RC-246
TXR-7027

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

FEB 13 1989

007027

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Metam Sodium ID Number 039003: Registrant's Reply to the Previous Toxicology Branch Review Comments Concerning the Mutagenicity Studies with Metam Sodium
Caswell No. 780, HED Project No. 9-0201A

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Review of the Registrant's Response to the Previous Toxicology Branch Review Comments Concerning the Following Mutagenicity Studies with Metam Sodium (Toxicology Branch Memorandum 1/29/88 J. Chen)

1. Mutagenecity Test on Metam Sodium in the Rec-Assay with Bacillus subtilis. Hazleton Biotechnologies Veenendal Laboratory Report No. BASF 87/0163

A. Negative Control Showing Non-preferential Growth Inhibition

Registrant's Response: " In addition to the solvent negative controls also Kenamycin controls (25 ul/plate) were used. As was stated on page 8 (4th paragraph) of the metam sodium report: No differential toxicity between the strains H17 and M45 was observed with Kenamycin. The actual inhibition zones of Kenamycin in these are now provided in Table 1."

Reviewer's Comments: The provided supplemental data for the negative control compound (Kenamycin) used in this study (Table 1 provided) is considered adequate.

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B. Rationale for determining the upper limits of the test material Concentration

Registrant's Response: "For testing procedures criteria that are normally taken into consideration for determining the highest amount of test material used are solubility and cytotoxicity. For highly soluble non-toxic test compounds a maximum of 5 mg/plate of (pure) material is generally considered sufficient for microbial test systems. Because the end-point in the "Rec-assay" is the assessment of a possible differential toxicity between the strains H17 and M45 of Bacillus subtilis, our normal approach is to apply standard discs up to 10 mg per plate for solids or 150 ul per plate for liquids for freely soluble/ mixable test compounds. In situations where the test material is highly toxic to both strains, resulting in less than four concentrations of that material that have measurable data (inhibitions zones), tests are repeated at lower concentrations of the test material. In the Metam-Sodium study both the high toxicity of the test material and the data resulted in an additional trial in which the toxicity curve was covered with smaller concentration steps of the test material."

Reviewer's Comments: The provided rationale for selecting 10 mg/plate (or 150 ul/plate) as the highest dose in this study is considered to be justified.

C. Positive Control Responses and Cell Densities

Registrant's Response: "Unfortunately I am not aware of positive control compounds that work more consistently and effectively than the ones (NMS and Sterigmatocystin) we use. Also in the report of the Gene-Tox Program ... Bacterial cells of both strains of Bacillus subtilis are innoculated at similar densities and grown overnight under the same conditions. At present we are not able to estimate bacterial cell densities after growth overnight in our laboratory by spectrophotometric or other photometric methods. On the other hand, these methods have been shown to be inaccurate because they cannot discern between dividing, viable or dead cells. The suspension and preincubation procedures that we also use in our laboratory for the "Rec-assay", however, have shown that cell densities of both H17 and M45 are generally not that different after growth overnight."

Reviewer's Comments: The provided explanations for clarifying the positive control responses and the cell density problems under either the activated or non-activated system are considered to be reasonable. However, it may be appropriate to point out that the inconsistently positive results of sterigmatocystin shown in Table 4 (M45, 18.3 mm; H17, 18.0 mm; less than a differential of 4 mm zone inhibition) could be caused by the borderline concentration of this positive control compound).

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Recommendation: The test material, Metam Sodium, was not recombinogenic (i.e., damage of DNA) to Bacillus subtilis in the presence or absence of metabolic activation at the concentrations tested (0.1 through 150 ul/well or plate). The study is upgraded to acceptable.

2. Cytogenetic Study in Vivo of Metam Sodium in Chinese Hamsters, Bone Marrow Chromosome Analysis. BASF Aktiengesellschaft Dept. of Toxicology Report No. BASF 81/0236

A. Preliminary Test for Dose Determination

Registrant's Response: "... The acute oral toxicity test results were described completely, i.e. "irregular respiration and squatting posture about 15 minutes - 2 hours after test substance administration, in some cases apathy was observed and the general state of some animals was poor". Due to the absence of additional signs of toxicity, a more detailed description could not be made. The doses at which the animals just survive but show clear signs of toxicity, will commonly be selected at the highest dose. Therefore, and for reasons given below (see comments on the mitotic index, item 2.b.), this selection is generally not based on cell toxicity. The selection of the highest test dose, i.e. 600 mg/kg b.w. must be based mainly on the fact that the originally selected highest dose of 900 mg/kg b.w., first was not survived by all animals, and second did not allow a metaphase analysis to be made due to the poor quality of the chromosones in this group rather than on animal morbidity. Doses between 600 mg/kg and 900 mg/kg b.w. were not tested because these two doses are close together; therefore, in our opinion, 600 mg/kg b.w. can be regarded as an upper limit of metam sodium."

Reviewer's Comments: It is true that under a normal test condition, the highest test substance concentration selected for the in-vivo cytogenetic assay should show a cytotoxic effect but allow sufficient metaphases (good quality of chromosomes) for reliable analysis. The submitted explanations for selecting 600 mg/kg of metam sodium as the highest dose for this study are considered to be reasonable.

B. The Mitotic Index

Registrant's Response: "The figures of the mitotic index can be seen from table 1. There was no difference between all dose groups and the control group (solvent control). These findings confirm earlier own observations, according to which no influence on the mitotic index could be observed (even at lethal doses); therefore,

the mitotic index in in vivo cytogenetic studies might not be a suitable criterion for dose selection based on target cell cytotoxicity. However, the fact that in the 900 mg/kg b.w. group the target cells were reached (demonstrated by the poor quality of chromosmes due to test substance treatment) shows that metam sodium generally reached the bone marrow. Therefore, we might assume that the target will also be reached after administration of 600 mg/kg b.w."

Reviewer's Comments: The subritted report amendment (Table 1), providing necessary information concerning the mitotic index results of the metam sodium-treated groups in Chinese hamsters for this study, is considered acceptable. These findings have confirmed that metam sodium was not a mitotic inhibitor when compared to the negative control. The registrant's interpretation is considered to be justified.

Recommendation: The test material, Metam Sodium, was not clastogenic in the Chinese hamster bone marrow cytogenetic assay at the dose levels tested (150 through 600 mg/kg). The study is upgraded to acceptable.

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TABLE 1

RESULTS OF THE "RECOMBINATION" ASSAY

A. TEST CONTROL COMPOUND NAME OR CODE: Kanamycin

B. TEST INITIATION DATES: February 13, 17, 25, 1987

C. TEST COMPLETION DATES: February 18, 26, 1987

INHIBITION ZONES

	H17		M45	
NONACTIVATION	1	2	1	2
Kanamycin				
TEST DATE				
February 13, 1987	26	25	25	25
February 17, 1987	21	22	22	22
February 25, 1987	21	21	22	22

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Table 1

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Cytogenetic Study in Vivo of Metam Sodium in Chinese Hamsters,
Determination of the Mitotic Index

Group 1 aqua dest.	Group 2 600 mg/kg	Group 3 600 mg/kg	Group 4 600 mg/kg	Group 5 300 mg/kg	Group 6 150 mg/kg
24 h	6 h	24 h	48 h	24 h	24 h
7.5	4.2	8.3	8.4	6.9	6.5
8.0	5.3	6.0	8.6	7.0	6.5
7.8	4.9	8.3	6.1	4.9	7.5
4.7	6.7	9.1	5.5	8.2	8.3
7.8	8.6	9.3	6.5	6.2	6.3
7.7	6.0	8.3	8.5	5.4	10.7
8.0	4.9	10.0	9.5	10.2	6.5
10.8	8.7	8.0	10.1	10.2	5.9
7.2	4.5	9.7	5.8	7.2	9.9
7.9	9.7	5.3	10.3	7.1	10.7
x̄: 7.74	6.35	8.23	7.93	7.33	7.88

The mitotic index is based on 1500 cells/animal