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

007546

OCT 13 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ADBAC (Alkyl dimethyl benzyl ammonium chloride) Toxicology Data Submitted Under MRID Nos. 41012601 and 41012701 in Response to the Registration Standard EPA ID (Not Provided by RD)

TOX Chem No.: 16I
TB Project No.: 9-1027A (9-0063)
RD Record No.: (Not provided by RD)

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

TO: John H. Lee, PM 31
Antimicrobial Program Branch
Registration Division (H7505C)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: Chemical Specialties Manufacturers Association (CSMA), acting for the ADBAC Quat Joint Venture, Washington, DC.

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Request

Review and evaluate the following two mutagenicity studies, both performed by Hazleton Laboratories America (HLA), Kensington, MD:

1. Mutagenicity Test on Alkyl dimethyl benzyl ammonium chloride (ADBAC) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay (HPC/UDS), HLA Project No. 10238-0-447, Final Report dated January 25, 1989 (EPA MRID No. 41012601).
2. Mutagenicity Test on Alkyl dimethyl benzyl ammonium chloride (ADBAC) in the CHO/HGPRT Forward Mutation Assay, HLA Project No. 10238-0-435, Final Report dated January 23, 1989 (EPA MRID No. 41012701).

TB Conclusions

Study	Reported Results	TB Evaluation
1. HPC/UDS	Reported negative in a single test up to a questionably toxic dose (manufacturing-use product [MP] tested).	UNACCEPTABLE
2. CHO/HGPRT	Negative for inducing forward mutation at a dose range producing severe toxicity, both with/without activation (MP tested).	Provisionally ACCEPTABLE

ATTACHMENT (DEC)

Reviewed By: Irving Mauer, Ph.D., Geneticist, 9/25/89
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 10/6/89
Toxicology Branch I - IRS (H7509C)

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DATA EVALUATION REPORT

I. SUMMARY

MRID (Acc.) No.: 41012701
ID No.: (Not given)
RD Record: (Not given)
Shaughnessy No.: 069105
Caswell No.: 16I
Project No.: 9-1027A
(9-0063)

Study Type. Mutagenicity - gene mutation in mammalian cells *in vitro* (CHO/HGPRT).

Chemical: ADBAC (alkyl dimethyl benzyl ammonium chloride)

Sponsor: CSMA, Washington, DC

Testing Facility: Hazleton Labatories America, Kensington, MD

Title of Report: Mutagenicity Test on Alkyl Dimethyl Benzyl American Chloride (ADBAC) in the CHO/HGPRT Forward Mutation Assay.

Author: Robert R. Young

Study Number: HLA No. 10238-0-435

Date of Issue: January 23, 1989

TB Conclusions:

Reportedly negative for inducing forward mutation at the HGPRT locus of CHO cells exposed in the presence and absence of a mammalian metabolic activation system (rat S9), up to excessively toxic concentrations.

Classification (Core-Grade):

Provisionally ACCEPTABLE pending receipt from registrant that the test article (designated "80% MP") is the form required by FIFRA regulations for generic testing.

II. DETAILED REVIEW

A. Test Material - ADBAC - Quat 80% Composite (from CSMA)

Description: Slightly viscous, clear yellow liquid
Batch (Lot): 7293 K
Purity (%): 80 (said to be the "MP")
Solvent/Carrier/Diluent: Deionized water

B. Test Organism - Established cell line

Species: Chinese Hamster Ovary (CHO-K1)
Rat Strain: BH_4
Source: Dr. A.W. Hsie (Oak Ridge National Laboratory, TN)

C. Study Design (Protocol) - This study was designed to assess the forward mutagenic potential (production of HGPRT mutants) of ADBAC when administered *in vitro* to CHO-K1 cells. A copy of the procedures employed is appended to this DER (from the investigator's FINAL REPORT - ATTACHMENT A of this DER).

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 - Cells cultures cleansed of spontaneous $HGPRT^-$ (hypoxanthine-guanine phosphosphoribosyl transferase-deficient) mutants were first tested for cytotoxicity (over a 10-dose range of 1 to 5000 ug/mL), then exposed for 4 hours to test article (one culture per dose level), both in the absence and presence of a mammalian metabolizing activation system consisting of the S9 supernatant fraction of liver enzymes from S-D rats pretreated with Aroclor 1254, plus CORE, NADP energy-producing cofactors (=S9 Mix). Following treatment, cells were refed with fresh medium and incubated for 7 days (mutant "expression period"), with intermittant subculturing every 2 to 3 days to maintain logarithmic growth. At the end of the expression period, cultures were reseeded in a medium containing the selection agent (the DNA base analog, 6-thioguanine, TG) which permits survival of only $HGPRT^-$ mutants (TG-resistant). After 7 to 10 days selection, cultures are fixed and stained (Giemsa). Two separate trials with ADBAC were conducted.

The mutagens, 5-bromo-2-deoxyuridine (BUdR) and 3-methylcholanthrene (3-MC) served as positive controls in both experiments for, respectively, the nonactivation and S9 supplemented series.

The following assay parameters were analyzed by the Kastenbaum and Bowman* tables [which provide Poisson probabilities for significant differences at both the 95 percent ($p < 0.05$) and 99 percent ($p < 0.01$) confidence levels]:

1. Relative Cell Survival (RCS, %)

$$= \frac{\text{Av. treated colonies}}{\text{Av. no. control colonies}} \times 100$$

2. Relative Population Growth (RPG, %)

$$= \frac{\text{Av. incr. in treated cell popu.}}{\text{Av. incr. in control cell popu.}} \times 100$$

3. Absolute Cloning Efficiency (ACE, %)

$$= \frac{\text{Av. no. viable colonies/dish}}{200} \times 100$$

4. Mutant Frequency (MF)

$$= \frac{\text{Total mutant clones}}{\text{No. dishes} \times (2 \times 10^5 \times \text{ACE})}$$

The laboratory provided the following criteria for accepting a completed assay for evaluation:

1. The mean ACE of (negative) controls should be preferably above 70 percent, but never below 50 percent.

2. Background (vehicle control) MFs from this lab's experience with the assay range between 0 and 10×10^{-6}; MFs greater than 15×10^{-6} are suspect of confounding (adventitious) factors.

3. Positive control MFs should be significantly ($p < 0.01$) elevated over concurrent negative controls. (An assay can be acceptable when a positive control is missing or lost, but only if the test article is clearly genetically active.)

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*Kastebaum, M.A. and Bowman, K.O.: Tables for determining the statistical significance of mutation frequencies. Mutation Res. 9:527-549, 1970.

4. Test article concentrations should include those providing up to 90 percent toxicity (10-15% clonal survival relative to negative controls), or at least 75 percent of "an excessively toxic concentration" (according to the author).

5. At least eight mutant selection dishes and two cloning efficiency dishes must provide MF data.

6. At least five concentrations of the test article should be tested, but no less than three.

For a test substance to be considered positive, all of the following evaluation criteria should be met:

1. An MF significantly different ($p < 0.05$) than control as determined from the Kastenbaum and Bowman tables, but must be at the same time greater than 15×10^{-6}.

2. A dose-related (or toxicity-related) increase in MF, preferably over at least three sequential concentrations.

3. An increase in MF but only at a single (high) dose close to the toxic level which is more than twice the value indicating a significant response.

4. Relations between MF and toxicity must be carefully interpreted.

E. Results - The preliminary rangefinding assay in nonactivation cultures showed that ADBAC was lethal at 20 ug/mL and higher, but only moderately cytotoxic (53% relative cell survival, RCS) at the next lower dose, 10 ug/mL. In S9-supplemented cultures, ADBAC caused total cell killing at 50 ug/mL and above, but was nontoxic at the next lower dosage, 20 ug/mL and below (Report Table 1 and 2, Figure 1). From these results, 10 doses were selected for the first mutation trial (1 to 65 ug/mL, -S9; 1 to 100 ug/mL, +S9).

As predicted from the rangefinder, the test article caused 100 percent toxicity at 20 ug/mL in the first nonactivated assay and RCS's of < 10 percent at 16 ug/mL, about 45 percent at 13 ug/mL and approximately 85 percent at 10 ug/mL, but was nontoxic below 10 ug/mL (Table 3, appended to this DER as ATTACHMENT B). None of the five treatments evaluated, however, induced MFs greater than the concurrent vehicle control (7.2×10^{-6}). In the collateral series using metabolic activation, ADBAC caused severe toxicity (< 10% RCS to lethality) at 30 ug/mL and higher, but was relatively

nontoxic at 20 ug/mL and below (Report Table 5, also appended here). As with the nonactivation series, none of the five surviving concentrations induced an MF different from background (6.9×10^{-6}).

For the independent repeat test (Trial II), slightly narrower dose ranges were chosen; 1 through 24 ug/mL for the nonactivated series, and 10 through 50 ug/mL, +S9. Similar cytotoxicity patterns to Trial I were encountered in this second experiment, significant dose-related decreases in cell survivals becoming evident without activation at 10 ug/mL (with insufficient cells to evaluate above 20 ug/mL), but in the presence of S9 only at 28 ug/mL and above, and near-lethality encountered at the HDT, 50 ug/mL (Report Tables 4 and 6, also appended here). In this trial, however, while MFs in activated cultures treated with ADBAC varied randomly with dose and toxicity within the negative control range of values (Table 6), statistically significant increases in MF over the combined concurrent controls (5.6×10^{-6}) were recorded in nonactivated cultures treated at the upper range of toxic doses (see Table 4), namely, 12.9 at 18 ug/mL ($p < 0.01$), 11.5 at 20 ug/mL ($p < 0.05$), and 14.6 at 24 ug/mL ($p < 0.01$). The author considers these higher-than-background values to reflect normal assay variation at cytotoxic levels, since they were within the acceptable range of variation for vehicle control MFs for this lab (0 to 14×10^{-6}), and were not found in Trial I at equitoxic dose levels.

Therefore, the author concluded that ADBAC was nonmutagenic in the presence and absence of metabolic activation, as determined from forward mutation at the HGPRT locus in CHO cells exposed to levels causing excessive toxicity.

TB EVALUATION

In all respects save one, this study would satisfy FIFRA data requirements for mammalian cell mutation, since it was performed in a scientifically adequate manner with appropriate controls, such that the negative results generated in repeat trials may be considered valid. The one reporting deficiency withholding a judgment of ACCEPTABLE lies in the test material employed, stated in the contract lab report as the "80 percent MP of the ai"; since testing for mutagenicity under FIFRA section 158.340 requires the technical grade of the active ingredient (TGAI), the registrant must provide assurance that the product tested here is the form of the substance so designated by FIFRA regulations.

Attachments

ATTACHMENT A

Procedures

Page ___ is not included in this copy.

Pages 9 through 25 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed By: Irving Mauer, Ph.D., Geneticist, 9/25/89
Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 10/6/89
Toxicology Branch I - IRS (H7509C)

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DATA EVALUATION RECORD

I. SUMMARY

MRID (Acc.) No.: 41012601
ID No.: (Not given)
RD Record: (Not given)
Shaughnessy No.: 069105
Caswell No.: 16I
Project No.: 9-1027A
(9-0063)

Study Type: Mutagenicity - DNA damage/repair *in vitro* (Rat HPC/UDS)

Chemical: ADBAC (alkyl dimethyl benzyl ammonium chloride)

Sponsor: CSMA, Washington, DC

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

Title of Report: Mutagenicity Test on Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay.

Author: Maria A. Cifone

Study Number: HLA No. 10238-0-447

Date of Issue: January 25, 1989

TB Conclusions:

Reportedly negative in a single test up to 6.37 ug/mL, a concentration said to produce minimal cytotoxicity.

Classification (Core-Grade): UNACCEPTABLE

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

II. DETAILED REVIEW

A. Test Material - ADBAC - Quat Code No. 349006 007546

Description: Clear, pale-yellow liquid
Batch (Lot): 7293 K
Purity (%): 80 (stated to be the MP)
Solvent/Carrier/Diluent: Deonized Water (DW)

B. Test Organism - Primary hepatocyte cultures

Species: Rat
Strain: Fischer-344
Age: "Adult" (males)
Source: Charles River Breeding Labs

C. Study Design (Protocol) - This study was designed to assess the genotoxic potential of ADBAC when administered to rat hepatocytes in vitro for determination of unscheduled DNA synthesis (UDS). 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. A Statement of Quality Assurance measures (inspections/audits) was also provided.

D. Procedures/Methods of Analysis - Following preliminary cytotoxicity testing (doses of 1 to 5000 ug/mL), hepatocytes obtained by perfusion from an adult male Fischer-344 rat were established as monolayer cultures (HPC) on plastic coverslips in dishes containing the standard supplemented growth medium, WME, (Williams E, with 50% FCS added, as well as 2 mL of L-glutamine, and 2.4 uM dexamethasome, plus antibiotics), and exposed to graded doses of test substance (5 cultures each dose) together with 5 uCi/mL tritiated-thymidine (^{3}H-TdR, 20 Ci/m mole). After 20 hours incubation, cultures were harvested and processed for the determination of UDS (as given below). The mutagen, 2-acetylaminofluorene (AAF, 4.48×10^{-7} M = 0.10 ug/mL), known to induce UDS in rat HPC, served as positive control.

Two of the five cultures per treatment were used to monitor toxicity, by trypan blue exclusion for cell viability.

The cells in the remaining three cultures per treatment were swollen in 1 percent sodium citrate, fixed in methanol:acetic acid, and dried for 24 hours. The coverslips were then mounted onto standard microscope slides (cell side up), coated with a photographic emulsion

(Kodak NTB-2), and stored at 4 °C in light-tight slide boxes for 7 to 10 days. The emulsion-covered slides were then developed (Kodak D-19), fixed, and stained with modified H&E.

Fifty cells on coded slides per treatment were scored under oil immersion for nuclear and background (cytoplasmic) silver grains, and this measure of UDS was expressed as net nuclear grain count (NNGC). Only cells with normal morphology were selected for grain counting. The few nuclei so blackened with grains as to be too numerous to count (TNTC) were considered cells in replicative ("scheduled") DNA synthesis rather than undergoing repair ("unscheduled") synthesis, and were excluded from data processing. Mean NNGCs from the three coverslips per treatment (totals of 150 nuclei each), as well as mean percentage of cells with six or more net nuclear grains, and mean percentage of cells having 20 or more net nuclear grains were tabulated, as were relative cell survivals from the two toxicity slides per treatment.

No statistical treatment of the data generated in this study was performed; rather the authors relied upon published results and their own lab experience to evaluate the genotoxic (UDS) potential of the test article.

The author considers a UDS assay acceptable for evaluation if all of the following criteria are met:

1. The initial perfused hepatocyte viability must preferably exceed 70 percent, but be not less than 50 percent.

2. Monolayer cell culture viability before treatment must also exceed 70 percent, preferably approach 90 percent.

3. Mean cell viability after treatment should not be less than 50 percent of the cell number at the initiation of treatment.

4. Mean NNGC in negative or solvent control must be in the range -5 to 1, and no more than 10 percent of cells should contain six or more grains, nor 1 percent of control cells have 20 or more grains. This laboratory reports that the mean negative control NNGC is -0.2, with approximately 1.3 percent of nuclei having 6+ grains, but no nuclei with 20+ grains.

5. Postive controls must be clearly responsive for UDS, e.g., 0.1 ug/mL AAF typically induces mean NNGC of 13, with 83 percent of nuclei having 6+ grains, and over 20 percent having 20+ grains.

6. A minimum of two replicate test cultures with at least 50 cells each must provide grain count data.

7. A minimum of six dose levels of the test substance must be represented; repeats are only necessary to achieve this minimum number of concentrations.

8. The HDT must produce cytotoxicity, as demonstrated by altered cell morphology, or be at the level of insolubility (in culture medium), or be the limit dose of 5000 ug/mL (or 5000 nL/mL).

For a substance to be considered positive by this laboratory, it must:

1. Increase mean NNGC to at least six above the concurrent negative control value, and/or
2. Induce at least 10 percent of nuclei with 6+ net grains, and/or

3. Increase the percent of nuclei with 20+ grains to 20 percent or more.

Also considered desirable for genetic activity (but not essential) are a dose-related increase in UDS over at least two consecutive concentrations with increasing toxicity at higher dose levels. The author considers an isolated increase at a treatment level far removed from toxic doses as "spurious" (biologically irrelevant).

E. Results - Only one trial of the UDS assay with the test article was performed at doses ranging from 0.319 to 10.6 ug/mL. ADBAC was lethal at concentrations above 8.5 ug/mL and excessively toxic at 8.5 [Data not shown in the Final Report, as acknowledged by the authors]. Toxicity was minimal at the next lowest dose, 6.37 ug/mL (providing 98+% cell survival), but morphology of many cells was ". . . slightly different from the solvent control cells" [NB: This alteration, however, was not further defined in the Report.]

Therefore, 6.37 ug/mL ADBAC was the top dose analyzed for UDS, plus five lower doses [see summary tabulation appended to this DER as ATTACHMENT B]. None of these treatments produced nuclear grain counts significantly

different from the solvent control, nor was a dose-related trend evident. By contrast, the positive control, AAF was genetically active, inducing a 20-fold increase over the solvent value in grain count, with the vast majority of cells containing 6+ grains and over 35 percent with 20+ grains.

The authors concluded that ADBAC did not induce UDS in rat hepatocytes, as measured by nuclear labeling in autoradiographic preparations (silver grain counts).

TB EVALUATION

This study appears to satisfy data requirements for the DNA damage/repair component ("other genotoxic effects") of mutagenicity testing. However, assessment of this test must be deferred until several deficiencies are satisfied, most importantly:

1. An adequate repeat of the entire experiment, preferably with hepatocyte cultures established from a female rat, in order to confirm the initial negative. This report should include concentrations above the highest so far evaluated for UDS (i.e., 6.37 ug/mL), in order to exclude the induction of any positive responses up to levels of demonstrable cytotoxicity.

2. Since mutagenicity testing with the TGAI (technical grade of the active ingredient," viz., alkyl dimethyl-benzyl ammonium chloride) is required by FIFRA section 158.340, assurance must be provided by the registrant that the article tested in this study (identified as the "80% manufacturing-use product") is the form of the substance so designated by FIFRA regulations.

Attachments

ATTACHMENT A

Procedures

Page ___ is not included in this copy.

Pages 32 through 59 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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