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DATA EVALUATION RECORD

NABAM

Mutagenicity--Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

STUDY IDENTIFICATION: Sernau, R. C. and Cavagnaro, J. Unscheduled DNA synthesis rat hepatocyte assay with Nabam. (Unpublished study No. 752-110 prepared by Hazleton Corporation, Vienna, VA, for Alco Chemical Corporation, Chattanooga, TN; dated September 17, 1985.) Accession No. 259441.

APPROVED BY:

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Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 4-10-86

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1. CHEMICAL: Nabam.

2. TEST MATERIAL: Nabam was described as a green liquid containing 30% of the active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA synthesis assay in primary rat hepatocytes.

4. STUDY IDENTIFICATION: Sernau, R. C. and Cavagnaro, J. Unscheduled DNA synthesis rat hepatocyte assay with Nabam. (Unpublished study No. 752-110 prepared by Hazleton Corporation, Vienna, VA, for Alco Chemical Corporation, Chattanooga, TN; dated September 17, 1985.) Accession No. 259441.

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7. CONCLUSIONS:

A. Under the conditions of the assay for unscheduled DNA synthesis (UDS), Nabam, tested at five doses ranging from 0.1 to 10 µg/mL, induced a significantly increased response at 5 µg/mL only; although not significant, increased grain counts were observed at all the lower doses and the increases appeared to be dose dependent. The highest dose (10 µg/mL) was cytotoxic with a relative survival of 34%. The positive control, 2-acetylamino-fluorene (2-AAF) at 0.05 µg/mL, demonstrated the sensitivity of the assay to detect a mutagenic effect. Therefore, Nabam is considered presumptively positive in the UDS rat hepatocyte assay.

B. This study is inconclusive, but the data indicate that the test material is presumptive positive.

8. RECOMMENDATIONS: It is recommended that the assay be repeated at the same dose levels to demonstrate reproducibility of the assay. In addition, to ensure a linear dose response and to fill the data gap, it is suggested that additional concentrations be included in the assay between the ranges of 1 and 5 µg/mL (5-fold difference) and 5 µg/mL (noncytotoxic) and 10 µg/mL (cytotoxic). In addition, the criterion used by the authors to assess a positive response "a mean net grain count of at least three standard deviations greater than the solvent control" appears to be somewhat insensitive; a value of two standard deviations greater than the solvent control would be more appropriate; therefore, it is recommended that individual net nuclear grain counts be submitted so that other UDS criteria may be applied to the data.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: A 30% solution of Nabam was described as a green liquid and labeled "T.S.q. 30% Alco/Vinings/Uniroyal." The test material was diluted in sterile, glass-distilled water, the solvent control.

2. Indicator Cells: Primary rat hepatocytes were obtained from the liver of a male Fischer 344 rat, which was purchased from Charles River Breeding Laboratories, Inc.

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[1] Only items appropriate to this DER have been included.

3. Cell Preparation:

   a. Perfusion Technique. The liver was cleansed with modified Hanks' balanced salt solution (M-HBSS) containing 0.5 mM EGTA. The liver was then perfused with Type IV collagenase at 100 U/mL in M-HBSS for 15-20 minutes. The liver was excised and placed in a chilled beaker of M-HBSS to mechanically release the hepatocytes.

   b. Hepatocyte Harvest/Culture Preparation: Recovered cells were washed, centrifuged, and resuspended in complete Williams' Medium E (WME) containing HEPES buffer, 10% fetal calf serum, L-glutamine, and antibiotics and dispensed (0.3×10^6 cells/mL) onto plastic coverslips in triplicate. The cultures were placed in a humidified, 37°C, 5% CO_2/air incubator for a 2.5-hour attachment period.

4. Selection of the High Dose: Nabam was assayed in an initial cytotoxicity test at an unspecified number of doses ranging from 0.05 to 1000 µg/mL.

5. UDS Assay:

   a. Treatment: Attached cells were washed with WME and refed with WME containing 10 µCi/mL of [^{3}H]thymidine, and the appropriate levels of the test material, the solvent, and positive or medium controls were added to triplicate cultures. The cultures were incubated overnight in a 37°C, humidified, 5% CO_2/air atmosphere. At the conclusion of the treatment period, cells were washed with M-HBSS; one of the triplicates for each treatment group was used to determine cytotoxicity by trypan blue exclusion. One hundred cells per dose were evaluated for cell viability. The two remaining replicates were transferred to new dishes for slide preparation. The pH of the medium containing the highest test dose was visually evaluated to determine if the test material altered the pH conditions.

   b. UDS Slide Preparation: Treated hepatocytes, attached to coverslips, were exposed to 1% sodium citrate for 10 minutes, fixed in methanol:acetic acid (3:1), mounted, and dried.

   c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB2 emulsion at 42°C and dried for several days at 4°C in a light-tight dessicated slide box. Slides were then developed in Kodak DS-19, fixed, stained with Giemsa:Dulbecco's phosphate-buffered saline (1:25), coded, and counted.

d. Grain Counting: The nuclear grains of 150 morphologically normal cells for each dose of the test material, solvent, and positive and medium controls were counted under oil immersion using an automated colony counter interfaced with a television camera mounted on a microscope. The mean net nuclear grain counts were determined by subtracting the nuclear grain count of each cell from the mean cytoplasmic grain count of three nuclear-sized areas within the cytoplasm of each cell counted

6. Evaluation Criteria: A test material was considered positive if it induced, at two doses, increases in mean grain counts that were >3 times the standard deviation of the solvent control plus the mean grain count of the solvent control. If this increase occurred at one dose the response was considered significant.

B. Protocol: A protocol was not presented.

12. REPORTED RESULTS:

A. Selection of the High Dose: An unspecified number of test material doses ranging from 0.05 to 1000 µg/mL was initially evaluated for cytotoxicity. The results were not presented; however, the authors stated that based on the findings, 100 µg/mL was selected as the highest test dose for the UDS assay.

B. UDS Assay: Seven doses (0.1, 0.5, 1.0, 5.0, 10, 50, and 100 µg/mL) were tested in the UDS assay. No cells survived treatment with 50 or 100 µg/mL. All remaining doses were processed for autoradiography.

The test material at dose levels of 0.1, 0.5, 1.0, 5.0, and 10 µg/mL resulted in a relative survival (RS) of ≥95% at doses from 0.1 to 5 µg/mL; a 34% RS was found at the 10-µg/mL level. Nabam at doses from 0.1 to 1 µg/mL showed increases in the mean nuclear grain counts; although these increases were not significant, a dose-related effect was suggested. At the 5-µg/mL dose, Nabam induced a statistically significant increase in the mean nuclear grain count over the solvent control.

Results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "A significant increase in the mean nuclear grain count was observed at one dose level. The remaining four dose levels showed increases in the grain counts suggesting a dose-related response. These increases were not significant according to the criteria established in the protocol."

TABLE 1. Results of the UDS Primary Rat Hepatocyte Assay with Nabam

Treatment	Dose (µg/mL)	Cells Scored	Relative Survival (%)	Mean Net Nuclear Grain Count±SD
Solvent Control				
Distilled Water		150	100	3.2± 3.6
Media Control				
WME		150	--	0.5± 2.4
Solvent for Positive Control				
DMSO		150	100	0.9± 1.9
Positive Control				
2-AF	0.05	150	98	20.5±11.0*
Test Material				
Nabam	0.1	150	99	5.9± 5.1
	0.5	150	99	4.6± 4.1
	1.0	150	101	7.8± 5.8
	5.0	150	95	18.5±10.9**
	10.0	150	34	9.4± 7.5

*Significant positive response; 3 times the S.D. plus the mean count of the solvent control, DMSO.

**Significant positive response; 3 times the S.D. plus the mean count of the solvent control, water.

B. A quality assurance statement was signed and dated July 15, 1985.

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14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that at doses of 0.1, 0.5, 1.0, and 50 µg/mL, Nabam caused a dose-related increase in the mean net nuclear grain count, with a statistically significant response at the 5-µg/mL dose. The dose-related effect declined at the highest level (10 µg/mL) due to cytotoxicity (37% RS); however, the mean net nuclear grain count (9.4) for the highest dose was 3 times higher than the solvent control count (3.2). The authors did not consider these results to be significant, because the responses did not meet their established criteria for a positive effect. However, we assess that Nabam produced a presumptive positive response and that the assay would have likely demonstrated a definitive dose-related response with more than one dose showing a significant increase had the authors included additional doses below and above 5 µg/mL, the dose at which a definitive and significant positive UDS response was reported.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-10.

APPENDIX A

Materials and Methods

NABAM

Page _____ is not included in this copy.

Pages 9 through 12 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.