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DATA EVALUATION REPORT

Study Type: Subchronic Oral Toxicity Study Guideline 82-1

Test Animal: Dog

ID #: MRID No.: 418184-01 DP Barcode No.: D164205
Caswell No.: 454E HED Project No.: 1-1237

Test Material: Sulfluramid (96.5% linear and 3.5% branched isomers) - (Batch # AN90247)

Synonym: N-ethyl perfluoro-octanesulfonamide

Doses: 0 ppm, 33 ppm, 100 ppm, 500 ppm, and 1500 ppm(20 mg/kg/day)

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

Study Number: WIL-157002

Study Period: February 6, 1990 to May 22, 1990 (In Life Study)

Testing Facility: WILL Research Laboratories, Inc., 1407 Montgomery Twp. Rd 805 Ashland, OH 44805

Title of Report: 90-Day Subchronic Dietary Toxicity Study in Dogs with Sulfluramid

Author: E. Crosby Tompkins, Ph.D.

Report Issued: November 21, 1990

Conclusions:

Oral administration of sulfluramid (96.6% linear and 3.4% branched isomeric mixture - MRD-89-472) in dogs via the diet at 33ppm, 100 ppm, and 500 ppm) and in capsular form at 20 mg/kg/day (initially 1500 ppm in the diet) for varying times up to 103 days produced the following major effects:

- o Increased mortality in the 1500 ppm:20 mg/kg/day males.
- o Reduction of body weight in the 500 ppm and 1500 ppm: 20 gm/kg/day groups

- o Increased liver weight in the 500 ppm and 1500 ppm: 20 gm/kg/day males
- o Increased blood urea nitrogen in the 500 ppm and 1500 ppm: 20 gm/kg/day groups
- o Increased renal hyperemia and nephrosis in the 1500 ppm: 20 mg/kg/day males
- o Reduction of epididymides and testes weights in the 500 ppm and 1500 ppm:20 gm/kg/day groups
- o Increased epididymal and testicular lesions affecting the seminiferous tubules of the testes of the 100 ppm, 500 ppm, and 1500 ppm:20 mg/kg/day dose groups.
- o Reduced percent motile sperm, and the reduction of epididymal sperm and testicular spermatid concentration of the 500 ppm and 1500 ppm: 20 mg/kg/day dose groups.

The NOEL for Sulfluramid, 96.6% linear and 3.4% branched, isomeric mixture was determined to be 33 ppm, when fed in the diet of dogs for a period of at least 90 days. The LOEL is 100 ppm based on the increased epididymal and testicular lesions. The doses employed in this study were sufficient to produce compound-related effects.

Sulfluramid appears to be a direct acting testicular toxin in the dog with primary effect on the late spermatids and possibly Sertoli cells.

CLASSIFICATION: Core-Minimum

Study Title: 90-Day Subchronic Dietary Toxicity Study in Dogs with Sulfluramid

Author: E. Crosby Tompkins, Ph.D.

Testing Facility: WILL Research Laboratories, Inc., 1407 Montgomery Twp. Rd 805 Ashland, OH 44805

Report Issued: November 21, 1990 Study No.: WIL-157002

Test Material: Sulfluramid (96.5% linear and 3.5% branched isomers) Batch #AN90247

Test Animal: Dogs (Outbred Beagle)

1. OBJECTIVE

The objective of this study was to evaluate the toxicological potential of Sulfluramid when administered to Dogs for 90 days.

2. MATERIALS AND METHODS

The dosage analysis, in-life, necropsy, and pathologic phases of this study were conducted at the WIL Research Laboratories, Inc., in Ashland, Ohio.

Test Material

- Physical Description: A white powder with 96.5% linear and 3.5% branched isomers. (Batch#AN90247 I)
- Source: Griffin Corporation, Valdosia, GA 31603
- Storage: Room temperature (pure material) and in the freezer for mixtures. The test compound was stable for 10 days.

Test Animals

- Species: Outbred Beagle dogs
- Source: Schering-Plough Research, Lafayette, NJ
- Total Number: 26 males and 18 females
- Age: Approximately 7 - 8 weeks old at start of study
- Body Weight: ♂ = 7214 - 10285 g; ♀ = 5515 - 9710 g on day 0
- Caging: In individual suspended stainless steel cages
- Acclimation period: 21 days

Feed and Water

Purina Certified Canine Chow #5007 meal (Ralston Purina Co., St. Louis MO.) and water were provided ad libitum.

Environmental Parameters: Air temperature = 65° - 74°F; Relative Humidity= 28% to 82%; 12 hours dark/light cycle; 10 to 15 fresh air exchanges per hour.

Group Arrangement

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

Dose Group	Dosage	# Males	# Females
Control	0 ppm	8[$]	4
Add-on Dose	33 ppm	4[@]	-
Low Dose	100 ppm	4	4
Mid Dose	500 ppm	4	4
High Dose	1500 ppm:20 mg/kg/day*	8[#]	4

$ = After two weeks four control males were assigned to 33 ppm dose group; @ = Four control males were assigned to this group after two weeks of study; # = Four dogs died within 2 weeks; * = Sulfluramid was removed from diet at the beginning of the 3rd week and capsules (20 mg/kg/day) were administered at the beginning of the 5th week of study.

Dosing Regimes

Initially four dogs/sex/group (with an additional 4 males each in the control and the high-dose groups) were administered with the test compound in the diet at dosages of 0, 100, 500, and 1500 ppm.

After two weeks of compound administration, four of the eight high-dose males died. The remaining high-dose dogs were taken off the treated diet for a two-weeks recovery period. During this time they were given a 120 ml of Ensure liquid nutrition by gastric intubation once and fed with Purina certified Canine diet nuggets and Alpo Beef Chunks. This was necessary because the dogs were not eating well, were losing weight and were in poor health. During the remainder of the study, the high-dose groups were fed with Purina certified Canine diet nuggets. A 20 mg/kg/day (equivalent to 1.6X the mg/kg/day being administered to the 500 ppm group) sulfluramid in capsule was administered daily for a total of 75/76 consecutive days.

Because four of the eight high-dose males died, four of the eight control males were assigned to a new 33 ppm dose group and they were maintained at this dose level for 90 days.

The 100 ppm and 500 ppm dose groups were fed with sulfluramid in the diet for a total of 103/104 consecutive days. Due to inappetence during study weeks 5-6, the 500 ppm dogs were fed with canned dog food for four consecutive days.

The controls were maintained on the basal diet for a total of 103/104 consecutive days. A summary of the study design is presented in Appendix A.

Study Duration

Surviving animals were terminated during week 15 of study.

Diet Preparation

Sulfluramid was mixed in the basal diet to the appropriate dose level and fed to the test dogs for a period of at least 13 weeks. Diet mixtures were prepared weekly, and the capsules were prepared daily. Individual dosages were adjusted weekly based on the most recent body weight.

Diet Analyses

Samples of the diet mixtures were collected for homogeneity for the first preparation. The remaining sample was stored for 10 days under laboratory conditions for analysis of stability. Fresh samples of diet mixtures were analyzed for test material concentration weekly for the first week and then at study week 8, 9, and 13. Samples of the test article were collected and analyzed at the start and at termination of the study for stability.

Clinical Observations

The dogs were checked twice daily for mortality, moribundity and signs of toxicity. Dogs were also checked during and one hour after dosing. 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 for scheduled and moribund sacrificed dogs. The mean body weights were calculated for each week and the mean body weight changes were calculated for the corresponding interval.

Food Consumption

Individual food consumption was recorded daily and the weekly averages were reported for the corresponding body weight intervals. Food intake was calculated as g/dog/day and g/kg/day.

Individual Compound Intake

Individual compound intake was calculated and reported as mg/kg/day.

Clinical Pathology Evaluation

Hematologic and clinical chemistry parameters were evaluated for all dogs prior to study initiation (study day -6), and on weeks 1, 3, 5, and 14 during the treatment period. Blood collections were conducted prior to feeding periods.

Blood samples were collected from the jugular vein of the dogs. It was not noted whether the dogs were under anesthesia during this procedure.

a. Hematology

All guidelines-required hematological parameters (✓) were evaluated as follows:

- ✓ Hemoglobin
- ✓ Hematocrit
- ✓ Erythrocyte count
- ✓ Prothrombin time
- ✓ Platelet count
- o Reticulocytes (not evaluated)
- ✓ Leukocyte count (total & differential)
- ✓ Mean Corpuscular Volume (MCV)
- ✓ Mean Corpuscular Hemoglobin (MCH)
- ✓ Mean Corpuscular Hemoglobin Conc. (MCHC)
- ✓ Activated Partial Thromboplastin Time

b. Clinical Chemistry

Clinical chemistry parameters including those required by EPA guidelines (✓) were evaluated as follows:

- ✓ Alkaline phosphatase
- ✓ Blood urea nitrogen (BUN)
- ✓ Creatine kinase
- ✓ Alanine aminotransferase
- ✓ Aspartate aminotransferase
- ✓ Glucose
- ✓ Total protein
- ✓ Cholesterol
- o Gamma glutamyltransferase
- ✓ Albumin
- ✓ Total bilirubin
- ✓ Creatinine
- ✓ Calcium
- ✓ Phosphorus
- ✓ Sodium and Potassium
- ✓ Chloride
- o Globulin
- o A/G Ratio

c. Urinalysis

Urinalysis was conducted on all dogs prior to study initiation (days -7 or -5) and on weeks 5 and 14 of the study. Urine was collected using a catheter for the majority of the dogs. Due to the inability to obtain samples with a catheter, metabolism cages were used for three dogs (one each from the control, 500 ppm and 1500 ppm groups). The following parameters including those required by the USEPA guidelines (✓) were analyzed:

- ✓ Volume
- ✓ Color and appearance
- ✓ Glucose
- ✓ Sediment
- ✓ Ketones
- ✓ Bilirubin
- ✓ Occult blood
- o Leukocytes
- ✓ pH
- ✓ Protein
- ✓ Specific Gravity
- o Nitrites

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 dogs were subjected to gross macroscopic examination. They were anesthetized with pentobarbital followed by exsanguination, and then necropsied. One 500 ppm moribund dog was sacrificed by cardiac puncture.

Tissues required by USEPA guideline (√) were harvested from all dogs and were fixed in 10% neutral buffered formalin as follows:

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

The right testis was fixed in 4% paraformaldehyde for microscopic evaluation. The right testis with epididymis was used to determine the spermatid count, epididymal sperm number, and sperm motility and morphology.

Organ Weights

Organs indicated by a (@) on the above Table were weighed. The heart, lung and spleen weights (#) as required by EPA guidelines were not taken. No explanation was given in the study report.

Histopathological Evaluation

All preserved tissues noted above were subjected to histopathological evaluations, except parathyroid of one high-dose (#695), thymus gland of one control (#661) and one 500 ppm dose (#670), and the heart of one control (#682) dog.

Statistical analysis

Standard statistical analyses were conducted including one-way analysis of variance followed by Dunnett's test. Two-tailed tests was 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.
- o A Flagging Statement was provided.

RESULTS AND DISCUSSIONS

a. Analyses of Test Article and Diet

The diet mixtures were homogeneous and the test article was stable for 10 days 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

Two high-dose males (673 & 676) were found dead and two high-dose males (675 & 680) were sacrificed *in extremis*. Adverse clinical signs prior to death or moribund sacrificed dogs included hypoactivity, labored breathing, prostration, emaciation, and mucoid and black stool.

c. Clinical Sign Observations

Pertinent clinical signs observed for the first two weeks (prior to the transfer of 4 dogs to the 33 ppm group) are summarized below:

Clinical Signs	0 ppm	33 ppm	100 ppm	500 ppm	1500ppm: 20 mg/kg/day
	M/F	M/F	M/F	M/F	M/F
# Dogs in Study	8/4	na	4/4	4/4	8/4
Hypoactivity	0/0	na	0/0	0/1	4/0
Abdominal area red	0/0	na	1/2	1/1	3/2
Inguinal area red	0/0	na	2/1	1/1	6/2
Labored breathing	0/0	na	0/0	0/0	3/0
Decreased Feces	0/0	na	0/1	0/1	8/4

M/F = Males/Females; na = Not applicable

In the first two weeks, adverse effects including hypoactivity, labored breathing, decreased feces, and reddening of abdominal and inguinal areas were noted primarily in the high-dose group.

Pertinent clinical sign data after the second week of study to termination are summarized as follows:

Clinical Signs	0 ppm	33 ppm	100 ppm	500 ppm	1500ppm: 20 mg/kg/day
	M/F	M/F	M/F	M/F	M/F
# Dogs in Study	4/4	4/0	4/4	4/4	4/4
Hypoactivity	0/0	0/na	0/0	1/0	0/2
Decreased Feces	0/0	0/na	0/0	0/2	3/4
Abdominal area red	0/0	2/na	1/2	1/0	2/2
Inguinal area red	0/0	2/na	2/1	1/0	2/2
Exfoliation abd.	0/0	1/na	1/0	3/2	0/3
Exfoliation ing.	0/0	0/na	1/0	3/2	0/3
Desquamation abd.	0/0	0/na	0/0	4/2	0/3
Desquamation ing.	0/0	0/na	0/0	4/2	1/3

M/F = Males/Females; abd. = Abdominal; ing. = Inguinal; na = not applicable.

Increased clinical signs noted were dermal effects including reddening, desquamation and/or exfoliation of the abdominal and inguinal areas in the treated groups. Dermal effects (desquamation, exfoliation and reddish coloration) were noted in 2/4, 2/4 and 4/4 males, and in 2/4, 2/4, 3/4 females of the 100 ppm, 500 ppm and high-dose groups, respectively. These dermal effects do not appear be related to treatment, because progression from one condition to another was not noted, the frequency and duration were inconsistent in these groups, and only one 500 ppm male and one high-dose female had these effects throughout the study.

Three high-dose dogs which survived to termination were hypoactive on one occasion prior to dosing and on four occasions, one hour following compound administration, within the first two weeks of study. One 500 ppm male was hypoactive on one occasion prior to dosing and on four occasions one hour following compound administration. Hypoactivity was noted in one 500 ppm male and two high-dose females after 2 weeks of study. Since no clear trend existed, increased hypoactivity in the high-dose group appears to be unrelated to treatment.

Decreased defecation was noted in two 500 ppm females, and in three males and four females of the high-dose groups. This finding is judged to be related to treatment.

d. Body weight data

The mean absolute body weights are presented in Appendix B. The decrease of high-dose female mean body weights was statistically significant from week 8 through 14. Percent body weight differences from the control ranged from -26.4% to -28.0%. Although not statistically significant, body weights of the high-dose males were decreased by -10.9% to -14.5% for weeks 9 to 14. Mean body weights of the 500 ppm groups were also decreased and these reductions were statistically significant from weeks 3 through 14 in the males, and week 5 in the females. Percent body weight reduction from the control ranged from -16.0% to -31.2% for the males and from -14.2% to -28.3% for females for weeks 3 to 14. In the 100 ppm dose females, the percent body weight reductions were between -8.0% to -12.5% from weeks 3 through 14, but they were not statistically significant.

The body weight gains are presented in Appendix C. Statistically significant weight decreases were noted in the high-dose males and females during the first two weeks of study. Overall (weeks 0 -14) body weight decreases for the high- dose males (-604 g) and females (-1265 g) were statistically significant. Body weight losses in the 500 ppm dose males and females were noted for the first five weeks of study, and they were statistically significant from weeks 1-2 through 4-5. Overall (weeks 0-14) body weight losses for the 500ppm dose males (-739 g) and females (-705 g) were also statistically significant. Based on the above data, the body weight gain decrease in the 500 ppm and high-dose groups is judged to be treatment-related.

e. Food Consumption Data

The food consumption data are presented in Appendix D (mean food consumption intake in gm/dog/day) and in Appendix E (summary mean weekly food consumption in gm/kg/day). The mean food consumption intake (gm/dog/day) for the 500 ppm and high-dose males and females was lower than the controls throughout the study. The reductions were statistically significant for weeks 2 to week 10 for the 500 ppm and high-dose groups. It is noted, however, that the food consumption reductions in the high-dose group were consistently less than the 500 ppm group after week 3 of study. This was probably due not only to dosage reduction from 1500 ppm (equivalent to 38 mg/kg/day) to 20 mg/kg/day of sulfluramid but also because the latter was given in capsular form. The statistically significant food consumption reduction in the 500 ppm and high-dose dogs is judged to be treatment-related.

The calculated compound intakes were 0.91-1.11 mg/kg/day, 2.52-3.69 mg/kg/day, 10.1-13.30 mg/kg/day and 23.18-28.48 mg/kg/day for the 33 ppm, 100 ppm, 500 ppm, and 1500 ppm:20 mg/kg/day groups, respectively.

g. Clinical Pathology

1. Hematology (Appendix F)

Slight depressions were noted in red blood cell, hematocrit, and hemoglobin values in the 500 ppm females and high-dose males and females from week three and in the 500 ppm males at week 5 of the study period. The slight reduction of red blood cells, hematocrit, and hemoglobin values noted in the high-dose groups appears to be related to treatment. Some of the leukocyte differential values of the treated groups differed significantly from the control, but none of these differences were large, persistent over time or noted in both sexes. Also, since no trends were apparent, these differences were not judged to be treatment-related.

2. Clinical Chemistry (Appendix G)

In general total protein, albumen and globulin values for the 500 ppm and high-dose females and to a lesser extend the males were decreased (some were statistically significant). The albumen/globulin ratios did not vary much as compared to the control and only on two occasions (week 5 of the high-dose males and week 14 of the 500 ppm females) were the A/G ratios statistically significant. Changes of the protein, albumen, and globulin in the high-dose dogs are judged to be related to treatment.

Elevation of Blood urea nitrogen was noted from week 1 to termination of the study for the 500 ppm and the high-dose groups (♂ and ♀) as compared to the control. All of the 500 ppm and some of the high-dose values were statistically significant. Elevation of BUN was more severe, when the compound was administered in the mixed diet (1500 ppm) than when it was given via capsule (20 mg/kg/day). Elevation of BUN in the 500 ppm and the high-dose groups is judged to be related to treatment.

Calcium and phosphorus serum levels were depressed (some were statistically significant) in the 500 ppm and in the high-dose groups. Milder effect in the high-dose after week two of study was probably due to the reduction of the dosage from 1500 ppm (equivalent to 38 mg/kg/day) to 20 mg/kg/day. Changes of phosphorus and calcium levels in the 500 ppm and high-dose groups are judged to be treatment-related. Other clinical chemistry changes were artifactual or judged to be unrelated to treatment.

3. Urinalysis

Summary and individual urinalysis data are presented in Tables 53 through 62 and in Tables 122 through 131, respectively. Volume

and specific gravity of the urine were comparable among the groups. An acidic urine was noted in the 500 ppm males and females and in the high-dose males as compared to the pretest values. The pH of the urine samples are summarized as follows:

	0 ppm	33 ppm	100 ppm	300 ppm	1500 ppm[a]
	M/F	M/F	M/F	M/F	M/F
Week -1	7.1/7.0	@/na	6.8/6.5	6.3/7.0	6.9/5.8
Week 5	7.5/7.5	8.0/na	8.3/6.3	6.3/5.3	5.8/6.0
Week 14	8.0/8.3	8.0/na	8.3/7.3	6.0*/6.5*	6.3/6.0**

* = significance at $p < 0.05$; ** = significant at $p < 0.01$;
a = dose reduced to 20 mg/kg/day from week 5; M/F = Males/Females;
na = not applicable; @ = Dogs not placed on study yet.

At week 14, the pH values of the 500 ppm dogs and the high-dose females were statistically significant as compared to the controls. As the investigators indicated, the acidic trend in urine pH was probably due to the decrease in food intake rather than an indication of a toxic effect.

Ophthalmologic Examination

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

Macroscopic and Microscopic Evaluations

All dogs were subjected to gross macroscopic examinations. Pertinent observable gross macroscopic findings are presented in Appendix K. Treatment-related gross abnormalities, all observed in the high-dose group included dark red contents in the cecum, ileum, and the stomach (colon, duodenum, and jejunum not shown in the summary Table in Appendix K), red areas in the ileum, stomach and the lung, and hemorrhage in the heart, stomach and thymus. Serous atrophy of the heart in one found dead and one moribund sacrificed dog appeared to be due to starvation rather than direct compound toxicity effects. Other macroscopic findings appear to be unrelated to treatment.

The histopathological findings of all dogs on study are presented in Appendix L. Increased incidences of the following observations were judged to be related to treatment: renal hyperemia and nephrosis, aspermia and giant-cell formation in the testes/epididymis, suppurative or granulomatous pulmonary inflammations and atrophied spleen and thymus gland, and lymph node hyperplasia, all in the high dose group. It should be noted that the two found dead high-dose males (#673 and #676) showed giant cell formation in the testes and aspermatogenesis was seen in one of the dogs (#673).

Organ Weights

Mean absolute organ weights, mean relative organ/body weight and organ/brain weight ratios are presented in Appendices H, I, and G, respectively. A significant decrease of testicular and epididymal weights (absolute and relative to brain and final body weight) were noted in the 500 ppm and high-dose groups. Most values of the 500 ppm dose group were statistical significant. This finding was also supported by the gross and microscopic data.

The mean ovarian weights (absolute and relative to brain and final body weight) were decreased (not statistically significant) in the high-dose group as compared to the control. However, since no microscopic adverse findings were noted, the observed ovarian weight decrease is judged to be related to the nutritional state of the females rather than to treatment.

There was an increase of absolute liver weight in the treated males as compared to the control. Increased liver/body weight and liver/brain weight ratios were noted in the 100, 500, and the high-dose groups, but only the liver/body weight ratio of the 100 ppm female, and the 500 ppm and the high-dose animals were statistically significant. The liver weight increase in the 500 ppm and the high-dose males are judged related to treatment. The slight liver weight increase of the females is equivocal. No dose-related microscopic abnormalities were noted in the liver. Other organ weight differences noted are not judged to be treatment-related.

Testicular Lesions, and Sperm and Spermatid Analysis

The summary incidence and mean severity grades of testicular lesions, summary epididymal sperm and testicular spermatid analysis and epididymal histopathologic evaluations are presented in Appendices, M, N, O, and P, respectively.

As discussed earlier, significant reductions in testicular weights in the 500 ppm and in epididymal weights in the 500 ppm and the high-dose groups were noted. These reductions are judged to be related to treatment.

Appendices M and N show that Sulfluramid affected the seminiferous tubules of the testes of the 100 ppm, 500 ppm, and the high-dose groups. Few seminiferous tubules of the 500 ppm and high-dose groups were normal. In the 100 ppm dose group there was a significant reduction in the number of normal seminiferous tubules. Testicular lesions in the 100 ppm dose group included increased incidences of failure to release late spermatids, cellular displacement, and Sertoli cell vacuolation. Increase incidence of testicular lesions in the 500 ppm and high-dose groups included degenerating cells, cellular disruption, and a decrease in

the number of late spermatids as well as lesions noted in the 100 ppm dose group. The testicular lesions severity was lowest in the 100 ppm dose group as compared to the 1500ppm:20 mg/kg/day dogs. Increased number and severity of testicular lesions in the 100 ppm, 500 ppm, and the high-dose groups are considered to be related to treatment.

Appendix O shows that the percent motile sperm, and the epididymal sperm and testicular spermatid concentration of the 500 ppm and the high-dose groups were significantly reduced, while the percent abnormal epididymal sperm was higher than the controls. These effects are judged to be related to treatment.

The histopathologic evaluations of the epididymides from both the head and the tail areas, also suggest that the 500 ppm and the high-dose groups had either some degree of reduced spermatogenic activity or some degree of increased spermatogenic cell degeneration or both, that are related to treatment.

Based on the results discussed above sulfluramid appears to be a direct acting testicular toxin in the dog with primary effect on the late spermatids and possibly Sertoli cells.

CONCLUSIONS

Sulfluramid (96.6% linear and 3.4% branched isomeric mixture - MRD-89-472) was administered to dogs in their diet (ppm dose groups) or by capsule (20 mg/kg/day) for varying times up to 103 days at dose levels as follows:

Dose Group	Dosage	# Males	# Females	Dosing period
Control	0 ppm	8$	4	103 days
Add-on Dose	33 ppm	4@	-	90 days
Low Dose	100 ppm	4	4	103 days
Mid Dose	500 ppm	4	4	103 days
High Dose	1500 ppm and 20mg/kg/day	8#	4&	14/75/90 days

$ = After two weeks four control males were assigned to 33 ppm dose group; @ = Four control males were assigned to this group after two weeks of study; # = Eight dogs were given 1500 ppm of sulfluramid in the diet for the first 2 weeks and four died within 2 weeks; the remaining four surviving male dogs were given 20 mg/kg/day of sulfluramid in capsular form for 75 days; & = dogs were given 1500 ppm of sulfluramide for the first 2 weeks and then were given 20 mg/kg/day of sulfluramid in capsular form for 90 days.

Administration of sulfluramid to dogs produced treatment-related increase (↑) or decrease (↓) of the following parameters:

Parameters		0 ppm	33 ppm	100 ppm	500 ppm	1500 ppm
Mortality	↑					♂
Defecation	↑				♀	♂,♀
Body Weight	↓				♂,♀	♂,♀
Food Consumption	↓				♂,♀	♂,♀
Erythrocyte, Hematocrit & Hemoglobin	↓					♂,♀
Protein, Albumen and Globulin	↓					♂,♀
Blood Urea Nitrogen	↑				♂,♀	♂,♀
Serum Calcium and Phosphorus	↓				♂,♀	♂,♀
Liver Weight	↑				♂	♂
GI Tracts: Red Contents	↑					♂,♀
Ileum/Lungs/Stomach:Red Areas	↑					♂,♀
Lymph Node Hyperplasia	↑					♂
Spleen and Thymus Atrophy	↑					♂
Renal Hyperemia and Nephrosis	↑					♂
Epididymides and Testes Weights	↑				♂	♂
Epididymal & Testicular Lesions	↑			♂	♂	♂
Aspermia and Giant Cells Formations in Testes	↑					♂
Spermatogenesis	↓				♂	♂
Sperm Motility and Count	↓				♂	♂

a = dosage reduced to 20 mg/kg/day from week 5; ↑ = increased; ↓ = decreased.

As shown in the above summary Table, administration of sulfluramid in dogs produced the following major effects:

- o Increased mortality in the 1500 ppm:20 mg/kg/day males.
- o Reduction of body weight in the 500 ppm and 1500 ppm: 20 gm/kg/day groups.

- o Increased liver weight in the 500 ppm and 1500 ppm: 20 gm/kg/day males
- o Increased blood urea nitrogen in the 500 ppm and 1500 ppm: 20 gm/kg/day groups.
- o Increased renal hyperemia and nephrosis in the 1500 ppm: 20 mg/kg/day males
- o Reduction of epididymides and testes weights in the 500 ppm and 1500 ppm:20 gm/kg/day groups
- o Increased epididymal and testicular lesions affecting the seminiferous tubules of the testes of the 100 ppm, 500 ppm, and 1500 ppm:20 mg/kg/day dose groups.
- o Reduced percent motile sperm, and the reduction of epididymal sperm and testicular spermatid concentration of the 500 ppm and 1500 ppm: 20 mg/kg/day dose groups.

The NOEL for Sulfluramid 96.6% linear and 3.4% branched isomeric mixture was determined to be 33 ppm, when fed in the diet of dogs for a period of at least 90 days. The LOEL is 100 ppm based on the increased epididymal and testicular lesions. The doses employed in this study were sufficient to produce compound-related effects.

Sulfluramid appears to be a direct acting testicular toxin in the dog with primary effect on the late spermatids and possibly Sertoli cells.

CLASSIFICATION: Core-Minimum

APPENDICES

APPENDIX A: Study Design (copied from p. 18 of the study report).

APPENDIX B : Mean Body Weights in Kg (values rounded off) at Various Times for the Control, 33 ppm, 100 ppm, 500 ppm and 1500 ppm Dose Groups (derived from p. 70-80 of the study report)

APPENDIX C : Mean Body Weight Gains in 100 gms (values rounded off) at Various Times for the Control, 33 ppm, 100 ppm, 500 ppm and 1500 ppm Dose Groups (derived from p. 81-89 of the study report)

APPENDIX D: Mean Food Consumption Intake in gm/dog/day (values rounded off) at Weekly Interval for the Control, 33 ppm, 100 ppm, 500 ppm and 1500 ppm Dose Groups (derived from p. 94-102 of the study report)

APPENDIX E: Summary Mean Weekly Food Consumption in gms/kg/day (values rounded off) for the Control, 33 ppm, 100 ppm, 500 ppm and 1500 ppm Dose Groups (derived from p. 103-111 of the study report)

APPENDIX F: Summary Hematology Data (derived from Tables 21 through 44 and Tables 90 through 113 of the study report)

APPENDIX G: Summary of the Clinical Chemistry Data (derived from p. 114 - 121 of the study report)

APPENDIX H: Mean Organ Weights in Grams (derived from p.271-276 of study report; some values were rounded off)

APPENDIX I: Mean Organ/Body Weight Ratios (derived from p.277-287 of study report; some values were rounded off)

APPENDIX J: Summary of Pertinent Gross Necropsy Data (Derived from p. 244-247 of the study report)

APPENDIX K: Summary of Pertinent Histopathological Data (Derived from p. 248 - 272 of the study report)

APPENDIX L: Summary of Pertinent Microscopic Data (Copied from p. 248-270 of the study report)

APPENDIX M: Summary Incidence Data of Testicular Lesions (Copied from p. 910 of the study report)

APPENDIX N: Summary Mean Severity Grades for Testicular Lesions (Copied from p. 911 of the study report)

APPENDIX O: Summary Epididymal Sperm and Testicular Spermatid Analysis (Copied from p. 908 of the study report)

APPENDIX P: Summary Epididymal Histologic Evaluations (Copied from p. 914 of the study report)

Sulfluramid

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Pages 19 through 33 are not included.

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