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DATA EVALUATION REPORT I

STUDY TYPE: 84-4 Unscheduled DNA Synthesis - Rat Hepatocytes

TOX CHEM NO. 585

MRID NO: 411746-01

TEST MATERIAL: Nabam

SYNONYMS: AQUATREAT DN-30 (30% NABAM IN WATER)

STUDY NUMBER(S): 752-110

SPONSOR: Nabam Task Force
1015 15th St. NW
Washington, D.C. 20005

TESTING FACILITY: Hazelton Biotechnologies Corp.
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

TITLE OF REPORT: Supplement to: Unscheduled DNA Synthesis Rat Hepatocyte Assay with Nabam

STUDY DIRECTOR: Cavagnaro, J.

REPORT ISSUED: 7/20/89

CLASSIFICATION: Acceptable. The test material (a 30% solution of Nabam in water) was demonstrated to cause an increased incidence of cells with $\geq$ 6 net nuclear grains at all dose levels (0.1 to 10 μg/ml) and an increased incidence of cells $\geq$ 20 net nuclear grains at the three highest dose levels (1.0 to 10 μg/mL). These findings indicate that Nabam is positive under the conditions of this assay. This study satisfies the requirements (84-4) for a UDS mutation assay in rat primary hepatocytes.

BACKGROUND:

This study was previously reviewed (refer to the attached DER from a memorandum dated 5/30/86). At that time it was concluded that: "This study is inconclusive, but the data indicate that the test material is (a) presumptive positive." The recommendation was that the assay be redone. However, the report as initially received by the Agency did not include individual cell net grain counts. This information has now been received and examined, and from it the numbers of cells with $\geq$ 6 and $\geq$ 20 net nuclear grains at each dose level have been determined.

CONCLUSIONS:

1. Relative to the findings for the negative (solvent) control, there were significant increases in numbers of cells with $\geq$ 6 net nuclear grains for all dose levels of Nabam, and there were also significant increases in numbers of cells with $\geq$ 20 net grains at the three highest dose levels of Nabam.

2. It is concluded that exposure to an aqueous solution containing 30% Nabam results in induction of UDS in rat hepatocytes, and that the test material was mutagenically active under the conditions of this assay.

3. It is noted that the nuclear labeling in the negative control cultures was higher than the recommended level of less than one grain per nucleus for the rat hepatocyte UDS assay (Mitchell, A. et al., Mutation Research 123: 363-410, 1983). This is a possible indication that the conduct of the study was such that maximum sensitivity of the assay was not assured.

4. The classification of this study is upgraded to acceptable.

A. MATERIALS:

Refer to the attached DER.

B. STUDY DESIGN:

Refer to the attached DER.

The raw data from the original study were transcribed and submitted to EPA. These raw data were not present in the original report received by the Agency. It is noted that there are a number of occurrences (such as on p. 7) where it is indicated that the raw data were illegible.

There is a signed and dated Quality Assurance Statement on page 4 of this report, which includes the statement that "The transcription of the raw data for this study was audited by

the Quality Assurance Department of Hazleton Laboratories America, Inc."

C. RESULTS:

The following table giving a summary of the assay results is contained in the first review of this study:

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.

The following numbers of cells with ≥ 6 and ≥ 20 net nuclear grains have been calculated from the copies of raw data in MRID 411746-01:

			Number of cells scored	≥ 6 net grains	≥ 20 net grains
Solvent Control					
Distilled water		9A	50	19	0
		9B	50	9	0
		9C	50	13	0
		Total	150	41	0
Media Control					
WME		6A	50	0	0
		6B	50	0	0
		6C	50	5	1
		Total	150	5	1
Solvent for Positive control - DMSO		2A	50	0	0
		2B	50	2	0
		2C	50	4	0
		Total	150	6	0
Positive Control					
2-AAF	0.05	8A	50	48	17
		8B	50	49	27
		8C	50	46	24
		Total	150	143	68
Test Material					
Nabam	0.1	1A	50	11	0
		1B	50	19	0
		1C	50	40	2
		Total	150	70	2
	0.5	4A	50	23	0
		4B	50	25	0
		4C	50	10	0
		Total	150	58	0
	1.0	3A	50	25	1*
		3B	50	40	4
		3C	50	30	1
		Total	150	95	6

* Cell no. 15, originally reported with 18 net granules, was recalculated as having 20 by this reviewer (refer to p. 13 of the report).

	5.0	7A	50	44	17
		7B	50	48	33
		7C	50	43	7
		Total	150	135	57
	10.0	5A	50	34	6
		5B	50	42	7
		5C	50	28	2
		Total	150	104	15

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Using Fisher's Exact Test, the following values for p were obtained by this reviewer for the incidences of nuclei with ≥ 6 and ≥ 20 net nuclear grains at the different concentrations of Nabam in pairwise comparison with the solvent control findings:

		>6 net grains	>20 net grains
Nabam	0.1 μg/mL	3.89×10^{-4}	not significant
"	0.5 μg/mL	2.46×10^{-2}	not significant
"	1.0 μg/mL	$< 10^{-8}$	1.48×10^{-2}
"	5.0 μg/mL	$< 10^{-8}$	$< 10^{-8}$
"	10.0 μg/mL	$< 10^{-8}$	2.11×10^{-5}

D. DISCUSSION:

In the review of this study as originally received (see the attached DER) it was concluded that: "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... Therefore, Nabam is considered presumptively positive in the UDS rat hepatocyte assay." However, no additional criteria for UDS were utilized (number of nuclei/exposure level showing ≥ 6 and/or ≥ 20 net nuclear grains). These are indicated in Brusick, D. Principles of Genetic Toxicology. Plenum Press, 1980, pp. 224-228. This reference states (p. 227):

> The test article should be considered active in the UDS assay at applied concentrations that cause (1) An increase in the mean nuclear grain count to at least six grains per nucleus in excess of the concurrent negative control value; and/or (2) the percentage of nuclei with six or more grains to increase above 10% of the examined population, in excess of the concurrent negative control; and/or (3) the percentage of nuclei with 20 or more grains to reach or exceed 2% of the examined population... Generally, if the first condition is satisfied, the second and often the third condition will also be met. However, satisfaction of only the second or third condition can also indicate UDS activity...all three of the above conditions should be considered in an evaluation."

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The following are the percentages of cells (out of the total of 150) that scored ≥ 6 and ≥ 20 net nuclear grains at each dose level:

	>6	>20
Solvent control (distilled water)	27.3	0.0
Media control (WME)	3.3	0.7
Solvent (DMSO) for positive control	4.0	0.0
Positive control (2-AAF, 0.05 µg/mL)	95.3	45.3
Nabam (0.1 µg/mL)	46.7	1.3
" (0.5 µg/mL)	38.7	0.0
" (1.0 µg/mL)	63.3	4.0
" (5.0 µg/mL)	90.0	38.0
" (10.0 µg/mL)	69.3	10.0

Clearly, at all dose levels of Nabam there was a greater than 10% higher incidence of cells ≥ 6 net nuclear grains as compared to the solvent control and further, at the 3 highest dose levels of Nabam, the incidence of cells with ≥ 20 net nuclear grains was more than 2%. It is therefore concluded that exposure to Nabam results in induction of UDS in rat hepatocytes, and that the test material is mutagenically active under the conditions of this assay. The study classification is upgraded to acceptable.