CASWELL FILE

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

010181

APR 2 1 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Ames Study (TA98 Strain Only) with Nabam; Analytical Data for Nabam and ETU in Aqueous Solution

TO: Stowe/Waldrop, PM 71
SRRD (H7508W)

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 4/14/93
Toxicology Branch 2
HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 4/15/93
Section Head, Review Section II
Toxicology Branch 2
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch 2 Marcia van Gemert 4/19/93
HED (H7509C)

DP Barcodes: D173307 and D189209 Submissions: S409891 and S437081

Chemical: 014503

Action Requested:

Review of an Ames study for Nabam (in MRID 421512-01) involving only strain TA98 in the presence of rat S9; review of ETU and Nabam analytical data (in MRID 426837-01).

Comments and Recommendations:

1. The Ames study in MRID 423871-01 involved exposure of Salmonella typhimurium strain TA98 to Nabam in the presence of rat S9. The Agency previously reviewed an Ames study on Nabam and found it acceptable (review document 008303) except for the findings involving the TA98 strain in the presence of rat S9.

2. TA98 was exposed to dose levels ranging from 0.302 to 600 µg/plate (first assay) and from 0.302 to 1200 µg/plate (second assay). This reviewer spoke with a representative from Jellinek

et al. and confirmed that these concentrations are expressed in terms of the active ingredient (Nabam), rather than the formulation (Aquatreat DN-30, approximately 30% Nabam). Under the conditions of the assay, there was no indication of an increased number of revertants at the histidine locus at any dose level (testing done in the presence of rat S9 only).

3. This study, with its negative findings, is acceptable. This study, when taken together with the report in MRID 410768-01 (and the ETU and Nabam analytical data in MRID 426837-01) satisfies the 84-2(a) gene mutation guideline data registration and/or reregistration requirement for products containing/consisting of technical Nabam.

4. The sister chromatid exchange in MRID 423036-01 (involving exposure of CHO cells to Nabam in the absence of S9 activation) has been previously classified as acceptable (review document 010094) because of the positive findings.

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Guideline Series 84: **MUTAGENICITY**

Reviewed by: Byron T. Backus, Ph.D. Byron T. Backus
Section II, Toxicology Branch II (H7509C) 4/15/93
Secondary reviewer: K. Clark Swentzel K. Clark Swentzel 4/15/93
Section II, Tox Branch II (H7509C)

DATA EVALUATION REPORT I

CHEMICAL: Nabam; Aquatreat DN-30 Tox. Chem. No.: 014503

STUDY TYPE: Salmonella/mammalian activation gene mutation assay (TA98 only) with rat S9 activation

MRID NUMBER: 423871-01; 426837-01

SYNONYMS/CAS No.: 014503

SPONSOR: Alco Chemical

TESTING FACILITY: Hazleton Washington, Inc
5516 Nicholson Lane
Kensington, MD 20895

TITLE OF REPORT: Mutagenicity Test on Aquatreat DN-30 (30% Nabam in Water) in the Salmonella/Mammalian Microsome Reverse Mutation Assay (Ames Assay) with a Confirmatory Assay.

AUTHOR: Lawlor, T. E.

STUDY NUMBER: HWA Study No. 14806-0-401R

REPORT ISSUED: 18 December, 1991

BACKGROUND: In a memorandum dated March 25, 1991 (Caswell file document 008303) it was stated that a previously reviewed Ames study on Aquatreat DN-30 was acceptable, except that retesting was necessary with TA98 in the presence of (preferably rat) S9.

CONCLUSION(S) - Executive Summary:

This assay was performed using strain TA98 only in the presence of S9 mix. In the initial assay doses ranging from 0.302 to 600 µg/plate were used (doses expressed in terms of the active ingredient Nabam; in terms of the test material, Aquatreat DN-30, the dose range was 1 to 2000 µg/plate). In the subsequent confirmatory assay doses of 0.302 to 1200 µg/plate were used. There was no indication of a positive mutagenic response in strain

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TA98. An acceptable level of cytotoxicity (reduced numbers of revertants/plate; reduction in background lawn over half the plate) was observed at 1200 µg/plate.

Classification: Acceptable. This study, in combination with the previously submitted information in MRID 259612, satisfies the 84-2(a) Guideline requirements for a gene mutation assay.

A. MATERIALS

1. Test Material: Aquatreat DN-30 (30% Nabam in water), described as a "clear, colorless liquid" (on some occasions it has been described as a green or greenish-yellow liquid), which was received 10/09/91. No lot number is reported.

   From information in MRID 426837-01 ("A Determination of Nabam and Ethylene Thiourea in Aqueous Preparations; Supplement to MRID 42303601, Sister Chromatid Exchange Assay, and to MRID 42151201, Ames Assay (Guideline 84-2)") ETU was present at 4.72-6.93% of measured Nabam content in recently prepared solutions containing 1.81-44.9 µg/ml Nabam; ETU was present at 1.49% of measured Nabam content in a solution containing 10,550 µg/ml Nabam. Refer to appended page 1.

   The solvent used was deionized water.

2. Control Materials:

   Negative: Deionized water in the presence of S9.
   Solvent/final concentration: n/a
   Positive (activation only): 2-aminoanthracene at 2.5 µg/plate.

3. Activation: S9 derived from

_X_ Aroclor 1254	_X_ induced	_X_ rat	_X_ liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other

   Describe S9 mix composition (if purchased, give details):

H_2O	0.70 ml
1M NaH_2PO_4/Na_2HPO_4, pH 7.4	0.10 ml
0.25M glucose-6-phosphate	0.02 ml
0.1 NADP	0.04 ml
0.825M KCl/0.2M $MgCl_2$	0.04 ml
S9 homogenate	0.10 ml

4. Test organisms: S. typhimurium strains
   ___ TA97 _X_ TA98 ___ TA100 ___ TA102 ___ TA104
   ___ TA1535 ___ TA1537 ___ TA1538 ; list any others:

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used (µg/plate):

Non-activated conditions: n/a
Activated conditions: 0.302, 1.3, 6.7, 33.3, 100, 300, 600 µg/ml (first assay); 0.302, 1.3, 6.7, 33.3, 100, 300, 600, and 1200 µg/ml (second - or confirmatory - assay). There is no indication within this report as to what the purity of the test article was (but this is adequately addressed in MRID 426837-01). The report states (p. 8 and 22) that "doses were corrected for purity of the test article;" this reviewer called Jellinek, Schwartz & Connolly, Inc. in order to get a clarification of this statement. The response made (after a consultation with the performing laboratory) was that these doses are expressed on the basis of Nabam content (so a reported dose of 0.302 µg/ml is equivalent to approximately 1 µg/ml of DN-30 Aquatreat).

B. TEST PERFORMANCE

1. Type of Salmonella assay:
- _X_ standard plate test
- ___ pre-incubation (___ minutes)
- ___ "Prival" modification (i.e. azo reduction method)
- ___ spot test

Protocol and Evaluation of Test Results: Refer to appended pages 2 through 5.

2. Preliminary cytotoxicity assay: Not done; from p. 8 "The doses in the mutagenicity study were selected based on the results of a previous Salmonella mutation assay performed on this test article.

3. Mutagenicity assay: Refer to appended pages 6 and 7. There was no doubling in the number of revertants at any dose level. At higher dose levels (300-1200 µg/plate) there were decreased numbers of revertants and, at 1200 µg/plate, a decrease in background lawn over at least part of the plates.

The positive control elicited an appropriate response.

The numbers of revertants in the vehicle controls in the presence of S9 mix, while rather low by this reviewer's standards, was within acceptable limits for this strain.

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4. On p. 3 there is a signed and dated "Good Laboratory Practice Compliance Statement." There is a Quality Assurance Statement on p. 4.

5. Reviewer's discussion/conclusions: The findings of this study adequately demonstrate that exposure to Nabam does not cause an increased number of revertants in S. typhimurium TA98 at the histidine locus in the presence of rat S9. When this information is combined with previously submitted material, the 84-2(a) gene mutation data requirement is satisfied for Nabam.

   Interestingly, the test laboratory has revised the acceptable range of revertants for TA98 from 15-50/plate (refer to Caswell document 008303) to 8-60/plate (refer to p. 37 of MRID 421512-01).

Nathan Revised

Page _____ is not included in this copy.

Pages 7 through 13 are not included in this copy.

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__✓__ FIFRA registration data.

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