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

008033

JUL 12 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Identification Nos: 6836-18, 90-Day Feeding Study in Dogs and 6836-53, Mutagenicity Studies with a Quaternary Ammonium Sanitizer-BARDAC®22

Tox Chem No.: 331A
Record No.: 202248
HED Project Nos.: 9-1193; 9-1193A

FROM: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 10/17/89
Review Section I
Toxicology Branch I - IRS
Health Effects Division (H7509C)

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

THRU: Roger Gardner, Acting Section Head
Review Section I Roger Gardner 7-2-90
Toxicology Branch I - IRS
Health Effects Division (H7509C)

You will find appended the Data Evaluation Reports (DERs) for the dog subchronic feeding study and three mutagenicity studies on BARDAC®22. The conclusions reached in these reviews are summarized as follows:

1. Subchronic feeding study in the dog.

   This study is classified Core supplementary pending receipt of the following information from the registrant.

   A) methods and results of statistical treatment of the data;

   B) summary table for food consumption data;

   C) statement enumerating organs that were evaluated histopathologically;

   D) advise as to whether a determination of platelet count or clotting time was undertaken;

E) submit historical control data, male and female, on kidney focal-and peripelvic focal lymphocytic aggregates.

Interim conclusions are that hematological, clinical chemistry and urinalysis data indicate no adverse effects at any dose level. For male dogs, the overall study NOEL=15 mg/kg/day based upon decreased body weight gain and the occurrence of "lymph node: blood in sinusoids" at 50 mg/kg/day (the highest dose tested). To facilitate determination of the LEL/NOEL in female animals, the registrant should submit the requested information on kidney lymphocytic aggregates.

2. Salmonella/Mammalian-Microsome Assay

The reviewer of this study concluded that the test material could be evaluated only up to a limited dose level (125 mg/plate) in Salmonella. Though not mutagenic at that does, the study is considered Core-inconclusive since the test organism was inappropriate for assaying the test article, and otherwise unacceptable because of major deficiencies with respect to current Agency regulations. The reviewer does not indicate any means of upgrading the study.

3. Analysis of metaphase chromosomes obtained from bone marrow of rats treated with the test material.

The Tox Branch reviewer of this study concluded the study to be unacceptable due to major procedural and reporting deficiencies. The reviewer recommended repeating the study employing i.p. injection of test compound using at least three single dose levels with timed sampling (up to 72 hours) in addition to a repeat dose schedule (e.g. 5 days) at the (best) effective dose found in acute testing.

4. Chromosomal aberrations assay with Chinese hamster ovary cells in vitro.

The reviewer concluded that the study cannot be reviewed because the final report is incomplete (missing pages). The registrant should be advised to submit the complete study.

Attachments

Reviewed By: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 9/14/89
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Edwin Budd, Section Head
Section I, Toxicology Branch I - IRS (H7509C) Budd 9/29/89

008033

DATA EVALUATION REPORT

Study Type: Subchronic Toxicity (90-Day) Dog Study

HED Project No.: 9-1193 TOX Chem No.: 331A

MRID No.: 402629-01

Test Material: BARDAC® 22

Synonym(s): Quaternary Ammonium Sanitizer; Didecyl Dimethyl Ammonium Chloride

Study Number: 2224a

Sponsor: Lonza, Inc.
Fair Lawn, NJ

Testing Facility: Food and Drug Research Laboratories, Inc.

Title of Report: 90-Day Feeding Study in Dogs With A Quaternary Ammonium Sanitizer BARDAC® 22.

Author: David E. Bailey, Ph.D.

Report Issued: April 7, 1975

Classification: Core-Supplementary

Conclusions:

May be considered for upgrading to Core-Minimum upon submission of the following:

1. Methods and results of statistical treatment of the data;

2. Summary table for food consumption data;

3. Statement enumerating organs that were evaluated histopathologically;

4. Data obtained in the study on platelet count or clotting time, if available;

5. Historical control data, both sexes, on the incidence of kidney focal- and/or peripelvic focal lymphocytic aggregates.

Hematological, clinical chemistry, and urinalysis data indicate no adverse effects at any dose level. Certain hematological and clinical parameters specified in the Guidelines evidently were not assayed. These included platelet count or measure of clotting time, serum calcium, phosphorus, albumin and creatinine. The registrant should be asked if platelet count was determined and, if so, submit the data. These particular deficiencies, however, should not preclude the study being rated Core Minimum when the requested information has been received and evaluated.

For male dogs, the overall study NOEL = 15 mg/kg/day based upon decreased body weight gain at 50 mg/kg/day (the highest dose tested) and upon the occurrence of "lymph node: blood in sinusoids," also at the high dose in male dogs.

To facilitate determination of the LEL/NOEL in females animals, the registrant should submit the requested information on kidney lymphocytic aggregates.

4

Special Review Criteria

A. Materials

1. Test Compound: BARDAC®-22 (a.i. - didecyl dimethyl ammonium chloride)

Description: Clear light yellow liquid

Lot No.: B-2754

Purity: 50% Didecyl dimethyl ammonium chloride [redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

2. Test Animals: Adult Beagle Dog

Age: Not indicated

Weight: 7 to 10 kg

Source: Food and Drug Research Labs, Waverly Division

B. Study Design - (The following is quoted or paraphrased from the study, pages 6 and 7, and the protocol, pages 33 and 34)

"For the purpose of this study, 32 beagle dogs (16 dogs of each sex) weighing 7 to 10 kg were selected from the Food and Drug Research Laboratories colony and individually housed in metal cages in temperature and humidity controlled laboratory facilities. All dogs had been immunized and treated for parasites and were free from visible defects. Four males and four females were assigned to each of the study groups, consisting of vehicle control and dosed groups of 5.0, 15.0, and 50.0 mg/kg/day. Following a housing period of several weeks, during which time pretest procedures specified in the protocol, including ophthalmological examinations, were performed, the exposure to the test material was initiated.

"The test material was incorporated into the diet (Purina Dog Chow) as an acetone solution prepared at appropriate concentrations for each dosage level. After thorough mixing in a mixer, the diet was air dried. The control diet was sham prepared in the same manner. The dried diet was fed to each dog once daily, six days per week, and any food not consumed within 1 hour was removed from the cage and weighed to provide a record of food consumption.

5

"All hematologic, biochemical and urinary examinations as required were performed once prior to the initiation of test material administration and after 30 and 90 days of treatment. During the 13th week, the animals were sacrificed by an overdose of sodium pentobarbital (i.v.) and subjected to postmortem examination for any evidence of gross pathological changes. The fresh weights of liver, kidneys, heart, thyroids, adrenals, spleen and gonads were recorded. Specimens of tissues and organs of all animals were fixed in formalin and selected tissues were processed histologically for microscopic examination of the H and E stained sections."

The protocol indicates (page 37) that statistical evaluations of the data will be performed on body weights, food consumption, clinical values, etc. However, there is no evidence in the body and tables of the write-up for the study that statistical analyses were performed.

C. Methods and Results

1. Clinical Observations

Although no summary table was developed in the study for clinical observations, Table 7 (page 24) presents individual clinical and/or gross findings. Inspection of the table does not reveal, in the opinion of this reviewer, any dose-related clinical findings for either sex. All dogs of both sexes survived to terminal sacrifice.

2. Body Weight/Food Consumption

Body weight data (Table 2, page 11) indicate that for the 13-week study period, males and females of the high-dose group gained less weight than did the controls (net male weight gains were: 3.0, 1.4, 1.7, and 0.2 kg for the control-, low-, mid-, and high-dose groups, respectively. For the same respective groups, females had mean gains of 1.4, 1.5, 1.3, and 0.1 kg). In terms of the overall weight gains for the 13-week period, it appears that both males and females suffered a weight gain deficit at the high dose. However, an inspection of the data for weight gains which occurred between various time points during the course of the study revealed that for males and females, a principal portion of the deficit occurred during the first 2 weeks of dosing, which could be indicative of a palatability problem. Males, but not females, exhibited another remarkable deficit at the high dose during the last 3 weeks of dosing which may or may not have been a meaningful

6

effect of the test material. The study author acknowledges a palatability problem occurring early in the study, which led the investigators to add garlic to the diet in an attempt to improve palatability. The study report does not provide a summary table presenting mean values for food consumption analogous to that presented for body weight data. However, inspection of individual food consumption data (Appendix III, page 45) clearly reveals for both males and females a compromise in food consumption for the high-dose group during at least the first several days of the study. This supports the view of poor palatability. Hence, there is no convincing argument based upon body weight changes that there was a toxic manifestation of the test material. Nevertheless, to be conservative, the high dose should be considered as an effect level based upon reduced body weight gain in both sexes at this dose.

In order to enable an adequate assessment of the food consumption data, the registrant should be advised to submit a summary table for the data analogous to that for mean body weight data for dogs (Table 2, page 11).

3. Ophthalmological Examinations

Ophthalmological examinations were performed on each dog pretest and prior to terminal sacrifice. The examinations did not disclose any compound-related effects, as affirmed by Stephen Bistner, D.V.M. (page 39).

4. Laboratory Investigations

Blood and urine were collected before treatment and at 30 and 90 days for hematology and clinical analysis.

a. Hematology - The hematology parameters assayed were hemoglobin, erythocytes, white blood cell count, hematocrit, differential count (band cell, polymorphonuclear neutrophils, lymphocytes, eosinophils, monocytes, basophils). Apparently lacking was a measure of clotting potential or platelet count.

Inspection of Table 3 (page 12) discloses that there were no dose-related effects on any of the parameters examined. Possible exceptions could be slight decreases in polymorphonuclear

neutrophils in the high-dose male group at 30 and 90 days. However, these relative numerical reductions are not considered by this reviewer to be a meaningful consequence of dosing.

Thus, for hematology parameters, NOEL = 50 mg/kg/day.

b. Clinical Chemistry - The clinical chemistry parameters assayed were glucose, blood urea nitrogen, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase, total protein, bilirubin, chloride, sodium, and potassium. Deviations from EPA Guidelines suggested assays were: calcium, phosphorus, albumin, and creatinine. For nonrodents, Guidelines indicate animals should be fasted for a period (not more than 24 hours) before taking blood samples. This reviewer is unable to locate any statement to the effect that fasting was imposed.

This reviewer is of the opinion that there were no remarkable dose-related effects on any of the clinical chemistry parameters indicated as examined (Table 4, page 14).

Hence, for clinical chemistry parameters, NOEL = 50 mg/kg/day.

c. Urinalysis - The urinalysis parameters assayed were pH, specific gravity, albumin, glucose, ketones, bile, and occult blood. The Guidelines do not require urinalysis on a routine basis in subchronic studies except when there is an indication based on expected or observed toxicity.

Among the urinalysis parameters examined, there were no remarkable findings for either sex (Table 5, pages 16 and 17). Thus, NOEL = 50 mg/kg/day.

5. Sacrifice and Pathology

a. Macroscopic examinations - All animals were sacrificed on schedule and were subjected to gross pathological examination. There were no gross pathologic findings related to dosing.

b. Organ Weights - The study protocol indicates (page 35) that at terminal sacrifice, the fresh

weights of liver, kidneys, heart, thyroids, adrenals, spleen, and gonads were to be weighed. Yet, Table 6 (page 18) indicates that in addition to the above-named organs, the following were also weighed: pituitary, brain, heart, and prostate/uterus.

In terms of organ weights and the ratios of each to the terminal body weight, the study author expresses the view that there were no treatment-related responses in any of the organs (page 8). Upon inspection of organ weight data as recorded in Table 6 (page 18), this reviewer shares the opinion expressed by the study director with the possible exception of the spleen in male dogs, where there may have been minor, dose-related decreases in weight; and among females where kidney and pituitary weights in the high-dose group may have been elevated:

	Organ Weight (% of Control)		
	Low	Mid	High
Spleen (Male)	84	79	74
Kidneys (Female)	128	112	137
Pituitary (Female)	111	110	122

Wide variations in individual organ weights for each of the above three organs lead this reviewer to the opinion that these findings should not be viewed as adverse consequences of dosing.

c. Histopathology - Presumably, all of the organs listed under b above as having been weighed were examined histopathologically. However, statements in the text are not definitive that each of these organs was so examined (page 7). Hence, the registrant should be advised to state which organs were examined histopathological, for purposes of comparison to Guideline requirements.

Among male animals, there were two dogs (i.e., 50 percent) in the high-dose group which exhibited "lymph node: blood in sinusoids." In the case of one of these two dogs (2413M), the lymph node was specified as mesenteric. The other (2416M) had no such specification rendered. This particular phenomenon was not observed in male dogs of any of the other groups.

Whether the lymph node phenomenon in the two high-dose male dogs was a consequence of dosing is speculative, but conservatively should be identified as an effect at 50 mg/kg/day (Table 7, pages 24 through 31). Hence, NOEL = 15 mg/kg/day for histopathologic effects among male animals.

With respect to females, histopathologic findings of the kidney present evidence of a possible effect of dosing. Such findings are summarized as follows by dog identification number:

Control (2430):

"slight focal lymphocytic aggregates"

Low-dose (2435):

"slight peripelvic focal lymphocytic aggregates"

Mid-dose (2438, 2440):

"slight peripelvic focal lymphocytic aggregates"

High-dose (2441, 2442, 2444): "

"slight peripelvic focal lymphocytic aggregates"

(2443):

"mild peripelvic focal lymphocytic aggregates"

If "focal" and "peripelvic focal" lymphocytic aggregates of the kidney are to be viewed as one phenomenon, then the incidences of this finding for the four groups would be: 25%, 25%, 50% and 100% of female dogs under test for the control, low-, mid- and high-dose groups, respectively. In the event that "peripelvic focal" is viewed as a distinct finding, then the incidences of this finding would be 0%, 25%, 50% and 100% of female dogs examined, a clear dose-response. It should be noted that among male animals there was one control dog (2402) and one high-dose animal (2413) that exhibited trace peripelvic focal lymphocytic aggregates.

In discussing histopathologic findings, the study author concluded that there was no morphologic evidence of injurious effects of the test material. The author notes that the most common findings were renal, but considered these and other microscopic findings to be minimal, not pathologically serious nor unusual in stock animals.(P.4)

10

Kidney lymphocytic aggregates exhibited a dose-response, wherein each female dog in the high-dose group exhibited the phenomenon. This reviewer is aware that this particular finding may not be unusual and may be of little tox concern, but without historical control data, such a conclusion would be an order of magnitude more uncertain.

11

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

008033

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Bardac 2280 (didecyldimethyl-ammonium chloride)
Toxicity Data Submitted Under MRID Nos. 40282201, 40705801, and 40705802
ID No. 6836-53

TOX Chem No.: 331A
TB Project No.: 9-1193A (9-6300)
RD Record No.: 202248

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

08/23/89

TO: Brian Dementi, Ph.D., Toxicology Branch I (IRS)
Health Effects Division (H7509C)

THRU: Karl Baetcke, Ph.D., ~~Head~~ CHIEF SUPPORT
Toxicology Branch I - Insecticide, Rodenticide Branch
Health Effects Division (H7509C)

9/29/89

Registrant: Lonza, Fair Lawn, NJ

Request

Review and evaluate the following mutagenicity studies:

1. Salmonella/Mammalian-Microsome Assay with Bardac 22, performed by the Institute of Toxicology, Swiss Federal Institute of Technology, Zurich (No study number), Report dated July 16, 1982 (EPA MRID No. 40282201).

2. P0151: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells in vitro, performed by Inveresk Research International (IRI), IRI Project No. 735717 (Report No. 4236), Report dated October 1986 (EPA MRID No. 40705801).

12

3. Analysis of Metaphase Chromosomes Obtained from Bone Marrow of Rats Treated with PO151, performed by the Huntingdon Research Center (HRC), No. LZA 74/8761, Report dated April 1, 1987 (EPA MRID No. 40705802).

TB Conclusions

(Detailed reviews are appended to this memorandum.)

Study	Reported Results	TB Evaluation
1. Ames Assay	Negative up to 125 ug/plate.	Inconclusive (UNACCEPTABLE). Inappropriate test organism (test substance is a bactericide).
2. CHO chromosome in vitro	--	[Cannot be reviewed because Final Report is incomplete (pages missing).]
3. Rat bone marrow	Negative at a single acute dose of 600 mg/kg.	UNACCEPTABLE due to major deficiencies.

Attachments (DERs)

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

08/23/89

9/29/89

008033

DATA EVALUATION REPORT

I. SUMMARY

MRID (ACC) No.: 40705802
ID No.: 6836-53
RD Record: 202248
Shaughnessy No.: 069149
Caswell No.: 331A
Project No.: 9-1193A
(9-0063)

Study Type: Mutagenicity - Chromosome damage in vivo (Aberrations in rat bone marrow)

Chemical: PO151 (didecyldimethyl-ammonium chloride)

Synonyms: Bardac 2280

Sponsor: Lonza, Fair Lawn, NJ

Testing Facility: Huntingdon Research Center (HRC), England

Title of Report: Analysis of Metaphase Chromosomes Obtained from Bone Marrow of Rats Treated with PO151.

Authors: J. Allen, R.J. Proudlock, and P.C. Brooker

Study Number: LZA 24/8761

Date of Issue: April 1, 1987

TB Conclusions:

UNACCEPTABLE due to major procedural and reporting deficiencies.

II. DETAILED REVIEW

A. Test Material - PO151 (Bardac 2280)

Description: Yellowish liquid
Batch (Lot): E 06130085
Purity (%): 50.3
Solvent/Carrier/Diluent: Sterile distilled water (DW)

B. Test Organisms - Rodent

Species: Rat
Strain: Sprague-Dawley (SPF-CD)
Age: (Not stated)
Weights - Males: (Not stated)
Females: (Not stated)
Source: Charles River UK, Kent

C. Study Design (Protocol) - This study was designed to assess the chromosome damaging potential of Bardac 2280 when administered by gavage to rats once and bone marrow sampled 6, 24, and 48 hours later.

A signed Statement of Confidentiality Claim was provided.

A signed Statement of Compliance with EPA GLPs was provided.

A signed Quality Assurance Statement was provided.

D. Procedure/Methods of Analysis - Following dose-selection (toxicity) testing at doses up to 1200 mg/kg, six groups of five males and five females each were gavaged with a single dose of 600 mg/kg test substance and femoral bone marrow collected 6, 24, and 48 hours later, following injection with colchicine 2 hours prior to sacrifice. A seventh group was given 40 mg/kg cyclophosphamide (CP, as positive control), and sacrificed at 24 hours.

Bone marrow was processed by conventional cytological techniques to yield microscope slide preparations of cells arrested in metaphase. Coded slides were examined under a 100X oil immersion objective and a total of 50 diploid metaphases per animal scored for chromosome aberrations according to standard guidelines.

E. Results - In preliminary toxicity testing, dose-related clinical toxicity, including death, was observed, beginning at 400 mg/kg and increasing in severity up to 1200 mg/kg (Report Appendixes 1 and 2). From these results,

the single dosage of 600 mg/kg was selected for the main test.

At no sampling time in the main test were any aberrant cells (metaphases showing chromosome damage) encountered in test groups (nor in vehicle controls), in contrast to a significant increased incidence of aberrations ($p < 0.001$) in the CP group (Report Table 1, attached to this DER, which summarized individual animal data from Report Tables 2 to 8).

F. TB Evaluation - UNACCEPTABLE. The study cannot be accepted in its present form for the following reasons:

1. Only one dose was employed (and that by oral administration only);

2. We are not assured that the technical grade (TGAI) was the test substance;

3. The severe clinical toxicity recorded above 600 mg/kg appears to reflect the consequences of (merely) administration of the test compound by oral intubation. No evidence is presented of systemic absorption of the material (or its metabolites) and transport to the target (bone marrow) in sufficient concentrations to cause cytotoxicity. By contrast, the positive compound caused only mild clinical signs but was active in bone marrow cells. (Ip injection would have obviated any question of absorption and transport.) [It is unusual (if not incredible!) not to have found at least one abnormal cell among 3000 metaphases examined (not even so much as a gap turned up!). Acknowledged practitioners of this assay record about 1 percent abnormal metaphases in control populations of rodents.*]

   We recommend repeating this assay, by ip injection of test compound, at at least three single dose levels with timed sampling (up to 72 hours) in addition to a repeat dose schedule (e.g., 5 days) at the (best) effective dose found in acute testing.

Attachments

---

*E.g., David Salsburg and Henry E. Holden, "A Statistical Examination of Historical Controls for Mouse Bone Marrow Cytogenic Assays," Environ. Mutag. 7, Suppl. 4:55-62 (1985).

16

ATTACH - I

M & M

DIDECYL DIMETHYL AMMONIUM CHLORIDE

Page_____ is not included in this copy.

Pages 18 through 24 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product 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.

X 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
Toxicology Branch I - IRS/HED (H7509C)
Secondary Reviewer: Karl Baetcke, Ph.D., Chief
Toxicology Branch I - IRS/HED (H7509C)

8/23/89

8/23/89

008033

DATA EVALUATION RECORD

I. SUMMARY

MRID (ACC) No.: 40282201
ID No.: 6836-53
RD Record: 202248
Shaughnessy No.: 069149
Caswell No.: 331A
Project No.: 9-1193A
(9-0063)

Study Type: (84-2) Mutagenicity - Gene mutation in bacteria (Salmonella/Ames)

Chemical: Bardac 2280 (didecyldimethyl-ammonium chloride)

Synonyms: PO151

Sponsor: Lonza, Fair Lawn, NJ

Testing Facility: Institute of Toxicology, Swiss Federal Institute of Technology/University of Zurich

Title of Report: Salmonella/Mammalian-Microsome Assay with Bardac 22.

Authors: U. Friedrich and F.E. Würgler

Study Number: (Not given)

Date of Issue: July 16, 1982

TB Conclusions:

Bardac 2280 is a bactericidal compound, hence could only be tested to limited dose levels in Salmonella (only up to concentrations of 125 ug/plate). No mutagenicity (increased reversions) were recorded.

Classification (Core-Grade):

Inconclusive, since the test organism is inappropriate for assaying the test article. (Otherwise, UNACCEPTABLE, because of major deficiencies to current Agency PAGs.)

25

II. DETAILED REVIEW

A. Test Material - Bardac 22

Description: (Not stated)
Batch (Lot): (Not stated)
Purity (%): (Not stated)
Solvent/Carrier/Diluent: Phosphate-buffered DW
Source: Lonza (Basel)

B. Test Organisms - Bacteria

Species: Salmonella typhimurium
Strains: TA98, TA100, TA1535, TA1537, TA1538
Source: B.N. Ames, UCal (Berkeley)

C. Study Design (Protocol) - This study was designed to assess the genotoxic potential of Bardac 22 when administered in vitro to Salmonella typhimurium his$^-$ mutants. A copy of the "materials and methods" section from the investigator's report is attached to this DER.

A signed Statement of No Confidentiality Claim was provided.

A signed Statement of Compliance with EPA GLPs was provided (prior to GLPs).

A signed Quality Assurance Statement was provided.

D. Procedure/Methods of Analysis - Following preliminary dose-selection testing (at six concentrations up to 2000 ug/plate), triplicate cultures of the histidine-deficient (his$^-$) Salmonella typhimurium strains TA98 and TA100 were exposed to Bardac 22 at concentrations of 31.25, 62.50, 125, 250, 500, and 1000 ug/plate, while strains TA1535, TA1537, and TA1538 were treated at concentrations of 3.9, 7.8, 15.6, 31.2, 67.5, and 125 ug/plate, in the absence or presence of a mammalian activation system consisting of rat liver microsomes (S9) plus cofactors. Reference mutagens* appropriate

---

*Presented in the final report (page 9) as follows:
- Without activation: 7.5 ug nitrofluorene (dissolved in 50 uL DMSO) per plate for strains TA98 and TA1538; 2.5 ug sodium azide (dissolved in 50 uL H_2O bidest.) per plate for strains TA100 and TA1535; 100.0 ug 9-aminoacridine (dissolved in 50 uL DMSO) per plate for strain TA1537:
- With activation: 5.0 ug benzo(a)pyrene (dissolved in 50 uL DMSO) per plate for strains TA98 and TA100.

to each strain and activation condition (positive controls) were employed concurrently.

After 48 hours incubation at 37 °C, revertent colonies were counted (visually or, on those plates with 70+ colonies per plate, by an electronic counter), background growth ("lawn") checked, and mean colony counts recorded in tabular form (Report Tables 2, 3, and 4).

No criteria for either assay acceptance or for defining positive and negative results were presented.

E. Results - [Summary Tables from the Final Report are attached to this DER.] In preliminary toxicity testing (Report Table 1), 62.5 ug/plate of the test substance was lethal in nonactivated TA98 and TA100 cultures, but permitted 65 percent cell survival in TA98 and 7 percent in TA100 with activation (+S9). The other three strains were even more sensitive to the bacteriocidal action of Bardac 22, since 31.5 ug/plate was the highest dose allowing survival.

At no test dose permitting cells to survive in any strain, however, did the number of revertents increase over vehicle control (Tables 2 and 3). In contrast, the reference mutagens generated values in TA98 and TA100 4X to 35X control (Report Table 4). [However, we note that no activated cultures of TA1535, TA1537, or TA1538 were tested for response to the reference mutagen, benzo(a) pyrene.]

The authors concluded Bardac 22 showed no mutagenicity in this assay.

F. TB Evaluation - While we agree that the test compound showed no mutagenic activity up to the limited doses that could be assayed, the study can only be judged INCONCLUSIVE for genetic activity, since Bardac 22 is a bactericidal substance, and thus inappropriate for testing in Salmonella (or any bacterial organism). Notwithstanding this assessment, this assay would be considered UNACCEPTABLE according to current Agency PAG 84-2, since:

1. The nature, purity, and other characteristics of the test substance were not given;

2. There was no repeat experiment to verify the presumptive negative;

3. No individual culture data were included; and

4. A complete set of positive control assays was lacking (no testing with 3 of the 5 strains under activation conditions (+S9)).

Attachments

ATTACHMENT I

MATERIALS & Methods

DIDECYL DIMETHYL AMMONIUM CHLORIDE

Page_____ is not included in this copy.

Pages 30 through 36 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product 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.

X 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.