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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metam Sodium: Review of a Chronic Toxicity / Carcinogenicity Study in Rats and Chronic Toxicity Study in Dogs Submitted by the Registrant.

P.C. Code: 039003
Submission: S468984
MRID Nos: 432758-01 and 432758-02
DP Barcode: D204958

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 8/29/94
Review Section I, Toxicology Branch II
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TO: Tom Myers / PM 51
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THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y M Ioannou 8/29/94
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) Marcia Van Gemert 8/29/94

Registrant: Metam Sodium Task Force

Action Requested: Review of a chronic toxicity / carcinogenicity study conducted in rats and a chronic toxicity study conducted in dogs submitted in support of reregistration of metam sodium.

(1)

1) MRID # 432758-01

Title: Metam Sodium: 1 Year Oral Toxicity Study in Dogs

Summary:

In a chronic toxicity study in dogs (MRID # 432758-01), metam sodium was administered by gelatin capsule to groups of 4 male and female beagle dogs at doses of 0, 0.05, 0.1, and 1.0 mg/kg/day for 52 weeks.

There were no deaths nor treatment-related clinical signs of toxicity. Group mean body weight and body weight gain were not significantly affected at any dose level. An increase in kaolin-cephalin time at the 1.0 mg/kg/day dose was observed in male and female dogs, but the toxicological significance of this observation is questionable. Group mean ALT levels at 1.0 mg/kg/day gradually increased in female dogs over the course of the study until study termination, where the mean value was 3x control. This increase was due mainly to changes in one female dog. In the female dog with elevated ALT, microscopic examination showed slight increases in hepatocyte and macrophage/Kupffer cell pigmentation, slight mononuclear cell infiltration, slight telangiectasis, and a positive reaction for hemosiderin.

The systemic LEL: > 1 mg/kg/day (males); **= 1 mg/kg/day** (females; increased ALT and microscopic changes in the liver).

The systemic NOEL ≥ 1 mg/kg/day (males); **= 0.1 mg/kg/day** (females)

This study is classified **core minimum** and satisfies the guideline requirements for §83-1, Chronic Toxicity Study in Dogs

2) MRID # 432758-02

Title: Metam Sodium: Two Year Drinking Study In Rats

Summary:

In a two year combined chronic toxicity/carcinogenicity study (MRID # 432758-02), Metam Sodium technical (43.14% a.i.) was administered in drinking water to groups of 64 male and female rats for either 52 weeks or 104 weeks at dose levels of 0, 0.019, 0.056, and 0.19 ppm (**1.3 mg/kg, 3.9 mg/kg,** and **12.0 mg/kg** for male rats; **2.3 mg/kg, 6.2 mg/kg,** and **16.2 mg/kg** for female rats).

At 0.19 mg/ml, male and female rats showed decreased group mean body weight gain for weeks 1-13 (12% decrease in males, 16% decrease in females) and weeks 1-105 (18% for males, 20% for females). Decreased food consumption, food efficiency, and water consumption were significantly affected at the

0.19 mg/ml dose in both sexes. Effects on hematology (decreased red blood cells, hemoglobin, hematocrit) and clinical chemistry (decreased cholesterol and triglycerides) were also observed in both sexes at the 0.19 mg/ml dose level. Increased number of liver masses and increased incidence of fat vacuolation of the liver were observed in male rats at the 0.19 mg/ml dose, as was increased incidence of wasting of voluntary muscle. Microscopic abnormalities of the nasal cavity (increased incidence of rhinitis, hypertrophy of Bowman's ducts/glands, atrophy and adenitis of Steno's gland, and olfactory epithelium hyperplasia and degeneration), heart and aorta (decreased mineralization), voluntary muscle (increased severity of degenerative myopathy), and sciatic nerve (increased severity of degeneration) were observed in male and/or female rats at the 0.19 mg/ml dose level. **The LEL of 0.19 mg/ml is based on the changes in body weight gain, food efficiency, hematologic and clinical chemistry alterations, and macro- and microscopic abnormalities observed at this dose in both sexes. The NOEL is 0.056 mg/ml.**

Evaluation of tumor data presented in this study demonstrated that metam sodium shows no carcinogenic potential in rats.

This study is classified core **minimum** and **satisfies** the guideline requirements for §83-5, Combined Chronic Toxicity and Carcinogenicity Study in Rats.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 8/26/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JMI 8/26/94
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity/Dogs (83-1)

EPA I.D. NUMBERS: P. C. CODE: 039003
MRID NUMBER: 432758-01

TEST MATERIAL: Metam Sodium

STUDY NUMBER: PD0905

TESTING FACILITY: Zeneca Central Toxicology Laboratory
Cheshire, United Kingdom

SPONSOR: Metam Sodium Task Force

TITLE OF REPORT: Metam Sodium: 1 Year Oral Toxicity Study in Dogs

AUTHOR(S): A. Brammer

REPORT ISSUED: May 23, 1994

EXECUTIVE SUMMARY: In this chronic dog study (MRID # 432758-01), metam sodium was administered in gelatin capsules to four male and four female beagle dogs per group at dosages of 0, 0.05, 0.1 or 1.0 mg/kg/day for 52 weeks. The study was conducted in two randomized blocks, each comprising two male and two female replicates consisting of one dog per treatment group.

There were no deaths nor treatment-related clinical signs of toxicity. Group mean body weights of the treated animals were comparable to the control groups over the course of the study.

The only consistent statistically significant change in any of the hematology parameters was an increase in the kaolin-cephalin time in the 1.0 mg/kg/day males and females. The toxicological significance of this finding without other alterations in coagulation or hematology parameters is questionable. The group mean ALT levels in the 1.0 mg/kg/day group females gradually increased over the course of the study until week 52 when the value was three times that of the control level. However, the difference was due to one female whose level peaked at 400 IU/l during weeks 45 and 52. This animal also had a slight increase in AST.

The only treatment-related finding on necropsy was on microscopic examination of the liver of the female from the 1.0 mg/kg/day group with the ALT elevation. This animal had a slight increase in hepatocyte and macrophage/Kupffer cell pigmentation, slight

mononuclear cell infiltration, slight telangiectasis and a positive reaction for hemosiderin.

LOEL - males > 1 mg/kg/day; females = 1 mg/kg/day
NOEL - males ≥ 1 mg/kg/day; females = 0.1 mg/kg/day

The study is **Core Minimum** and **satisfies** the guideline requirements (83-1) for a chronic toxicity study in the dog.

I. MATERIALS

A. Test Material

Name: Metam sodium
Chemical Name: Not provided
Purity: 43.148% w/w
Batch Reference: BAS/005/00N 90-2
Description: Yellow colored aqueous solution
Storage Conditions: Under argon at room temperature, in the dark, in a vented area

Initially, samples were sent to BASF for analysis to confirm stability and then subsequently to Zeneca Agrochemicals, Jealott's Hill for the remainder of the study. Appendix B of the study report contains results of these analyses which show that the values obtained from Jealott's Hill were generally slightly lower than those received from BASF. However, all concentrations were within an acceptable range of the nominal concentration.

B. Administration: gelatin capsules

C. Test Animals

Species: Purebred beagle dogs
Source: Zeneca Pharmaceuticals, Cheshire, UK
Age: 17-20 weeks old when received
Weight: Males - Approximately 12.2 kg; Females - approximately 10.3 kg at commencement of treatment
Housing: In pairs or threes in indoor pens for the first seven days and then individually
Environmental Conditions: Temperature: usually 19-25^0 C
Relative humidity: not provided
Photoperiod: 12 hours light/dark
Air changes: 10 per hour
Food and Water: Males - 400 g daily of an expanded dry diet (Laboratory Diet A); Females - 350 g of diet; water *ad libitum*
Acclimation Period: Four to five weeks

All dogs were vaccinated and treated regularly for possible nematode and ear mite infestation.

II. METHODS

A. Dosage and Administration

Sixteen (16) male and 16 female dogs were "randomly allocated to treatment groups" as follows:

Group	Dosage Level (mg/kg/day)	Number of Dogs Male	Female
1	0 (Control)	4	4
2	0.05	4	4
3	0.1	4	4
4	1.0	4	4

The study report indicates that the dose levels selected were based on the results of a previous dog study (CTL Report Number CTL/P/3679). In a June 23, 1992 memo from Yiannakis M. Ioannou, Toxicology Branch II, to Christine Rice/Tom Myers (PM 52), Accelerated Reregistration Branch, the dose levels of 0, 0.05, 0.2 and 0.5 mg/kg/day were suggested for females in the chronic toxicity study. For male dogs, the high dose suggested was at least 1.0 mg/kg/day with the mid and low dose levels as deemed appropriate by the registrant. These recommendations were based on limited data from a study in which dogs were dosed with 0, 1, 5 or 10 mg/kg/day for 90 days.

The appropriate weight of the chemical was placed in a capsule with a capacity of 5 ml which was then placed into a capsule with a 10 ml capacity. Control animals received the vehicle only at a volume similar to that of the high dose group. Dosing solutions of 0.5, 1.0 or 10 mg/ml were prepared daily; analyses of these solutions for chemical stability and achieved chemical concentration were done five times during the study.

B. Experimental Design

The study was conducted in two randomized blocks each comprising two male and two female replicates. Each replicate consisted of four dogs, one per treatment group. Replicates 1, 2, 5 and 6 were started on August 1992 and 3, 4, 7 and 8 on September 1992.

The study protocol required the following observations and examinations at the indicated times or frequencies.

clinical signs of toxicity - twice daily (after dosing and at end of working day)
"thorough examination" - weekly
record of fecal consistency - daily
clinical examination (including cardiac and pulmonary auscultation and indirect ophthalmoscopy) by veterinarian - prestudy and during weeks 13, 26, 39 and 52
body weights - before feeding throughout prestudy period, on day 1, and thereafter at weekly intervals
food consumption - daily for at least 2 weeks prestudy and throughout the treatment period
hematology, clinical chemistry - weeks -1, 4, 13, 26 and 52; additional samples were taken for alanine aminotransferase (ALT) and aspartate aminotransferase (AP) on weeks 8, 19,

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32, 39 and 45
urinalysis - weeks -1, 26 and 52
gross necropsy - all animals
histopathology - designated organs and tissues from all animals

C. Pathological Parameters

For hematology and clinical chemistry evaluations, blood was drawn from the jugular vein before feeding. Urine was collected by catheterization.

The CHECKED (X) hematology parameters were examined.

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Prothrombin Time
__Reticulocyte count
__Total plasma protein (TP)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
X Kaolin-cephalin time (activated partial thromboplastin time)

* EPA guideline requirement

The CHECKED (X) clinical chemistry evaluations were done.

Electrolytes:
X Calcium*
X Chloride*
__Magnesium*
X Phosphorus*
X Potassium*
X Sodium*

Enzymes:
X Alkaline phosphatase (AP)
__Cholinesterase
X Creatine kinase*
__Lactic acid dehydrogenase
X Serum alanine aminotransferase (ALT, also SGPT)*
X Serum aspartate aminotransferase (AST, also SGOT)*

Other:
__Albumin*
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
__Globulins
X Glucose*
X Total Bilirubin*
X Total Protein*
X Triglycerides

* EPA guideline requirement

The CHECKED (X) urinalysis parameters were measured.

__Appearance*
X Volume*
X Specific gravity*
X pH
X Sediment (microscopic)*
X Protein*
X Glucose*
X Ketones*
X Bilirubin*
X Blood*
__Nitrate

* EPA guideline requirement

The following CHECKED (X) tissues were preserved at the routine necropsy; the (XX) organ(s) in addition were weighed.

Digestive System	Cardiovasc./Hemat. System	Neurologic System
__Tongue	X Aorta*	XXBrain*
X Salivary glands*	X Heart*	X Periph. nerve*
__Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes (Optic n.)*
X Jejunum*	X Thymus*	Glandular
X Ileum*	Urogenital System	XXAdrenals*
X Cecum*	XXKidneys*	__Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	XXTestes*	XXParathyroids*
XXLiver*	XXEpididymides	XXThyroids*
X Gall bladder*	X Prostate/urethra	Other
X Pancreas*	__Seminal vesicle	X Bone*
Respiratory System	X Ovaries	X Skeletal muscle*
X Trachea*	X Uterus*	X Skin
X Lung*	__Vagina	X All gross lesions and masses

* EPA Guideline requirement

In addition to the routine hematoxylin and eosin stains on the tissue sections, selected sections of the liver were stained by the Perl's prussian blue method for hemosiderin, the Gordon and Sweet method for reticulin and the Masson trichrome method for connective tissue.

D. Statistical Analyses

A description of the statistical analyses from the study report is attached to the DER. Data from the two replicates were combined for the analyses.

E. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming nc data confidentiality. A signed "EPA Flagging Criteria" document indicated that the study neither meets nor exceeds the criteria of 40 CFR 158.34.

III. RESULTS

A. Analyses of Dosing Solutions

Analyses of five samples of the 0.5, 1.0 and 10 mg/ml dosing solutions demonstrated that the concentrations of metam sodium were within acceptable ranges during the study. (Tables 2 and 3, pages 30-31 of the study report.)

B. Mortality

No animals died during the study.

C. Clinical Signs and Physical Examinations

There were no treatment-related clinical or ophthalmoscopic abnormalities observed during the study. Salivation at dosing was sporadically noted in animals from each of the treatment groups but was regularly observed in three animals; a male and female in the 0.1 mg/kg/day group and a female in the 1.0 mg/kg/day group. One male dog in the 0.1 mg/kg/day group was noted to be thin from week 12 to the end of the study, however, the animal appeared healthy in every other respect. One male in the 1.0 mg/kg/day group had an increased incidence of fluid feces once weekly during weeks 12-29 of the study.

D. Body Weight and Body Weight Gain

The study report states that group mean body weights for females in the 0.1 and 1.0 mg/kg/day groups were reduced in comparison to the controls during the latter half of the study. The % difference from control progressed to 8% by the end of the study for both groups. The finding is considered to be due to one female in each group which lost weight at the end of the study. The study report states that this was incidental to treatment. The female in the 0.1 mg/kg/day group gained 2.6 kg (10.1 to 12.7 kg) from weeks 1-13, but then lost 0.6 kg by the end of the study (final weight = 11.8 kg). However, overall weight gain in this animal was comparable to that of other females in this group. The animal in the 1.0 mg/kg/day group gained 2.5 kg (9.5 to 12.0 kg) from weeks 1-21 but then lost slightly at the end of the study (final weight = 12.2 kg). However, overall weight gain in this animal was also comparable to other females in the group.

The treated male groups also had small reductions in weight gain at the end of the study. One male in the 0.1 mg/kg/day group had a marked weight reduction which was considered incidental to treatment. This animal gained 1.3 kg from weeks 1-15 (13.8 to 15.1 kg), but then began to lose weight gradually until it returned to its week 1 weight at week 31, where it essentially remained for the rest of the study.

Body weight and body weight data are presented in Table 1. Only body weight was statistically analyzed (on a weekly basis) in the study report.

Table 1
Body Weight and Body Weight Gain in Dogs
Administered Metam Sodium Orally for 52 Weeks[a]

	Dosage Levels (mg/kg/day)							
	Males				Females			
	0	0.05	0.1	1.0	0	0.05	0.1	1.0
Mean Body Weight (kg)								
Week 1	12.1	12.1	12.2	12.2	10.3	10.4	10.5	10.1
Week 13	14.9	14.6	14.7	14.8	12.0	12.2	11.9	11.7
% Control Value	-	98	99	99	-	102	99	98
Week 26	16.1	15.6	15.7	15.7	13.0	13.2	12.6	12.2
% Control Value	-	97	98	98	-	102	97	94
Week 53	16.9	16.2	16.4	16.3	13.8	14.0	12.9	12.7
% Control Value	-	96	97	96	-	101	93	92
Mean Body Weight Gain (kg)								
Weeks 1-13	2.8	2.5	2.5	2.6	1.7	1.8	1.4	1.6
% Control Value	-	89	89	93	-	106	82	94
Weeks 13-26	1.2	1.0	1.0	0.9	1.0	1.0	0.7	0.5
% Control Value	-	83	83	75	-	100	70	50
Weeks 26-53	0.8	0.6	0.7	0.6	0.8	0.8	0.3	0.5
% Control Value	-	75	88	75	-	100	38	63
Weeks 1-26	4.0	3.5	3.5	3.5	2.7	2.8	2.1	2.1
% Control Value	-	88	88	88	-	104	78	78
Weeks 1-52	4.8	4.1	4.2	4.1	3.5	3.6	2.4	2.6
% Control Value	-	85	88	85	-	103	69	74

a Body weights were extracted from Table 7 (pages 44-57) of the study report; body weight gains and % control values were calculated by the reviewer.

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E. Food Consumption

Food intake in the treated groups was comparable to the controls.

F. Clinical Pathology

Hematology

The only consistent statistically significant change in any of the hematology parameters was increases in the kaolin-cephalin time in the 1.0 mg/kg/day group males (statistically significant at Weeks 4, 13 and 26) and females (significant at Weeks 4, 13 and 52). The toxicological significance of this finding is questionable.

Clinical Chemistry

The study report states that group mean ALT levels were increased in the 1.0 mg/kg/day group females from week 8. However, the effects were animal specific, accounting for the lack of statistical significance. Review of the individual animal data shows that one female (animal # 229) had a gradual increase in ALT values over the course of the study that peaked at 400 IU/l for weeks 45 and 52. The other females in this group had values that were slightly elevated over the pretreatment levels and only slightly exceeded the control levels. The 52-week AST level for female # 229 was also slightly increased. One male in the 1.0 mg/kg/day group had a gradual increase in ALT levels over the course of the study with a 52-week value of 134 IU/l. The group mean values for the major liver enzymes in the dog (ALT and AST) are presented in Table 2.

Table 2
Group Mean Liver Enzyme Levels in Dogs
Administered Metam Sodium for One Year[a]

	Dosage Levels (mg/kg/day)							
	Males				Females			
	0	0.05	0.1	1.0	0	0.05	0.1	1.0
ALT (IU/l)								
Pre-Treatment	18.8	16.0	14.0	13.8	19.0	18.5	17.0	20.3
Week 4	22.5	21.8	16.8	16.0	23.8	20.8	19.8	24.8
8	22.0	26.5	16.8	19.5	22.5	24.0	22.0	30.5
13	24.3	31.3	25.8	21.5	29.5	28.3	22.0	38.0
19	31.3	32.5	24.8	24.5	29.0	28.0	26.5	40.8
26	29.3	36.5	24.3	28.0	33.3	27.0	29.3	103.3
32	35.3	36.5	22.0	33.5	28.3	34.0	24.8	102.8
39	33.0	37.5	21.8	38.8	26.0	34.5	24.5	90.0
45	36.0	42.3	24.0	38.8	29.5	32.0	26.3	128.5
52	42.0	54.0	29.3	56.5	32.8	34.5	29.3	125.0
AST (IU/l)								
Pre-treatment	16.0	15.0	13.8	13.0	17.5	17.0	18.0	16.3
Week 4	16.3	18.5	16.3	15.0	22.0	17.8	22.0	20.3
13	18.0	21.5	22.5	16.0	21.8	23.0	20.3	21.5
26	21.8	22.3	22.8	17.5	21.3	20.3	25.3	26.8
52	28.5	32.5	27.3	24.3	26.5	28.5	29.5	28.8

a Extracted from Table 10 (pages 105-106 and 123-126) of the study report

Urinalysis

There were no treatment-related changes.

G. Necropsy Findings

Gross Necropsy

There were no treatment-related changes on post-mortem macroscopic examination.

Organ Weights

Organ weights for the treated groups were comparable to the control groups.

Histopathology

The study report states that treatment-related findings were confined to the liver of one female dog (animal # 229) in the 1.0 mg/kg/day group and comprised a slight increase in hepatocyte and macrophage/Kupffer cell pigmentation, slight mononuclear cell infiltration and slight telangiectasis. The individual animal necropsy report also shows that this animal had a positive reaction for hemosiderin by Perl's technique. However, one control group female also had hepatic changes consisting of monocellular infiltration, minimal hepatocyte pigmentation and increased macrophage/Kupffer cell pigmentation.

H. Conclusion from Study Report

"The study report concluded that the oral administration of metam sodium to dogs for 1 year at dose levels of 0.05 - 1.0 mg/kg/day caused no overt toxicity, although changes were seen in individual animals. Toxicologically significant elevations in plasma ALT activity and histopathological changes in the liver were observed in one female in the 1.0 mg/kg/day group. The no-observable effect level over a 1 year period was 0.1 mg/kg/day."

I. DISCUSSION

In this chronic dog study (MRID # 432758-01), metam sodium was administered in gelatin capsules to four male and four female beagle dogs per group at dosages of 0, 0.05, 0.1 or 1.0 mg/kg/day for 52 weeks. The study was conducted in two randomized blocks, each comprising two male and two female replicates consisting of one dog per treatment group.

There were no deaths nor treatment-related clinical signs of toxicity, apart from sporadic salivation on dosing in the treated groups.

Group mean body weights of the treated animals were comparable to the control groups, however body weight gains for all the treated males and the two highest dosage group females were less than 90% of the control value during most of the study. The toxicological significance of this finding is questionable for the following reasons. First, body weights for the treated animals were comparable to the control groups (>90% of the control value) for the study duration. Second, the dogs were approximately six months old when treatment commenced. Most dogs reach 50% of their mature weight by 4 months of age. Rapid growth continues until 6-9 months

and adult weight is approached at approximately 1 year of age.[1] Therefore, it would be expected that weight gain during the study would not be significant. Third, the difference in body weight gain was due to one animal/sex in the 1.0 mg/kg/day group. Food consumption was comparable between the treated and control groups.

The only consistent statistically significant change in any of the hematology parameters was an increase in the kaolin-cephalin time in the 1.0 mg/kg/day males and females. The toxicological significance of this finding without other alterations in coagulation or hematology parameters is questionable. The group mean ALT levels in the 1.0 mg/kg/day group females gradually increased over the course of the study until week 52 when the value was three times that of the control level. However, the difference was due to one female (# 229) whose level peaked at 400 IU/l during weeks 45 and 52. This animal also had a slight increase in AST. The group mean ALT level in the 1.0 mg/kg/day group males was only slightly increased over the control value at 52 weeks. One male in the group had a gradual increase in the parameter over the course of the study that peaked at 134 IU/l at week 52. The increases were not statistically significant in either the 1.0 mg/kg/day group males or females.

The only treatment-related finding on necropsy was on microscopic examination of the liver of female #229 from the 1.0 mg/kg/day group. This animal had a slight increase in hepatocyte and macrophage/Kupffer cell pigmentation, slight mononuclear cell infiltration, slight telangiectasis and a positive reaction for hemosiderin. However, one control group female also had hepatic changes consisting of monocellular infiltration, minimal hepatocyte pigmentation and increased macrophage/Kupffer cell pigmentation.

J. CONCLUSION

The lack of clear-cut, consistent findings in this study make the assignment of a Lowest Observed Effect Level (LOEL) difficult. Although, the hepatic changes (increased ALT and AST levels and microscopic findings) were limited to one female in the 1.0 mg/kg/day group, they are consistent with changes reported in other studies in dogs with this chemical. In the 90-day oral study in dogs (MRID # 426000-01), one female in the 1.0 mg/kg/day group also had a significantly increased ALT level. Therefore, this dose should be considered an effect level for females.

LOEL - males $>$ 1 mg/kg/day; females = 1 mg/kg/day
NOEL - males $\geq$ 1 mg/kg/day; females = 0.1 mg/kg/day

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[1] Lewis LD, Morris ML and Hand MS. Small Animal Nutrition III. Mark Morris Associates, Topeka, Kansas (1987).

Reviewed by: Timothy F. McMahon, Ph.D. 8/25/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 8/25/94
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Combined Carcinogenicity/Chronic Toxicity - rats
Guideline: 83-5

EPA ID Numbers: MRID number: 432758-02 P.C. Code: 039003

Test material: Metam Sodium

Chemical name: Sodium N-methylthiocarbamate

Project I.D. : PR0838

Sponsor: Metam Sodium Task Force

Testing Facility: Zeneca Central Toxicology Laboratory
Cheshire, UK

Title of report: Metam Sodium: Two Year Drinking Study in Rats

Author(s): N J Rattray

Study Completed: May 23, 1994

Executive Summary:

In a two year combined chronic toxicity/carcinogenicity study (MRID # 432758-02), Metam Sodium technical (43.14% a.i.) was administered in drinking water to groups of 64 male and female rats for either 52 weeks or 104 weeks at dose levels of 0, 0.019, 0.056, and 0.19 ppm (**1.3 mg/kg**, **3.9 mg/kg**, and **12.0 mg/kg** for male rats; **2.3 mg/kg**, **6.2 mg/kg**, and **16.2 mg/kg** for female rats).

At 0.19 mg/ml, male and female rats showed decreased group mean body weight gain for weeks 1-13 (12% decrease in males, 16% decrease in females) and weeks 1-105 (18% for males, 20% for females). Decreased food consumption, food efficiency, and water consumption were significantly affected at the 0.19 mg/ml dose in both sexes. Effects on hematology (decreased red blood cells, hemoglobin, hematocrit) and clinical chemistry (decreased cholesterol and triglycerides) were also observed in both sexes at the 0.19 mg/ml dose level. Increased number of liver masses and increased incidence of fat vacuolation of the liver were observed in male rats at the 0.19 mg/ml dose, as was increased incidence of wasting of voluntary muscle. Microscopic abnormalities of the nasal cavity (increased incidence of rhinitis,

hypertrophy of Bowman's ducts/glands, atrophy and adenitis of Steno's gland, and olfactory epithelium hyperplasia and degeneration), heart and aorta (decreased mineralization), voluntary muscle (increased severity of degenerative myopathy), and sciatic nerve (increased severity of degeneration) were observed in male and/or female rats at the 0.19 mg/ml dose level. **The LEL of 0.19 mg/ml is based on the changes in body weight gain, food efficiency, hematologic and clinical chemistry alterations, and macro- and microscopic abnormalities observed at this dose in both sexes. The NOEL is 0.056 mg/ml.**

Evaluation of tumor data presented in this study demonstrated that metam sodium shows no carcinogenic potential in rats.

Dosing was considered adequate in male and female rats based upon the decrease in body weight gain, food efficiency, changes in hematology and clinical chemistry, and microscopic pathology observed at the 0.19 mg/ml dose level.

This study is classified core **minimum** and **satisfies** the guideline requirements for §83-5, Combined Chronic Toxicity and Carcinogenicity Study in Rats.

I. MATERIALS AND METHODS

A. Test Material:

Metam Sodium technical; purity: 43.15% (written verification provided on page 426 of the report)
description: yellow colored aqueous solution
sample reference No: BAS/005/00N 90-2

B. Test Animals

Six batches of approximately 50 male and 50 female rats were delivered weekly for 6 weeks beginning in March of 1991. The strain was listed as Hsd/Ola: Wistar Tox strain obtained from Harlan Olac Ltd. Shaws Farm, Blackthorn, Bicester, Oxon, UK. Rats were delivered as weanlings (22 days old) and were placed into a single animal room for the entire study. Age: approximately 4 weeks at receipt. Acclimation period: approximately 2 weeks. Weight range (at time of dosing): males, 133-256g; females, 99-186g.

C. Animal Husbandry

Rats were housed by sex in multiple rat racks constructed of square-section aluminum. Cages were constructed of stainless steel, with solid sides, and 14 gauge wire mesh front, back, and floor. Cages were suspended over stainless steel collecting trays lined with absorbent paper sheets. Rats were initially housed not more than 6 per cage, and four per cage following assignment to experimental groups.

Food (Powdered CT1 Diet) was supplied in glass jars of approximately 300g capacity and was available *ad libitum* except during collection or urine. Drinking water was supplied in 250ml polycarbonate bottles fitted with glass nozzles and was also available *ad libitum* except during urine collection periods. Temperature during the study was designed to be maintained at 19-23 ^{o}C, with an overall range of 16-28 ^{o}C observed for the study as a whole. Humidity was designed to be maintained at 45%, with an overall recorded range of 25-86%.

D. Drinking Water Preparation

All experimental drinking water preparations were prepared daily in batches of 10 liters. For each dose level, the appropriate amount of metam sodium was added to the purified drinking water. This was then thoroughly mixed and dispensed into polycarbonate drinking bottles. This preparation supplied enough water to last each cage one day, after which bottles were removed, emptied, refilled, and replaced with drinking water from a new, freshly prepared batch.

E. Stability and Homogeneity

Amounts of metam sodium required for 10 liter batches of drinking water preparations at each dose level (corrected for purity) are summarized below (page 429 of the report):

DRINKING WATER PREPARATION

Group	Conc. of metam sodium in water (mg/ml)	Quantity of Test Substance to Prepare 10l of water (g)	Colour Code
1	0	0	Blue
2	0.019	0.43	Green
3	0.056	1.29	Yellow (Gold)
4	0.19	4.3	Red

Concentration of metam sodium in drinking water was determined by reacting portions of stock solution with cupric chloride-acetic acid reagent and measuring the resultant yellow solutions spectrophotometrically at 420nm.

During the pre-experimental period, drinking water analysis was performed twice to confirm the acceptability of the preparation procedure. Stability over a 24 hour period was also determined. After the start of treatment, drinking water was analyzed twice weekly for the first 2 weeks, once a week for the next 2 weeks, and thereafter, one batch was analyzed monthly to confirm established concentrations of metam sodium. Concentration was determined from samples taken from the bulk preparation immediately prior to administration to test animals.

Results of test article analysis in drinking water as well as 24 hour stability were presented in Appendices H and I, pages 439-470 of the report. A total of 30 measurements were made of drinking water concentration of metam sodium at each dose level over the course of the study. The following data are the mean analyzed concentration and mean percent of nominal at each dose.

Dose (mg/ml)	Mean conc.	Mean % Nominal
0.019	0.018	96.7
0.056	0.054	97.7
0.19	0.187	98.4

As shown, mean concentrations of metam sodium in drinking water prior to administration were close to nominal values.

Data on dose solution stability were actually the same data presented in the mouse carcinogenicity study on metam sodium (MRID # 432335-01) and are reproduced below:

Table 1
Dosing Solution Stability of Metam Sodium in Drinking Water[a]

Group	Nominal Conc. (mg/ml)	Analyzed Conc. (mg/ml)[b]	% Nominal
1	Control	ND	
2	0.019	0.016	87.6
3	0.074	0.067	91.3
4	0.23	0.221	96.2

[a]data taken from Appendix H of MRID # 432335-01. [b]analyzed concentration and % nominal represent the mean of 12 measurements made at 24 hours post-dose preparation.

Appendix J, pages 471-478 of the present study, summarized the doses received by male and female rats over the course of this study, with corresponding mean values for the overall study period. The above table shows that at the low dose, degradation of approximately 12% from original concentration occurs over a 24 hour period, with lesser degradation observed at the two higher doses. Starting concentrations of test material at each dose were indicated above on page 4 of this review. The report stated the mean intake of test chemical at each dose as follows:

Males: **1.5 mg/kg** (low dose); **4.3 mg/kg** (mid dose); **12.5 mg/kg** (high dose)

Females: **2.7 mg/kg** (low dose); **6.8 mg.kg** (mid dose); **16.8 mg/kg** (high dose)

These doses can be adjusted for degradation occurring over a 24 hour period as has been the practice for calculating doses of metam sodium received in other toxicology studies submitted to the Agency:

Males: **1.3 mg/kg** (low dose); **3.9 mg/kg** (mid dose); **12.0 mg/kg** (high dose)
Males: **1.3 mg/kg** (low dose); **3.9 mg/kg** (mid dose); **12.0 mg/kg** (high dose)

Females: **2.3 mg/kg** (low dose); **6.2 mg.kg** (mid dose); **16.2 mg/kg** (high dose)

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F. Experimental Design and Dosing

The following experimental design was used for this study:

Group #	Dietary Level (mg/ml)	Sacrifice Interval (weeks) 52 (Tox phase) M	52 (Tox phase) F	104 (Onco phase) M	104 (Onco phase) F
1 (Control)	0	12	12	52	52
2 (Low)	0.019	12	12	52	52
3 (Mid)	0.056	12	12	52	52
4 (High)	0.19	12	12	52	52
5 (Sentinel)	0			12	12
6 (Sentinel)	0.19			12	12

In this design, 52 animals/sex were assigned to treatment with metam sodium technical in drinking water for a period of 104 weeks, while an additional 12 rats/sex received metam sodium in drinking water for 52 weeks. Microbiological sentinels (12/sex) were given either 0 mg/ml or 0.19 mg/ml metam sodium and were housed in the same racks as the experimental animals. The duration of treatment for sentinel rats was not stated. Treatment of rats commenced between March 19, 1991 and April 25, 1991.

Dose selection for the present study was based on results of a 90-day toxicity study in rats previously performed in the same laboratory. Results of this study were not summarized in the present report.

G. Statistical Analysis

Analysis of variance was used for statistical evaluation of food and water consumption for the first 12 weeks of the study, organ weights, hematology, and clinical chemistry. Body weight was analyzed by analysis of covariance.

Differences from control were tested by comparing each treatment group least squares mean with the control least squares mean using Student's t-test based on the error mean square in the analysis.

Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Mortalities resulting from animals killed during the study or accidental deaths were considered censored observations.

Incidence of individual tumors and overall incidence of each tumor type were considered by comparing each treated group and the control group at certain time points and during selected time intervals using Fisher's Exact Test. A test for trend was conducted in addition using the Cochran-Armitage Test. Tumor analyses were conducted separately for males and females.

H. Compliance

A signed statement of no data confidentiality claims was provided.

A signed statement of GLP compliance was provided.

A signed statement of quality assurance was provided.

A signed statement of flagging studies for potential adverse effects was provided. According to the report, under the criteria of 40 CFR 158.34, this study neither meets nor exceeds any of the applicable criteria.

II. OBSERVATIONS AND RESULTS

A. **Mortality**

Rats were observed prior to study initiation to ensure that they were physically normal and that they exhibited normal activity. During the study, all rats were examined daily for changes in clinical condition and behavior. Once a week, a detailed examination of each rat was made. Any rats requiring euthanasia or found dead were given a post mortem examination. Survival in male and female rats is summarized in the following Table (Table 2), and is based upon data obtained from Table 5 of the report.

TABLE 1

Survival in Rats Given Metam Sodium in the Diet for 104 Weeks[a]

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
1	64/64	64/64	64/64	64/64	64/64	64/64	64/64	64/64
% alive	100	100	100	100	100	100	100	100
13	64/64	64/64	63/64	64/64	64/64	64/64	64/64	64/64
% alive	100	100	98	100	100	100	100	100
27	64/64	63/64	63/64	63/64	63/64	64/64	64/64	63/64
% alive	100	98	98	98	98	100	100	98
53	61/64	62/64	62/64	63/64	62/64	60/64	62/64	61/64
% alive	95	97	97	98	97	94	97	95
77[b]	44/52	42/52	41/52	46/52	45/52	42/52	44/52	45/52
% alive	85	81	79	88	86	81	85	86
105[b]	11/52	15/52	20/52	16/52	27/52	25/52	23/52	29/52
% alive	21	29	38	31	52	48	44	56

[a]data taken from Table 5, pages 75-84 of the report. Numbers represent the total rats alive over the total rats in the dose group.

[b]excludes rats sacrificed at the 52 week time point (both sexes, N = 12).

* $p < 0.05$ vs control.

The data above indicate no significant effects of treatment on mortality in male and female rats. At week 105, however, mortality in control male rats appeared unacceptably low, i.e. less than 25% alive at week 105. Mortality in female rats at all dose levels was much less at week 105 in relation to male rats.

There were no historical control data provided with which to make a comparison of mortality in this strain of rat. It is of interest to note in this respect that the number of male rats found dead were 7, 3, 2, and 1 for the 0, 0.019, 0.056, and 0.19 mg/ml dose levels, respectively.

B. Clinical Observations

Cageside observations for indications of toxic effects were made once daily, and a detailed clinical examination performed weekly.

Clinical observations were summarized in Table 4, pages 55-72 of the report. These data indicated that treatment-related effects were observed only in male rats, and included reduced hindlimb function (observed in 5, 6, 2, and 13 control, low dose, mid dose, and high dose rats, respectively), stains around the nose (observed in 16, 19, 19, and 23 control, low dose, mid dose, and high dose rats, respectively), and thin appearance (observed in 15, 18, 15, and 31 control, low dose, mid dose, and high dose rats, respectively).

C. Body Weights

Body weight measurements were taken immediately prior to test article administration at study initiation, once every week for the first 14 weeks of the study, and then once every 2 weeks until study termination. Group mean body weights at selected times are presented in **Table 2**.

TABLE 2

Group Mean Body Weights (grams) in Male and Female Rats Given Metam Sodium in the Diet for 104 Weeks[a]

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
1	184.9± 26.6	182.6± 23.0	183.6± 24.8	181.4± 26.8	129.6± 15.0	130.9± 14.9	129.8± 14.2	130.7± 15.8
13	440.3± 38.7	439.7± 41.4	438.6± 40.6	407.0±** 30.5	240.2± 20.3	238.3± 18.9	231.5±** 17.1	223.5±** 18.7
27	526.2± 46.4	526.6± 51.2	523.1± 52.1	479.7±** 35.7	268.0± 21.2	267.1± 21.3	259.4±** 18.8	248.7±** 19.8
53	579.7± 56.9	581.5± 63.3	577.0± 54.0	532.0±** 40.7	311.9± 33.7	308.6± 33.9	295.3±** 27.6	274.6±** 24.9
N	44	42	41	46	45	42	44	45
77	581.3± 57.9	565.3± 65.3	574.9± 59.3	526.0±** 48.2	350.4± 37.8	345.6± 48.5	332.7±* 33.6	306.5±** 29.4

Table 2, cont.

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
N	11	15	20	16	27	25	23	29
105	510.5± 45.0	498.3± 55.2	492.0± 40.8	448.0±** 56.0	342.9± 35.9	346.8± 47.2	326.2± 37.8	301.4±** 31.3

[a]data taken from Table 5, pages 75-84 of the report. * $p < 0.05$ vs control; ** $p < 0.01$ vs control.

Statistically significant effects were noted on group mean absolute body weight at the 0.19 mg/ml dose level for both male and female rats, and at the 0.056 mg/ml dose level in female rats. In male rats, group mean body weight was decreased by 8% vs control at week 13, while body weight in female rats was decreased by 7% vs control at this time point ($p < 0.01$). At subsequent intervals, body weight at the 0.19 mg/ml dose level was decreased by approximately 8% in male rats, and by approximately 12% in female rats. At the 0.056 mg/ml dose level, group mean body weight in female rats was decreased by 3-5% over the course of the study ($p < 0.05$).

Effects of test article treatment on group mean body weight gain in male and female rats are summarized in the following Table (**Table 3**):

TABLE 3

Group Mean Body Weight Gain in Male and Female Rats Given Metam Sodium in the Diet for 104 Weeks[a]

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
1	184.9± 26.6	182.6± 23.0	183.6± 24.8	181.4± 26.8	129.6± 15.0	130.9± 14.9	129.8± 14.2	130.7± 15.8
weight gain (g)								
1-13	255.4	257.1	255.0	225.6	110.6	107.4	101.7	92.8
% cont.	-	100	100	88	-	97	92	84
1-53	394.8	398.8	393.4	350.6	182.3	177.7	165.5	143.9
% cont.	-	101	100	89	-	97	91	79
1-105	325.6	315.7	308.4	266.6	213.3	215.9	196.4	170.7
% cont.	-	97	95	82	-	101	92	80

[a]data calculated from Table 2, above.

Effects on body weight gain were observed at the 0.19 mg/ml dose level in both male and female rats, where weight gain was decreased by 12-18% in males vs control, and by 16-20% in females vs control over the course of this study. At the 0.056 mg/ml dose level, body weight gain was unaffected in male rats, and was decreased by approximately 8-9% in female rats over the course of the study.

C. Food Consumption and Efficiency

Food consumption was measured weekly for the first 14 weeks of the study for each cage of rats. Food consumption was measured again on week 16, and thereafter every fourth week for the remainder of the study. Food efficiency was calculated for the first 12 weeks of the study.

Group mean food consumption data are presented in **Table 4** below:

TABLE 4

Group Mean Food Consumption (g/rat/day) in Male and Female Rats Given Metam Sodium in the Diet for 104 Weeks[a]

Weeks of Study	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
1	25.3± 1.1	25.0 0.7	24.9± 0.9	21.4±** 2.5	18.4± 0.8	18.0± 0.7	17.4±** 0.6	15.9±** 0.8
% cont.	-	99	98	84	-	98	94	86
13	26.5± 1.3	25.7* 0.9	26.0± 1.2	24.0±** 0.9	18.0± 1.0	17.8± 0.9	16.8±** 0.9	16.4±** 1.0
% cont.	-	97	98	90	-	99	93	91
Mean 1-13	26.9	26.4	26.4	24.3	18.5	18.4	17.5	16.7
% cont.	-	98	98	90	-	100	94	90
52	25.9± 1.8	25.3± 1.0	24.8± 3.1	24.5* 1.3	19.4± 1.5	19.0± 1.0	17.7±** 1.0	16.5±** 1.3
% cont.	-	100	96	96	-	98	91	85
104	24.2± 3.2	24.1± 2.6	25.6± 4.2	21.4±* 2.4	18.0± 2.5	18.3± 3.2	17.8± 2.9	17.1± 1.3
% cont.	-	100	105	88	-	102	99	95

[a]data from Table 7, pages 101-106 of the report.

In both male and female rats, group mean food consumption values (measured by cage) were decreased by 10% relative to control for weeks 1-13 of the study. At weeks 52 and 104, decreases were also observed in both sexes at the 0.19 mg/ml dose level, ranging from 4-15% below control values. The comparison of food consumption values at the 0.056 and 0.19 mg/ml dose levels appears to indicate a dose-related effect in both sexes.

Food efficiency calculations were performed by the registrant and are shown in the following table, taken from page 107 of the report:

TABLE 5

Group Mean Food Efficiency (%) in Male and Female Rats Given Metam Sodium in the Diet for 104 Weeks [a]

		Conc. of metam sodium in water (mg/ml) 0(Control)	0.019	0.056	0.19
Males					
Weeks 1-4	MEAN	18.19	18.62	18.32	17.76
	S.D.	1.70	1.34	1.64	1.84
	N	16	16	16	16
Weeks 5-8	MEAN	9.61	9.93	9.88	9.45
	S.D.	0.63	0.75	0.56	0.58
	N	16	16	16	16
Weeks 9-12	MEAN	6.25	6.18	6.42	6.15
	S.D.	0.67	0.65	0.42	0.41
	N	16	16	16	16
Overall (Weeks 1-12)	MEAN	11.31	11.54	11.49	11.04*
	S.D.	0.82	0.73	0.70	0.73
	N	16	16	16	16
Females					
Weeks 1-4	MEAN	12.47	12.09	11.98	11.28**
	S.D.	1.66	1.40	1.37	1.89
	N	16	16	16	16
Weeks 5-8	MEAN	5.42	5.44	5.51	5.36
	S.D.	0.80	0.79	0.74	0.98
	N	16	16	15	16
Weeks 9-12	MEAN	3.27	3.19	3.10	3.27
	S.D.	0.39	0.66	0.77	0.54
	N	15	16	16	16
Overall (Weeks 1-12)	MEAN	7.12	6.93	6.85	6.61**
	S.D.	0.71	0.72	0.66	0.98
	N	15	16	15	16

[a]data taken from Table 7a, page 107 of the report.

Efficiency of food utilization was decreased in male rats at the high dose for weeks 1-12 of the study. While this was labeled as statistically significant, this represented a decrease of only 3% from control. In female rats, larger decreases in food efficiency were observed, and at the additional time of weeks 1-4 of the study in contrast to males. For this time period, efficiency was decreased by almost 10% at the high dose vs control. For the 1-12 week study period, efficiency in female rats at the high dose was decreased by 7%.

Weight gain decreases in male rats at the 0.19 mg/ml dose level for weeks 1-13 of the study (12%) were only slightly in excess of the decreases in food consumption at this dose (10%), which might explain the slight decrease in food efficiency at the 0.19 mg/ml dose for the period of 1-12 weeks. Females, however, showed a weight gain decrease of 16% for weeks 1-13 of the study at the 0.19 mg/ml dose, and a food consumption decrease of only 10%. These observations taken together would indicate test article related toxicity, but are somewhat mild in terms of effect.

D. Intake of Metam Sodium

Mean intake values for metam sodium (in mg/kg) are shown below, based upon the evaluation of test article stability and decomposition observed in this study and summarized on pages 4 and 5 of this review.

TABLE 6

Group Mean Achieved Dosage of Metam Sodium in Male and Female Rats Over 104 Weeks[a]

Dose Group (mg/ml)	Average Intake (weeks 1-104) (mg/kg/day)	
	males	females
0.0	-	-
0.019	1.3	2.3
0.056	3.9	6.2
0.19	12.0	16.2

[a]data taken from pages 4 and 5 of this review.

E. Water Consumption

Water consumption was recorded for each cage on a daily basis, and is reported on a weekly basis. Summary is made below for selected time points:

Table 7
Water Consumption (ml/rat/day) in Male and Female Rats Receiving Metam Sodium in Drinking Water[a]

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
week 1	35.0± 6.5	32.3± 5.4	30.0±** 3.5	22.3±** 1.4	29.7± 3.7	29.2± 3.5	25.2±** 4.2	17.3±** 1.3
week 13	39.1± 4.8	36.5± 4.9	37.1± 5.6	25.8±** 1.5	45.2± 6.4	40.1±** 6.3	31.0±** 4.9	20.5±** 1.7
weeks 1-13	42.4± 3.2	40.6± 3.5	38.3± 3.0	26.3± 1.53	43.7± 5.0	41.6± 4.5	33.2± 3.0	20.0± 1.3
week 26	35.3± 4.0	33.3± 4.2	33.7± 3.4	25.7±** 1.5	43.9± 5.5	36.2±** 6.2	30.7±** 6.0	20.4±** 1.5
week 52	33.7± 3.9	31.2± 3.1	31.0± 6.1	26.0±** 1.3	44.2± 3.8	37.6±** 5.5	33.2±** 5.8	22.8±** 2.7
week 78	40.6± 8.2	38.2± 7.1	36.7± 7.4	32.9±** 4.5	40.0± 6.9	41.7± 8.9	34.6±* 4.3	24.6±** 2.3
week 104	53.8± 8.0	53.3± 11.8	56.7± 15.2	41.8±* 11.8	38.8± 7.4	44.1± 18.1	33.9± 8.3	27.8±* 6.4

[a]data taken from Table 6, pages 85-100 of the report. *p < 0.05 vs control; **p < 0.01 vs control.

The above data show significant decreases in water consumption in both male and female rats at the 0.056 and 0.19 mg/ml dose levels. Female rats appeared to be affected in a greater manner than male rats at these two dose levels. For example, mean water consumption for weeks 1-13 of the study was decreased in treated male rats by 4.2%, 9.6%, and 37.9% from control at the 0.019, 0.056, and 0.19 mg/ml dose levels, respectively. In females, the corresponding decreases were 4.8%, 24.0%, and 54.2%. Thus, at a given dose level, the decrease in water consumption appeared greater for female rats, and further, significant decreases in water consumption were observed at dose levels in female rats where there was no significant effect in male rats (weeks 26, 52, and 78 above, for example). It is worth noting that such large decreases in water consumption for both sexes at the high dose could have potentially adverse effects on normal physiology, as in many instances, the decrease in water consumption approached or exceeded 50% vs control.

F. **Ophthalmoscopic Examination**

An indirect ophthalmoscopic examination was performed on the eyes of all animals from the control and high dose groups prior to treatment and again at 52 weeks of the study. The eyes of control and high dose females were examined during the week prior to scheduled termination.

According to the data presented in the report (Tables 8, pages 109-111), there did not appear to be any significant relationship between treatment with metam sodium and ocular abnormalities. It is, however, mentioned that examination at pre-study (52 males and 51 females examined) showed focal opacities in 2 female rats assigned to the high dose group, and none in male rats. At week 52 (examination of 52 males and 49 females), focal opacities were observed in 5 high dose females vs 2 in control, and linear opacities were observed in 3 high dose females vs 1 in controls. At terminal sacrifice, there were no ophthalmoscopic abnormalities observed in treated animals.

F. Clinical Pathology

Blood samples for hematology and serum chemistry were obtained at weeks 14, 27, 53, and 79 from the tail vein of 13 designated male and 13 designated female rats per main study group. Any designated rat which died or was killed prior to sampling time was replaced, if considered necessary, by an alternative animal in order to maintain acceptable group size. Blood was obtained by cardiac puncture from interim kill and main study animals at termination. Samples (1ml) were introduced into tubes containing EDTA for hematology measurements and into tubes containing lithium heparin for clinical chemistry. Blood films were prepared from all animals and a differential white cell count done with an assessment of red cell morphology on control and high dose animals. Bone marrow smears were taken from interim and main study animals at termination. Reticulocyte counts were done on all animals found to be anemic.

a) Hematology

The following CHECKED hematological parameters were examined:

x total leucocyte count*	- total plasma protein*
x erythrocyte count*	x leukocyte differential*
x hemoglobin (HGB)*	x mean corpuscular HGB
x hematocrit (HCT)*	x mean corpusc. HGB conc.
x platelet count	x mean corpusc. volume
- packed cell volume	- methemoglobin
- reticulocyte count	x prothrombin time

*EPA guideline requirement "-" not analyzed

Hematological findings were summarized in Table 9, pages 112-139 of the report. Significant observations are summarized below (Table 8):

Table 8
Hematological Findings in Male and Female Rats
Receiving Metam Sodium in Drinking Water[a]

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
week 14								
HGB (g/dl)	15.9± 0.5	16.2±* 0.6	15.6± 0.5	15.4±* 0.5	15.1± 0.7	15.5± 0.4	15.2± 0.5	14.9± 0.6
HCT	0.491± 0.021	0.504± 0.022	0.485± 0.020	0.480± 0.032	0.474± 0.023	0.476± 0.020	0.472± 0.027	0.460±* 0.027
RBC (10^{12}/l)	8.36± 0.38	8.50± 0.46	8.35± 0.41	8.14± 0.63	7.53± 0.50	7.55± 0.46	7.52± 0.48	7.23±* 0.49
week 27								
HGB	15.2± 0.5	15.3± 0.6	15.0± 0.4	14.8±* 0.4	14.7± 0.3	14.7± 0.4	14.4±* 0.5	14.2±** 0.4
HCT	0.443± 0.015	0.447± 0.016	0.437± 0.014	0.431±* 0.014	0.418± 0.013	0.416± 0.014	0.408± 0.016	0.405±* 0.015
RBC	9.58± 0.47	9.60± 0.46	9.59± 0.33	9.33± 0.41	8.37± 0.21	8.41± 0.28	8.22± 0.35	8.07±** 0.31
week 53								
HGB	15.0± 0.5	14.9± 0.6	14.8± 0.5	14.5±* 0.3	14.4± 0.4	14.5± 0.4	14.3± 0.7	14.0± 0.7
HCT	0.446± 0.016	0.443± 0.015	0.439± 0.016	0.432±* 0.011	0.418± 0.015	0.423± 0.017	0.419± 0.023	0.405± 0.019
RBC	9.45± 0.42	9.21± 0.41	9.37± 0.36	9.07±** 0.24	8.14± 0.27	8.25± 0.25	8.12± 0.47	7.79±* 0.41

Table 8, cont.

Weeks of Study	Males (mg/ml) 0.0	0.019	0.056	0.19	Females (mg/ml) 0.0	0.019	0.056	0.19
week 79								
HGB	14.8± 0.4	14.4± 0.9	14.4± 0.7	14.2±* 0.7	14.6± 0.3	14.6± 0.5	14.4± 0.8	14.1± 0.5
HCT	0.454± 0.012	0.440± 0.026	0.442± 0.021	0.434±* 0.024	0.433± 0.010	0.439± 0.016	0.433± 0.027	0.422± 0.017
RBC	9.26± 0.29	8.83± 0.65	9.03± 0.51	8.81±* 0.24	8.31± 0.32	8.26± 0.23	8.19± 0.48	7.86±** 0.34
week 105								
HGB	14.4± 2.2	13.3± 1.2	11.9±** 1.9	12.6±** 1.1	13.9± 1.3	13.8± 0.7	13.5± 0.8	13.2±* 1.0
HCT	0.450± 0.072	0.419± 0.043	0.382±** 0.055	0.401±* 0.039	0.434± 0.038	0.432± 0.022	0.423± 0.026	0.415±* 0.032
RBC	8.71± 1.12	8.20± 0.85	7.31±** 1.30	7.80±* 0.96	8.17± 0.85	8.04± 0.45	7.86± 0.46	7.52±** 0.64

[a]data taken from Table 9 of the report, pages 112-139. *p < 0.05 vs control. **p < 0.01 vs control.

The most consistent changes observed in this study were changes in hemoglobin (HGB), hematocrit (HCT), and red blood cells (RBC). At the 0.19 mg/ml dose level, both male and female rats showed a consistent decrease of 3 to 4% from control in mean HGB and RBC throughout the study. Mean HCT was decreased as well, but not always in both sexes and not always at all times. The largest decreases observed in these parameters occurred at week 105, where HGB was decreased 12% in high dose males and 5% in high dose females, with HCT and RBC following a similar pattern. Although the changes shown above achieved statistical significance, the degree of change was small (~ 3-5%). Thus, the toxicological significance of these findings is debatable.

b) **Clinical Chemistry:**

The following CHECKED parameters were measured:

- x glucose*
- x albumin*
- - A/G ratio (calculated)
- x creatinine
- x total bilirubin*
- - direct bilirubin
- - indirect bilirubin
- x urea nitrogen*
- x total protein*
- x cholesterol*
- x triglycerides
- - electrophoretic protein fractions

- x calcium*
- x inorganic phosphate*
- x sodium*
- x potassium*
- x chloride*

- x AST(SGPT)*
- x ALT(SGOT)*
- x alkaline phosphatase
- x creatine kinase*
- - lactate dehydrogenase
- - sorbitol dehydrogenase
- - gamma glutamyl trans-peptidase
- - ornithine carbamyl transferase

- - plasma ChE
- - red cell ChE
- x urea

*EPA guideline requirement

"-" not examined

A summary of findings in blood chemistry measurements were presented in Table 10, 140-73 of the report.

Early in the treatment period (weeks 14 and 27), both alanine and aspartate aminotransferase were decreased at the high dose in female rats. At week 14, alanine and aspartate aminotransferase were decreased by 15% and 11% respectively, and at week 27, by 27% and 32% respectively. At week 53 and beyond, there were no significant differences from control.

At weeks 79 and 105, both plasma cholesterol and triglycerides were decreased in female and male rats at the 0.19 mg/ml dose level. For cholesterol, the decreases ranged from 4-10% in males, and 17-19% in females. For triglycerides, the decrease ranged from 22-43% in females, and 18-32% in males. There were no corresponding decreases in total plasma protein (only a 3% decrease observed in high dose females at week 27).

c) **Urinalysis:**

During weeks 13, 26, 52, and 78, individual urine samples were collected over a 16-18 hour period from 13 designated male and 13 designated female rats per dose group. At study termination, urine was collected from the designated female rats, but from all surviving male rats. During the collection period, rats were housed in individual metabolism cages and denied access to food and water.

The following CHECKED parameters were examined:

x appearance*	x glucose*
x volume*	x pH
x specific gravity*	- bilirubin*
x protein*	x urobilinogen
x ketone*	- nitrite
x blood*	- total reducing substances
x sediment analysis*	

*EPA guideline requirement "-" not examined

Results of urinalysis were presented in Table 11 of the report, pages 174-183. Qualitative tests were summarized in Table 12.1, pages 185-186, and sediment analysis presented in Table 12.2, pages 188-211. Significant findings are summarized in the following Table (Table 9):

Table 9
Urinalysis in Male and Female Rats Administered Metam Sodium in Drinking Water for 104 Weeks[a]

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
volume								
week 52	7.38± 2.61	7.83± 2.57	6.85± 2.34	5.47±* 1.74	7.48± 1.65	6.69± 2.77	5.09±* 1.61	5.24±* 1.95
week 78	10.49± 3.43	10.54± 2.78	10.03± 2.77	8.65± 3.24	8.75± 2.41	8.05± 2.28	8.13± 2.95	6.21±* 1.82

Table 9, cont.

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
urine pH								
week 13	6.58± 0.14	6.50± 0.20	6.47± 0.19	6.31±** 0.12	6.15± 0.24	6.06± 0.17	6.10± 0.23	6.01± 0.22
week 26	6.56± 0.21	6.44± 0.22	6.49± 0.10	6.33±** 0.14	6.17± 0.20	6.10± 0.12	6.09± 0.16	6.09± 0.10
urine protein								
week 26	32.82± 15.56	29.12± 10.29	28.24± 11.71	22.81±* 6.80	2.02± 1.76	1.24± 0.24	1.27± 0.43	1.47± 0.96
week 52	113.2± 52.39	88.43± 41.41	82.92± 35.30	70.41±* 26.68	19.85± 21.38	11.50± 8.80	4.08±** 1.12	6.08±** 3.56
week 78	220.9± 95.07	221.17± 94.00	209.51± 87.77	146.54±* 62.46	64.46± 65.00	37.48± 23.34	14.80±** 8.52	18.84±** 12.92

[a]data taken from Table 11, pages 174-183. *p < 0.05 vs control; ** p < 0.01 vs control.

Changes in urine volume during weeks 52 and 78 were observed, as well as changes in urine pH (decreased during weeks 13 and 26 in males at 0.19 mg/ml) and protein (decreased in males and females during weeks 26, 52, and 78). The decreases in urine protein, which appeared dramatic at the 0.19 mg/ml dose level, were explained in the report to be the result of abnormally high control values. When these were removed (as appears to be the case for the data presented on pages 182-183 of the report), statistical significance was observed in male and female rats at week 78 at the 0.19 mg/ml dose level (34% reduction in males, 33% reduction in females).

Qualitative testing of urine showed a trend towards the presence of blood in urine with increasing dose of test chemical, as evidenced by the increase in the number of animals with more prominent grading for blood in urine. The results of this analysis are shown below, taken from pages 185 and 186 of the report: [Qualitative grading was based on a positive arbitrary scale of increasing presence of a urinary parameter (+, ++, or +++)]:

GROUP URINE QUALITATIVE TESTS

MALES

		CONTROL					0.019mg/ml					0.056mg/ml					0.19mg/ml				
	WEEK	13	26	52	78	TERM	13	26	52	78	TERM	13	26	52	78	TERM	13	26	52	78	TERM
BLOOD	-VE	13	13	13	13	16	11	13	12	12	18	12	12	12	12	23	12	13	12	13	23
	TRACE	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	-	-	-	-	-
	+	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	++	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	1
	+++	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	1	-	1	-	1

NML = NORMAL CDY = CLOUDY BDP = BLOOD PRESENT VISUALLY URO/GEN = UROBILINOGEN

FEMALES

		CONTROL					0.019mg/ml					0.056mg/ml					0.19mg/ml				
	WEEK	13	26	52	78	104	13	26	52	78	104	13	26	52	78	104	13	26	52	78	104
BLOOD	-VE	13	13	12	13	13	13	13	12	13	13	13	13	13	13	12	12	12	11	11	11
	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	+++	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	2	1

NML = NORMAL BDP = BLOOD PRESENT VISUALLY URO/GEN = UROBILINOGEN

The report stated that the finding of blood in urine was low in incidence and not considered of biological significance. However, there is an apparent relationship to dose of metam sodium, and thus it could be considered an effect of treatment.

Urine was also analyzed qualitatively for the presence of crystals, renal epithelial cells, bacteria, mucus, mixed cell casts, white blood cells, yeast cells, wax casts, squamous epithelial cells, granular casts, and sperm. There did not appear to be any effect of treatment on the incidence or severity of any of these in male or female rat urine.

G. Macroscopic Observations

Sacrifice of animals was performed on 12 rats/sex/group after 52 weeks of treatment (during week 53) and on all surviving animals after 104 weeks of treatment. All animals found dead or sacrificed in extremis during the study were subjected to a full post mortem examination as soon as possible after death and always within 24 hours. Rats were weighed, anesthetized with halothane, and exsanguinated.

Summary of macroscopic observations was presented in the report (Tables 14A-14B, pages 222-282). Table 14A summarized data for unscheduled deaths and the interim sacrifice, while Table 14B summarized macroscopic findings in all animals combined.

A summary of apparent treatment-related findings is made below (Table 9):

Table 9a

Macroscopic Findings in Metam Sodium Treated Rats[a]

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
Intercurrent Deaths								
Liver								
No. examined	42	37	33	36	28	28	29	23
mass	2	1	2	6	0	0	1	0
enlarged	0	3	2	6	0	0	0	0
pale spots	3	3	6	13	0	0	0	1
Lung								
No. examined	42	37	33	36	28	28	29	23
pale	1	0	2	3	0	0	0	0
Pituitary								
No. examined	42	37	33	36	28	28	29	23
mass	5	2	2	3	17	15	17	9
Voluntary muscle								
No. examined	42	37	33	36	28	28	29	23
wasted	1	1	0	9	0	0	1	1

[a]data taken from Table 14A, pages 222-241 of the report.

For those animals dying or sacrificed during the study, male rats at the 0.19 mg/ml dose level showed an increased incidence of abormal liver pathology (mass, pale spots, enlarged) and increased incidence of animals with wasted voluntary muscle. There was an apparent decrease in the incidence of animals with pituitary masses for both sexes.

In female rats, there were none of the effects observed in males.

The following table (Table 9b) shows the incidence of macroscopic observations in all animals on study, as the deviations shown above were not observed in increased incidence in those rats surviving to study termination:

Table 9b

Macroscopic Findings in Metam Sodium Treated Rats[a]

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
				All Animals				
Adrenal gland								
No. examined	64	64	64	64	64	64	64	64
speckled	4	4	4	6	8	7	2	10
Ears								
No. examined	3	1	1	3	0	1	3	0
traumatized pinna[b]	0	3	3	5	1	0	4	3
Eye								
No. examined	64	63	64	64	64	63	64	64
pale	8	5	12	11	1	0	2	1
Liver								
No. examined	64	64	64	64	64	64	64	64
mass	4	1	4	8	0	2	3	2
enlarged	0	5	7	7	1	0	2	1
pale spots	3	3	8	15	0	0	0	1
mottled	1	2	4	4	1	0	1	1
Lung								
No. examined	64	64	64	64	64	64	64	64
pale	1	1	2	5	0	0	0	0
Pituitary								
No. examined	64	64	64	64	64	64	64	64
mass	7	3	4	3	33	36	34	25
Voluntary muscle								
No. examined	64	64	64	64	64	64	64	64
wasted	1	2	0	9	0	0	1	1

[a]data taken from Table 14B, pages 261-282 of the report.

[b]number with findings exceeded the number examined; no explanation given.

For all animals considered together, deviations in control incidence of macroscopic pathology occurred primarily in male rats at the 0.19 mg/ml dose level, and included eye effects (pale), liver effects (enlarged, mass, pale spots), lung effects (pale), and voluntary muscle effects (wasting). The only lesion which appeared to occur in both sexes was a slight increase in the incidence of traumatized pinnae, occurring at the high dose.
The incidence of pituitary masses as observed macroscopically appeared decreased at the high dose in both sexes compared to control incidence.

H. Organ Weights

Organ weights were recorded in the 12 rats/sex/dose sacrificed at week 53, and in those rats surviving to study termination. The weight of the liver, kidneys, adrenals, brain, and testes were recorded. Organ / body weight ratios were also calculated.

Results were presented in Tables 13.1 for interim kill animals and in Table 13.2 for terminal kill animals. Significant findings are summarized below:

Table 10

Organ Weights at 52 and 104 Weeks in Metam Sodium Treated Rats[a]

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
No. examined	11	12	11	12	10	11	12	12
52 Weeks								
adrenals	0.061± 0.018	0.059± 0.016	0.057± 0.009	0.055± 0.005	0.064± 0.012	0.068± 0.012	0.063± 0.010	0.087± 0.105
% cont.	-	97	93	90	-	106	98	136
adrenal/ b.w.	0.010	0.009	0.010	0.010	0.020	0.021	0.021	0.031
kidneys	3.71± 0.59	3.66± 0.29	3.61± 0.38	3.26±** 0.18	2.22± 0.22	2.20± 0.39	2.06± 0.16	2.29± 0.26
% cont.	-	99	97	88	-	99	93	103
kidney/ b.w.	0 62	0.58	0.61	0.61	0.69	0.67	0.69	0.83

Table 10, cont.

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
No. examined	11	15	20	16	26	25	23	29
104 Weeks								
adrenals	0.071± 0.014	0.069± 0.013	0.205± 0.563	0.086± 0.089	0.080± 0.036	0.085± 0.018	0.105± 0.149	0.077± 0.025
% cont.	-	97	288	121	-	106	131	96
adrenal/ b.w.	0.014	0.014	0.043	0.021	0.023	0.025	0.034	0.026
kidneys	5.36± 1.25	5.31± 1.47	5.76± 1.72	4.69± 1.03	2.88± 0.60	2.79± 0.45	2.82± 0.53	2.65± 0.54
% cont.	-	99	107	88	-	97	98	92
kidney/ b.w.	1.07	1.10	1.20	1.08	0.84	0.81	0.87	0.88

[a]data taken from Tables 13.1 and 13.2, pages 212-221 of the report.
*$p < 0.05$ vs control.

At both the interim sacrifice time point as well as the terminal kill time point, the only changes noted in organ weights were those of the adrenal glands and kidneys. However, the effects on organ weight were not consistent between sexes. For example, adrenal weight in male rats at the 0.19 mg/ml dose level from the interim sacrifice group was decreased by 10% from control, while adrenal weight in female rats at the same dose level and time point was increased by 36% from control. A fairly strong dose-response relationship could be observed in both cases. At the terminal sacrifice point, no significant changes were observed in adrenal weight

Kidney weight was noted to be decreased significantly in male rats at the 0.19 mg/ml dose level from the interim sacrifice time point (decrease of 12% from control), but no effects were observed in female rats. At the terminal sacrifice time point, the same percentage decrease was observed in male rats at the 0.19 mg/ml dose level, but was not considered statistically significant by the study author.

I. Microscopic Observations

Samples of the following tissues were preserved in 10% buffered formalin, except skin, testis, epididymis, and mammary gland, which were placed in Davidson's fixative, and eye and Harderian gland, which were placed in Davidson's solution.

Digestive	Respiratory	Urogenital
x oral cavity	x trachea	x kidneys*
x salivary glands*	x lungs*	x urinary bladder*
x esophagus*	x nasal cavity	x testes*
x stomach*		x epididymides*
x duodenum*	Cardiovascular	x seminal vesicle*
x jejunum*	x aorta*	x prostate
x ileum*	x heart*	x ovaries
x cecum*	x bone marrow	x uterus*
x colon*	x lymph nodes*	x vagina and cervix
x rectum*		
x liver*		
x pancreas*		
x gall bladder*		

Neurologic	Glandular	Other
x brain*	x adrenals *	x bone (sternum)
x peripheral nerve*	x lacrimal gland	x bone marrow
x spinal cord (3 levels)*	x mammary gland	x skeletal muscle
x pituitary*	x parathyroids*	x skin*
x eyes	x thyroids*	x lesions and tumors*
	x Harderian gland	x spleen*
	x thymus*	

*EPA guideline requirement "-" not examined

The above tissues were prepared for microscopic examination by dehydration and embedding in paraffin wax, cutting thin sections, and staining with hematoxylin and eosin. Special stains were used as appropriate.

1) Non-Neoplastic Observations

A number of observations were recorded for animals in the interim sacrifice, unscheduled sacrifice, and terminal sacrifice groups. Because findings were numerous but similar, the observations for intercurrent deaths, terminal sacrifice, and animals surviving to termination are combined in one table (Table 11):

Table 11

Non-Neoplastic Microscopic Findings in Metam Sodium Treated Rats[a]

All Animals

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
adrenal gland								
No. examined	64	64	64	64	64	64	64	64
vascular ectasia								
total	4	6	7	12	54	48	50	44
minimal	3	5	7	9	13	12	9	8
slight	1	1	0	2	25	29	28	23
moderate	0	0	0	1	14	7	13	12
marked	0	0	0	0	2	0	0	1
aorta								
No. examined	64	63	64	64	62	64	64	64
mineralization								
total	15	14	7	2	0	0	0	0
slight	6	2	4	0				
moderate	7	6	2	2				
marked	2	6	1	0				
Eye								
No. examined	64	63	64	64	63	63	64	64
cataractous change								
total	6	6	7	8	17	15	14	10
minimal	4	4	6	8	10	7	9	7
slight	1	0	1	0	5	5	4	1
moderate	0	0	0	0	2	2	1	1
marked	1	0	0	0	0	1	0	1

Table 11, cont.

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
Harderian gland								
No. examined	64	63	64	64	63	63	64	64
increased porphyrin								
total	35	21	29	30	10	15	17	14
minimal	29	13	15	12	1	**12**	**9**	**9**
slight	6	8	**14**	**18**	9	3	8	5
Heart								
No. examined	64	64	64	64	64	64	64	64
vascular mineralization								
total	11	4	2	2	0	0	0	0
minimal	0	0	1	0				
slight	5	2	0	1				
moderate	5	1	1	1				
marked	1	1	0	0				
Liver								
No. examined	64	64	64	64	64	64	64	64
fat vacuolation								
total	28	26	**31**	**32**	6	5	8	7
minimal	8	9	**14**	**11**	3	4	3	4
slight	9	4	4	**10**	1	1	2	2
moderate	6	6	7	6	0	0	2	1
marked	5	7	6	5	2	0	1	0
spongiosis/peliosis hepatis with altered hepatocytes								
total	7	5	**15**	**13**	1	0	3	2
minimal	0	3	6	1	0	0	3	0
slight	5	1	7	**12**	1	0	0	2
moderate	2	1	2	0	0	0	0	0

Table 11, cont.

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
Nasal cavity								
No. examined	63	64	64	64	64	64	64	64
rhinitis								
total	1	4	2	9	1	3	4	4
minimal	0	2	2	2	0	2	1	2
slight	1	2	0	5	0	1	3	1
moderate	0	0	0	2				
hypertrophy of Bowman's ducts/ glands								
total	0	0	2	**39**	0	0	0	**43**
minimal	0	0	2	**13**	0	0	0	**29**
slight	0	0	0	**12**	0	0	0	**13**
moderate	0	0	0	**14**	0	0	0	**1**
olfactory epithelium hyperplasia								
total	0	0	0	**7**	0	0	0	**2**
minimal	0	0	0	**5**				
slight	0	0	0	**2**				
olfactory epithelium degeneration								
total	1	0	0	**28**	0	0	1	**5**
minimal	1	0	0	**8**	0	0	1	**3**
slight	0	0	0	**18**	0	0	0	**2**
moderate	0	0	0	**2**				
Steno's gland atrophy								
total	11	12	15	**49**	13	14	17	**43**
minimal	8	9	11	**34**	12	14	14	**31**
slight	3	3	4	**14**	1	0	3	**12**
moderate	0	0	0	**1**				

Table 11, cont.

	Males (mg/ml)				Females (mg/ml)			
	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
Steno's gland adenitis								
total	14	4	13	**23**	1	1	**10**	**9**
minimal	12	3	13	**21**	1	1	**7**	**9**
slight	0	1	0	**2**	0	0	3	0
moderate	2	0	0	0				
Sciatic Nerve								
No. examined	64	64	64	64	63	64	64	63
degeneration								
total	47	42	45	47	37	34	36	39
minimal	28	25	24	13	24	20	22	19
slight	15	6	15	**20**	13	14	12	17
moderate	1	8	4	**11**	0	0	2	3
marked	3	3	2	3	0	0	0	0
Spleen								
No. examined	64	64	64	64	64	64	64	64
increased hemosiderin								
total	2	2	2	**8**	10	6	8	**20**
slight	2	2	2	**8**	10	6	8	**20**
Voluntary muscle								
No. examined	64	64	64	64	64	64	64	64
degenerative myopathy								
total	37	39	36	44	24	17	15	27
minimal	26	27	23	9	24	16	13	18
slight	7	7	7	**10**	0	1	1	**5**
moderate	2	3	5	**15**	0	0	1	**3**
marked	2	2	1	**10**	0	0	0	1

[a]data taken from Tables 15a and 15b, pages 283-395 of the report.

In those animals killed or dying during the study period as well as in those surviving to study termination, microscopic abnormalities were observed at the 0.19 mg/ml dose level in the **adrenal gland** (increased vascular ectasia), **aorta and heart** (decreased mineralization), **eye** (cataractous change), **nasal cavity** (rhinitis, hypertrophy of Bowman's ducts/glands, olfactory epithelium hyperplasia and degeneration), **liver** (spongiosis/peliosis hepatis with altered hepatocytes), Steno's gland atrophy, adenitis), **spleen** (hemosiderin), **sciatic nerve** (degeneration), and **voluntary muscle** (degenerative myopathy). The changes observed in the adrenal gland, aorta, heart, and liver were confined primarily to male rats, while the remaining changes were observed in both sexes. According to the report, the lesion in Bowman's ducts and glands was characterized by cellular hypertrophy and in some animals, degeneration of the olfactory epithelium was observed, which consisted of vacuolation, reduction in the number of sensory cells, pyknotic sensory cells, focal loss of apical cytoplasm, and occasional pigmented cells.

The incidence of rhinitis, increased in males given 0.19 mg/ml, was not considered to be treatment-related, but could have exacerbated the changes in Bowman's glands. However, there were several animals with moderate hypertrophy of Bowman's glands/ducts without rhinitis.

The incidence of degenerative myopathy overall was not increased in treated male rats, but the severity of this change was increased at the 0.19 mg/ml dose level, and the most marked cases correlated with the wasted muscle observed macroscopically (Table 9a).

According to the report, there were no microscopic correlates to the enlarged livers or pale spots observed macroscopically. However, increased incidence of fat vacuolation and spongiosis/peliosis hepatis with altered hepatocytes was observed in male rats at the 0.19 mg/ml dose level.

In the interim sacrifice rats of both sexes, the only change of note was in atrophy of Steno's gland, which was graded as slight and was increased in both high dose males and females.

Those animals surviving to study termination showed similar effects in the nasal cavity at the 0.19 mg/ml dose as those which died during the study. However, there was one change noted in those rats surviving to termination which was not evident in any other format, and this was the observation of bile duct proliferation. In just this sub group, the incidence of this lesion graded as 'slight' increased from 7/11 in control to 16/20 at the 0.56 mg/ml dose level and 13/15 at the 0.19 mg/ml dose level.

2) Neoplastic Observations

Separate summaries are provided for neoplastic observations in those animals sacrificed during the study, those surviving to study termination, interim sacrifice animals, and all animals combined (Table 12):

Table 12

Neoplastic Observations in Male and Female Rats Administered Metam Sodium in Drinking Water[a]

	Males (mg/ml)				Females (mg/ml)			
Intercurrent	0.0	0.019	0.056	0.19	0.0	0.019	0.056	0.19
Liver								
No. examined	42	37	33	36	28	28	29	23
hepatocellular adenocarcinoma	2	2	2	**5**	0	0	1	0
Interim Sacrifice								
Pituitary gland								
No. examined	11	11	10	12	8	10	11	12
adenoma	2	1	2	**5**	3	5	6	3
Terminal Sacrifice								
Pituitary gland								
No. examined	11	15	20	15	26	25	23	29
adenoma	6	10	**16**	**12**	24	25	22	25
All Animals								
Liver								
No. examined	64	64	64	64	64	64	64	64
hepatocellular adenocarcinoma	3	2	4	5	0	0	2	0
hepatocellular adenoma	2	1	3	4	1	1	1	2
Pituitary								
No. examined	64	63	63	62	62	63	63	64
adenoma	28	30	**33**	**35**	51	50	53	46

[a]data taken from Tables 17a-17b, pages 400-420 of the report.

In addition to the above data, hemangioma and hemangiosarcoma were considered in all animals irrespective of site, as was performed in the mouse carcinogenicity study (MRID # 432335-01). The sites of hemangioma in the present rat study included the cervical lymph node, mesenteric lymph node, thymic lymph node, and subcutaneous tissue. Sites for hemangiosarcoma included the mesenteric lymph node, subcutaneous tissue, tail, liver, lung, and uterus. However, the preponderance of tumors were observed only in male rats.

Hemangioma (all sites, male rats)

Control - 9/64 animals
0.019 mg/ml dose - 4/64 animals
0.056 mg/ml dose - 4/64 animals
0.19 mg/ml dose - 8/64 animals

Hemangiosarcoma (all sites, male rats)

Control - 0/64 animals
0.019 mg/ml dose - 3/64 animals
0.056 mg/ml dose - 7/64 animals
0.19 mg/ml dose - 3/64 animals

The question of whether these tumors were observed in separate rats was addressed in the review of this study by the California Department of Environmental Protection (Earl Meierhenry, personal communication). This review showed that of the benign hemangiomas found, one rat in the low dose group was found to have this tumor type at 2 sites (mesenteric and thymic lymph nodes). Of the malignant hemangiosarcomas found, 2 rats in the low dose group were found to have this tumor type at 2 and 3 sites (liver and lung; liver, lung, and mesenteric lymph node, respectively).

The only other observations of tumorigenicity in this study involved the liver (adenocarcinoma) and pituitary (adenoma), both of which appeared slightly increased in male rats at the 0.19 mg/ml dose level. However, this did not appear to be a strong dose-response relationship.

III. DISCUSSION

In the present study, the chronic toxicity and carcinogenicity of Metam Sodium technical was examined in male and female Hsd/Ola: Wistar Tox rats. Test chemical was administered in the drinking water at nominal doses of 0, 0.019, 0.056, and 0.19 mg/ml for either 52 weeks (interim sacrifice group) or 104 weeks (terminal sacrifice group). When corrected for decomposition of test material, doses of metam sodium received by male rats were calculated as **1.3 mg/kg** (low dose); **3.9 mg/kg** (mid dose); and **12.0 mg/kg** (high dose). For female rats, these doses were calculated as **2.3 mg/kg** (low dose); **6.2 mg.kg** (mid dose); **16.2 mg/kg** (high dose). Standard measurements on body weight, food consumption, clinical pathology, ophthalmology, urinalysis, and histopathology were performed as appropriate for this study.

There were no apparent detrimental effects of treatment with metam sodium on survival in male and female rats at any dose level used in this study, although survival in control male rats was below an acceptable level at study termination (i.e. < 25% alive). However, the occurrence of this at such a late time point (post 78-weeks) is not thought to compromise the conclusions of the study. Clinical signs observed in the present study were confined to male rats, where the incidence of reduced hindlimb function, staining around the nose, and thin appearance were increased at the 0.19 mg/ml dose level.

Statistically significant effects were noted on group mean absolute body weight at the 0.19 mg/ml dose level for both male and female rats, and at the 0.056 mg/ml dose level in female rats. Group mean body weight gain for weeks 1-13 was decreased by 12% in male rats and 16% in female rats at the 0.19 mg/ml dose level, but was not significantly affected at lower doses. For weeks 1-53, a similar effect was noted, with decreases of 11% in males and 21% in females at the 0.19 mg/ml dose level. For the study period as a whole, body weight gain was decreased 18% in male rats and 20% in female rats at the 0.19 mg/ml dose level.

Decreases in food consumption were also observed in this study, with a decrease from control of 10% in male and female rats at the 0.19 mg/ml dose level during weeks 1-13. Larger or smaller decreases were observed subsequent to this time point. Food efficiency was also reported as significantly decreased in male and female rats for weeks 1-12 of the study. The decrease in food consumption for male rats for weeks 1-13 of the study at the 0.19 mg/ml dose (10%) is approximately equal to the decrease in weight gain (12%), but for females, the decrease in weight gain (16%) exceeds the decrease in food consumption (10%). Based on the combined observations of body weight gain, food consumption, and food efficiency, a mild toxic effect of metam sodium could be inferred at the 0.19 mg/ml dose level. Water consumption was also measured in this study and was found to be significantly affected at the 0.19 mg/ml dose level for both sexes, and at the 0.056 mg/ml dose level for females. The decrease was dose-related, and equaled or exceeded 50% of control values at the 0.19 mg/ml dose level. The significance of such a dramatic decrease in water consumption on toxicity of metam sodium (as related to effects on normal physiology) was not addressed in this study.

Ophthalmological examination at pre-study (52 males and 51 females examined) showed focal opacities in 2 female rats assigned to the high dose group, and none in male rats. At week 52 (examination of 52 males and 49 females), focal opacities were observed in 5 high dose females vs 2 in control, and linear opacities were observed in 3 high dose females vs 1 in controls. At terminal sacrifice, there were no ophthalmoscopic abnormalities observed in treated animals.

Hematological examinations showed effects on red blood cells (RBC), hematocrit (HCT), and hemoglobin (HGB) at the 0.19 mg/ml dose level. These effects consisted of decreases in these parameters of from 3-5% vs control, and were observed up to week 79 of the study, usually in both sexes. At week 105, the decreases in these parameters were slightly greater in high dose males vs high dose females (HGB: 12% in males, 5% in females; HCT: 11% in males, 4% in females; RBC: 10% in males, 8% in females). The increased hemosiderin observed in the spleen of high dose males and females could be coupled to the decrease in RBC, HCT, and HGB and be interpreted as a mild hemolytic anemia at this dose.

Treatment with metam sodium produced effects on the clinical chemistry parameters of cholesterol (decreases of 10% and 17% in high dose males and females at week 105; decreases of 4% and 19% in high dose males and females at week 79), triglycerides (decrease of 18% and 22% in high dose males and females at week 79; decrease of 32% and 43% in high dose males and females at week 105), alanine transaminase (decrease of 15% in high dose females at week 14; decrease of 27% and 24% in high dose and mid dose females at week 27), and aspartate transaminase (decrease of 11% in high dose females at week 14; decrease of 32% in high dose females at week 27). There was also an effect on glucose in mid- and high dose females at week 105 (increases of 16% and 14%, respectively), but this was not observed at earlier time points. The most significant effects appeared to be on cholesterol and triglycerides.

Urinalysis showed a decreased volume in high dose males and females at week 52 (decreases of 25% and 30%, respectively), and week 78 (29% decrease in high dose females). Specific gravity was increased slightly (0.5%) in high dose females at week 52, but increased by 6% over control in high dose females at week 78. The qualitative analysis of urine showed that in both sexes, the presence of blood appeared to increase in prominence as the dose of metam sodium was increased. Both the number of animals displaying this symptom as well as the severity of the symptom appeared increased at the high dose, and some dose-response relationship was apparent.

Macroscopic examination of rats in this study (all animals combined) showed an increase in liver masses in male rats at the high dose (8 recorded vs 4 in control). Of the eight recorded masses, four were identified as adenocarcinomas, three identified as adenomas, and one identified as an hepatoblastoma. The incidence of enlarged liver as well as pale spots was also increased at both the mid and high dose for male rats, although there were no definitive microscopic correlates. Fat vacuolation was, however, increased at the high dose, possibly providing some correlate for the enlarged liver appearance at the high dose. Wasting of voluntary muscle was observed to be increased at the high dose in male rats, and could be correlated with an increase in the severity and incidence of degenerative myopathy. The severity (but not incidence) of sciatic nerve degeneration was also shown to be increased at the high dose in male rats.

Significant microscopic findings were observed in the nasal cavity of high dose male and female rats in this study.The changes observed included rhinitis, hypertrophy of Bowman's ducts/glands, atrophy and adenitis of Steno's gland, and olfactory epithelium hyperplasia and degeneration. Insofar as the incidence was so greatly increased at the high dose, this is considered an effect of treatment. As stated in the report, the lesions observed are indicative of a systemic effect due to the posterior location of Bowman's and Steno's glands in the nasal passage, and because both glands are metabolically active (meaning presumably the capacity to biotransform the parent chemical). Of interest is the observation of decreased mineralization of the aorta and heart observed in high dose males vs control.

Based on examination of the tumor data presented in this study, there is no evidence for tumorigenicity of metam sodium in rats.

Based upon the decrease in body weight gain and food efficiency in both sexes, as well as the observed hematological, clinical chemistry, and microscopic pathology changes, the **LEL is considered to be 0.19 mg/ml. The NOEL is considered to be 0.056 mg/ml.**

Dosing was considered adequate in male and female rats based upon the decrease in body weight gain, food efficiency, changes in hematology and clinical chemistry, and microscopic pathology observed at the 0.19 mg/ml dose level.

IV. Classification

This study is classified **core minimum** and satisfies the guideline requirement for §83-5, Combined Chronic Toxicity and Carcinogenicity Study in Rats.