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

JAN 29 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Memorandum

Subject: Metam-Sodium, Sodium-N-methyl-dithiocarbamate, EPA Identification No. 039003. TOX CHEM NO. 780

From: John H.S. Chen, D.V.M. John H.S. Chen 1/21/88
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

To: Geralding Werdig, PM 50
Data Call-In Program
Registration Division (TS-767C)

Thru: Robert B. Jaeger, Section Head
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C) RBJ 1/21/88 1/29/88

Registrant: Stauffer Chemical Co. Letter of August 17, 1987

Action Requested: Review of the five mutagenicity studies with Metam-Sodium (Acc No. 403056-01; 403056-02; 403056-03; 403056-04; 403056-05) to determine if the chronic studies are needed.

Recommendation:

1. The Registrant should be apprised of the deficiencies noted in the following studies which are identified in the detailed review:

   A. Cytogenetic Study In Vivo of Metam-Sodium in Chinese Hamsters Bone Marrow Chromsome Analysis, Single Oral Administration. BASF Aktiengeselleschaft Dept. of Toxicology, FRG Report No. BASF 87/0238, June 30, 1987. Unacceptable.

   B. Mutagenicity Test on Metam-Sodium in the Rec-Assay with Bacillus subtilis. Hazleton Biotechnologies Veenendal Laboratory, The Netherlands. Report No. HBC E-9642-0-404 (BASF 87/0163), March 27, 1987. Unacceptable.

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2. The following mutagenicity studies have been reviewed and are acceptable as described:

A. Gene Mutation Category

Report on the Study of Metam-Sodium in the Ames Test. BASF Aktiengeselleschaft Dept. of Toxicology. FRG Report No. BASF 87/0208, June 5, 1987. Negative response at 20-2500 ug/plate either with or without metabolic activation. Acceptable.

B. Structural Chromosomal Aberration Category

In Vitro Cytogenetic Investigation in Human Lymphocytes with Metam-Sodium. BASF Aktiengeselleschaft Dept. of Toxicology, FRG Report No. BASF 87/0116, March 9, 1987. Positive response either at 20 ug/ml without metabolic activation or at 20-40 ug/ml with metabolic activation. Acceptable.

C. Other Genotoxic Effect Category

Mutagenicity Test on Metam-Sodium in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Hazleton Laboratories America, Inc. Report No. HLA 9736-0-447 (BASF 87/0240), July 1, 1987. Negative response at 0.5-250 nl/ml. Acceptable.

3. Although the chromosome-damaging effect of Metam-Sodium was found in one of the three acceptable mutagenicity studies, this clastogenic effect of Metam-Sodium in vitro using human lymphocytes must be further evaluated in vivo Chinese hamster bone marrow cytogenetics. Therefore, Toxicology Branch recommends that the deficient mutagenicity studies cited in our conclusion #1 must be resolved before a decision can be made about whether chronic studies are needed for Metam-Sodium.

84-2 UDS in Rat Primary Hepatocytes

Reviewed by: John H.S. Chen John H Chen 1/19/88
Section I, Toxicology Branch (TS-769C)
Tertiary Reviewer: I. Mauer Irving Mauer 1/22/88
Section VI, Toxicology Branch (TS-769C)
Reviewed by Section Head: R.B. Jaeger RBJ 1/21/88
Section I, Toxicology Branch (TS-769C)

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DATA EVALUATION REPORT

Study Type: UDS in Rat Primary Hepatocytes **TOX. CHEM. NO.:** 780

Accession No.: 403056-01 **MRID NO.:**

Test Material: Metam Sodium (Batch No. ZH 130595; 42.2% Purity)

Synonyms:

Study Number (s): BASF 87/0240 (HLA 9736-0-447)

Sponsor: BASF Corporation Chemical Division

Testing Facility: Hazleton Laboratories America, Inc.

Title of Report: Mutagenicity Test on Metam Sodium in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

Author(s): M.A. Cifone, Ph.D.

Report Issued: July 1, 1987

Conclusions:

Metam Sodium was inactive in the unscheduled DNA synthesis in primary rat hepatocyte at the dose levels tested.

Dose levels tested: 0.5, 1, 2.5, 5, 10, 50, 100, and 250 nl/ml

Classification of Data: Acceptable

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Title of Report: Mutagenicity Test on Metam Sodium in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay
BASF 87/0240, July 1, 1987

I. Materials and Methods:

1. Test Materials

The stability of the test compound, Metam Sodium (Batch ZH130585; 42.2% Purity), throughout the study period was proven analytically. Solutions were made in Williams' Medium E (WME). 2-Acetyl aminofluorene (2-AAF) at 0.1 ug/ml was used as the positive control.

2. Medium

Williams' Medium E supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 2.4 uM dexamethasone, 90 U/ml penicillin, 90 ug/ml streptomycin sulfate and 140 ug/ml gentamicin. The culture medium which was referred to as WME contained no dexamethasone and serum components.

3. Indicator Cells

The hepatocyte obtained from an adult male Fischer 344 rat (150-300 g) was used in this assay. The cells were obtained by perfusion of the liver in situ with a collagenase solution (50-100 Unit/ml). Monolayer cultures were established on plastic coverslips in culture dishes for initiation of the UDS assay. All cultures were maintained as monolayers at about 37° C in a humidified atmosphere containing 5% CO_2.

4. Dose Selection

Attached primary cells were exposed to a wide range of concentrations of Metam Sodium (0.025 through 1000 nanoliters/ml) for 21 hours. After exposure, cells were refed with WME, trypsinized and counted (trypan blue exclusion method). The relative survivals were determined by comparing the dosed groups to the control.

5. UDS Assay

This assay was based on the procedures described by Williams (Cancer Res. 37, 1845-1851, 1977). The freshly isolated liver cells attached on coverslips (0.5 X 10^6 viable cells) were used. Following the addition of metam sodium (0.5, 1, 2.5, 5, 10, 50, 100, and 250 nl/ml) and ^{3}H-thymidine (20 uCi/mmole) in the culture dishes with 2.5 ml WME containing 1% fetal bovine serum, the culture dishes were incubated for 18-20 hours at 37° C. After incubation, the treated cultures were washed with WME, swelled with 1% sodium citrate and fixed with acetic acid: ethanol (1:3). The cover slips were air dried, mounted on glass slide (cells up), dipped in an emulsion of Kodak NTB2 and dried. The coated slides were stored for 7-10 days at 4° C in light tight box containing packets of drierite. The emulsions were then developed in D19 (Eastman Kodak), fixed and stained with Williams' hematoxylin and eosin procedure. The cells were examined microscopically and the field was displayed on the video screen of an electronic counter.

6. Measurement of UDS

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UDS was measured by counting nuclear grains and subtracting the average number of grains in three nuclear-sized areas adjacent to each nucleus (background count). This net nuclear grain count was determined for 50 randomly selected cells on each coverslip. The mean net nuclear grain count was determined from the triplicate coverslips (150 total nuclei) for each treatment condition.

7. Evaluation Criteria

A test material was considered positive if it induced a significant increase (over the control) in net nuclear grain count exceeding 7.71, or at least 16% of the nuclei containing 6 or more grains, or at least 2% of the nuclei containing 20 or more grains.

II. Reported Results:

1. Preliminary Cytotoxicity Assay (Table 1 attached)

The cells were exposed to 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 50, 100, 250, 500, and 1000 nl/ml of Metam Sodium for 21 hours, resulting in a percent relative survival (% RS) range of 17% at 100 nl/ml to 55.3% at 50 nl/ml. Based on these data, 250 nl/ml was selected as the highest dose for the UDS assay.

2. UDS Assay (Table 1 attached)

The test material was assayed at 0.5, 1, 2.5, 5, 10, 50, 100, and 250 nl/ml. The results of the UDS assay indicated that the test material did not cause any significant changes in the nuclear labeling of primary rat hepatocytes at the concentrations tested (0.5 through 250 nl/ml).

III. Evaluation and Recommendation:

1. The positive control, 2-AAF at 0.1 ug/ml induced marked increase in net nuclear grain count exceeding 7.86 (also 69.3% of the nuclei containing 6 or more grains; 9.3% of the nuclei containing 20 or more grains). These results indicate that the cell population employed was responsive and the methodology was adequate for the detection of UDS in rat primary hepatocytes.

2. The test material has been tested to cytotoxicity level (i.e., 250 nl/ml).

3. The nuclear labeling in the negative (solvent) control was found within the normal range of net nuclear grain count per nucleus (also 6% of the nuclei containing 6 or more grains; none of the nuclei containing 20 or more grains) established by the testing laboratory.

4. The average net nuclear count per nucleus for the treated hepatocyte cultures with Metam Sodium was in a range of 0.44 to 2.69 at the concentrations tested (0.5 through 250 nl/ml).

5. Under the test conditions reported, the test material failed to induce any significant changes in the nuclear labeling of rat hepatocytes at the dose levels tested. Therefore, the test material was inactive in the unscheduled DNA synthesis in primary rat hepatocyte. The study is acceptable.

TABLE 1

SUMMARY OF DATA FROM RAT HEPATOCYTE UDS ASSAY

CLIENT: Metam-Sodium Task Force HLA ASSAY NO.: 9736 ASSAY INITIATION DATE: March 10, 1987

CLIENT'S CODE: Metam-Sodium Batch ZH 130 5b5 ZNT No. 65/232 SOLVENT: WME Medium TECHNICIAN: Andrea Ham

Test Condition	Concentration	UDS* grains/ nucleus	Avg.† % nuclei with ≥ 6 grains	Avg.† % nuclei with ≥ 20 grains	Survival†† at 21 hrs, %
Negative Control	--	1.71	6.0	0.0	100.0
Positive Control (2-AAF)	0.1 µg/ml	9.57	69.3	9.3	98.5
Test Material:					
Metam-Sodium	250 nl/ml	2.38	12.0	0.0	***
Metam-Sodium	100 nl/ml	0.49	0.0	0.0	17.3
Metam-Sodium	50 nl/ml	0.44	0.0	0.0	55.3
Metam-Sodium	10 nl/ml	2.05	9.3	0.0	83.7
Metam-Sodium	5.0 nl/ml	1.28	4.7	0.0	93.4
Metam-Sodium	2.5 nl/ml	1.02	1.3	0.0	96.0
Metam-Sodium	1.0 nl/ml	2.69	10.7	0.0	102.6
Metam-Sodium	0.5 nl/ml	0.75	0.7	0.0	96.4

* UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
Average values for triplicate coverslips.
† Survival = Number of viable cells per unit area relative to the negative control x 100%.
2-AAF = 2-acetyl aminofluorene.
ND = Not determined
Mean cytoplasmic grain count for solvent controls = 3.12.
***Cells took up the vital dye trypan blue at the time of cytotoxicity determination, but the autoradiography showed that the cells had a normal grain distribution indicating that the cell died late in the treatment period.

9736-0-447

84-2 Rec-Assay with Bacillus subtilis
Reviewed by: John H.S. Chen
Section I, Toxicology Branch (TS-769C)
Tertiary Reviewer: I. Mauer
Section VI, Toxicology Branch (TS-769C)
Reviewed by Section Head: R.B. Jaeger
Section I, Toxicology Branch (TS-769C)

John H.S. Chen 1/19/88

1/20/88

RBJ 1/22/88

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DATA EVALUATION REPORT

Study Type: Rec-Assay with Bacillus subtilis

TOX. CHEM. NO.: 780

Accession No.: 403056-02

MRID NO.:

Test Material: Metam Sodium (Batch No. ZH 130585; 42.2% Purity)

Synonyms:

Study Number (s): BASF 87/0163 (HBC E-9642-0-404)

Sponsor: BASF Corporation Chemical Division

Testing Facility: Hazleton Biotechnologies Veenendal Laboratory, The Netherlands

Title of Report: Mutagenicity Test on Metam Sodium in the Rec-Assay with Bacillus subtilis

Author(s): Dr. A.J.W. Hoorn

Report Issued: March 27, 1987

Conclusions:

Metam Sodium was not recombinogenic (i.e., damage of DNA) to Bacillus subtilis organisms (H17 and M45) at the concentrations tested.

Concentrations tested: 0.1, 1, 5, 10, 25, 50, 100, and 150 ul/Well

Classification of Data: Unacceptable

Deficiencies found: lack of negative control; unclear rationale for dose selection; inconsistent responses for positive controls.

Title of Study: Report on the Mutagenicity Test on Metam Sodium in the Rec-Assay with Bacillus subtilis
HBC Study No. E-9642-0-404, March 27, 1987

I. Materials and Methods:

1. Test Materials

The test compound, Metam Sodium (Batch No. ZH130585; 42.2% Purity) was stored in the dark at 4°C until required. Solutions were made in sterile deionized water. Solutions of methylmethane sulfonate in sterile deionized water and sterigmatocystin in DMSO were prepared prior to use and served as positive controls.

2. Indicator Microorganisms

The Bacillus subtilis strains H17 (Rec$^+$) and M45 (rec$^-$) used in this study were obtained from Dr. T. Kada, National Institute of Genetics, Mishima, Japan. The damage to DNA is repaired through cellular recombination functions in wild type (H17) cells but not in recombinationless mutant (M45) cells. The indicator strains were kept at 4°C on nutrient broth agar plate. For each experiment, an inoculum from the stock cultures plates was grown overnight at 37°C in trypticase soy broth, appropriately diluted and used.

3. Media

The plates for the assay consisted of nutrient broth plates with 1.5% bactoagar (NB plates). The overlay agar consisted of 0.6 gms of purified agar with 0.1M NaCl.

4. In-Vitro Metabolic Activation System

The mammalian metabolic activation system consisted of rat liver homogenate (S-9) from the Aroclor 1254-treated male Sprague-Dawley rats and the cofactor solution described by Ames et al.,(Mutation Res. 31: 347-364, 1975). The S-9 mix (per ml) contained the following components: S-9, 100 ul; 4 uM NADP; 5 uM D-glucose-6-phosphate; 8 uM $MgCl_2$; 33 uM KCl; 100 uM Sodium phosphate buffer (pH 7.4).

5. Rec-Assay

The procedure used was based on the method decribed by Slater et al., (Cancer Res. 31: 970-973, 1971). The assay was briefly described as follows: Both overnight cultures of these two strains of B. subtilis were mixed with overlay agar and then poured onto the NB agar plates. After the top agar has set, wells of uniform diameter were made in the center of each agar plate. These wells were sealed at the bottom with the overlay agar. For the nonactivation assays, 0.1 ml a 0.2M phosphate buffer, pH 7.4, was added to each well. Then, volumes of the appropriately

5. Rec-Assay - continued

diluted test compound and control compounds were added to the wells of the appropriate plates. For the activation assays, 0.5 ml of the S-9 mix was added to the overlay agar. Volumes of the appropriately diluted test compound and control compounds were added to the wells of the appropriate plates. The plates were incubated at 37°C for 24-48 hours. The zones of inhibition were measured and recorded in mm's on standard printed forms.

6. Evaluation Criteria

In this well diffusion assay, a positive result must demonstrate a significant preferential inhibition or killing of the repair deficient (M45) strain of B. subtilis (greater than a differential of 4 mm of zone inhibition). If a test compound produces a positive response in a single test that cannot be reproduced in additional runs, the initial positive test data lose significance.

II. Reported Results:

1. Dose Selection

Because the sponsor did not specify dose levels, the initial tests were performed with standard dose levels ranging from 0.1 ul to 150 ul per plate (i.e., 0.1, 1, 5, 10, 25, 50, 100 and 150 ul/plate).

2. Recombinogenic Activity (Tables 1, 2, 3, 4, 5 and 6 attached)

In the first trial (Tables 1 and 2), the test compound did not induce significant dose - related differential toxicity (less than 4 mm of zone inhibition) between the strains H17 and M45 of B. subtilis either in the presence or absence of metabolic activation. The repeat tests (Tables 3 and 4) failed again to induce any dose-related differential toxicity greater than 4 mm of zone inhibition at the same dose levels ranging from 0.1 to 150 ul per plate under the activation or nonactivation assay system. The results of the second repeat test (Table 5) showed dose-related increases (1 to 3 mm of zone inhibition) between 0.1 and 2.5 ul per plate. At higher concentrations increases were observed that were considered spurious because of a lacking dose-effect relationship (Table 5). With metabolic activation the results were negative (Table 6). Therefore, metam sodium was not recombinogenic to Bacillus subtilis indicator organisms under these conditions tested.

III. Evaluation and Recommendation:

The evaluation of recombinogenic activity (i.e., damage of DNA) in the Rec- Assay with Bacillus subtilis cannot be accomplished due to the following reporting deficiencies:

1. The described procedure for the preparation of test cultures of Bacillus subtilis (H17 and M45) appears incomplete. According to the acceptable procedure for performing the bacterial DNA damage or repair test (EPA Health Effect Test Guidelines 560/6-83-001), positive, negative, and vehicle controls should be concurrently run in each assay. The negative control which must show non-preferential growth inhibition was missing in each of these studies. Chloramphenicol is an example of the negative control used.

2. Rationale for determining the upper limit of the test material concentration are unclear and must be clarified. Among the criteria to be taken into consideration for determining the test material concentration are cytotoxicity and solubility.

3. The positive controls (MMS and Sterigmatocystin) did not demonstrate consistently positive responses in this study (See Tables 1, 2, 4, and 5). Therefore, it is questionable whether the test cultures were properly prepared for each of these studies. It should be borne in mind that in evaluating the differential growth inhibition of repair proficient (H17) and repair deficient strains of B. subtilis, both of these bacterial cell suspensions must be standardized to equally desired density of viable cells prior to testing (References: Slater et al., Cancer Res. 31: 970, 1971; Rosenkranz et al., Chemical Mutagens Vol. 6, 109-147, 1980., Plenum Press).

Therefore, the study is unacceptable in the present form. However, it may be upgraded on resolution of the reporting deficiencies.

Metam-sodium toxicology review

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84-2 Chinese Hamsters Bone Marrow Cytogenetic Assay
Reviewed by: John H.S. Chen
Section I, Toxicology Branch (TS-769C) John H.S. Chen 4/9/88
Tertiary Reviewer: I. Mauer
Section VI, Toxicology Branch (TS-769C) 11/20/88
Reviewed by Section Head: R.B. Jaegor
Section I, Toxicology Branch (TS-769C) RBJ 1/24/88

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DATA EVALUATION REPORT

Study Type: Chromosomal Aberrations (In-Vivo) **TOX. CHEM. NO.:** 780

Accession No.: 403056-05 **MRID NO.:**

Test Material: Metam Sodium (Batch No. ZH130585; 42.2% Purity)

Synonyms:

Study Number (s): BASF 87/0238

Sponsor: BASF Corporation Chemical Division

Testing Facility: BASF Aktiengesellschaft Dept. of Toxicology, FRG

Title of Report: Cytogenetic Study in Vivo of Metam Sodium in Chinese Hamsters, Bone Marrow Chromosome Analysis

Author(s): Dr. H.P. Gelbke and Dr. G. Engelhardt

Report Issued: June 30, 1987

Conclusions:

Metam Sodium was not clastogenic in the Chinese hamsters bone marrow cytogenetic assay at the concentrations tested.

Concentrations tested: 150, 300, and 600 mg/kg

Classification of Data: Unacceptable

(Deficiency: The upper limit of Metam Sodium used in this study was inadequate)

Title of Report: Cytogenetic Study in Vivo of Metam Sodium in Chinese Hamsters Bone Marrow Chromosome Analysis, Single Oral Administration
BASF 87/0238, June 30, 1987

I. Materials and Methods:

1. Test Materials

The solutions of Metam Sodium (Batch No. ZH130585; 42.2% Purity) were made in aqua dest. The stability of the test material throughout the study period was proved analytically. Solution of Cyclophosphamide in aqua dest was prepared prior to use and served as positive control.

2. Test Animals

Male and female chinese hamsters (7 to 13 weeks old; 31.7 g) strains SH (Chin) Ki, SPF, Savo-Ivanovas, D-7964 Kisslegg, FRG, were used for the cytogenetic investigations. Before the beginning of the experiment, the animals were transferred to Makrolon cages (Type M-1) and housed individually under environmentally controlled conditions. Standardized pelleted feed (Kliba Haltungsdiat, Klingentalmuhle AG, CH4303) and drinking water from bottles were available ad libitum. Five male and five female animals were used per treatment group for the cytogenetic study (Table on page 15 attached).

3. Dose Determinations

From a pretest results (acute oral toxicity), deaths were observed at a dose of 1470 mg/kg body weight. All animals survived at 1210 mg/kg body weight but it showed some signs of toxicity such as irregular respiration and squatting posture about 15 minutes to 2 hours. Therefore, a dose of 1200 mg/kg body weight was originally selected as the highest dose for this study. However, in a later cytogenetic test, 8 out of 20 animals died after the treatment with 1200 mg/kg of Metam Sodium. Thus, it was decided to reduce the top dose down to 900 mg/kg body weight. Again, few animals died and the slides prepared from the remaining hamsters could not be evaluated due to a poor quality of the chromosomes (only few metaphases were found). Therefore, a dose of 600 mg/kg body weight was administered as the highest dose in the present cytogenetic investigation (300 and 150 mg/kg were selected as lower doses).

4. Test Procedure and Administration

Treatment consisted of a single oral administration. The volume of administration was 10 ml/kg body weight. Five male and 5 female animals per sacrifice interval (6, 24, and 48 hours) were given the predetermined dose of Metam Sodium by the oral route. For control purposes, male and female animals were given the solvent aqua dest by the same route. The positive control, Cyclophosphamide (40 mg/kg), was administered once orally to male and female animals in a volume of 10 ml/kg body weight (24 hours posttreatment).

5. Preparation of the Bone Marrow

The bone marrow was prepared according to the method described by Schmid, W. and G.R. Staiger (Mut. Res., 1, 99-108, 1969). About 2 hours prior to sacrifice, the animals were intraperitoneally injected with 3.3 mg Colcemid/kg. Bone marrow cells were aspirated from each of two femurs into a centrifuge tube containing Hank's solution. Following centrifugation to pellet the cells, the resultant cell pellets were treated with 1% sodium citrate for 20 minutes at 37 C. After this hypotonic treatment, the cells were sedimented by centrifugation and fixed in a fixative (methanol:glacial acetic acid, 3:1) for 30 minutes. After cell fixation, the cells were resuspended in fresh fixative and this suspension was kept overnight at 4 C. Drop preparation of cell suspension was made on ice-cold slide. These slides were flammed to facilitate spreading the chromosomes and stained with a solution of Giemsa and Titrisol (10 ml Giemsa, 200 ml Titrisol, pH 7.2) for 10 minutes and rinsed in aqua dest.

6. Microscopic Evaluation

One hundread metaphases from each of the males and females of every test group were analyzed for chromosomal aberrations. Morphological observations were scored as follows:

Chromatid gap	Multiple aberration
Chromatid break	Intrachanges
Chromosome break	Interchanges
Chromatid fragment	Polyploidy (changes in the number
Chromosome fragment	of chromosomes)
Chromatid deletion	Aneuploidy (metaphases with missing and
Chromosome deletion	additional chromosomes)

7. Statistical Evaluation

The exact test according to Fisher was applied to register significant differences between the relative frequencies of a characteristic of two groups. The relative frequencies of metaphases with aberrations per animal were used as a criterion of the rank determination for the asymptotic U test according to Mann-Whitney. The two tests were calculated at the levels of 95% and 99%. The statistic calculations were carried out in the computer center of BASF Aktiengesellschaft.

II. Reported Results:

1. Clinical Examination

The doses of 600 mg/kg, 300 mg/kg and 150 mg/kg body weight led to irregular respiration in test animals about 30 minutes, 1 hour and 2 hours after test material administration respectively. The single oral administration of either the solvent or positive control did not cause any evident signs of toxicity.

2. Summarized Aberration Data (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18 attached) - gaps excluded

Time of Sacrifice hrs	Treatment (mg/kg)	No. of Animals Per Group	No of Metaphases Examined	No. of Metaphases w/Aberration	% of Aberration Per Group
6	0	5 M	500	1 (A)	
		5 F	500	0	0.1
	600	5 M	500	2	
		5 F	500	1	0.3
24	0	5 M	500	1 (A)	
		5 F	500	0	0.1
	150	5 M	500	1 (A)	
		5 F	500	1 (B)	0.2
	300	5 M	500	1 (A)	
		5 F	500	0	0.1
	600	5 M	500	9 (A,B,C)	
		5 F	500	0	0.9*
	40(CPP)	5 M	500	147 (G,H,I)	
		5 F	500	114 (G,H,I)	26.1**
48	0	5 M	500	1 (A)	
		5 F	500	0	0.1
	600	5 M	500	1 (A)	
		5 F	500	0	0.1

* Significantly greater than the control value $P<0.05$
**Significantly greater than the control value $P<0.01$
M = Male; F = Female; CPP = Cyclophosphamide; (A) = Chromatid break; (B) = Chromosome break; (C) = Chromatid fragment; (D) = Chromosome fragment; (E) = Chromatid deletion; (F) = Chromosome deletion; (G) = Multiple aberrations; (H) = Intarchages; (I) = Interchanges.

Findings:

i. The positive control, Cyclophosphamide, induced significant increase of chromosomal aberrations in bone marrow cells of Chinese hamsters ($P<0.01$) at the sacrifice interval of 24 hours.

ii. Significant increase of chromosomal aberrations was also observed in the bone marrow cells of animals treated with 600 mg/kg Metam Sodium ($P<0.05$). However, the significance at a value of 0.9% aberrant cells excluding gaps was due to the low spontaneous rate of 0.1% aberrant metaphases excluding gaps and was of no biological relevence since 0.9% were within the range of that of the historical control data. There were no significant differences in the types and frequencies found between the solvent control group and the lower dose groups (i.e., 300 and 100 mg/kg).

iii. There was no recorded change in ploidy in the treatment groups relative to the solvent controls.

III. Evaluation and Recommendation

1. The positive control, Cyclophosphamide at 40 mg/kg, adequately demonstrated the sensitivity of the Chinese hamster bone marrow cell system to detect a clastogenic effect.

2. The number of cells with chromosomal aberrations in the negative (solvent) control (0.1% metaphases) was found within the acceptable range established by the testing laboratory.

3. Although a brief description for dose determination was included in this report, detailed results of these preliminary toxicity tests (either acute oral toxicity or cell cytotoxicity) were not given. The target cell cytotoxicity of the test compound was described at 900 mg/kg body weight (See details in the BASF Report 87/0238, Page 13). But, there was no toxicity evidenced either by target cell cytotoxicity or by animal morbidity at 600 mg/kg body weight except the observed symptom of irregular respiration in all dosed groups. Therefore, it is questionable whether an upper limit of Metam Sodium was chosen for this study.

4. According to the acceptable procedure for performing the In Vivo Mammalian Bone Marrow Cytogenetic Assays (EPA Health Effect Test Guidelines 560/6/83-001 or OECD Guidelines for Mutagenicity Tests), the highest dose tested should produce some indication of toxicity as evidenced by animal morbidity or target cell toxicity. The LD_{50} is a suitable guide. The mitotic index, which also reflects cytotoxic effect of test compound, should be submitted. Therefore, the study is unacceptable in the present form. However, the study may be upgraded on resolution of the reporting deficiency.

Metam-sodium toxicology review

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84-2 In Vitro Cytogenetic Assay in Human Lymphocytes
Reviewed by: John H.S. Chen John H.S. Chen 1/19/88
Section I, Toxicology Branch (TS-769C)
Tertiary Reviewer: I. Mauer
Section VI, Toxicology Branch (TS-769C) 1/20/88
Reviewed by Section Head: R.B. Jaeger RBJ 1/21/88
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chromosomal Aberrations (In-Vitro) TOX. CHEM. NO.: 780

Accession No.: 403056-04 MRID NO.:

Test Material: Metam Sodium (Batch No. ZH 130585; 42.2% Purity)

Synonyms:

Study Number (s): BASF 87/0116

Sponsor: BASF Corporation Chemical Division

Testing Facility: BASF Aktiengesellschaft Dept. of Toxicology, FRG

Title of Report: In Vitro Cytogenetic Investigation in Human Lymphocytes with Metam Sodium

Author(s): Dr. H.P. Gelbke

Report Issued: March 9, 1987

Metam Sodium was clastogenic in the cultured human lymphocytes either at 20 ug/ml without metabolic activation or at 20 and 40 ug/ml with metabolic activation.

Concentrations tested: 1, 5, 10, and 20 ug/ml without S9 mix;
10, 20, and 40 ug/ml with S9 mix.

Classification of Data: Acceptable

Title of Report: In Vitro Cytogenetic Investigations in Human Lymphocytes with Metam Sodium
BASF 87/0116, March 9, 1987

I. Materials and Methods:

1. Test Materials

The solutions of Metam Sodium (Batch No. ZH130585; 42.2% Purity) were made in aqua dest. Solutions of Mitomycin-C and Cyclophosphamide in aqua dest were prepared prior to use and served as positive controls.

2. Preparation of Human Lymphocyte Cultures

Human venous blood was drawn aseptically into sterile syringes that contained heparin to prevent clotting. About 0.5 ml of heparinized blood was added to 6.0 ml of culture medium (chromosome medium 1A with phytohemagglutinin) in each centrifuge tube and the cultures were incubated with closed caps at 37°C for 48 hours.

3. Toxicity and Dose Determinations

The doses were determined from an appropriate pretest with cultures exposed to a wide dose range of the test compound (1 through 40 ug/ml). The top dose was determined by the solubility of Metam Sodium in aquest dest and the toxicity of this solution to dividing lymphocytes. All cultures were incubated at 37°C for 2 hours.

4. Treatment of Cultures

Each 48-hour culture was exposed to the test compound for 24 hours at 37°C without S-9 mix. Colcemid was added 2-3 hours before harvesting the dividing lymphocytes. In the case of assay with metabolic activation system, freshly prepared S-9 mix was added to the appropriate cultures with test material for an exposure of 2 hours at 37°C. After this exposure, cells were washed with unsupplemented culture medium and then re-incubated in complete culture medium for further 22 hours. Three hours before termination, cell division was arrested by the addition of colcemid to each culture (1.33 ug/ml). The cells were swollen with hypotonic solution (Hank/Aquest dest 1:4) at 37°C for 20 minutes, then fixed in methanol:glacial acetic acid fixative (4:1 v/v), dropped onto slides, and air-dried.

5. The Metabolic Activation System

The metabolic activation system comprised rat liver enzymes and an energy-producing system. The enzymes contained in a preparation of liver microsomes (S-9 fraction) from male Sprague-Dawley rats treated previously with Aroclor 1254. The S-9 fraction was added to a cofactor mixture to form the activation as described by Ames et al (Mutation Res. 31, 347-364, 1975). The concentrations of the cofactors in the S-9 mixture were: 8 mM $MgCl_2$; 33 mM KCl; 5 mM Glucose-6-Phosphate; 4 mM NADP; 100 mM Phosphate Buffer (PH 7.4).

6. Staining and Scoring of Slides

Slides were stained with Giemsa and Titrisol (10 ml Giemsa, 190 ml Tritrisol, PH 7.2) for subsequent scoring of chromosome aberration frequencies. One hundread metaphases of each culture were scored for the test compound, negative and solvent controls. Only fifty metaphases of each culture were scored for the positive controls. Morphological observations were scored as follows:

Chromatid gap	Multiple aberrations
Chromatid break	Intrachanges
Chromosome break	Interchanges
Chromatid fragment	Polyploidy (changes in the number of chromosomes)
Chromosome fragment	
Chromatid deletion	Aneuploidy (metaphases with absent or additional chromosomes)
Chromosome deletion	

7. Mitotic Index

A mitotic index based on 1500 cells was determined for at least the top two doses that yield metaphase cells and for the solvent and negative controls.

8. Statistical Evaluation

The exact test according to Fisher was used to answer the questions whether there are significant differences between control groups and dose groups with regard to the rate of structural aberrant metaphases (Lienert, G.A. Verlag Anton Hain, Meisenheim am Glan,1975). A significant increase in the number of aberrant cells against the untreated controls and solvent controls at least at 95% was determined. The statistical calculations were carried out in the computer center of BASF Aktiengesellschaft.

II. Reported Results:

1. Preliminary Toxicity Test (Tables on page 8 and 19 attached)

According to the results obtained from a pretest for determining the highest doses in this study, 20 ug/ml in the experiment without S-9 mix or 40 ug/ml in the experiment with metabolic activation were selected. This selection was based on the quality of metaphases and not on the mitotic index. The reason for this is that the concentration at the 20 ug/ml under the nonactivation condition had less scorable metaphases due to cytotoxicity of the test material (i.e., only 100 cells instead of 200 planned metaphases were evaluated).

2. Main cytogenetic Test (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 attached)

Summarized Incidence of Chromosomal Aberrations (Tables 1 and 2 - gaps excluded)

Treatment ug/ml	Total No. of Cells Examined	No. of Aberrations Examined	No. of Aberrations Per Cell	Mitotic Index
W/O S-9 Mix				
0 (Solvent)	200	2 (A)	0.01	100
1 (Metam Sodium)	200	3 (A,C)	0.015	104.9
5 (" ")	200	2 (A,C)	0.01	144.4
10 (" ")	200	7 (A,B,C,D)	0.035	161.3
20 (" ")	100	10 (A,B,H,G)	0.10**	143.5
0.3 (Mit)	100	51 (G,H,I)	0.51**	27.7
W/S-9 Mix				
0 (Solvent)	200	2 (A,H)	0.01	100
10 (Metam Sodium)	200	4 (A,B)	0.02	100.1
20 (" ")	200	10 (A,B,C,H)	0.1*	117.5
40 (" ")	200	17 (A,B,G,H)	0.085**	157.0
6 (CPP)	100	15 (H,I)	0.15**	84.4

* Significantly greater than the control value $P<0.05$
**Significantly greater than the control value $P<0.01$
Mit = Mitomycin-C; CPP = Cyclophosphamide; (A) = Chromatid break; (B) = Chromosome break; (C) = Chromatid fragment; (D) = Chromosome fragment; (E)=Chromatid deletion; (F)=Chromosome deletion; (G) = Multiple aberrations; (H) = Intrachange; (I) = Interchange.

Findings:

i. The positive controls, Mitomycin-C and Cyclophosphamide, induced significant positive responses ($P<0.01$) under the nonactivation and activation conditions respectively.

ii. Metam-Sodium showed a dose-dependent trend and demonstrated a statistically significant increase in the number of chromosomally damaged cells in the experiments both with (20 and 40 ug/ml; $P<0.05$ and $P<0.01$) and without metabolic activation (20 ug/ml; $P<0.05$). Therefore, the test material had a chromosome-damaging (clastogenic) effect in vitro using human lymphocytes.

iii. There was no significant increase in the numerical chromosome aberrations (i.e., heteroploidy) in the treatment groups when compared to the corresponding control values.

III Evaluation and Recommendation:

1. The positive controls, mitomycin-c at 0.3 ug/ml and cyclophosphamide at 6 ug/ml, adequately demonstrated the sensitivity of the cultured lymphocyte system to detect a clastogenic effect.

2. The number of cells with chromosomal aberrations in the negative (solvent) control (1% of metaphases) was found within the acceptable range established by the testing laboratory.

3. The highest-dose level of Metam Sodium, 20 ug/ml (-S9), has demonstrated cytotoxicity to dividing lymphocytes (only 100 metaphases available for chromosomal analysis).

4. Although the preliminary assessment of cell cycle delay was not conducted in this study (Reference: EPA Health Effects Test Guidelines EPA 560/6-83-001), the single harvest time (22 hours posttreatment; 70 hours-total culture time) for cells exposed to the test material appeared adequate for the detection of chromosomal aberrations in cultured human lymphocytes.

5. The assay was conducted in a manner to generate valid results. Metam Sodium was considered clastogenic in the cultured human lymphocytes either at 20 ug/ml without metabolic activation (at toxic dose level) or at 20 ug/ml and 40 ug/ml with metabolic activation (at non-toxic level). The study is acceptable.

Metam-sodium toxicology review

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84-2 Salmonella Mutagenicity Test

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12/17/87
12/17/87
12/21/87

006570

DATA EVALUATION REPORT

Study Type: Gene Mutation in Bacteria

TOX. CHEM. NO.: 780

Accession No.: 403056-03

MRID NO.:

Test Material: Metam-Sodium (Batch No. ZH 130585; 42.2% Purity)

Synonyms:

Study Number (s): BASF 87/0208

Sponsor: BASF Corporation Chemical Division

Testing Facility: BASF Aktiengesellschaft Dept. of Toxicology, FRG

Title of Report: Report on the Study of Metam Sodium in the Ames Test

Author(s): G. Engelhardt

Report Issued: June 5, 1987

Conclusions:

Metam Sodium was nonmutagenic to TA92, TA98, TA100, TA1535, TA1537 and TA1538 strains of Salmonella typhimurium (both in the standard plate test and in the preincubation test) either with or without metabolic activation at the concentrations tested (20 through 2500 ug/plate).

Concentrations tested: Standard Plate Test: 20, 100, 500, 1000, 1500, 2000 and 2500 ug/plate; Preincubation Test: 4, 20, 100, 200, 300, 400, 500, 1000 and 2500 ug/plate.

Classification of Data: Acceptable

Title of Report: Report on the Study of Metam Sodium (Sodium-N-methyl-dithiocarbamate) in the Ames Test
BASF 87/0208, June 5, 1987

I. Materials and Methods:

1. Test Materials

The test compound, Metam Sodium (42.2% purity; 56.2% H2O; 1% Dimethyl-thiourea) was stored at 4° C. Solutions were made in aqua dest and were freshly prepared for each experiment. Solutions of 2-aminoanthracene, N-methyl-N-nitro-N-nitroso-guanidine, 4-nitro-O-phenylendiamine, 9-aminoacridine chloride monohydrate and mitomycin-C in DMSO were prepared prior to use and served as positive controls.

2. Bacteria

Six histidine-requiring strains of Salmonella typhimurium (TA1535, TA100, TA92, TA1537, TA1538 and TA98), which were checked and confirmed for their characteristic spontaneous reversion rate, were used in this study.

3. In-vitro Metabolic Activation System

The mammalian metabolic activation system consisted of rat liver homogenate from Aroclor 1254-treated male Sprague-Dawley rats and cofactor solution described by Ames et al. (Mutation Res. 31, 347-364, 1975). The S-9 mix (10 ml) contained 3 ml of S-9 fraction and 7 ml of cofactor solution (8 mM MgCl2; 33 mM KCl; 5 mM Glucose-6-phosphate; 4 mM NADP; 100 mM Phosphate buffer, PH 7.4).

4. Mutagenicity Test

A. Standard Plate Test

The experimental procedure was based on the method of Ames Salmonella Mammalian Microsomal Mutagenicity Test (Mutation Res. 31, 374-364, 1975). The mutagenicity of Metam Sodium was evaluated by the Ames test either in the presence or absence of metabolic activation.

B. Preincubation Test

The experimental procedure was based on the method described by Yahagi et al. (Mutation Res. 48, 121-130, 1977) and Matsushima et al. (In: Norpoth, K.H. and R.C. Garner, Short-Term Test Systems for Detecting Carcinogens., Springer Verlag Berlin, Heidelberg, New York, 1980). This test was briefly described as follows: 0.1 ml test solution, 0.1 ml bacterial suspension and 0.5 ml S-9 mix were incubated at 37°C for the duration of 20 minutes. Subsequently, 2 ml of soft agar was added and, after mixing, the samples were poured onto the Vogel-Bonner agar plates within approx. 30 seconds.

4. Preincubation Test - continued

Mutations were quantified on triplicate plates for each strain by counting the his$^+$ revertant colonies after 48 hours of incubation at 37° C on a selective agar plate. Positive control compound for each strain and solvent control were run concurrently with the test compound.

5. Evaluation Criteria

A substance to be characterized as positive in the Ames test has to fulfill the following requirements:

A. doubling of the spontaneous mutation rate (over the control value);
B. dose-response relationship;
C. reproducibility of the results.

II. Reported Results: (Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 attached)

1. Toxicity

Following the exposures to test cultures with Metam Sodium on a selective agar plate for 48 hours at 37° C, a bacteriotoxic effect (reduced his$^-$ background growth, decrease in the number of his$^+$ revertants) was observed in the standard plate test depending on the strain and test conditions from about 500-2500 ug/plate onward (TA 92), from about 1500-2500 ug/plate onward (TA100, TA1538) or at doses greater than 2000 ug/plate (TA98, TA1535, TA1537). In the preincubation test, bacteriotoxicity was found from about 100-500 ug/plate onward (all tester strians without S-9 mix; TA1535 with S-9 mix) or at doses greater than 2500 ug/plate (TA100, TA92, TA1537, TA1538, TA98 with S-9 mix).

2. Mutagenicity

No increase in the number of revertant colonies (less than 2-fold) over concurrent control value was observed for any of tester strains following exposure to the test compound (Standard Plate Test: 20, 100, 500, 1000, 1500, 2000 and 2500 ug/plate; Preincubation Test: 4, 20, 100, 200, 300, 400, 500, 1000 and 2500 ug/plate) both in the standard plate test and in the preincubation test either in the presence or absence of S-9 mix.

III. Evaluation and Recommendation:

1. The specific procedures for confirming the genotypes of TA1535, TA1537, TA1538, TA100, TA98 and TA92 strains of Salmonella typhimurium, which were based on the individual sensitivity test recommended by the Ames test, are considered adequate.

III. Evaluation and Recommendation: continued

2. The spontaneous revertant colonies for each of the six tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Ames test (Mutation Res. 31, 347-364, 1975).

3. The strain specific control compounds (N-methyl-N-nitro-N-nitroso-guanidine, 4-nitro-o-phenylendiamine, 9-aminoacridine chloride monohydrate and mitomycin C) and the positive control (2-aminoanthracene), to ensure the efficacy of the activation system, have given strongly positive responses as expected.

4. The high-dose level of the test compound (2500 ug/plate) has demonstrated bacteriotoxicity in treated cultures (TA92, TA98, TA100, TA1535, TA1537, TA1538) either in the standard plate test or in the preincubation test.

5. Under the test conditions reported, the assay was conducted in a manner to generate valid results. Therefore, the test compound, Metam Sodium, was not mutagenic in the Ames Salmonella/Mammalian Microsomal Mutagenicity Test (both in the standard plate test and in the preincubation test) either with or without metabolic activation at the concentrations tested (20 through 2500 ug/plate). The study is acceptable.

Metam-sodium toxicology review

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