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

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MAY 14 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Metam Sodium - Review of a 90-Day Study in Mice

Tox. Chem. No.: 780
HED Proj. No. : 2-0826

TO: Susan Lewis, PM 21
Herbicide-Fungicide Branch
Registration Division (H7505C)

FROM: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

J. M. Ioannou 5/7/9

THRU: Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C)

Marcia van Gemert 5/8/92

Registrant: Metam Sodium Task Force

Action Requested: Review a 90-day drinking water study in mice with Metam Sodium

Toxicology Branch II has completed the review of a 90-day study with Metam Sodium titled:

"Metam Sodium: 90-Day Drinking Water Study in Mice with a 28-day Interim Kill" (MRID No.: 421173-01)

Conclusions:

Metam Sodium was administered to male and female mice in the drinking water at the dose levels of 0.018 mg/ml (2.7 mg/kg/day for males; 3.6 mg/kg/day for females), 0.088 mg/ml (11.7 mg/kg/day for males; 15.2 mg/kg/day for females), 0.35 mg/ml (52.4 mg/kg/day for males; 55.4 mg/kg/day for females), and 0.62 mg/ml (78.7 mg/kg/day for males; 83.8 mg/kg/day for females) for 90 days. The systemic toxicity NOEL was 0.018 mg/ml (2.7 mg/kg/day for males and 3.6 mg/kg/day for females) and the LEL was 0.088 mg/ml (11.7 mg/kg/day

for males and 15.2 mg/kg/day for females) based on urinary bladder lesions (eosinophilic granules, cystitis and mucosal hyperplasia) in both sexes and decrease in hematology parameter values (HGB, RBC, HCT) in female mice. Based on decreased body weight gains the MTD appears to have been achieved at 0.35 mg/ml (52.4 mg/kg/day) in males and at 0.62 mg/ml (83.8 mg/kg/day) in females.

The study was classified as core-supplementary due to deficiencies listed in the data evaluation report.

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DATA EVALUATION REPORT

Metam Sodium

Study Type: Subchronic Oral Toxicity: 90-Day Study

Study Title: Metam Sodium: 90-Day Drinking Water Study in Mice with a 28-Day Interim Kill

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

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May 1, 1992

Principal Author	Betty Shindel, M.P.H.	Date 5-1-92
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Contract Number: 68D10085
Work Assignment Number: 1-77
Clement Number: 91-248
Project Officer: James Scott

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EPA Reviewer and Section Head: Mike Ioannou Signature: M Ioannou
Review Section I, Toxicology Branch II (HED) Date: 5/6/92

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity: 90-Day Study

TEST MATERIAL: Metam sodium TOX CHEM. NUMBER: 780

SYNONYMS: Carbam; SMDC MRID NUMBER: 421173-01

STUDY NUMBER: PM0808

REPORT NUMBER: CTL/P/3185

SPONSOR: Metam Sodium Task Force (no further information provided on address of sponsor)

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire UK

TITLE OF REPORT: Metam Sodium: 90-Day Drinking Water Study in Mice with a 28-Day Interim Kill

AUTHOR: A.J. Whiles

REPORT ISSUED: 09/26/91

CONCLUSIONS: Metam sodium was administered to male and female mice in the drinking water at the dose levels of 0.018 mg/mL (2.7 mg/kg/day for males; 3.6 mg/kg/day for females), 0.088 mg/mL (11.7 mg/kg/day for males; 15.2 mg/kg/day for females), 0.35 mg/mL (52.4 mg/kg/day for males; 55.4 mg/kg/day for females), and 0.62 mg/mL (78.7 mg/kg/day for males; 83.8 mg/kg/day for females).

The systemic toxicity NOEL was 0.018 mg/mL (2.7 mg/kg/day for males; 3.6 mg/kg/day for females) and the LEL was 0.088 mg/mL (11.7 mg/kg/day for males; 15.2 mg/kg/day for females) based on urinary bladder lesions in both sexes and decreases in hematology parameters (HGB, RBC, HCT) in females. The NOEL is tentative because of poor stability of metam sodium in water over a 24-hour period.

Based on decreased body weight gains the MTD appears to have been achieved at the dose level of 0.35 mg/mL (52.4 mg/kg/day) in males and at 0.62 mg/mL (83.8 mg/kg/day) in females.

CORE CLASSIFICATION: Core Supplementary. Testing of clinical blood chemistry parameters and ophthalmological examinations were not performed as specified in Guideline 82-1. The significant instability of metam sodium in water over a 24-hour period limits the accuracy of assigning a NOEL because it is impossible to determine the amount of test article ingested. Homogeneity of the test article in drinking water solutions was not determined; therefore, it is not certain if a homogeneous mix was established.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Metam sodium

Supplier batch reference number: BAS 005 00 N

Testing facility reference numbers: Y06930/007, Y06930/008

Source: BASF Limburgerhoff, West Germany

Purity: 45.13% w/w (assumed purity as stated in materials and methods section); impurities not identified

Physical description: Yellow liquid

Density: 1.218 g/cm^3

Storage conditions: According to the certificate of analysis, the test article was to be refrigerated until analysis. According to the materials and methods section, the test article was stored in the test facility's dispensary at room temperature in the dark, in a sealed glass bottle.

2. Test Animals

Species: Mouse

Strain: C57/10JfAP/Alpk

Source: Barriered Animal Breeding Unit (BABU), ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire UK

Sex and numbers: Males, 75; females, 75

Age: 29-33 days old on arrival

Mean body weights at week 1: Males, 20.1 grams; females, 17.1 grams. Mean body weights prior to initiation of the study were not reported.

Animals were acclimated for 1 week prior to initiation of the study. During the acclimation period, animals were housed by sex in groups of approximately 5 per cage. The temperature and humidity of the animal room were measured once daily. Temperature ranged from 19° to 25°C, and relative humidity ranged from 55% to 79%. An alternating 12-hour light/dark cycle was maintained in the animal room. The ventilation system provided a minimum of 12 air changes/hour. The animal room was designated for this study only. Mice received feed (powdered CT1 diet) ad libitum. The CT1 diet was manufactured by Special Diets Services, Ltd., Stepfield, Witham, Essex, UK. Dietary constituents of CT1 diet were provided in the report, and all batches of CT1 diet

passed contaminant specifications. Drinking water containing the test article was provided ad libitum via drinking bottles.

A randomized block design was used to allocate the animals to cages and racks. During the acclimation period, the animals were randomly assigned by sex (based on body weight) to appropriate test groups. Mice were housed 5/cage. The initial body weight ranges by which the animals were randomly assigned to respective dose groups were not reported. Animals were identified by a numbered ear tag.

Dose Preparation and Dose Administration: Table 1 provides a description of the experimental design of this study.

Doses of test article were administered to treated animals via drinking water on a daily basis for 90 days. Five animals/sex/group were killed and examined after an interim period of 29 days. The doses administered were 0.018, 0.088, 0.35, and 0.62 mg/mL. The rationale for dose selection was based on results from a previous 14-day drinking water study in mice which was not available for review. The results of the 14-day drinking water study were not discussed in the study report. Drinking water was purified by reverse-osmosis and adjusted to a pH of 9.0 using sodium hydroxide and hydrochloric acid. According to the study author, metam sodium is stable under alkaline conditions. According to the materials and method section, each dose was prepared by adding a weighed amount of the test article adjusted for an assumed purity of 45.13% w/w to an appropriate amount of drinking water. The test article/drinking water preparation was then dispensed into drinking bottles. Dosing preparations were prepared daily, except for control and 0.62-mg/mL dose preparations which were used over a 2-3 day period at the beginning of the study.

3. Test Article Analyses for Purity and Stability

According to the materials and methods section, concentrations and stability of metam sodium in water, purified by reverse-osmosis and adjusted to pH 9.0 using sodium hydroxide solution, were determined from separate preparations after completion of the study and were not determined from actual samples used in the study. According to the certificate of analysis included in the study report, the concentration of metam sodium was 525.54 g/L, representing 103.0% of the theoretical concentration of 510 g/L.

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TABLE 1. Experimental Design of Drinking Water Study in Mice Administered Metam Sodium for 90 days with a 29-day Interim Kill

Group	Conc. of Metam Sodium in drinking water (mg/mL)	No. of Animals/Group		No. of Days of Treatment
		Males	Females	
1	0.000	10	9*	90
2	0.018	10	10	90
3	0.088	10	10	90
4	0.350	10	10	90
5	0.620	10	10	90
6	0.000	5	5	29
7	0.018	5	5	29
8	0.088	5	5	29
9	0.350	5	5	29
10	0.620	5	5	29

*One 90-day female control died before start of study.

Twenty-four-hour stability data for metam sodium/drinking water solutions in polypropylene drinking bottles were provided and indicated that metam sodium was significantly unstable in water; a large reduction in concentration of metam sodium was observed after 24 hours. The largest reduction in stability of the test article was seen in the lowest dosage group (0.018 mg/mL). Data for the percent of initial concentration of test article remaining after 24 hours (data from two different preparation dates) for each dosage group are presented below.

Group (mg/mL)	Initial concentration of test article (mg/mL)	% of initial concentration of test article after 24 hours
0.018	0.020-0.022	29.1-31.5%
0.088	0.081-0.082	38.3-39%
0.35	0.32-0.40	68.8-85.0%
0.62	0.58-0.60	76.6-79.3%

The homogeneity of the test material in the drinking water was not examined; therefore, it is not certain if a homogeneous mix was established.

4. Statistical Methods

ANOVA was used to analyze initial body weight, weekly water and food consumption, total food consumption, hematology, and organ weights, separately for males and females. The study report stated that hematology results from the sexes combined were examined to determine whether differences between control and treated groups were consistent between sexes. ANOCOVA was used to analyze weekly body weight by covariance on initial body weight, and organ weight by covariance on final body weight, separately for males and females. A two-sided Student's t-test was used to determine if there were statistically significant differences between each treatment group least square mean and the control group least square mean at the 5% and 1% level.

5. General Observations

(a) Mortality/moribundity/survival

Each animal was observed daily for mortality and moribundity. Animals were also observed for mortality and moribundity during the 10-day acclimation period. No treatment-related mortality or moribundity was observed during the study period. One control female died before the start of the study. One control female was killed *in extremis* at week 8 because of irregular breathing, subdued behavior, and piloerection. The study author stated that this mouse had lung congestion. Two treated female mice in the 0.35-mg/mL dose group were killed *in extremis* during weeks 7-12 because of hydrocephalus. The study author stated that hydrocephalus is a common spontaneous abnormality in young C57/10JfAP/Alpk mice and is not considered to be treatment related.

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(b) Clinical observations

Animals were observed daily for clinical signs of toxicity. There were no treatment-related signs of clinical toxicity in treated animals. Clinical observations consisted of damaged tail, torn left ear, swollen ears, hair loss, piloerection, reduction in whiskers, and subdued behavior. The frequency of clinical observations in treated animals was similar to that of controls.

(c) Body weight/food and water consumption/test article intake

Body weight: Table 2 summarizes mean body weight data. Body weight was measured at the time of group assignment, on day 1 before administration of the test article, daily on days 2-8, and weekly thereafter. For week 13, mice were weighed at the beginning and end of the week. The reviewers could not locate initial body weight data prior to the beginning of the study report. The earliest data available for individual body weights were for week 1. There were statistically significant decreases in mean body weight in males at doses of 0.35 and 0.62 mg/mL during weeks 2-13 as compared to male controls. For females there were statistically significant decreases in mean body weight during weeks 3-6 and 8 at 0.35 mg/mL and during weeks 2-6, 8, 9, 12, and 13 at 0.62 mg/mL as compared to female controls. For females at a dose of 0.088 mg/mL, there was a statistically significant decrease in mean body weight at week 8; however, the decrease was not of toxicological significance because it was not consistent over time.

Body Weight Gains (g) by Week 8 and 13 on Study

Dose	Males		Females	
(mg/mL)	Week 8	Week 13	Week 8	Week 13
0.000	6.7 (100.0)[a]	9.3 (100.0)	5.5 (100.0)	6.8 (100.0)
0.018	7.3 (109.0)	10.2 (109.7)	5.5 (100.0)	6.8 (100.0)
0.088	6.9 (103.0)	9.2 (98.9)	5.0 (90.9)	7.1 (104.4)
0.350	5.2 (77.6)	6.9 (74.2)	5.1 (92.7)	7.1 (104.4)
0.620	5.3 (79.1)	7.6 (81.7)	4.5 (81.8)	6.1 (89.7)

[a]Numbers in parenthesis denote percent of control

Statistically significant increases in mean body weight were observed for females at 0.088 mg/mL during weeks 10 and 11. There were increases in mean body weight for females at 0.35 and 0.62 mg/mL during week 11; however, the increases were not significant. The study author stated that there was no rational explanation for the dramatically increased growth of females given 0.088, 0.35, or 0.62 mg/mL metam sodium during week 11 of the study or for the subsequent body weight loss recorded during week 12.

TABLE 2. Mean Body Weights (g ± SD) at Selected Intervals for Mice Administered Metam Sodium in Drinking Water for 90 Days[a]

Metam Sodium Concentration (mg/mL)	Week 1	2	4	8	13
			Males		
0.000	20.4±1.3	21.9±1.4	24.0±1.3	27.1±1.8	29.7±2.1
0.018	20.0±1.6	21.7±1.8	23.6±1.7	27.3±1.4	30.2±1.7
0.088	20.3±1.6	22.4±1.3	24.6±1.7	27.2±1.6	29.5±2.0
0.350	20.0±1.1	20.7±1.5*	22.5±1.0**	25.2±1.2**	26.9±1.1**
0.620	19.9±1.3	19.9±1.3**	21.2±1.2**	25.2±1.6**	27.5±2.0*
			Females		
0.000	17.6±1.1	18.3±1.0	20.2±1.3	23.1±0.8	24.4±0.8
0.018	17.2±1.1	18.3±1.1	20.4±1.1	22.7±1.3	24.0±1.0
0.088	16.8±1.4	17.8±1.6	19.5±1.3	21.8±1.3*	23.9±1.3
0.350	16.8±1.4	17.1±1.4	18.7±1.5**	21.9±1.0*	23.9±0.8
0.620	16.9±1.0	16.5±1.2**	18.5±1.0**	21.4±0.7**	23.0±0.7**

[a]Data extracted from Study CTL/P/3185, Table 3, pp. 39-40.

* Significantly different from control group mean; p≤0.05.
** Significantly different from control group mean; p≤0.01.

Food consumption: Food consumption data were recorded weekly throughout the study period for each cage of mice. There were no statistically significant changes in weekly food consumption in treated males or females at doses of 0.018, 0.088, or 0.35 mg/mL as compared to their respective controls. At the highest dose of 0.62 mg/mL, there were statistically significant decreases in weekly food consumption for males during weeks 1, 3, and 13, and for females during weeks 1, 3, and 9.

Water consumption: Table 3 summarizes mean water consumption data. Water consumption data were recorded daily throughout the study period for each cage of mice. There were statistically significant decreases in total water consumption (weeks 1-13) in males at a dose of 0.62 mg/mL, and in females at doses of 0.35 and 0.62 mg/mL, as compared to their respective controls. Weekly water consumption was statistically significantly decreased in males at 0.088 mg/mL (week 4), 0.35 mg/mL (weeks 1, 3, and 4), and 0.62 mg/mL (weeks 1-4) as compared to male controls. Weekly water consumption was statistically significantly decreased in females at 0.018 mg/mL (week 1), 0.088 mg/mL (weeks 1-4 and 6), 0.35 mg/mL (weeks 1-7), and 0.62 mg/mL (weeks 1-8, 10, and 13), as compared to female controls.

Test article intake: The group mean test article intake values for the 0.018-, 0.088-, 0.35-, and 0.62-mg/mL dose groups over a 90-day period were 2.7, 11.7, 52.4, and 78.7 mg/kg/day in males, respectively, and 3.6, 15.2, 55.4, and 83.8 mg/kg/day in females, respectively. The study author stated that these values can only be considered to be approximations of the mean doses received because of the relative instability of metam sodium in drinking water.

(e) Ophthalmoscopic examination

This study deviated from Guideline 82-1 by not performing ophthalmological examinations on any of the animals. Guideline 82-1 requires that an ophthalmological examination be performed prior to administration of the test substance and at the termination of the study, preferably in all animals, but at least in the high-dose and control groups.

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TABLE 3. Mean Water Consumption (mL/day ± SD) at Selected Intervals in Mice Administered Metam Sodium in Drinking Water for 90 Days[a]

Metam Sodium Concentration (mg/mL)	Week 1	Week 4	Week 8	Week 13	Total Water Consumption (Weeks 1-13)
			Males		
0.000	4.34±0.26	3.89±0.17	3.56±0.26	3.70±0.28	344.12±27.32
0.018	4.31±0.16	3.95±0.21	3.80±0.08	3.95±0.07	354.67±16.59
0.088	3.92±0.34	3.44±0.32**	3.31±0.44	3.33±0.05	309.35±21.47
0.350	2.92±0.19**	3.30±0.06**	3.83±0.04	3.93±0.24	330.60±10.23
0.620	2.15±0.16**	2.68±0.04**	3.10±0.26	3.52±0.12	272.12±11.71*
			Females		
0.000	4.26±0.15	4.34±0.19	4.57±0.81	5.91±0.65	423.71±28.49
0.018	3.94±0.18**	3.93±0.36	4.44±0.46	5.35±1.91	391.98±39.29
0.088	3.46±0.10**	3.55±0.27**	3.60±0.36	4.25±0.40	336.35±34.77
0.350	2.62±0.17**	3.06±0.08**	3.64±0.26	3.81±0.21	304.96±24.49*
0.620	2.09±0.10**	2.70±0.16**	3.01±0.10*	3.13±0.09*	252.86± 1.57**

[a]Data extracted from Study CTL/P/3185, Table 5, pp. 43-44.

* Significantly different from control group mean; p≤0.05.
** Significantly different from control group mean; p≤0.01.

6. Clinical Pathology

Blood samples were collected from all mice by cardiac puncture at the end of the 90-day study. Hematology parameters were analyzed for all terminal kill animals with the exception of the leukocyte differential count and red cell morphology analyses which were only performed on mice from the control and highest dose groups. Checked (X) parameters were examined.

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Erythrocyte morphology
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

* = Recommended by Subdivision F (November 1984) Guidelines

Table 4 summarizes data on selected hematology parameters.

Analysis of hematology parameters was not performed for interim kill (day 29) animals.

For the terminal kill (day 90) animals, treatment related changes in hematology parameters in females consisted of statistically significant decreases in HGB, HCT, and RBC at doses of 0.088, 0.35, and 0.62 mg/mL as compared to female controls. Treatment related changes in hematology parameters in males consisted of statistically significant decreases in HGB and RBC at doses of 0.35 and 0.62 mg/mL and HCT at a dose of 0.62 mg/mL, as compared to male controls.

Statistically significant changes in MCH consisted of increases for females at doses of 0.35 and 0.62 mg/mL and decreases for males at doses of 0.018 and 0.088 mg/mL, as compared to their respective controls. These changes in MCH were not considered to be treatment related because no dose response was observed and the changes were in opposite directions for males and females. There was a statistically significant increase in MCV for males at 0.35 and 0.62 mg/mL and for females at 0.62 mg/mL. There was a statistically significant decrease in MCHC for males at 0.088, 0.35, and 0.62 mg/mL. There was a statistically significant decrease in platelet count for males at doses of 0.088 and 0.35 mg/mL. The study author stated that increases in MCV and MCH were not treatment related because they were compensating adjustments for reduced hemoglobin and red blood cell counts. In summary, the lowest doses at which dose-related hematological changes were noted were 0.35 mg/mL (decreased HGB and RBC) for males and 0.088 mg/mL (decreased HGB, HCT, and RBC) for females. However, the changes in hematological parameters were slight and no historical control data for this particular strain of mouse were provided to determine if the changes were within normal biological range.

TABLE 4. Selected Hematology Parameters (Mean ± S.D.) for Mice Administered Metam Sodium in Drinking Water for 90 Days[a]

Group	Dose conc. (mg/mL)	RBC ($1x10^{12}$/L)	HGB (g/dL)	HCT	MCH (pg)	MCHC (g/dL)	MCV (fL)
				Males			
1	0.000	8.55±0.18	15.4±0.3	0.468±0.008	18.0±0.4	32.9±0.7	54.8±0.6
2	0.018	8.64±0.45	15.2±0.8	0.471±0.026	17.6±0.2*	32.3±0.4	54.6±0.5
3	0.088	8.67±0.22	15.2±0.2	0.474±0.010	17.5±0.3**	32.0±0.3**	54.7±0.5
4	0.350	8.25±0.48*	14.9±0.7*	0.462±0.026	18.1±0.4	32.3±0.7*	55.9±0.7**
5	0.620	8.03±0.24**	14.6±0.2**	0.453±0.015*	18.1±0.4*	32.2±0.9*	56.3±0.7**
				Females			
1	0.000	9.00±0.35	15.4±0.4	0.486±0.015	17.1±0.8	31.7±1.1	54.1±1.5
2	0.018	8.91±0.46	15.3±0.3	0.477±0.025	17.2±0.7	32.2±1.2	53.6±1.1
3	0.088	8.59±0.26*	14.8±0.5*	0.465±0.016*	17.3±0.5	31.9±1.1	54.1±0.9
4	0.350	8.15±0.44**	14.4±0.3**	0.446±0.022**	17.8±0.7*	32.4±1.2	54.6±0.7
5	0.620	8.13±0.33**	14.3±0.4**	0.451±0.023**	17.6±0.5*	31.8±1.3	55.7±0.8**

[a]Data extracted from Report No. CTL/P/3185, Table 6, pp. 46-48.

*Significantly different from control group 1; $p \leq 0.05$
**Significantly different from control group 1; $p \leq 0.01$

(b) Blood (clinical) chemistry

Analyses of blood clinical chemistry parameters were not performed for interim or terminal kill animals. This study deviated from Guideline 82-1 requirements by not conducting blood clinical chemistry analyses at the end of the study period.

(c) Urinalysis

Urinalysis was not performed. Guideline 82-1 does not require performance of urinalysis. The Guideline states that urinalysis is to be performed only when useful data might be obtained based on expected or observed toxicity.

7. Sacrifice and Pathology

A gross necropsy was performed on all animals. Animals were anesthetised with halothane BP vapor, and killed by exsanguination using a cardiac puncture. Microscopic examinations were performed on the checked (X) tissues below for terminal kill animals from the control and highest dose (0.62 mg/mL) groups. For terminal kill animals from the 0.018-, 0.088-, and 0.35-mg/mL dosage groups, microscopic examinations were performed only on the bladder, kidney, and liver; on rare occasions the skin, spleen, lung, and brain were also examined. Microscopic examinations were performed on the bladder, brain, liver and lung of animals that died during the study. Macroscopic examinations were performed on the tissues checked (X) below for all terminal kill animals. Also, macroscopic examinations were performed on the eye, mammary gland, harderian glands, oral cavity, bone (knee joint and femur), voluntary muscle, sciatic nerve, and spinal cord for all terminal kill animals. For all interim kill animals, macroscopic examinations were performed only on the adrenal gland, brain, epididymis, kidney, liver, spleen, stomach, and testis. Macroscopic examinations were performed on the brain, lung, and skull of animals that died during the study. The double-checked (XX) organs were also weighed for all animals following terminal and interim kills.

Three types of fixative were used: Bouin's solution was used for skin and mammary gland; Davidson's solution was used for eyes and Harderian gland; and 10% neutral buffered formol saline was used for all remaining tissues.

Digestive System	Cardiovascular/Hematologic	Neurologic
X Stomach*	X Aorta*	XX Brain
X Colon*	X Heart*	Sciatic nerve*
X Duodenum*	X Bone marrow (sternum)	Spinal cord
X Jejunum*	X Lymph nodes*	(three levels)
X Ileum*	X Spleen*	Eyes
X Cecum*	X Thymus*	X Pituitary*
X Colon*		
X Rectum*	Urogenital	Glandular
X Salivary glands*		
X Esophagus*	XX Kidneys*	XX Adrenal
XX Liver*	XX Testes*	Mammary gland
X Pancreas*	X Urinary bladder*	X Thyroid*
X Gall bladder	X Epididymides	X Parathyroid*
	X Prostate	Harderian gland
Respiratory	X Seminal vesicle	X Preputial glands
	X Ovaries	
X Trachea	X Uterus*	
X Lung*	X Cervix	

Other

Oral cavity
X Nasal cavity
Bone (knee joint and femur)
Skeletal muscle
X Skin*
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic pathology

Terminal kill: Accentuated lobular pattern of the liver was observed for treated animals at doses of 0.35 (3/18 animals) and 0.62 mg/mL (1/20 animals). The study author did not consider the accentuated lobular liver pattern to be treatment related because there was no clear dose-response relationship and no associated histopathological findings in the liver. Black discoloration of the spleen (localized melanosis) occasionally occurred in treated animals, but a dose response relationship was not established. The study author did not consider this effect to be compound related because it was typical of mice of this age and strain. Other reported macroscopic abnormalities were observed in 1-2 treated animals only and consisted of cystic kidney, distended bladder, flaccid testis, dark lung, swollen head, and depressed and/or red areas of the brain.

Interim kill: The accentuated lobular pattern of the liver observed in terminal kill animals at 0.35 and 0.62 mg/mL was also observed in interim kill animals of the same dose groups with a frequency of 4/10 animals at 0.35 mg/mL, and 3/10 animals at 0.62 mg/mL. There were no macroscopic abnormalities reported in any of

the tissues for any of the control animals. Other macroscopic abnormalities in interim kill animals were observed in 1-2 treated animals only and consisted of discolored spleen, adrenal gland with red spot(s), enlarged hepatic lymph node, and sore(s) on the tail.

(b) Organ weights and organ/body weight ratios

Table 5 summarizes selected organ weight data. Organ weight data consisted of absolute organ weight, organ to body weight ratio (based on terminal body weight at end of week 13), and organ weight adjusted for final body weight by the statistical method of ANOCOVA.

Terminal kill

Liver: There were statistically significant increases for males and females in absolute liver weight at doses of 0.35 and 0.62 mg/mL, and in liver weight adjusted for body weight at doses of 0.088, 0.35, and 0.62 mg/mL, as compared to their respective controls. However, no clear dose-response relationship was observed.

Kidney: For males and females at 0.35 and 0.62 mg/mL, there were statistically significant increases in kidney weight adjusted for body weight. There was a statistically significant increase in females at 0.35 mg/mL. These increases appeared to be incidental because a dose-response relationship was not observed.

Brain: Statistically significant decreases occurred for brain weight adjusted for body weight in males at 0.018 and 0.088 mg/mL, and absolute brain weight in males at 0.088 mg/mL. However, the decreases were incidental because a dose-response relationship was not observed.

Other organs: There were no statistically significant changes in adrenal and testes weights as compared to controls.

The reviewers found a discrepancy in the study report in the recording of terminal body weight for females at doses of 0.018 and 0.088 mg/mL in the organ weight summary tables as compared to the body weight summary tables. However, the discrepancy was slight and did not affect interpretation of the organ to terminal body weight ratios.

Interim kill

Liver: There were statistically significant increases in liver weight adjusted for body weight for males and females at 0.35 mg/mL and 0.62 mg/mL as compared to their respective interim controls. The only statistically significant increase in absolute liver weight was in females at a dose of 0.62 mg/mL. A dose-response relationship was not observed.

TABLE 5. Selected Organ Weight Data for Mice Administered Metam Sodium in Drinking Water for 90 Days[a]

Metam Sodium Conc. (mg/mL)	Liver			Kidney		
	Absolute Weight (g)	Organ to Body Weight Ratio (%)	Organ Weight Adjusted for Final Body Weight (g)	Absolute Weight (g)	Organ to Body weight Ratio (%)	Organ Weight Adjusted for Final Body Weight (g)
			Males			
0.000	1.38±0.10	4.65±0.30	1.32	0.387±0.028	1.305±0.053	0.376
0.018	1.38±0.12	4.57±0.27	1.30	0.392±0.030	1.300±0.068	0.375
0.088	1.48±0.15	5.00±0.26	1.43*	0.386±0.030	1.309±0.047	0.377
0.350	1.59±0.12**	5.91±0.31	1.70**	0.396±0.026	1.468±0.069	0.417**
0.620	1.59±0.20**	5.77±0.46	1.67**	0.395±0.032	1.442±0.114	0.411**
			Females			
0.000	1.15±0.08	4.71±0.20	1.09	0.322±0.021	1.317±0.066	0.315
0.018	1.08±0.08	4.56±0.21	1.08	0.327±0.020	1.378±0.044	0.327
0.088	1.15±0.14	4.82±0.34	1.15*	0.321±0.020	1.357±0.067	0.322
0.350	1.39±0.07**	5.83±0.28	1.38**	0.348±0.019*	1.456±0.061	0.346**
0.620	1.29±0.12**	5.58±0.38	1.35**	0.340±0.020	1.479±0.083	0.350**

[a]Data extracted from Report No. CTL/P/3185, Table 6, pp. 57, 58.

*Significantly different from control group mean; $p \leq 0.05$.
**Significantly different from control group mean; $p \leq 0.01$.

Adrenals: There was a statistically significant increase in absolute adrenal weight and adrenal weight adjusted for body weight in females at 0.35 mg/mL as compared to interim female controls. However, the increase was not dose-related and appeared to be incidental.

Other organs: There were no statistically significant changes in kidney or brain weights as compared to controls.

(c) Microscopic pathology

Terminal kill

Liver: Microscopic findings reported for the liver consisted of slight periportal hepatocyte vacuolation, slight centrilobular hepatocellular vacuolation, slight microvesicular change in centrilobular hepatocytes, and minimal inflammatory cell infiltration. None of the reported microscopic abnormalities of the liver were considered to be treatment related by the study author or the reviewers because the same frequencies of abnormalities were observed in both treated and control animals and no dose-response was seen.

Bladder: Microscopic findings of the urinary bladder were reported for males and females at doses of 0.088, 0.35, and 0.62 mg/mL. Cystitis was reported in 8/8 females and 10/10 males from the 0.35-mg/mL group, and 3/10 females and 8/10 males from the 0.62-mg/mL group. Mucosal hyperplasia was reported in 7/8 females and 10/10 males from the 0.35-mg/mL group, and 8/10 females and 9/10 males from the 0.62-mg/mL group. Eosinophilic granules in the bladder epithelium were reported for 10/10 males and 10/10 females at 0.088 and 0.62 mg/mL, and 7/10 males and 8/8 females at 0.035 mg/mL. A dose-response relationship was not observed in the severity of cystitis, mucosal hyperplasia, and eosinophilic granules in the epithelium. Cystitis, eosinophilic granules in bladder epithelium, and bladder mucosal hyperplasia were also reported in 1 or 2 of the two females from the 0.35 mg/mL group which died during the study.

Kidney: A polycystic kidney (marked severity) was reported in 1/10 males at a dose of 0.35 mg/mL and 1/10 females at a dose of 0.62 mg/mL.

Pancreas: Minimal lymphocytic infiltration and a dilated duct in the pancreas were observed in 1/10 males at a dose of 0.62 mg/mL. No information was provided for the pancreas in females in the summary of microscopic findings. According to individual animal data, there was no appreciable disease of the pancreas in females.

Other organs: There were no microscopic abnormalities reported for the adrenal gland, brain, preputial gland, salivary gland, spleen, testis or stomach of treated animals that were not observed with similar frequency in the control animals.

Interim kill: Microscopic examinations were performed on the interim kill animals from the control and highest dose (0.62 mg/mL) groups. The adrenal gland, brain, kidney, spleen, stomach, and liver were routinely examined; on rare occasions the epididymis, gall bladder, and testis were also examined.

Liver: At a dose of 0.62 mg/mL, one male had slight centrilobular hypertrophy of the liver, and two females had minimal inflammatory cell infiltration of the liver which was not observed in any of the control animals.

Stomach: One female at 0.62 mg/mL had a cystic gland in the stomach; this observation was not reported for any of the control animals.

Other organs: There were no microscopic abnormalities reported for the adrenal gland, brain, kidney, spleen, testis, epididymis, and gall bladder of treated animals that were not observed with similar frequency in the control animals.

The reviewers have no other comments regarding the materials and methods sections.

A signed Good Laboratory Practice Compliance Statement and a signed Quality Assurance Statement were included.

B. DISCUSSION

The study deviated from Guideline 82-1 by not performing clinical blood chemistries or ophthamological examinations. The reviewers could not locate initial body weight data from day 1 in the study report; week 1 data were the earliest data available for individual body weights. The reviewers located a slight discrepancy in the recording of terminal body weight for females at doses of 0.018 and 0.088 mg/mL in the organ weight summary tables as compared to the body weight summary tables. However, the discrepancy was minor and did not affect interpretation of organ to terminal body weight ratios. The title of the report indicates that the study design included a 28-day interim kill; however, the study refers to the interim kill as being performed after 29 days of treatment. The purity of the test article was not verified.

The study author assigned a NOEL of 0.018 mg/mL for systemic toxicity of metam sodium in males and females and a LOEL of 0.088 mg/mL. However, the reviewers conclude that the NOEL of 0.018 mg/mL is a tentative NOEL. The NOEL is considered tentative based on significant instability of the test article, lack of homogeneity data for the test article/drinking water solutions, lack of historical control data for this strain of mice (especially to evaluate the significance of histopathology findings in the urinary bladder), and failure to perform urinalysis to further investigate possible urinary bladder toxicity. The significant instability of metam sodium in water after

24 hours, especially at a concentration of 0.018 mg/mL (29.1-31.5% of initial concentration remained after 24 hours), limits the conclusions that can be drawn from this study because it is not possible to determine the actual amount of test article administered to the animals. Stability data are limited to 24 hours; the high-dose animals received the same test article/drinking water solution over a 2-3 day period at the beginning of the study. It would have been useful for the study report to include stability data on the time-course of decay for the test article in water over a 24-hour period. The reviewers noticed a discrepancy with regard to the storage conditions of the test article that may have affected its stability. According to the certificate of analysis, the test article was to be refrigerated until analysis; however, according to the materials and methods section, the test article was stored in the test facility's dispensary at room temperature in the dark, in a sealed glass bottle. Another difficulty in assessing the amount of test article ingested in drinking water, resulted from the statistically significant dose-related decrease in total water consumption (weeks 1-13) in males and females at doses of 0.35 and 0.62 mg/mL; there was also evidence of decreased total water consumption at 0.088 mg/mL, but the decrease was not statistically significant.

No treatment-related mortality, moribundity, or clinical signs of toxicity were observed in any of the treated animals during the 90-day study period. Treatment-related statistically significant decreases in mean body weight were observed in males and females at doses of 0.35 and 0.62 mg/mL as early as weeks 2-3 and persisted throughout most if not all of the remainder of the 13-week study period. The study author stated that there was a statistically significant reduction in overall body weight in animals given metam sodium for 90 days -- up to 13% and 11% in male and female mice, respectively, at 0.62 mg/mL and up to 9% and 8% in male and female mice, respectively, at 0.35 mg/mL. A maximum tolerated dose (MTD) was approximated based on decreases in body weight gain at the dose level of 0.35 mg/mL in males and at 0.62 mg/mL in females. Mean body weight gain in males from the 0.35-mg/mL dosage group was 77.6% and 74.2% of control at weeks 8 and 13, respectively. Mean body weight gain in females from the 0.62-mg/mL dosage group was 81.8% and 89.7% of control at weeks 8 and 13, respectively. Statistically significant decreases in weekly food consumption occurred only at 0.62 mg/mL for males during weeks 1, 3, and 13, and for females during weeks 1, 3, and 9. Water and food consumption determinations were based on 5 animals/cage. Therefore, no accurate individual data on these parameters were available, and individual body weight changes could not be correlated with individual food consumption changes.

Treatment-related changes in hematology parameters were observed at doses as low as 0.088 mg/mL in females and 0.35 mg/mL in males. At doses of 0.088, 0.35, and 0.62 mg/mL in females, statistically significant dose-related decreases in hemoglobin (HGB), hematocrit (HCT), and red blood cell count (RBC) were observed. At 0.35 and 0.62 mg/mL, dose-related statistically significant decreases were observed in males for RBC and HGB. A decrease was seen in HCT; however, it did

not become statistically significant until a dose of 0.62 mg/mL. Other statistically significant changes in hematology parameters were not considered to be treatment related because they occurred in a sporadic manner that was not dose related. At 0.35 and 0.62 mg/mL, statistically significant increases occurred in mean corpuscular volume (MCV) in males and mean corpuscular hemoglobin (MCH) in females. However, the study author did not consider these increases to be treatment related but considered them to be compensating adjustments for the reduced hemoglobin and red blood cell counts.

The results of microscopic examination of the urinary bladder indicate that the bladder may be a target organ of metam sodium in male and female mice. Eosinophilic granules in the bladder epithelium of males and females were observed at doses of 0.088, 0.35, and 0.62 mg/mL. Cystitis was observed in males and females at doses of 0.35 and 0.62 mg/mL. Mucosal hyperplasia was observed in males and females at doses of 0.35 mg/mL and 0.62 mg/mL. The severity and incidence of eosinophilic granules, cystitis, and mucosal hyperplasia did not increase with increasing doses. The study author was inconsistent in his interpretation of microscopic findings in the urinary bladder by stating that the bladder lesions were apparently treatment-related, but then stating that they were equivocal. It is difficult to determine if the bladder lesions were treatment-related without historical histopathology control data for this strain of mouse.

Increases in liver weight and macroscopic abnormalities of the liver were reported for male and female mice 29 and 90 days after test article administration. For males and females at 0.088, 0.35, and 0.62 mg/mL after 90 days, and males and females at 0.35 and 0.62 mg/mL after 29 days, there were statistically significant increases in relative liver weight as compared to their respective controls. Macroscopic abnormalities consisting of pale color and accentuated lobular pattern in the liver were observed in males and females at 0.35 and 0.62 mg/mL after 29 and 90 days. At a dose of 0.62 mg/mL, there was a statistically significant increase in absolute liver weight for females at 29 and 90 days and for males at 90 days. However, there was no clear dose-response with regard to increased liver weights or increased severity of abnormal macroscopic findings, and there were no significant effects observed upon microscopic examination of the liver to support the toxicological significance of the increase in liver weight. If the study design had included testing of clinical blood chemistries it could have provided useful information in assessing metam sodium's potential for liver toxicity.

In summmary, the systemic toxicity NOEL for metam sodium was 0.018 mg/mL (2.7 mg/kg/day for males; 3.6 mg/kg/day for females). The LEL of 0.088 mg/mL (11.7 mg/kg/day for males; 15.2 mg/kg/day for females) was based on lesions in the bladder of males and females after 90 days and statistically significant decreases in hematology parameters (HGB, RBC, HCT) in females after 90 days. The NOEL is tentative because poor stability of metam sodium in water over a 24-hour period makes it difficult to determine the amount of test article administered in the drinking water.

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
90-Day Oral Toxicity Species: mouse ICI Centrap Tox. Lab. # PM0808; 9/26/91	Metam Sodium 45.13% ai	42117 3-01	Metam Sodium was administered to male and female C57 mice in drinking water at dose levels of 0, 0.018, 0.088, 0.35 and 0.62 mg/ml, equivalent to 2.7, 11.7, 52.4 and 78.7 mg/kg/day for males and 3.6, 15.2, 55.4 and 83.8 mg/kg/day in females, respectively. The systemic ~~toxicity~~ NOEL was 0.018 mg/ml in both sexes. The LEL was 0.088 mg/ml based on urinary bladder lesions (eosinophilic granules, cystitis, mucosal hyperplasia) in both sexes and decreased HGB, RBC and HCT in female mice. Based on body weight gain decrements, the MTD appears to have been achieved at 0.35 mg/ml in males and 0.62 mg/ml in females.		Supplementary

CASWELL FILE

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