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

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APR 15 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: ADBAC [Alkyl Dimethyl Benzyl Ammonium Chloride]---
Company Response and Data Submitted Under MRID #
422908-01 and 422908-02

ID # 069105

Chemical: 016-I (069105)
RD Record: S-425912
HED Project: D182923

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I
Health Effects Division (H7509C)

03-29-93

~~TO:~~
THRU: Brian Dementi, Ph.D., DABT
Review Section III
Toxicology Branch-I
Health Effects Division (H7509C)

Brian Dementi 4/13/93

~~FOR:~~
TO: Larry Schnaubelt/Brigid Lowry, PM #72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I

Karl P. Baetcke 3/31/93

Registrant: ADBAC Quat Joint Venture (Huntington, Lonza, Mason, PPG, Sherex, and Stepan), submitted by the Chemical Specialties Manufacturers Association (CSMA), Washington, DC.

Request: Review and evaluate the following submissions from the registrant:

(1) Data from a mutagenicity assay, entitled:

Genotoxicity Test on Alkyl Dimethyl Ammonium Chloride (ADBAC) in the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures, performed at Hazleton Washington, Inc. (HWA), Vienna, VA, HWA Project #14778-0-447, Final Report dated April 15, 1992. (EPA MRID #422908-01)

(2) Addendum (dated April 15, 1992) to a previously submitted mutagenicity study (MRID # 422908-02), entitled:

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Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay, performed by Hazleton Labs. America (HLA), (HLA Study No. 10238-0-447), Report dated January 25, 1989 (EPA MRID No. 41012601), with Revision February 16, 1989,

which was judged UNACCEPTABLE for the following deficiencies: (DER attached to Memo: Mauer to Lee dated Oct. 13, 1989, HED Doc. # 007546):

(i) Repeat test required (to confirm initial negative).
(ii) Higher dose levels should be tested (up to demonstrable cytotoxicity).
(iii) The MP employed must be designated as the TGAI

(3) Another mutagenicity study, entitled:

Assessment of the Mutagenic Activity of Hyamine-3500 in the Mouse Micronucleus Test, performed by Scantox Biologisk Laboratorium A/S, Skensved (Denmark) for Lonza Inc., Fairlawn, NJ, Project #10753, Final Report dated December 16, 1985 (EPA MRID # 403111-01),

(4) Response to previous TOX-I review of the following mutagenicity study:

Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the CHO/HGPRT Foreward Mutation Assay, performed by Hazleton Labs., America (HLA), HLA Project # 10238-0-435, Final Report dated January 23, 1989 (EPA MRID # 41012701),

which was judged provisionally acceptable, pending receipt that the test article (designated 80% MP) was the formulation required by FIFRA regulations for generic testing (HED DOC #007546)

TB CONCLUSIONS:

ITEM (1): This genotoxicity (DNA damage/repair) assay (MRID #422908-01) is judged fully ACCEPTABLE in demonstrating negative results for UDS in primary rat hepatocyte cultures exposed up to cytotoxic concentrations, 6.46 ug/ml (see detailed review attached to this memo).

ITEM (2): The ADDENDUM (MRID #422908-02) provided acceptable supplemental information to the previously submitted Report judged UNACCEPTABLE, since

(i) Data from an adequate (ACCEPTABLE) repeat confirming the initial negative are available, as MRID 422908-01 (DER attached here).

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(ii) Cytotoxicity was demonstrated at non-genotoxic higher dosages (10 to 11 ug/ml).

(iii) The test substance employed was a homogenous composite of commercial grade (MP) materials from the six manufacturers participating in the ADBAC Quat Reregistration Program, and this 80% manufacturing-use product has been accepted by the Agency for generic testing to generate toxicology (as well as environmental fate, and wildlife) data (LETTER: Lee to CSMA, dated June 24, 1987).

ITEM (3): The mouse micronucleus assay (MRID #403111-01) is judged Provisionally ACCEPTABLE in demonstrating negative cytogenetic results in vivo at a dose adversely affecting erythropoiesis (i.e. cytotoxic), pending submission of data from the preliminary dose-selection investigations, as well as characterization of the test article.
ITEM (4): The proviso for fully accepting the CHO/HGPRT mutagenicity assay is removed (as stated above) by the acceptance by the Agency of the 80% MP for generic (TGAI) testing for the generation of toxicology data (Lee to CSMA, dated June 24, 1987).

ATTACHMENT: DER

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

03-22-93

3/31/93

DATA EVALUATION RECORD

MRID NUMBER No.: 40311101
PC No.: 069105
RD Record No.: S425912
EPA ID No.: 069105
Tox Chem. No.: 016-I
Project No.: D182923

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---Chromosome aberrations in vivo (Mouse MT)

CHEMICAL: ADBAC [alkyl dimethyl benzyl ammonium chloride]

SPONSOR: Lonza, Inc., Fairlawn, NJ (a member company in CSMA's ADBAC Quat Joint Venture)

TESTING FACILITY: Scantox, Skensved (Denmark)

TITLE OF REPORT: Assessment of the Mutagenic Activity of Hyamine-3500 in the Mouse Micronucleus Test

AUTHOR(S): Th Kallersen

STUDY NUMBER: 1075.3

DATE ISSUED: December 16, 1985

CONCLUSIONS: Negative for inducing micronuclei in polychromatic erythrocytes of mice treated at a singular cytotoxic dose (400 mg/kg).

TB-I EVALUATION: Provisionally acceptable; detailed data from the preliminary dose-selection study needs to be submitted.

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II. DETAILED REVIEW

A. TEST MATERIAL: Hyamine-3500

Description: (Liquid)
Batches (Lots): L-5383
Purity (%): 80%
Solvent/carrier/diluent: Distilled water (DW)

B. TEST ORGANISM: Rodent

Species: Mice
Strain: NMRI (SPF)
Age: 6-7 weeks
Weights - males/females: 25-30 g
Source: Gl. Bomholtgard Ltd., Ry (Denmark)

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the clastogenic potential of the test article in bone marrow cells when administered to mice, and the extent of micronucleus formation determined in polychromatic erythrocytes, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following "preliminary investigation" (for dose selection[1]), groups of mice (5/sex/group) were dosed once by oral gavage with 400 mg/kg test article, and sacrificed 24, 48 and 78 hours later. Two other groups (5/sex/group) were given equal volumes of either the diluent (DW) as solvent control, or the mutagen/clastogen, cyclosphosphamide (CP, 30 mg/kg) as positive control, and sacrificed at 24 hours.

Femoral bone marrow of sacrificed animals were prepared as cell smears on microscope slides, fixed in methanol and stained with May-Gruenwald/Giemsa. The following data were collected from these slide preparations:

Number of normochromatic erythrocytes (NCE) per 1000 erythrocytes.

Number of polychromatic erythrocytes (PCE) per 1000 erythrocytes.

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[1]Detailed data were not included in this Final Report.

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Number of micronuclei (MN) in 1000 normochromatic erythrocytes.

Number of micronuclei (MN) in 1000 polychromatic erythrocytes.

These results were analyzed by one-way ANOVA and/or non-parametric methods after transformation to normalized scores.

E. RESULTS: From preliminary dose-selection testing (only) summarized in this Report, the MTD was estimated at 400 mg/kg, since PCE/NCE ratios were significantly reduced; above 400 mg/kg "...mortality was too high." [As stated on p.8 of the Report].

In the main assay, one mouse of the 72-hour group was found dead on the third day after treatment. The PCE/NCE ratio was reduced in all test groups (as well as the positive controls), but no alteration in MN from solvent control value was found in any timed test group (Report Tables 1-3, attached here).

By contrast, the CP-group evidenced a significant increase in MN-PCE.

The investigator concluded that ADBAC was non-mutagenic in the mouse micronucleus test as performed in the lab.

F. TB EVALUATION: Provisionally acceptable as demonstrating no clastogenesis in bone marrow cells treated at a sufficiently high (cytotoxic) singular dose, pending submission (for the record) of detailed data from the preliminary dose-selection study.

ATTACHMENT: Data Tables

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40311101:Disk 6:MAUER:MB:3/19/93

Page ___ is not included in this copy.

Pages 9 through 11 are not included in this copy.

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_____ Description of quality control procedures.

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_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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

03-19-93

4/15/93

DATA EVALUATION RECORD

MRID NUMBER No.: 422908-01
PC No.: 069105
RD Record No.: S425912
EPA ID No.: 069105
Tox Chem. No.: 016I
Project No.: D182923

I. SUMMARY

STUDY TYPE: (84-4) Mutagenicity -- DNA damage/repair in vitro (HPC/UDS)

CHEMICAL: ADBAC [alkyl dimethyl benzyl ammonium chloride]

SPONSOR: ADBAC Quat Joint Venture/CSMA, Washington, D.C.

TESTING FACILITY: Hazleton Washington (HWA) Inc., Vienna, VA

TITLE OF REPORT: Genotoxicity Test on Alkyl Methyl Ammonium Chloride (ADBAC) in the Assay for Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures

AUTHOR: Marie E. McKeon

STUDY NUMBER: HWA #14778-0-447

DATE ISSUED: April 15, 1992

CONCLUSIONS: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocytes (HPC) exposed in vitro up to cytotoxic doses (6.46 ug/ml).

TB-I EVALUATION: ACCEPTABLE

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II. DETAILED REVIEW

A. Test Material: ADBAC

Description:	Clear yellow liquid
Batches (Lots):	7293K
Purity (%):	80%
Solvent/carrier/diluent:	Sterile Deionized Water (DW)

B. Test Organism: Mammalian primary hepatocytes

Species:	Rat
Strain:	Fischer 344
Weights - females (only):	190.4 g
Source:	Harlan Sprague Dawley, Inc.

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the genotoxic (DNA-damaging) potential of the test article to enhance unscheduled DNA synthesis (UDS), when administered in vitro to primary rat hepatocyte cultures (HPC), and measuring nuclear silver grain counts (an indication of repair synthesis), according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Monolayer (coverslip) hepatocyte cultures, established from cells obtained by perfusion of the liver in situ (from a single adult female F-344 rat), were exposed for 18.8 hr to tritiated thymidine (10 uCi/ml 3HTdr, of spec. act.= 48Ci/mMole), together with a graded series of 14 concentrations of the test article. In addition to a solvent (DW) control, other cultures were treated with the mutagen, 2-acetylaminofluorene (AAF, 4.48 x 10-7 M = 0.10 ug/ml). Each treatment was performed on five cultures, two of which were used for cytotoxicity measurements (by trypan blue exclusion). The remaining three cultures per treatment were re-fed with fresh medium containing 1 mM unlabeled thymidine, then exposed to 1% (hypotonic) sodium citrate (to swell cells), fixed in Carnoy's Fluid (acetic acid: ethanol::1:3), and finally mounted (cell side out) on standard glass microscope slides. The slides were dipped in liquid photographic emulsion (Kodak NTB-2),

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and stored in light-tight boxes at 4°C for 7 days. After storage, the preparations were developed in standard photographic fluids (D19), followed by staining with modified H and E.

Net nuclear silver grains (NNGC) were calculated from crude nuclear counts less the background of cytoplasmic counts. Mean NNGC was determined from the triplicate cover slips per treatment (150 total nuclei per treatment). A minimum of 6 dose levels (of the 14 initiated) were analyzed for UDS, according to strict sets of (accepted) criteria for assay acceptance, dosage range, and evaluation of response (positive/negative/"equivocal")

E. RESULTS: The test article was lethal at 8.61 ug/ml, and progressively cytotoxic at doses equal to or above 4.31 ug/ml (Report Table 1, attached here). Treatments producing moderate levels of cytotoxicity (62.7% relative survival and greater) as well as non-toxic doses were analyzed for UDS. None of these analyzable treatments induced silver grain labeling different from solvent control. In contrast, the mutagen 2-AAF induced large increases in nuclear labeling in the absence of significant toxicity.

The investigator concluded that ADBAC was negative for genotoxicity (repair) in primary rat liver hepatocyte cultures.

F. TB EVALUATION: ACCEPTABLE

ATTACHMENTS: Data Tables

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DER/MAU/4229081.IM/Jacqueline/Disc 1

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