurane, killed by exsanguination, and then necropsied. All moribund rats were also necropsied after they were killed by carbon dioxide asphyxiation.

Tissues harvested from all rats were fixed in 10% neutral buffered formalin as follows:

o Eyes	o Liver @	o Esophagus
o Brain @	o Spleen @	o Stomach
o Pituitary	o Pancreas	o Jejunum & Ileum
o Sciatic nerve	o Kidney @	o Duodenum
o Spinal cord (3X)	o Adrenals @	o Colon & Caecum
o Heart @	o Urinary Bladder	o Rectum
o Aorta	o Thymus	o Testes @
o Lung @	o Thyroid/Parathyroid	o Prostate @
o Trachea	o Salivary gland	o Epididymis
o Musculature	o Lymph nodes	o Seminal vesicles @
o Skin	o Bone marrow(sternum)	o Uterus
o Mammary Gland	o Harderian gland	o Ovaries @
o All lesions	o Lacrimal gland	o Oviduct & Vagina

Organ Weights

Organs indicated by a (@) on the above Table were weighed.

Histopathological Evaluation

All preserved tissues from the control and the high-dose groups were subjected to histopathological evaluations. Only the the liver, lung, stomach, kidneys, and gross lesions of the mid-dose group were subjected to histopathological evaluations. These fixed tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin-eosin. All other tissues harvested were stored for possible future evaluation.

Sperm Motilities and Spermatid Concentration

Sperm motility was determined by collecting the sperm from the left cauda epididymis. Sperm were placed in the TYP (Test Yolk Buffer) solution on a slide and then evaluated under a low power microscope (20X or 25X magnification), by counting the number of motile and non-motile sperm. The left testicular parenchyma was homogenized into a suspension in Triton X100 and distilled water. Then the homogenization-resistant spermatid nuclei were counted under a low power microscope (40X magnification). Spermatid concentration was calculated per gram of testis weight.

Statistical analysis

A variety of standard statistical analyses were conducted including Bartlett's test to determine if the dose groups have equal variance. For the parametric procedure, a standard one way ANOVA using F distribution to assess significance was used. Dunnett's test was used to determine the significant difference between the control and the treated groups. Kruskal-Wallis test (test of equality of means) was used for nonparametric procedures. Dunn's Summed Rank test was used to determine which treatment group differs significantly from the control. The Bartlett's test was conducted at the 1% level of significance, and all other tests were conducted at the 5% and 1% level of significance.

Compliance Statements

- o A signed Statement of Confidentiality Claim was provided.
- o A signed Statement of compliance with EPA GLP's was provided.
- o A signed Quality Assurance Statement was provided.

RESULTS AND DISCUSSIONS

a. Analyses of Test Article and Diet

The results of the pre-study analysis of the test material in the diet showed that the diet mixtures were homogeneous and they were stable for 28 days when stored in the freezer and for 7 days when stored at room temperature. The analyzed concentrations of the test article in the diet were within 11% of the nominal target values and the overall means for each dose group were within 5% of the target values. These results are acceptable.

b. Mortality

A total of ten high-dose rats did not survive to termination. This included eight (5♂ and 3♀) rats that were found dead, and two (1♂ and 1♀) rats that were sacrificed *in extremis*. Convulsions were noted in five rats that were found dead.

c. Clinical Signs Observations

Frequent clinical signs observed in all dose groups were scab formation, alopecia, maloccluded/broken/missing incisors, nasal and ocular discharges. Additional clinical signs observed primarily in the high dose groups were convulsions, agitations (during handling), hyperactivity, and emaciation. These are judged to be related to treatment and can be summarized as follows:

Clinical Signs	0 ppm	10 ppm	50 ppm	150 ppm
	M/F	M/F	M/F	M/F
Numbers of Rats in Study	15/15	10/10	10/10	15/15
Mortality	0/0	0/0	0/0	6/4
Agitations	0/0	0/0	0/0	4/0
Convulsions	0/0	0/0	0/0	3/2
Hyperactivity	0/1	0/0	0/2	3/3
Emaciation	4/1	1/1	1/1	5/12

M/F = Males/Females

d. Body weight data

The mean body weights are presented in Appendix A. As seen from this Appendix, both the high-dose males as well as the females exhibited a statistically significant decrease in body weight as compared to the control, starting from day 21 and day 7, respectively. There was a general trend for a decrease in body weight with increasing dose from day 21 through termination. The body weight gain for the high-dose rats at termination as compared to the initial body weight was lower than the control (-36% for the males and -49% for the females). The body weight decrease in the high-dose rats is judged to be related to treatment.

e. Food Consumption Data

The food consumption data are presented in Appendix B. As seen from this Appendix, a statistically significant decrease in food consumption in the males was restricted to weeks 5 and 7 of the study. A linear trend for decreasing food consumption with increasing dose was noted from week 4 through week 11 in the males. A statistically significant reduction of mean absolute food consumption was observed from week 7 to week 10 in mid-dose females, while in the high-dose females, this reduction was noted throughout the study period. A linear trend of food consumption reduction with increasing dose was noted in both sexes throughout the study period, but it only attained a statistical significance in the high-dose females. The food consumption decrease in the high-dose females is judged to be treatment-related.

g. Clinical Pathology

1. Hematology (Appendix E)

A statistically significant decrease was noted in the high-dose red blood cell, hematocrit, and hemoglobin values for both sexes as compared to the control. Also a linear dose response was observed in each of the three parameters listed above. The reduction of the red blood cell, hematocrit, and hemoglobin values were judged to be related to treatment. This is an indication of an anemic condition.

2. Clinical Chemistry (Appendix F)

Clinical chemistry data showed a statistically significant increase of albumin, bilirubin, blood urea nitrogen and alkaline phosphatase in the high-dose rats. A statistically significant increase of alanine transferase, phosphorus, and sodium was noted in the high-dose females, and an elevation of blood urea nitrogen and alkaline phosphatase was noted in the mid-dose males. A statistically significant decrease of cholesterol was noted in the high-dose group of both sexes and in the mid-dose females as compared to the control. A linear response to dose level was noted for each of the above listed parameters. Changes of alkaline phosphatase, bilirubin, and alanine transferase (in females) in the high dose group may be related to the microscopical abnormalities noted in the livers (see histopathological evaluations below), and they are judged to be related to treatment. Other clinical chemistry parameter changes were within historical control values or the changes were not biologically significant.

Ophthalmologic Examination

No compound-related ophthalmologic abnormalities were observed in all surviving rats evaluated at terminal sacrifice.

Gross Macroscopic Findings

All rats which died or were sacrificed in extremis or at the scheduled necropsy were subjected to gross macroscopic examinations. The number of rats with pertinent observable gross findings are as follows:

Clinical Signs	0 ppm	10 ppm	50 ppm	150 ppm
	M/F	M/F	M/F	M/F
Number of Rats in Study	15/15	10/10	10/10	15/15
Emaciation	2/1	0/0	2/0	6/9
Staining of the Fur	0/0	1/0	0/0	6/4
Thickened Liver	0/0	0/0	0/1	6/3
Liver Discoloration	0/0	0/1	0/1	3/3
Abnormal GI Tract Contents	0/0	0/0	0/0	5/3
Dark Areas on Stomach	0/0	0/0	0/0	4/4
Kidney Discolored	0/1	0/0	0/0	0/1
Undescended Testes	0/na	0/na	0/na	5/na
Uterus Distended	na/2	na/0	na/0	na/2

GI = Gastro-intestinal; M/F = Males/Females; n/a = not applicable. Derived from p. 32 - 62 of the study report.

As seen from the above Table, treatment-related gross abnormalities, all observed in the high-dose group, included emaciation, staining of the fur, thickened liver, liver discoloration, abnormal GI tract contents, dark areas on the stomach, and undescended testes in the males.

Organ Weights

Mean organ weights and mean organ/body weight ratio data are presented in Appendices C and D, respectively. A statistically significant organ weight decrease were noted in the high-dose group spleen of both sexes, the lung of the males, and the heart, and kidneys of the females. A statistically significant increase in mean liver weight was noted in the mid-dose females and in the high-dose males and females. In general, the above noted organs showed a linear response to dose (an increase for the liver and a decrease for the spleen, lung, heart, and kidneys).

As seen from Appendix D, a statistically significant increase in mean organ/body weight ratios were noted in both sexes for the high-dose brain, kidneys, and livers as well as the mid-dose liver. A statistically significant increase of the organ/body weight ratios were also noted for the kidneys, brain and ovaries of the mid-dose females, and for the testes of the high-dose males. In general, the above noted organs showed an increased linear response to dose.

Based on the above results, the increase of brain/body weight, kidney/body weight, and testis/body weight ratios in the high-dose rats are attributed to treatment-related inhibition of body weight gain. The increase of the absolute liver weight and the liver/body weight ratio in the mid- and high-dose rats are related to treatment.

Sperm Count and Sperm Motility:

The testicular spermatid counts and concentrations were similar in the treated groups when compared to the controls. The spermatid concentrations were 82.0, 84.7, 91.9, and 84 x 10^{-6} per gram of testicular parenchyma for the 0, 10, 50, and 150 ppm dose groups, respectively.

The number of the motile and non-motile sperm as well as the percent sperm motility were also comparable among the dose groups. The average percent motility values were 73.2, 71.3, 75.1, and 72.3% for the 0, 10, 50, and 150 ppm dose groups, respectively.

Histopathological Evaluation

Pertinent histo-pathological findings are summarized as Appendix G. As seen from this Appendix, increased incidence of centrilobular hepatocellular hypertrophy and an associated hepatocellular vacuolation were noted in the mid- and high-dose livers, and they are judged to be related to treatment. The vacuolated hepatocytes were primarily midzonal in the males and periportal in the females. Increased incidence of multifocal/pelvic mineralization in the kidneys of the mid- and high-dose females are of uncertain significance.

DISCUSSIONS AND CONCLUSIONS

Sulfluramid (96.6% linear and 3.4% branched isomeric mixture - MRD-89-472) was fed in the diet of Sprague-Dawley rats at dose levels of 0, 10, 50, and 150 ppm.

Ten high dose rats (6♂ and 4♀) died prior to study termination.

Treatment-related clinical signs included agitation (during handling), convulsions, hyperactivity, and emaciation in the high dose group (150 ppm).

Statistically significant body weight reductions in both sexes of the high-dose group (starting on day 21 in the males and on day 7 in the females) were judged to be related to treatment. Similar food consumption reduction (not always statistically significant) was also observed.

Based on the results of the mean absolute organ weight and organ weight/body weight ratio data, the increase of brain/body weight, kidney/body weight, and testis/body weight ratios in the 150 ppm dose rats are attributed to treatment-related inhibition of body weight gain. The increase of the absolute liver weight and the liver/body weight ratio in the 50 ppm and 150 ppm dose rats are related to treatment.

The reduction of the red blood cell, hematocrit, and hemoglobin values in the high-dose rats were judged to be treatment related.

The elevation of alkaline phosphatase, bilirubin, and alanine transferase (in females) in the high dose (150 ppm) group may be related to liver abnormalities, and they are judged to be related to treatment.

Treatment-related gross abnormalities, all observed in the high-dose group, included emaciation, staining of the fur, thickened liver, liver discoloration, abnormal GI tract contents, dark areas on the stomach, and undescended testes in the males.

The testicular spermatid counts and concentrations as well as the percent sperm motility were not affected by administration of Sulfluramid.

Treatment-related changes were noted in the mid- and high-dose livers, namely centrilobular hepatocellular hypertrophy and an associated hepatocellular vacuolation. The vacuolated hepatocytes were primarily midzonal in the males and periportal in the females.

The NOEL for Sulfluramid ~~96.6%~~ linear and ~~3.4%~~ branched isomeric mixture (MRD-89-472) was determined to be 10 ppm, when fed in the diet for period of 90 days. The LOEL is 50 ppm based on body weight, food consumption, hematology, clinical chemistry, organ weight, and histo-pathological data.

CLASSIFICATION: Core-Minimum

APPENDICES

APPENDIX A : Mean Body Weights in Grams (values rounded off) at Various Times for the Control, Low, Mid, and High-Dose Groups (derived from p. 64-65 of the study report)

APPENDIX B: Mean Food Consumptions in grams/week/rat (values rounded off) at various times for the Control, Low, Mid, and High-Dose Groups (derived from p. 68-69 of the study report).

APPENDIX C: Mean Organ Weights in Grams (some values were rounded off) for the Control, Low-, Mid-, and High-Dose Groups (derived from p.73-74 of study report)

APPENDIX D: Mean Organ/Body Weight Ratios (some values were rounded off) for the Control, Low-, Mid-, and High-Dose Groups (derived from p.75-76 of study report)

APPENDIX E: Summary of the Hematology Data (copied from p. 77-78 of the study report)

APPENDIX F: Summary of the Clinical Chemistry Data (copied from p. 79-81 of the study report)

APPENDIX G: Summary of Pertinent Histopathological Data (Derived from p.272-276 of the study report)

Sulfluramid

Page_____ is not included in this copy.

Pages 14 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Description of quality control procedures.

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