CASWELL FILE

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DATA EVALUATION RECORD

007689

I. SUMMARY

MRID (Acc) No.: 41221201
ID No.: TS-56478
RD Record: 253,253
Shaughnessy No.: 014503
Caswell No.: 585
Project No.: 9-2232

Study Type: Mutagenicity - Transformation in vitro (Balb/C-3T3 Cells)

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 in vitro Transformation of Balb/C-3T3 Cell Assay with an S9 Activation System.

Author: Brian C. Myhr

Study Number: HLA 10645-0-488

Date of Issue: August 25, 1989

TB Conclusions:

Reported as negative for inducing cell transformation in the presence of different lots of rat liver metabolic activation (S9) at doses into the severely toxic range (140 to 160 ug/mL).

Classification (Core-Grade):

UNACCEPTABLE as a comprehensive evaluation of the transforming potential of nabam, since no companion assay under nonactivation conditions was performed, nor mentioned by reference.

II. DETAILED REVIEW

A. Test Material - Aquatreat DN-30 (30% Nabam in Water)

Description: Clear, pale yellow liquid
Batch (Lot): 5243
Purity (%): 30
Solvent/Carrier/Diluent: Eagle's Minimum Essential Medium (EMEM)

B. Test Organism - Established cell line

Species: Mouse
Strain: Balb/C-3T3/Clone 1-13
Source: Dr. T. Kakanuga, National Cancer Institute, Bethesda, MD

C. Study Design (Protocol) - This study was designed to assess the cell transformation potential of Aquatreat DN-30 when administered in vitro. 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 (inspection/audits).

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing (15 concentrations of test substance*, in twofold increments up to 11,500 ug/mL), cell cultures were exposed for 4 hours in the presence of a rat liver S9 mammalian metabolic activation system to eight concentrations of test article (18 cultures per test treatment), or to control substances (EMEM - 36 cultures), or to the mutagen dimethylnitrosamine (DMN - 18 cultures). An additional toxicity control was provided by exposure to the membrane cell poison, ouabain, of three mixed cultures consisting of normal (wild-type) Balb/C-3T3 cells (ouabain-sensitive) and a mutant cell clone resistant to ouabain.

After the initial 4-hour treatment, all test cultures were transferred to fresh (test article-free) EMEM and incubated for 4 weeks (with refeeding twice weekly). The clonal survival cultures were refed with EMEM containing 4 mM ouabain and harvested 8 to 11 days later.

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*Based upon the content of the ai, nabam, in Aquatreat DN-30 (30%) viz., 345 mg/mL.

At the termination of posttreatment incubation, coded cultures were fixed and stained (10% Giemsa), and examined by eye and both microscopy for enumeration of transformed foci of cells (according to published conventions established by experts), as well as for colony survival.

Since (as has been the published experience of expert practitioners for this assay) numerous foci may appear at random in both treated and control cultures, the performing lab has used a $\log_{10}$ mathematical conversion of assay data to deal with this non-normal distribution, following which Bailey's modification of Student's t-test was applied to evaluate any significant differences of test chemical treatment-transforming activity from negative control, with three levels for interpreting such statistical analysis:

For Individual Treatments

- Strong positive response, as represented by $p < 0.01$;
- Weak positive response, as represented by $p \leq 0.05$; and
- Negative response, as represented by $p > 0.05$.

Further, in order to establish what this lab considers sufficient evidence of transformation dose-responsiveness, two or more dose levels attaining the 95 percent confidence level are required, or responses achieving the 99 percent level ($p < 0.01$) for one or more test material treatments.

E. Results - In preliminary dose range-finding, nabam was severely toxic (7% cell survival relative to solvent control) at 180 ug/mL and lethal at the next dose level tested (359 ug/mL) and above (Report Table 1). Cytotoxicity decreased in a dose-responsive mannor below 180 ug/mL, and was at control levels at 11.2 ug/mL despite considerable variability between doses and trials. On the basis of these results, eight concentrations were selected for the main assay, ranging between 12 and 288 ug/mL in order to cover the survival range between 10 and 100 percent.

Of the five trials of the transformation assay begun, only the third and final experiments* yielded usable transformation data; the other trials were aborted due to excessive toxicity for the DMN positive control, or to mold contamination. Data from the two completed experiments (appended to this DER as ATTACHMENT B) were provided in six tabulations included in the FINAL REPORT, namely, for scoring results of transformed foci in each culture (Report Tables 6 and 7), by summary of each trial (Report Tables 2 and 3) and resulting statistically calculations (Report Tables 4 and 5).

The first reported trial was also plagued with mold contamination at the two lowest doses (12 and 29 ug/mL) as well as at the severely toxic dose (161 ug/mL), resulting in too few cultures for statistical evaluation; doses higher than 161 ug/mL were lethal. Cultures treated with nabam at the remaining three intermediate doses (58, 98, and 127 ug/mL) showed moderate dose-related cytotoxicity (respectively, 29, 34, and 49 percent survival), but no evidence of transformation (induced increases over the transformed control value in focus formation). The increase at 161 ug/mL (a total of 29 foci, resulting in a untransformed mean of 4.14 foci per culture) was due to a single culture containing 22 foci (see Table 6, attached), considered by the investigator as an outlier caused by "colony splitting during the refeeding process."

The second experiment employed an adjusted dose range, in an attempt to obtain more cultures in the toxic range that could still be evaluated for transformation (see Table 3, attached), plus a different lot of S9. This trial confirmed the negative result of the first trial in that at five doses bracketing the toxic level which could be evaluated (from 12 to 115 ug/mL, resulting in survivals ranging from 93 to 13 percent), i.e., no evidence for transformation was found (see Tables 5 and 7, attached).

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*Performed with different lots of S9, respectively, R201 from Microbiological Associates (34.5 mg/mL protein), and #0257 from Molecular Toxicology Inc. (41.9 mg/mL protein), but at the same concentration, 40 uL/mL in both assays.

Hence, the author concluded that, under the conditions of repeat assays, nabam (as Aquatreat DN-30) was negative for transforming Balb/C-3T3 cells over an inclusive dose range of 12 to 127 ug/mL (the latter approaching excessive toxicity) in the presence of rat liver S9 metabolic activation.

F. TB Conclusion - This study was conducted according to adequate procedures under appropriate control conditions to validate the negative results for transformation obtained, but only in the presence of a metabolic activation system. Hence, a comprehensive assessment of nabam in this type of assay is incomplete, because it lacks the submission of a companion study without activation (or mentioned even by reference).

Attachments

ATTACHMENT A

Procedures

Napam Revised

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