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CASWELL FILE

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

007689

JAN 24 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Chemical (Caswell) No.: 585
RD Record No.: 253,252/253,253
HED Project No.: 9-2231/9-2232

MEMORANDUM

SUBJECT: Nabam - Mutagenicity Data Submitted under MRID Nos. 41177201 and 41221201
EPA ID No. TF-56478

FROM: Irving Mauer, Ph.D., Geneticist 01-02-90
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THRU: Karl P. Baetcke, Ph.D., Chief 1/18/90
Toxicology Branch I - Insecticide, Rodenticide Support
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Submitters: Jellinek, Schwartz, Connelly and Freshman, Washington, DC, representing Alco Chemical and other members of the Nabam Task Force (#56478)

Request

Review and evaluate the following mutagenicity studies submitted in response to data gaps identified in the Nabam Registration Standard (April 1987):

1. Mutagenicity Test on Aquatreat DN-30 (30% Nabam in Water) in the Rat Bone Marrow Cytogenetic Assay, performed by Hazleton Labs America (HLA), Kensington,

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MD, HLA #10645-0-452, Final Report dated July 3, 1989 (MRID No. 41177201; RD Record No. 253,252; HED Project No. 9-2231).

2. Mutagenicity Test on Aquatreat DN-30 (30% Nabam in Water) in the in vitro Transformation of Balb/C-3T3 Cell Assay with an S9 Activation System, performed by Hazleton Labs America (HLA), Kensington, MD; Final Report dated August 25, 1989 (MRID No. 41221201; RD Record No. 253,253; HED Project No. 9-2232).

TB Conclusions

Following are assessments of these studies (detailed reviews are appended to this memorandum):

Study	Reported Results	TB Conclusion
Chromosome damage in rat bone marrow cells	Negative for inducing structural aberrations in bone marrow cells of rats dosed orally up to 1200 mg/kg.	ACCEPTABLE
Cell transformation in vitro	Negative for inducing increased foci in Balb/C-3T3 cells under activation conditions (S9), up to toxic levels (140 to 160 ug/mL). No comparable assay without activation.	UNACCEPTABLE

Attachments (DERs)

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Reviewed By: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - IRS (H7509C)

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DATA EVALUATION RECORD

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I. SUMMARY

7/3/89

MRID (ACC) No.: 41177201
ID No.: TF-56478
RD Record: 253,252
Shaughnessy No.: 014503
Caswell No.: 585
Project No.: 9-2231

Study Type: Mutagenicity - Cytogenetics in vivo (Rat Bone Marrow)

Chemical: Nabam

Synonyms: Aquatreat® DN-30

Sponsor: Alco Chemical, Chattanooga, TN

Testing Facility: Hazleton Laboratories America (HLA), Kensington, MD

Title of Report: Mutagenicity Test on Aquatreat DN-30 (30% Nabam in Water) In the Rat Bone Marrow Cytogenetic Assay.

Author: James L. Ivett

Study Number: HLA 10645-0-452

Date of Issue: July 3, 1989

TB Conclusions:

Reported as negative for inducing chromosome aberrations when administered orally at single doses up to 1200 mg/kg test substance.

Classification (Core-Grade):

ACCEPTABLE as submitted.

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II. DETAILED REVIEW

A. Test Material - Aquatreat DN-30

Description: Clear, light-yellow liquid
Batch (Lot): 5243
Purity (%): 30
Solvent/Carrier/Diluent: Sterile deionized water (DW)

B. Test Organism - Rodent

Species: Rat
Strain: Sprague-Dawley
Age: 8 to 10 weeks
Weights - Males: 215.2 to 238.6 g
Females: 170.8 to 198.0 g
Source: Charles River, Raleigh, NC, and Harlan Inc., Frederick, MD

C. Study Design (Protocol) - This study was designed to assess the clastogenic (chromosome-damaging) potential of nabam when administered by oral gavage to Sprague-Dawley rats. A copy of the procedures employed is appended to this DER (from the investigator's FINAL REPORT), as ATTACHMENT A.

A statement affirming compliance with Agency GLPs was provided, as well as a Statement of Quality Assurance measures (inspections/audits).

D. Procedures/Methods of Analysis - Following dose range-finding assays, groups of male and female rats (5/sex/group) received test substance once by oral gavage (dose volume = 10 mL/kg in DW), and were sacrificed 6, 18, or 30 hours* later. (A secondary group of six males and five females was dosed at the HDT as replacements for any losses.) Control groups of five males and females each received 10 mL/kg DW only or cyclophosphamide (CP, 60 mL/kg) a proven clastogen in rats and sacrificed, respectively, 30 and 18 hours later.

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*To assure detection of all aberrations resulting from breaks occurring within the first cell cycle (normally 10 to 14 hours) following acute treatment, as well as those induced in cells delayed by treatment in their progression through the cell cycle.

Two hours before scheduled sacrifice, all animals were injected ip with the mitotic-arresting agent, colchicine (2 mg/kg), then killed by CO_2 asphyxiation. Bone marrow was collected and processed for metaphase chromosome analysis by conventional cytological techniques onto microscope slides. Coded (Giemsa-stained) slides were scored for the standard array of chromosomal aberrations in 50 metaphase spreads per animal, concurrently with determination of a mitotic index (number of cells in mitosis per 500 cells counted) as an indirect measure of cytotoxicity.

Data were collected for both number of structural chromosome aberrations (per cell, per animal), including (staining) gaps (which were counted but not included in statistical analyses), as well as numbers of cells with at least one structural change or with two or more such aberrations. Mean values from all test groups (male separately from female) were compared to that from DW controls by the Kruskal-Wallis Test, with alpha set at $p \leq 0.05$. A positive resonse was considered as a statistically significant dose-related increase in aberrations at three dose levels.

E. Results - Three dose rangefinding assays were performed (as reported in the Amendments to Study Protocol, included in ATTACHMENT A here), but the results of only the third trial were employed to select doses for the definitive study. In this final dose-selection trial, doses ranged from 1000 to 3450 mg/kg, administered once to groups of three males and three females each, which were observed for 30 hours postdose. The following mortalities were recorded in this trial:

Dose[1/]	Number Treated		Mortalities	
(mg/kg)	M	F	M	F
1000	3	3	0	0
1500	3	3	2	1
2000	3	3	2	3
2500	3	3	2	3
3000	3	3	3	3
3450	3	3	3	3

[1/]Of Aquatreat = 30 (30% ai)

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Except at 1000 mg/kg, all survivors at 72 hours, as well as those which died showed evidence of clinically adverse effects, which included languor, hunched-over demeanor and/or squint-eyed. Based on those results, the (combined) LD_{50} of this test article was calculated as 1614 (1216 to 1928) mg/kg (compared to the LD_{50} value of 810 mg/kg provided to the contractor by the sponsor). The dose levels selected for the definitive study were 120, 400, and 1200 mg/kg.*

In the main assay, clinical toxicity (diarrhea, lethargy) was observed only in the highest dose groups of the 18- and 30-hour harvest groups. However, compared to the large, significant increases 30 to 60 times greater than DW controls in chromosomally aberrant cells from CP-treated animals (Report Tables 1 and 8), no test article groups showed significant increases of aberration for either sex at any sacrifice time or dose level, nor significant cytotoxicity as determined by mitotic indices (recorded for individual test animals in Report Tables 2 through 7, and summarized in Report Tables 9 and 10 - the latter attached to this DER as ATTACHMENT B).

The author concluded that Aquatreat DN-30 (30% Nabam in Water) was not a clastogen in bone marrow cells of male and female rats under the conditions of this assay.

F. <u>TB Evaluation</u> - ACCEPTABLE.

* Calculated in terms of 100% a.i.

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ATTACHMENT A

Procedures

XVIII. STUDY PROTOCOL

Page _____ is not included in this copy.

Pages 9 through 35 are not included in this copy.

The material not included contains the following type of information:

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