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OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

**SUBJECT:** Metam Sodium: Review of an Acute Neurotoxicity Study Submitted by the Registrant.

P.C. Code: 039003  
Submission: S452536  
MRID No: 429778-01 and 429778-02  
DP Barcode: D196503

**FROM:** Timothy F. McMahon, Ph.D., Pharmacologist *T. McMahon* 3/10/94  
Review Section I, Toxicology Branch II  
Health Effects Division (7509C)

**TO:** Tom Myers / PM 51  
Special Review and Reregistration Division (7508W)

**THRU:** Yiannakis M. Ioannou, Ph.D., Section Head *Y. M. Ioannou* 3/10/94  
Review Section I, Toxicology Branch II  
Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief *M. Van Gemert* 3/10/94  
Toxicology Branch II  
Health Effects Division (7509C)

**Registrant:** Metam Sodium Task Force

**Action Requested:** Review of an acute neurotoxicity study submitted in support of reregistration of metam sodium.



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## I. Data Summary:

A study entitled, "An Acute Neurotoxicity Study of Metam Sodium in Rats" was submitted by the Metam Sodium Task Force for review. This study was conducted at WIL Research Laboratories, Ashland, Ohio and was completed in September of 1993.

An acute neurotoxicity study was conducted in male and female Sprague-Dawley rats using nominal dose levels of 50 mg/kg, 750 mg/kg (12 rats/sex/dose), and 1500 mg/kg (16 rats/sex/dose). Based on percent active ingredient (43.15%), **actual doses** were **0, 22, 324, and 647 mg/kg**. Viability, clinical signs, body weights, functional observational battery, and motor activity evaluations were performed.

Mortality was observed at the 1500 mg/kg (647 mg/kg actual dose) dose level, where a total of 5 males and 3 females were found dead during the course of the study. Signs of systemic toxicity were observed at the 750 and 1500 mg/kg dose levels, and included alterations in posture and palpebral closure, increased lacrimation and salivation, alterations in respiratory rate, decreased arousal, decreased rearing activity, increased time to first step, lack of approach, olfactory, and pupil responses, absent or reduced tail pinch response, reduced hindlimb strength, and decreased body temperature and body weight. Reductions in mean ambulatory and total motor activity were observed at the 50 mg/kg dose level and above. Inhibition of plasma and red cell cholinesterase was observed at the 1500 mg/kg dose level in male and female rats 24 hours post-dose. **The LEL of 22 mg/kg is based on reduced ambulatory and total motor activity observed in male and female rats. The NOEL is < 22 mg/kg and was not achieved in this study.**

This study is classified core minimum and satisfies the guideline requirement (§ 81-8) for an acute neurotoxicity study in rats.

## II. Recommendations

Based on the secondary review of this study by Dr. William Sette, Science Analysis Branch, the following recommendations are put forth:

- 1) The registrant will be required to supply cholinesterase measurements in the 90 day neurotoxicity study, based on the inconclusive nature of the results observed on cholinesterase inhibition in the acute study.
- 2) Data on NTE were requested in the DCI for metam sodium but were not supplied with the present study. The registrant is asked to supply an explanation and/or NTE data to satisfy this requirement.
- 3) Based on the significant effects observed on locomotor activity at the lowest dose level, a schedule-controlled operant behavior study is requested.

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Reviewed by: Timothy F. McMahon, Ph.D.  
Section I, Toxicology Branch II (7509C)  
Secondary Reviewer: William F. Sette, Ph.D. *William F. Sette 3/7/94*  
Peer Review Section, Science Analysis Branch (7509C)

### Data Evaluation Record

Study type: Acute Neurotoxicity - rats  
Guideline: § 81-8

EPA ID Numbers: MRID numbers: 429778-01 (range finding); 429778-02 (definitive)  
Submission: S452536  
DP Barcode: D196503  
PC Code: 039003

Test material: Sodium N-methyldithiocarbamate

Synonyms: Metam-sodium

Study number(s): WIL - 188009

Sponsor: Metam Sodium Task Force, Los Angeles, California

Testing Facility: WIL Research Laboratories, Inc., Ashland, Ohio

Title of reports: A Range Finding Acute Study of Metam Sodium in Rats (range -finding)  
An Acute Neurotoxicity Study of Metam Sodium in Rats (definitive)

Study Director: Ian C. Lamb

Studies Completed: September 22, 1993 (range-finding); September 27, 1993  
(definitive)

Executive Summary: A range-finding study was conducted with metam sodium to determine dose levels for the definitive acute neurotoxicity study and to estimate time of peak effect of dose administration within 8 hours of dosing in rats. Groups of 2 male and 2 female Sprague-Dawley rats received single oral doses of 150, 300, 600, 800, 1250, and 1500 mg/kg metam sodium (43.15% a.i.), while single male and female rats received single oral doses of 2000 mg/kg. Based on the results of this study, a definitive acute neurotoxicity study was conducted using dose levels of 50 mg/kg, 750 mg/kg (12 rats/sex/dose), and 1500 mg/kg (16 rats/sex/dose). Based on percent active ingredient (43.15%), **actual doses were 0, 22, 324, and 647 mg/kg.** Viability, clinical signs, body weights, functional observational battery, and motor activity evaluations were performed.

Mortality was observed at the 1500 mg/kg (647 mg/kg actual dose) dose level, where a total of 5 males and 3 females were found dead during the course of the study. Signs of systemic toxicity were observed at the 750 and 1500 mg/kg dose levels, and included alterations in posture and palpebral closure, increased lacrimation and salivation, alterations in respiratory rate, decreased arousal, decreased rearing activity, increased time to first step, lack of approach, olfactory, and pupil responses, absent or reduced tail pinch response, reduced hindlimb strength, and decreased body temperature and body weight. Reductions in mean ambulatory and total motor activity were observed at the 50 mg/kg dose level and above. Inhibition of plasma and red cell cholinesterase was observed at the 1500 mg/kg dose level in male and female rats 24 hours post-dose. **The LEL of 22 mg/kg is based on reduced ambulatory and total motor activity observed in male and female rats. The NOEL is < 22 mg/kg and was not achieved in this study.**

This study is classified core minimum and satisfies the guideline requirement (§ 81-8) for an acute neurotoxicity study in rats.

## I. MATERIALS AND METHODS

A. Test Material: Metam Sodium; purity: 43.15%  
bottle no: 098465; reference no. YO6930/008/009  
description: pale yellow liquid  
storage: Under argon at room temperature in the dark. Verification of homogeneity and 8-hour stability of dosing solutions was provided (pages 855-866).

B. Vehicle: deionized water

C. Test Animals: Species: rat, male and female Sprague-Dawley  
Source: Charles River Breeding Laboratories, Portage, MI  
Age: approximately 43 days old at study initiation.  
Weight range (day 0): males, 182-254g; females, 138-187g

### D. Animal Husbandry:

Conditions of animal husbandry appeared similar for both the range-finding study and definitive study. For the range finding study, a total of 54 rats were received (27/sex), while in the definitive study, 52 rats/sex were received. Rats were individually identified by a metal eartag displaying the animal number and were housed for the first three days three per cage in suspended stainless steel wire mesh cages. After three days, animals were housed individually, presumably in the same type of cage. Conditions of temperature and humidity were stated in the definitive study as 71-74 °F and 42-66%, respectively. In the definitive study, it was stated that animals were acclimated for 18 days prior to study initiation, which included a one week pretest period. In the range-finding study, animals were acclimated for a minimum of 7 days. Purina Certified Rodent Chow # 5002 and municipal water were provided *ad libitum*.

### E. Experimental Design and Dosing:

In the range-finding study, four sub-groups (A, B, C, and D) were employed in the determination of a benchmark dose for metam sodium. For group A, 2 rats/sex/dose received a single oral dose of metam sodium in deionized water at doses of 150, 300, 600, 800, 1250, and 1500 mg/kg, while 1 rat/sex received 1750 and 2000 mg/kg metam sodium. The data from this dose group were used to determine the time of peak effect from administration of metam sodium. For part B, 2 rats/sex were dosed at 50 mg/kg, and 3 rats/sex at 2000 mg/kg. Part C consisted of 2 rats/sex dosed at 1750 mg/kg. Part D consisted of 3 males and 2 females dosed at 100 mg/kg, and 3 rats/sex dosed at 1750 mg/kg. Post-dosing examinations were performed for group A for up to 8 hours, and up to 4 hours for groups B-D.

In the range-finding study, mortality was observed at the 1750 and 2000 mg/kg dose levels. Predominant clinical signs in animals which survived were salivation at 100 mg/kg and above, lacrimation at 150 mg/kg and above, alterations in gait at doses of 600 mg/kg and above, and ptosis at dose levels of 1250 mg/kg and above. Based on the most common clinical findings, the overall time of peak effect was estimated at 45 minutes.

post-dose. The apparent no observable effect level was estimated to be 50 mg/kg, and the benchmark dose was estimated at 1500 mg/kg.

In the definitive neurotoxicity study, animals were assigned by computer-based randomization to four separate dose groups as follows:

<u>Group #</u>	<u>Dose (mg/kg)<sup>a</sup></u>	<u>Nominal Dose (mg/kg)<sup>b</sup></u>	<u>No. of Animals</u>	
			<u>male</u>	<u>female</u>
1	0	0	12	12
2	50	22	12	12
3	750	324	12	12
4	1500	647	16	16

<sup>a</sup>based on 100% active ingredient

<sup>b</sup>based on 43.15% active ingredient.

According to the above table, it would appear that the actual doses received by the rats in this study were 0, 22, 324, and 647 mg/kg for the four dose groups. However, dose solution analysis as presented on pages 855-866 of the report appear to indicate that the concentrations of metam sodium found within the dose solutions are as originally stated in the report (0, 50, 750, and 1500 mg/kg). This would be true assuming 100% active ingredient for the technical material. As the technical material is actually 43.15% active ingredient, the actual doses received by the rats in this study must be considered as those listed above under nominal dose.

For dosing, a 16-gauge stainless steel gavage cannula was used in non-fasted rats as a single dose on study day 0. Individual doses were calculated based on the individual body weight recorded prior to administration.

#### F. Homogeneity and Stability:

As stated, homogeneity and stability data were presented in the text of the report (pages 855-866) for dose solutions at all concentrations used in the definitive study. Data were presented for analyses made prior to dosing as well as on the day of dosing. Results are summarized in the following table (Table 1):

**Table 1**  
Homogeneity and Stability of Metam Sodium Dosing Solutions<sup>a</sup>

	<u>Dose Conc. (mg/ml)</u>		
	<u>10</u>	<u>150</u>	<u>300</u>
<u>prep date 4/7/93<sup>b</sup></u>			
<u>Homogeneity</u>			
top	98.9	86.3	83.7
middle	105	88.7	83.3
bottom	108	103	85.5
overall mean	104	92.6	84.2
<u>8-Hr Stability</u>			
% of target	111	90.7	95.2
 <u>prep date 4/8/93<sup>b</sup></u>			
<u>Homogeneity</u>			
top	-	117	94.2
middle	-	113	82.0
bottom	-	85	92.7
overall mean		105	89.6
 <u>prep date 4/12/93<sup>b</sup></u>			
<u>Homogeneity</u>			
top	-	90	91.2
middle	-	93	89.8
bottom	-	92	97.3
overall mean		91.7	92.8

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<sup>a</sup>data taken from pages 855-866 of the report

<sup>b</sup>numbers represent the mean concentration as a % of target of duplicate determinations.



As illustrated, the first measurement of homogeneity showed that the dose solutions for the 50 and 750 mg/kg dose groups met the criteria for homogeneity, i.e. the top, middle, and bottom means differed from the overall mean by less than 10% and the overall mean was within  $\pm 10\%$  of the dose concentration. The criteria for homogeneity was not met for the 1500 mg/kg dose group.

Eight hour stability, conducted on the dose solutions prepared on 4/7/93, showed that concentrations were within  $\pm 10\%$  of the dose concentration for time zero.

Homogeneity analysis was repeated on 4/8/93 for the 750 and 1500 mg/kg dose groups. These results showed that neither dose solution met the requirements for homogeneity.

On the day of dose administration, homogeneity analysis was again conducted for the dose solutions prepared on this date. These data showed acceptable homogeneity for both the 750 and 1500 mg/kg dose solutions. Thus, according to the report, dose solutions were homogenous and stable for 8 hours at room temperature.

#### G. Statistical Analysis:

A copy of the statistical procedures used in this study is attached to this review.

#### H. Compliance:

A signed statement of compliance with Good Laboratory Practices was provided.

A signed statement of No Data Confidentiality Claims was provided.

A signed Quality Assurance Statement was provided.

A signed statement of EPA Flagging Criteria under 40 CFR §158.34 was provided. The report stated that the study neither meets nor exceeds any of the applicable criteria.

## II. OBSERVATIONS AND RESULTS:

**1) Clinical Observations and Mortality:** All animals were observed twice daily for mortality and/or moribundity, and were also given detailed clinical examinations once a day. On days when the Functional Observational Battery was performed, no additional clinical signs were recorded.

Data summarizing mortality and clinical signs in the animals in this study were presented in Table 1 of the report, beginning on page 45. Mortality occurred at the 1500 mg/kg dose level, where a total of 5 males and 3 females were found dead during the course of the study. Three males and 2 females were found dead on study day one, while the remaining males and females were found dead on study days 2 and 6, respectively. All remaining animals at 1500 mg/kg as well as all animals at the lower doses (including control) survived to study termination (study day 15).

Clinical signs in those animals dying during the study consisted of hypoactivity, alterations in gait, hypothermia, ptosis, and shallow respiration. These signs were also present in surviving animals.

In those animals surviving the study period, several treatment-related signs were observed at the 750 and 1500 mg/kg dose levels. A clear effect could be discerned at the 1500 mg/kg dose level for both sexes, and, according to the report, many of these signs were limited to the day after dosing (study day 1). Signs for the 750 and 1500 mg/kg dose levels, where they were primarily observed, are summarized below (Table 2):

**Table 2**  
**Clinical Signs in Metam Sodium Treated Rats in an Acute Neurotoxicity Study<sup>a</sup>**

	<u>males</u>		<u>females</u>	
	<u>750 mg/kg</u>	<u>1500 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
rocking, lurching, swaying	0/0	5/5	1/1	10/7
hypoactive	0/0	5/5	0/0	8/6
prostrate	0/0	2/2	0/0	3/3
ptosis (both eyes)	0/0	5/5	0/0	4/4
decreased defecation	0/0	7/6	0/0	18/11
decreased urination	0/0	5/5	0/0	19/12
small feces	0/0	4/2	3/3	12/7

<sup>a</sup>data taken from Table 1, pages 45-52 of the report. Numbers represent the total occurrence of each clinical sign over the number of animals observed with the specific sign.

In addition to the above, a predominant clinical sign at 750 and 1500 mg/kg for both sexes consisted of red, yellow, or orange staining on various body surfaces. Red, yellow, or orange material was observed for 10/12 males and 7/12 females in the 750 mg/kg dose group on day 1, and for 10/11 males and 13/13 females in the 1500 mg/kg dose group also on day 1. According to the report, this staining often persisted until day 2 or 3, and was considered treatment related based on the increased incidence compared to control. The incidence of decreased defecation, urination, and small feces was also considered treatment related at the 750 and 1500 mg/kg dose levels.

**2) Body Weight:** Individual body weights were recorded prior to study initiation (day - 10), and on study days 0, 7, and 14. Weights were also recorded during Functional Observational Battery procedures and prior to necropsy. Summary of group mean body weights for male and female rats is summarized below (Table 3):

**Table 3a**  
Group Mean Body Weight in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

<u>Study Day</u>		<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
<u>Males</u>					
0		216± 14.9	211± 10.7	217± 13.1	223± 17.6
7		267± 18.3	259± 15.5	241±** 19.4	231±** 15.7
14		319± 25.8	306± 17.3	299± 22.6	292±* 18.4
<u>Females</u>					
0		155± 9.9	160± 14.1	161± 7.9	165± 11.4
7		174± 11.7	178± 15.1	169± 12.2	166± 14.8
14		191± 14.9	198± 17.3	186± 12.2	189± 13.9

<sup>a</sup>data taken from Table 2, pages 53-54 of the report. N=12, except at 1500 mg/kg, where N=16 until day 7 for males and females. At day 7 and beyond, N at 1500 mg/kg = 11 for males, and =13 for females.

**Table 3b**  
Group Mean Body Weight Gain in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

Study Days

<u>Males</u>	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
0-7	51± 7.4	47± 12.9	24±** 11.6	4±** 11.6
7-14	52± 9.0	48± 8.6	58± 9.1	61±* 7.7
0-14	103± 15.8	95± 16.1	82±** 15.3	65±** 14.4
<u>Females</u>	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
0-7	18± 4.6	18± 2.9	8±** 8.5	2±** 7.1
7-14	17± 5.3	20± 5.9	17± 5.1	23±* 5.0
0-14	36± 6.8	39± 6.3	25±** 9.1	25±** 4.7

<sup>a</sup>data taken from Tables 3 and 3a, pages 55-58 of the report. N=12, except at 1500 mg/kg, where N=16 until day 7 for males and females. At day 7 and beyond, N at 1500 mg/kg = 11 for males, and =13 for females.

Absolute body weight over the course of the study was significantly affected only in male rats, where on day 7 a decrease of 10% in absolute group mean body weight was observed at the 750 mg/kg dose, and a decrease of 13% observed at the 1500 mg/kg dose. On day 14, a decrease of 8% was observed in group mean body weight for males at 1500 mg/kg. These decrease were statistically different from control.

Body weight gain, in contrast to body weight, was significantly affected in both sexes at both the 750 and 1500 mg/kg dose levels. In males, weight gain was decreased by 53% vs control for days 0-7 at 750 mg/kg, and was decreased by 92% vs control at 1500 mg/kg. From days 7-14, an increase of 17% was observed in weight gain for males at 1500 mg/kg. Overall weight gain for days 0-14 was decreased 20% in males at 750 mg/kg, and 37% at 1500 mg/kg.

In female rats, weight gain for days 0-7 was decreased 55% vs control at 750 mg/kg, and by 89% vs control at 1500 mg/kg. For days 7-14 of the study, an increase of 35% was seen in weight gain for females at 1500 mg/kg vs control. Overall weight gain for days 0-14 was decreased 30% for female rats at both the 750 and 1500 mg/kg dose levels vs control.

In the absence of food consumption data, the basis for body weight effects in treated rats is not clear. Such drastic effects at the 1500 mg/kg dose level (especially on weight gain) would suggest a treatment related effect, but the data at 750 mg/kg could also be due to decreases in food consumption. The report appeared to associate administration of metam sodium with these decreases from statements made in the conclusions (page 38). However, the true cause is not known.

### **3) Functional Observational Battery**

For animals in this study, a Functional Observational Battery was performed in a sound-proofed room equipped with a white noise generator set to operate at  $70 \pm 10$ dB, with one exception: home cage observations were performed in the animal room. According to the report, testing was performed by the same technicians without knowledge of the animal group assignment. All animals were observed for the following parameters (page 21 of the report, following):

a. Home Cage Observations

Posture  
Convulsions/tremors  
Feces consistency

Biting  
Palpebral (eyelid) closure

b. Handling Observations

Ease of removal from cage  
Lacrimation/Chromodacryorrhea  
Piloerection  
Palpebral closure  
Red/Crusty deposits  
Eye prominence

Ease of handling animal in hand  
Salivation  
Fur appearance  
Respiratory rate/character  
Mucous membranes/Eye/Skin color  
Muscle tone

c. Open Field Observations (evaluated over a 2 minute observation period)

Mobility  
Rearing  
Convulsions/Tremors  
Grooming  
Bizarre/Stereotypic behavior  
Time to first step (seconds)

Gait  
Arousal  
Urination/Defecation  
Gait score  
Backing

d. Sensory Observations

Approach response  
Startle response  
Pupil response  
Forelimb extension  
Air righting reflex

Touch response  
Tail pinch response  
Eyeblink response  
Hindlimb extension  
Olfactory orientation

e. Neuromuscular Observations

Hindlimb extensor strength  
Hindlimb foot splay

Grip strength-hind and forelimb  
Rotarod performance

f. Physiological Observations

Catalepsy  
Body temperature

Body weight

## a) Home Cage Observations

Measurement of home cage observations pretest showed no significant differences in the number of male or female rats displaying any abnormal behavior (Tables 4 and 5, pages 59-62 of the report). Following administration of metam sodium (day 0 of the study), treatment related signs of toxicity were observed at the 750 and 1500 mg/kg dose levels, and consisted of alterations in posture and palpebral closure. These alterations are summarized in the following table (Table 4):

**Table 4**  
Home Cage Observations (Day 0) in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

<u>Observations (Males)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
<u>posture</u>				
sitting or standing normally	8 (66.7)	8 (66.7)	0 (0)*	2 (18.2)*
sitting, head held low	1 (8.3)	0 (0)	6 (50)	2 (18.2)
flattened, limbs may be extended	0(0)	0(0)	5 (41.7)*	6 (54.5)*
<u>palpebral closure</u>				
eyelids wide open	9 (75.0)	9 (75.0)	1 (8.3)*	0 (0)*
eyelids slightly drooping	0 (0)	0 (0)	4 (33.3)	3 (27.3)
drooping eyelids (half-closed)	0 (0)	0 (0)	5 (41.7)*	5 (45.5)*
<u>Observations (females)</u>				
<u>posture</u>				
sitting or standing normally	10 (83.3)	12 (100)	1 (8.3)*	3 (23.1)*
sitting, head held low	0 (0)	0 (0)	10 (83.3)*	4 (30.8)
flattened, limbs may be extended	0(0)	0(0)	1 (8.3)	5 (38.5)*
<u>palpebral closure</u>				
eyelids wide open	10 (83.3)	12 (100)	1 (8.3)*	0 (0)*
eyelids slightly drooping	0 (0)	0 (0)	3 (25.0)	1 (7.7)
drooping eyelids (half-closed)	0 (0)	0 (0)	6 (50.0)*	6 (46.2)*
eyelids completely shut	2 (16.7)	0 (0)	2 (16.7)	6 (46.2)

<sup>a</sup>data taken from Tables 6 and 7, pages 63-66 of the report. N = 12 except at 1500 mg/kg, where N = 11.

**Note:** Table 6, page 64, and Table 7, page 66, show N= 13 for high dose males and females. This contrasts with N=11 for Table 6 page 63 and Table 7 page 65.

As shown by the above data, both alterations in posture and palpebral closure were observed in male and female rats at the 750 and 1500 mg/kg dose groups. The numbers of males and females with these observations were statistically significant in comparison to controls. It is noted that some animals in controls and at 50 mg/kg had completely shut eyelids; this, however, was attributed to the animals being asleep.

Data for the functional observational battery collected on days 7 and 14 showed no significant differences from control in the incidence of home cage parameters (Tables 8-11, pages 67-74 of the report).

### b) Handling Observations

Summary of treatment-related effects on handling observations is made below (Table 5):

**Table 5a**  
Handling Observations (Day 0) in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>.

<u>Observations (Males)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
<u>lacrimation</u>				
none	12 (100)	12 (100)	3 (25.0)*	1 (9.1)*
slight	0 (0)	0 (0)	6 (50.0)*	6 (54.5)*
severe	0 (0)	0 (0)	3 (25.0)*	4 (36.4)*
<u>salivation</u>				
none	12 (100)	12 (100)	0 (0)*	0 (0)*
slight	0 (0)	0 (0)	3 (25.0)	1 (9.1)
severe	0 (0)	0 (0)	9 (75.0)*	10 (90.9)*
<u>fur appearance</u>				
normal	12 (100)	12 (100)	2 (16.7)*	3 (27.3)*
slightly soiled	0 (0)	0 (0)	10 (83.3)*	6 (54.5)*
very soiled	0 (0)	0 (0)	0 (0)	2 (18.2)
<u>palpebral closure</u>				
eyelids wide open	12 (100)	11 (91.7)	5 (41.7)*	2 (18.2)*
eyelids slightly drooping	0 (0)	0 (0)	7 (58.3)*	6 (54.5)*
drooping eyelids (half-closed)	0 (0)	1 (8.3)	0 (50.0)	3 (27.3)
<u>respiratory rate</u>				
normal	12 (100)	11 (91.7)	11 (91.7)	6 (54.5)*
decreased	0 (0)	1 (8.3)	1 (8.3)	5 (45.5)*



**Table 5b**  
 Handling Observations (Day 0) in Metam Sodium Treated Rats  
 in an Acute Neurotoxicity Study<sup>a</sup>

<u>Observations (Females)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
<u>lacrimation</u>				
none	12 (100)	12 (100)	2 (16.7)*	0 (9.1)*
slight	0 (0)	0 (0)	6 (50.0)*	5 (38.5)*
severe	0 (0)	0 (0)	4 (33.3)	8 (61.5)*
<u>salivation</u>				
none	12 (100)	11 (91.7)	0 (0)*	0 (0)*
slight	0 (0)	1 (8.3)	2 (16.7)	1 (7.7)
severe	0 (0)	0 (0)	10 (83.3)*	12 (92.3)*
<u>fur appearance</u>				
normal	12 (100)	12 (100)	3 (25.0)*	4 (30.8)*
slightly soiled	0 (0)	0 (0)	8 (66.7)*	7 (53.8)*
very soiled	0 (0)	0 (0)	1 (8.3)	2 (15.4)
<u>palpebral closure</u>				
eyelids wide open	12 (100)	12 (100)	4 (33.3)*	1 (7.7)*
eyelids slightly drooping	0 (0)	0 (0)	6 (50.0)*	6 (46.2)*
drooping eyelids (half-closed)	0 (0)	0 (8.3)	1 (8.3)	5 (38.5)*
<u>respiratory rate</u>				
normal	11 (91.7)	12 (100)	11 (91.7)	5 (38.5)*
decreased	0 (0)	0 (0)	1 (8.3)	8 (61.5)*
increased	1 (8.3)	0 (0)	0 (0)	0 (0)

<sup>a</sup>data taken from Tables 14 and 15, pages 88-99 of the report. N = 12, except at 1500 ppm, where N = 13.

As with home cage observations, effects of treatment on handling observations was evident at the 750 and 1500 mg/kg dose levels for both sexes in this study. A statistically significant increase in the incidence and severity of lacrimation and salivation was observed in both sexes, as was an increase in soiled fur. Palpebral closure also appeared to be affected in both sexes at the 750 and 1500 mg/kg dose levels, with the incidence of eyelids slightly drooping and drooping eyelids increased at these dose levels. Respiratory rate was also observed to be decreased in both sexes, primarily at the 1500 mg/kg dose level.

Measurement of handling observations on days 7 and 14 of the study showed no significant treatment related effects in male or female rats, similar to what was observed for home cage observations in terms of duration of effect of treatment.

## c) Open Field Observations

Open Field Observations are summarized from the report below (Table 6). It is noted that pretest measurements showed an increased mean defecation count in high dose female rats, but there were no other differences noted at the pretest interval in either sex.

**Table 6**  
Open Field Observations (Day 0) in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

<u>Observations (Males)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
time to first step (sec.)	0.4±0.15	0.3±0.08	0.5±0.16	0.8±0.56**
<u>gait</u>				
normal	12 (100)	12 (100)	6 (50.0)*	2 (18.2)*
ataxia, sway, lurching	0 (0)	0 (0)	4 (33.3)	4 (36.4)*
<u>arousal</u>				
low (stuporous)	0 (0)	0 (0)	4 (33.3)	8 (72.7)*
normal	12 (100)	12 (100)	8 (66.7)	3 (27.3)*
rearing activity	5.6±2.19	5.4±3.03	2.9±1.83*	2.0±1.48**
<u>Observations (Females)</u>				
time to first step (sec.)	0.3±0.05	0.3±0.05	0.7±0.42*	0.8±0.52**
<u>gait</u>				
normal	12 (100)	12 (100)	4 (33.3)*	4 (30.8)*
ataxia, sway, lurching	0 (0)	0 (0)	3 (25.0)	3 (23.1)*
walks on tiptoes	0 (0)	0 (0)	5 (41.7)*	6 (46.2)*
<u>arousal</u>				
low (stuporous)	0 (0)	0 (0)	6 (50.0)*	8 (61.5)*
normal	12 (100)	12 (100)	6 (50.0)*	5 (38.5)*
rearing activity	7.4±3.12	8.1±3.50	3.7±2.81*	2.8±2.44**

a- data taken from Tables 22 and 23, pages 134-142 of the report. N =12 except at 1500 mg/kg, where N = 11 for males and N = 13 for females.

As with other measurements, observations on open field activity were affected primarily on day 0 of treatment and at the 750 and 1500 mg/kg dose levels in male and female rats. Treatment related effects included an increase in mean time to first step, alterations in gait and arousal and a decrease in rearing activity. These effects were observed in both sexes and reached statistical significance at the 1500 mg/kg dose level. In many cases, significance was also reached at the 750 mg/kg dose level. Observations at 7 and 14 days showed no significant differences from control.

#### d) Sensory Observations

**Table 7**  
Sensory Observations (Day 0) in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

<u>Observations (Males)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
<u>approach response</u>				
no reaction	0 (0)	0 (0)	3 (25.0)*	4 (36.4)*
slow approach	12 (100)	12 (100)	9 (75.0)	7 (63.6)*
<u>olfactory orientation</u>				
no reaction	0 (0)	0 (0)	3 (25.0)	5 (45.5)*
reaction present	12 (100)	12 (100)	9 (75.0)	6 (54.5)*
<u>pupil response</u>				
no response	1 (8.3)	0 (0)	5 (41.7)	2 (18.2)
response present	11 (91.7)	12 (100)	7 (58.3)	9 (81.8)
<u>tail pinch</u>				
no reaction	0 (0)	0 (0)	1 (8.3)	3 (27.3)
slow reaction	10 (83.3)	5 (41.7)	7 (58.3)	7 (63.6)
<u>Observations (Females)</u>				
<u>approach response</u>				
no reaction	0 (0)	0 (0)	2 (16.7)	3 (23.1)
slow approach	12 (100)	10 (83.3)	10 (83.3)	10 (76.9)
<u>olfactory orientation</u>				
no reaction	0 (0)	0 (0)	3 (25.0)	5 (38.5)*
reaction present	12 (100)	12 (100)	9 (75.0)	8 (61.5)*

**Table 7, cont.**

<u>pupil response</u>				
no response	0 (0)	0 (0)	3 (25.0)	4 (30.8)
response present	12 (100)	12 (100)	9 (75.0)	8 (69.2)
<u>tail pinch</u>				
slow reaction	3 (25.0)	4 (33.3)	9 (75.0)*	9 (69.2)*

<sup>a</sup>data taken from Tables 30-31, pages 168-175 of the report. \* p < 0.05 vs control. N = 12 except at 1500 mg/kg, where N = 11 for males and N = 13 for females.

Treatment related signs of toxicity in sensory observations were observed at the 750 and 1500 mg/kg dose levels in both sexes, and included an increase in the absence of an approach response, an increase in the absence of an olfactory response, an increase in the absence of a pupil response, and an increase in the incidence of no reaction or slow reaction to tail pinch.

The report stated that the percentages of males and females showing the absence of an approach response, the absence of a pupil reaction, and the absence of or slow reaction to tail pinch was outside the historical control range for these observations at the performing laboratory. In addition, the report stated that the percentages of males and females showing only a slight startle response at the 750 and 1500 mg/kg dose levels was outside the historical control range of the performing laboratory.

There were no apparent differences from control considered as effects of treatment in male and female rats for sensory observations on days 7 and 14 of treatment at the 750 and 1500 mg/kg dose levels. At the 50 mg/kg dose level, there were no apparent differences from control at any time point measured following treatment.

#### e) Neuromuscular Observations

**Table 8**  
Neuromuscular Observations (Day 0) in Metam Sodium Treated Rats  
in an Acute Neurotoxicity Study<sup>a</sup>

<u>Observations (Males)</u>	<u>Number (%) showing each observation</u>			
	<u>0 mg/kg</u>	<u>50 mg/kg</u>	<u>750 mg/kg</u>	<u>1500 mg/kg</u>
reduced hindlimb resistance; animal shows some weakness	0 (0)	0 (0)	2 (16.7)	2 (18.2)
<u>Observations (Females)</u>				
reduced hindlimb resistance; animal shows some weakness	0 (0)	0 (0)	1 (8.3)	4 (30.8)

<sup>a</sup>data taken from Tables 38-39, pages 198-201 of the report. N = 12 except at 1500 mg/kg, where N = 11 for males and N = 13 for females.

The only observation noted in the category of neuromuscular observations was a reduced hindlimb resistance for male and female rats at the 750 and 1500 mg/kg dose levels on day 0 of treatment. There were no apparent effects of treatment on fore and hindlimb grip strength, rotarod performance, and hindlimb footsplay.

#### f) Physiological Observations

According to the data presented (Tables 45 and 46, pages 213-214 of the report), reduced body temperature was recorded for male and female rats at the 750 and 1500 mg/kg dose levels on day 0 of treatment. In males, body temperature was reduced to 37.5 and 36.9 degrees Celsius at the 750 and 1500 mg/kg dose levels, respectively, from a value of 38.9 in controls ( $p < 0.01$ ). In females, body temperature at the 750 and 1500 mg/kg dose levels was recorded as 37.1 and 36.7 degrees Celsius, significantly different from the value of 39.1 in controls ( $p < 0.01$ ). Reductions in body temperature apparently persisted in male rats at the 1500 mg/kg dose level to day 7 of the study, and in female rats at the 750 and 1500 mg/kg dose levels.

On study days 7 and 14, body weight was reported to be decreased in male and female rats at the 750 and 1500 mg/kg dose levels in comparison to controls. The effects on body weight in male and female rats has been summarized above in Table 3a of this review.

#### 4) Locomotor Activity

Observations for locomotor activity were made on all animals during the pretest period, at the approximate time of peak effect (~ 45 min post-dosing), and on study days 7 and 14. Locomotor activity was measured automatically using the digiscan 'micro' animal activity system. Data were collected in one minute intervals and test sessions were 41 minutes in duration for each animal. The first minute of activity was deleted due to placement of the animal in the activity cage during this interval, which resulted in incomplete data for the first minute.

Activity was divided into 2 categories: total activity and ambulatory activity. Total activity was defined as a combination of fine motor skills (interruption of one or two adjacent photobeams), while ambulatory activity was defined as interruption of three or more consecutive photobeams.

Overall mean ambulatory and total motor activity was not significantly different from control in male and female rats at the pretest interval. On study day 0, however, total motor activity and ambulatory activity were significantly reduced in male rats at all dose levels compared to control. In female rats, total motor activity was reduced at all dose levels on day 0, while total ambulatory activity was reduced at 750 and 1500 mg/kg metam sodium on day 0. These changes are summarized below (Table 9):

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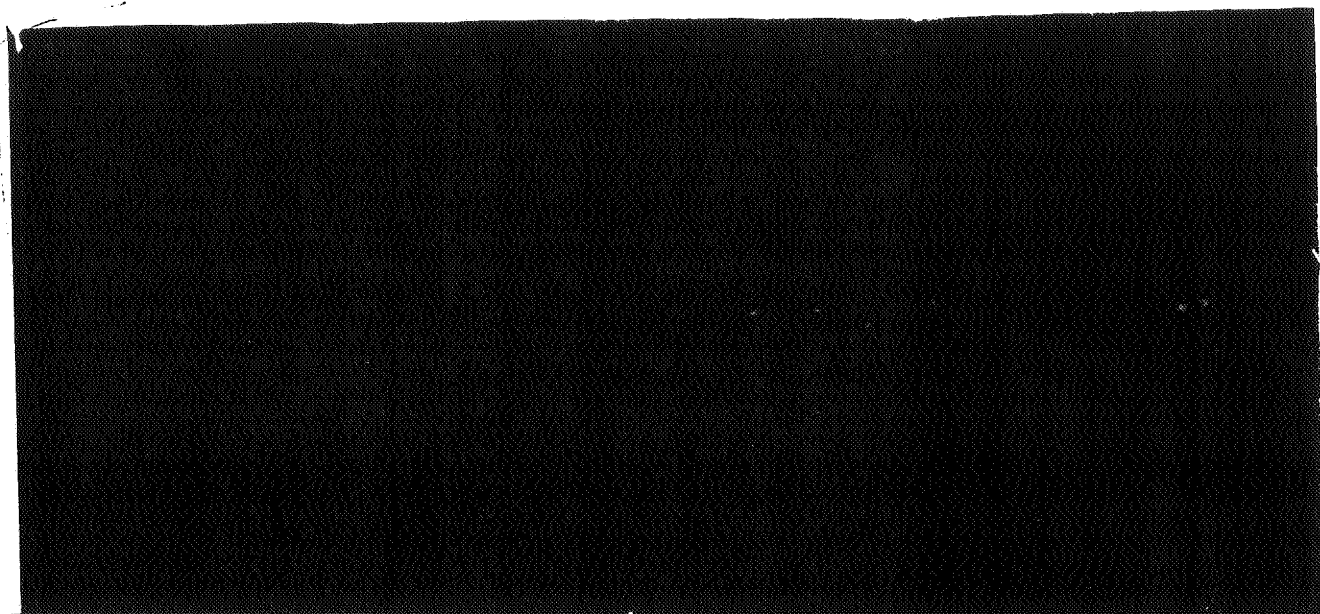
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The most noticeable reductions in both total motor activity and mean ambulatory activity occurred for male and female rats during the first subsession, where significant reductions were observed in both types of activity at all dose levels. In the second through fourth subsessions, significant reductions were observed only at the 750 and/or 1500 mg/kg dose levels. However, overall mean counts were reduced for the 4 subsessions combined, as shown above.

For days 7 and 14 of measurement, total motor and ambulatory activity were affected only in high dose females, where a decrease in total counts (from 926 to 655,  $p < 0.05$ ) was observed on day 7, and a decrease from 870 to 694 (n.s.) observed on day 14.

## 5) Pathology

### a) Unscheduled Deaths

As previously mentioned, a total of 5 males and 3 females of the 1500 mg/kg dose group died on study. Gross observations for these animals were made in Table 52, pages 244-245 of the report. In the males which died, reddened adrenal glands, reddened lymph nodes, reddened stomach mucosa, hemorrhagic thymus, and tan and/or brown matting were recorded for a single animal, while 2 males were found with pale lungs, distended stomach, external scabbing, and yellow matting. Three males were reported with red matting externally. Two males were reported with clear fluid contents of the thoracic cavity, while reddened cervical lymph node was reported in a single male rat.

In females, 2 of the three which died were reported with reddened adrenal glands and yellow matting. Dark red intestinal contents, reddened stomach mucosa, clear fluid contents of the abdominal cavity, and red matting were reported in a single female rat.

## b) Brain Weight and Dimensions

Brain weights and dimensions were presented in Table 53, pages 246-247 of the report for male and female rats. According to these data, there were no significant differences in brain weight and dimensions for treated vs control rats at study termination.

## c) Microscopic Examination

Summary of microscopic examination of the central and peripheral nervous system of rats was presented in Table 54, pages 248-255 of the report. A limited number of observations were noted in male and female rats at the control and 1500 mg/kg dose groups, and included the following: digestion chambers in the sciatic nerve and tibial nerve of control male rats (one of five examined); inclusion cyst in the lumbar spinal cord of one female rat at the 1500 mg/kg dose level; digestion chambers in the tibial nerve (one control and one 1500 mg/kg female and lumbar ventral root fibers (one control female).

The report noted that spontaneous nerve fiber degeneration, characterized by digestion chambers, had been characterized in both control and treated rats and should not be considered uncommon. One female rat at the 1500 mg/kg dose level was reported with a cyst in the lumbar region of the spinal cord. This lesion was considered to be congenital in origin.

## 6) Cholinesterase Measurement

A separate study, WIL-188010, was conducted in order to determine the effect of metam sodium on red cell, plasma, and brain cholinesterase. For this study, metam sodium was administered as a single dose of 1500 mg/kg in a dose volume of 5 ml/kg to a group of 10 male and 10 female Sprague-Dawley rats. A concurrent control received vehicle only at the same dose volume. On day 0, at the time of peak effect of metam sodium (45 minutes post-dose), blood was collected at the time of euthanization from 5 rats/sex/group. Blood was collected from the remaining 5 rats/sex/group at 24 hours post-dose. Cholinesterase activity was determined in the red cell, plasma, and the olfactory region, cerebral cortex, cerebellum, brain stem, hippocampus, and mid brain areas of the brain

Results, as summarized in the report (Tables 3 and 3a, pages 988-993; see following page) showed no significant inhibition of plasma or red cell cholinesterase on day 0 of treatment (45 minutes post-dose) with metam sodium following a 1500 mg/kg dose. Brain cholinesterase was also unaffected on day 0 as well as on day 1 (24 hours post-dose). At 24 hours post dose, plasma and red cell cholinesterase were decreased in treated male rats by 6% and 12%, respectively. In female rats, a similar effect was observed, with inhibition of 24% for plasma cholinesterase and 14% for red cell cholinesterase. These decreases were observed in relation to the concurrent (24 hour post-dose) control.



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## Discussion/Conclusions

The present study was conducted to determine the acute neurotoxic effects of metam sodium at dose levels of 0, 50, 750, and 1500 mg/kg. These dose levels were selected on the basis of a range-finding study with metam sodium, as outlined in this review.

Major clinical and systemic effects were observed at the 750 and 1500 mg/kg dose levels of metam sodium in male and female rats, and included the following:

- 1) Mortality in male and female rats at the 1500 mg/kg dose level;
- 2) alterations in posture and palpebral closure during home cage observations;
- 3) increases in lacrimation, salivation, altered palpebral closure, changes in fur appearance, and decreased respiratory rate during handling observations;
- 4) increased time to first step, gait alterations, decreased arousal, and decreased rearing activity during open field observations;
- 5) absent approach, olfactory, and pupil responses, and absent or reduced tail pinch response during sensory observations. A reduced startle response at 750 and 1500 mg/kg was also considered a possible effect of treatment.
- 6) reduced hindlimb extensor response strength during neuromuscular observations;
- 7) decreased body temperature on days 0 and 7 as well as decreased body weight at the 750 and 1500 mg/kg dose levels during physiological observations;
- 8) reductions in mean ambulatory and total motor activity observed at the 50, 750, and 1500 mg/kg dose levels for males and females on day 0. In females, decreases in mean ambulatory and total motor activity counts persisted to study days 7 and 14 at the 1500 mg/kg dose level, but some recovery was apparent during this time.
- 9) No treatment related effects on brain weight, dimensions, or nervous system pathology were observed in males or females.
- 10) Twenty four hours post-dose, inhibition of plasma and red cell cholinesterase was observed in both male and female rats at 1500 mg/kg metam sodium. Decreases of 6% and 12% were observed for plasma and red cell cholinesterase in male rats, and decreases of 24% and 14% for plasma and red cell cholinesterase for female rats. No inhibition of brain cholinesterase was observed.

Systemic LEL = 22 mg/kg (males and females; reduced ambulatory and total motor activity)

Systemic NOEL < 22 mg/kg; not achieved

**Classification:** core minimum

This study satisfies the data requirement (§81-8) for an acute neurotoxicity study in rats.