CASWELL FILE

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

006796

JUL 28 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Evaluation of Option 1, Tier 1 Data Submitted to Satisfy Antimicrobial Registration of Q9-DC5700

Caswell No.: 892B
TB Project No.: 8-0254

FROM: Henry Spencer, Ph.D., Pharmacologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK for HS: 7/22/88

TO: John Lee/James Wilson, PM Team 31
Disinfectants Branch
Registration Division (TS-767C)

THRU: Albin Kocialski, Ph.D., Supervisory Pharmacologist
Review Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ABK 7/22/88

and

Theodore Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

7/28/88

Background

The Disinfectants Branch has requested that the data base be evaluated with regard to fulfilling the data requirements for continued registration of the antimicrobial Q9-DC5700. The listing of studies submitted with their evaluations and additional requirements are listed in the following paragraphs.

Conclusions

As noted from the exposure categories (medium for clothing use) cited in FR Vol 52, No. 4 (1987), several studies are

data gaps in supporting the registration of Q9-DC5700. The registrant needs to address these data gaps with regard to each registered use and respective exposure.

As determined by the exposure categories, the following studies are still needed to fulfill data requirements:

Acute

1. Primary Dermal Irritation, and
2. Acute Inhalation Toxicity.

Subchronic

1. A 90-Day Dermal Toxicity Study;
2. A 90-Day Inhalation Toxicity Study;
3. A Teratology Study (2 Species); and
4. A 90-Day Feeding Study.

Other

A battery of mutagenicity studies with appropriate solvents.

All exposure categories require the following studies:

1. A 90-Day Dermal or Inhalation Study;
2. A Teratology Study (Rat); and
3. A Battery of Mutagenicity Studies.

Additionally, medium exposure categories also require:

1. A Subchronic Feeding Study;
2. A Second Species Teratology Study (Rabbit); and
3. A Dermal Absorption Study.

Of the following list of available studies, only those studies indicated with an asterisk were considered relevant to the evaluation of this product.

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*1.	Acute Toxicological Properties of Dow Corning Q9-DC5700,		
	a. Acute Oral LD_{50}	Core:	Minimum
	b. Acute Dermal LD_{50}	Core:	Minimum
	c. Primary Eye Irritation	Core:	Minimum
	d. Primary Skin Irritation	Core:	Supplementary
*2.	Human Repeated Insult Patch Test	Core:	Invalid (IBT)
3.	28-Day Subacute Dermal Toxicity With Treated Fabric in Albino Rabbits	Core:	Invalid (IBT)
*4.	Acute Vapor Inhalation Toxicity	Core:	Invalid (IBT)
*5.	Host-Mediated Assay for Detection of Mutagens	Core:	Invalid (IBT)
6.	Vaginal Irritation Study in Dogs	Core:	Invalid (IBT)
7.	32-Day Human Exposure to Treated Socks	Core:	Supplementary information
8.	3-Month Human Wear Test of Treated Athletic Socks	Core:	Supplementary information
*9.	Teratology Study in Rats	Core:	Invalid (IBT)
*10.	Mutagenicity Evaluation of Q9-DC5700	Core:	Unacceptable
*11.	Mammalian Cell Transformation Without Exogenous Activation	Core:	Unacceptable
*12.	Mammalian Cell Transformation With Exogenous Activation	Core:	Unacceptable
**13.	Durability (Leaching Study)	Core:	Minimum
**14.	Percutaneous Absorption in Rabbits	Core:	Supplementary
15.	Determination of Respirable Particle Size	Core:	Supplementary study

*16. Acute Aerosol Inhalation Toxicity — Core: Invalid (IBT)

Reviews of the following studies are included:

1. Mutagenicity - in vitro Mammalian cell transformation assay with exogenous metabolic activation. MRID No. 403852-13 (Unacceptable).

2. Mutagenicity - in vitro Mammalian cell transformation assay without exogenous metabolic activation. MRID No. 403852-12 (Unacceptable).

3. Mutagenicity - Salmonella/mammalian microsome reverse mutation. MRID No. 403852-11 (Unacceptable).

4. $AOLD_{50}$ (Rat) MRID No. 403852-01 (Minimum).

5. $AOLD_{50}$ (Rabbit) MRID No. 403852-01 (Minimum).

6. Primary Eye Irritation (Rabbit) MRID No. 403852-01 (Minimum).

7. Primary Skin Irritation (Rabbit) MRID No. 403852-01 (Supplementary).

Attachments

**Special study required by the Toxicology Branch.

Reviewed By: Albin B. Kocialski
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski ABK 4/17/88

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DATA EVALUATION REPORT

Study Type: Acute Oral Toxicity ($AOLD_{50}$ - Rat)

Toxicity Chemical No.: 892B

MRID No.: 403852-01

Test Material: Dow Corning Q9-5700. Lot No. AD-0003 (50% 3-[trimethoxysilyl] propyl dimethyl octadecyl ammonium chloride). Note: Product also contains methanol. EPA Registration No. 34292-1 (For Manufacturing Use Only).

Synonyms: Dow Corning 5700 Antimicrobial Agent

Study Number: AD-0003

Sponsor: Dow Corning Corporation

Testing Facility: Dow Corning Corporation
Midland, MI

Title of Report: Acute Toxicologic Properties of Dow Corning Q9-5700

Author: Edward J. Hobbs

Report Issued: August 27, 1987

Conclusion:

$AOLD_{50}$ (Both sexes) = 12.27 g/kg.

Category of Toxicity: Category IV

Classification: Minimum

Special Review Criteria (40 CFR 154.7): None

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Materials and Methods:

Forty young albino Sprague-Dawley rats equally divided as to sex were divided into four groups of 10 animals each (5/sex/dose). Rats were quarantined for 5 days prior to actual testing and examined for general good health. Animals were housed in stock cages and were allowed food (a standard laboratory rat diet) and water ad libitum. Animals were, however, fasted overnight prior to compound administration and weighed approximately 200 to 275 g at test time.

All four doses were administered undiluted by gavage. Following dosing, animals were housed individually and observed regularly for a period of 14 days. All mortalities and/or toxic signs were recorded. The $AOLD_{50}$ was calculated using the techniques of Weil, Thompson and Thompson and Weil.

Results:

Dose (g/kg)	Number Dead	Number Tested	Percent Dead	Time of Death Following Dose Administration
3.98	2	10	20	4 Days
7.95	2	10	20	1 and 4 Days
15.8	6	10	60	1 Hour
31.6	10	10	100	20 Minutes

Acute Oral LD_{50} = 12.27 g/kg.
Standard Deviation of LD_{50} = $\pm$ 0.16 g/kg.

Discussion:

The study submitted fulfills the intent of the regulatory requirement for this kind of study. It is noted here that several reporting deficiencies were observed. The following were not reported: individual body weights at 7 and 14 days, food consumption, and toxic signs.

Conclusion:

$AOLD_{50}$ both sexes = 12.27 g/kg.

Category of Toxicity: Category IV

Classification: Minimum

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Reviewed By: Albin B. Kocialski
Section VII, Toxicology Branch (TS-769C) ABK 3/19/88
Secondary Reviewer: Albin B. Kocialski

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DATA EVALUATION REPORT

Study Type: Acute Dermal Toxicity ($ADLD_{50}$ - Rabbit)

Toxicity Chemical No.: 892B

MRID No.: 403852-01

Test Material: Dow Corning Q9-5700. Lot No. AD-0003 (50% 3-[trimethoxysilyl] propyl dimethyl octadecyl ammonium chloride). Note: Product also contains methanol. EPA Registration No. 34292-1 (For Manufacturing Use Only).

Synonyms: Dow Corning 5700 Antimicrobial Agent

Study Number: AD-0003

Sponsor: Dow Corning Corporation

Testing Facility: Dow Corning Corporation
Midland, MI

Title of Report: Acute Toxicologic Properties of Dow Corning Q9-5700

Author: Edward J. Hobbs

Report Issued: August 27, 1987

Conclusion:

$ADLD_{50}$ was greater than 7.95 g/kg/body weight (bwt).

Category of Toxicity: Category III

Classification: Minimum

Special Review Criteria (40 CFR 154.7): None

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Materials and Methods:

Twelve young adult and healthy New Zealand strain albino rabbits weighing between 2.3 to 3.0 kg were selected for testing following a 7-day quarantine and observation period. Rabbits were housed individually and had free access to food (standard laboratory rabbit ration) and water. The animals were then divided into three groups of four animals each. Twenty-four hours prior to dermal application, each rabbit was clipped free of hair equivalent to 10 percent of the total body surface on the back. The animals were then returned to their cages overnight. The following day, all rabbits received epidermal abrasions 2 to 3 cm apart longitudinally over the area of exposure and then received skin applications of undiluted test material at three selected dose levels. After each application, the exposure site was covered by wrapping the trunk of the animal with an impervious cuff that was held in place with a cloth bandage for a period of 24 hours, after which time the test material was removed. The animals were observed during the 24-hour contact period and for a 14-day period thereafter. Observations were made for mortality, local skin reactions, and general toxic signs.

Results:

Dose (g/kg)	Number Dead	Number Tested	Percent Dead
2.00	0	4	0
3.98	0	4	0
7.95	0	4	0

Acute Dermal LD_{50} = > 7.95 g/kg.

Discussion:

The study submitted fulfills the intent of the regulatory requirement for this kind of study. It was reported by the study authors that:

> All animals exhibited normal behavioral patterns during the total observation period. Dose related local skin reactions ranged from slight to moderate erythema and edema, and followed with slight to moderate exfoliation in some of the middle and high dose animals at the seven day point.

However, individual animal data were not presented and sex of the animals was not reported.

Conclusion:

$ADLD_{50}$ was greater than 7.95 g/kg/bwt.

Category of Toxicity: Category III

Classification: Minimum

Reviewed By: Albin B. Kocialski
Section VII, Toxicology Branch (TS-769C) ABK 4/14/88
Secondary Reviewer: Albin B. Kocialski

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DATA EVALUATION REPORT

Study Type: Primary Eye Irritation - Rabbits

Toxicity Chemical No.: 892B

MRID No.: 403852-01

Test Material: Dow Corning Q9-5700. Lot No. AD-0003 (50% 3-[trimethoxysilyl] propyl dimethyl octadecyl ammonium chloride). Note: Product also contains methanol. EPA Registration No. 34292-1 (For Manufacturing Use Only).

Synonyms: Dow Corning 5700 Antimicrobial Agent

Study Number: AD-0003

Sponsor: Dow Corning Corporation

Testing Facility: Dow Corning Corporation
Midland, MI

Title of Report: Acute Toxicologic Properties of Dow Corning Q9-5700

Author: Edward J. Hobbs

Report Issued: August 27, 1987

Conclusion:

Dow Corning Q9-5700 produces eye damage and severe irritation. Eye damage appears to be almost immediate and irreversible.

Category of Toxicity: Category I

Classification: Minimum

Special Review Criteria (40 CFR 154.7): None

Materials and Methods:

Six young New Zealand albino rabbits were selected to evaluate the eye irritation potential of the test product. Two drops (equivalent to 0.1 mL) of test material were placed onto the left eyeball of each animal. This eye was then washed (within 30 seconds - exact time not reported) for 2 minutes in a flowing stream of tepid water. The right eye was treated similarly but was not washed.

Animals were immediately observed for a pain response: "Within 2 to 3 minutes after the unwashed eye was treated, each was observed for conjunctival and corneal response. Similar observations were made of both eyes at 1 hour, 24 hours, 48 hours, 7 days and 14 days after treatment. Both eyes are stained with fluorescein (5% water solution) at 1 hour, 24 hours, 48 hours, 7 days and 14 days. This necessitated the washing of both eyes to remove the excess stain."

Results:

Slight pain was reported for 5/6 animals with one animal showing a moderate amount of pain. Within 1.0 hour in all animals, eye damage (cornea) was reported as moderate. About 50 to 100 percent of the cornea was necrotic and lesions were shallow. Eye damage progressed with time to severe (marked necrosis over the whole area of the eye) to very severe (heavy necrosis with loss of the eye). The iris and conjunctivae were moderately to very severely affected. Effects did not reverse nor did they diminish during the 14-day observation period. There were essentially no differences observed between washed and unwashed eyes. The authors of the report concluded the following:

> Undiluted Dow Corning® Q9-5700 has a severe effect upon the eye. Direct contact will result in tissue destruction leading to permanent impairment of vision. Special and particular precautions must be taken to prevent contact with the eyes.

Conclusion:

Dow Corning Q9-5700 produces eye damage and severe irritation. Eye damage appears to be almost immediate and irreversible.

Category of Toxicity: Category I

Classification: Minimum

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Reviewed By: Albin B. Kocialski
Section VII, Toxicology Branch (TS-769C) ABK 4/19/88
Secondary Reviewer: Albin B. Kocialski

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DATA EVALUATION REPORT

Study Type: Primary Skin Irritation Test in Albino Rabbits

Toxicity Chemical No.: 892B

MRID No.: 403852-01

Test Material: Dow Corning Q9-5700. Lot No. AD-0003 (50% 3-[trimethoxysilyl] propyl dimethyl octadecyl ammonium chloride). Note: Product also contains methanol. EPA Registration No. 34292-1 (For Manufacturing Use Only).

Synonyms: Dow Corning 5700 Antimicrobial Agent

Study Number: AD-0003

Sponsor: Dow Corning Corporation

Testing Facility: Dow Corning Corporation
Midland, MI

Title of Report: Acute Toxicologic Properties of Dow Corning Q9-5700

Author: Edward J. Hobbs

Report Issued: August 27, 1987

Conclusion (Tentative):

Dow Corning Q9-5700 produces slight to moderate skin irritation.

Category of Toxicity (Tentative): Category III

Classification: Supplementary

Special Review Criteria (40 CFR 154.7): None

Materials and Methods:

Six white laboratory rabbits were shaved free of hair over the entire abdomen. All animals were then returned to their cages for several days to allow for healing of the skin. Applications were made "neat" as follows and were discontinued in the presence of skin burn.

- Application to the Ear--Approximately 0.5 mL of the test substance was applied to the ear in three separate applications over a period of 3 days.
- Application to the Intact Abdomen--Approximately 0.5 mL of the test material was applied under a 1-inch by 1-inch cotton pad and held in place by a cloth bandage. A total of three applications were made over a period of 3 days.
- Application to the Abraded Abdomen--An area of skin about 1-inch by 1-inch square was crosshatched with a hypodermic needle deep enough to penetrate the stratum corneum but not to produce more than a trace of bleeding.

Results:

Examination of the data indicates that undiluted Dow Corning Q9-5700 produces slight to moderate erythema and edema which can be accompanied with slight necrosis and slight scab formation. Based on the grading criteria used by the registrant, undiluted Dow Corning Q9-5700 appears to be a slight to moderate skin irritant.

Discussion:

Toxicology Branch requires some clarificatión as to the method of application prior to coming to a conclusion with regard to this study. The way the methods of application are written, it is unclear as to whether 0.5 mL of test compound is applied at each application site on each day for a total of 1.5 mL for the 3 days, or if 0.5 mL is divided up into three separate aliquots with each fraction applied each day for a total application volume of 0.5 mL.

If the former application method is the test method, then the product would be classified as a slight to moderate irritant and placed into Category III for skin irritation toxicity; however, if the latter method was the test method, then the category of toxicity would be at least II or I, and serious consideration would be given to repeating the study.

Conclusion (Tentative):

Dow Corning Q9-5700 produces slight to moderate skin irritation.

Category of Toxicity (Tentative): Category III

Classification (Tentative): Supplementary

94885:I:Kocialski:LHED-02:KENCO:04/08/88:05/18/88:DD:wb:vo:ek:rw
R:94890:Kocialski:LHED-2:KENCO:4/15/88:4/26/88:rw:EK:DD

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EPA 68-02-4225)

DYNAMAC No. 355-A
March 8, 1988

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DATA EVALUATION RECORD

DOW CORNING 5700 ANTIMICROBIAL AGENT

Mutagenicity--Salmonella/Mammalian Microsome Reverse Mutation

STUDY IDENTIFICATION: Isquith, A. Mutagenicity evaluation of Dow Corning 5700 [3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride]. (Unpublished study No. 1853-13 prepared and submitted by Dow Corning Corp., Midland, MI; dated January 3, 1978.) MRID No. 403852-11.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/8/88

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INERT INGREDIENT INFORMATION IS NOT INCLUDED

1. CHEMICAL: Dow Corning 5700 antimicrobial agent; 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride; TX-1559.

2. TEST MATERIAL: Four samples of TX-1559 were identified as:

Lab No.	Sponsor's Identification	Description
TX-1559A	Dow Corning 5700 (lot No. BN126008)	42% 3-(trimethoxysilyl)-propyldimethyl-octadecyl ammonium chloride [redacted] 50% methanol.
TX-1559B	Dow Corning X9-5706	Prepared hydrolyzate of Dow Corning 5700 (Ref. E-2054-149).
TX-1559C	Artificial sweat extract	Dow Corning 5700 treated Gold Cup style 7953 (75% orlon/25% nylon); Burlington socks. Extracted with artificial sweat [Blood and Other Body Fluids, P. L. Altman and D. S. Dittmar, FASEB, 1961] for 24 hours on a New Brunswick rotary shaker at 125 ppm.
TX-1559D	Artificial sweat extract	Sock control. Same as (C) above without Dow Corning 5700 treatment.

3. STUDY/ACTION TYPE: Mutagenicity-Salmonella/mammalian microsome reverse mutation.

4. STUDY IDENTIFICATION: Isquith, A. Mutagenicity evaluation of Dow Corning 5700 [3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride]. (Unpublished study No. 1853-13 prepared and submitted by Dow Corning Corp., Midland, MI; dated January 3, 1978.) MRID No. 403852-11.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 3.7.88

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 3-7-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicity
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 3-7-88

Henry Spencer, Ph.D.
EPA Reviewer

Signature: Henry Spencer
Date: 3/9/88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A.J.K.
Date: 3/11/88

7. CONCLUSIONS:

A. Four test samples, designated TX-1559A through D (see Test Material, Section 11.A.1, for descriptions) were assayed in spot and plate incorporation Salmonella typhimurium reverse mutation assays. Under the conditions of the plate incorporation assays, four nonactivated and S9-activated doses (50, 100, 250, and 500 µg/plate) of each sample were neither cytotoxic nor mutagenic. Doses evaluated in the spot tests were not specified. Although all samples were reported negative, the lack of cytotoxicity, performance of the assay with single plates, and the questionable responses of the tester strains to the positive controls indicate unacceptable evidence that TX-1559 is not mutagenic. (See Reviewer's Discussion and Interpretation of Study Results, Section 14).

B. The study is unacceptable.

8. RECOMMENDATIONS:

A. It is recommended that the repeat study be performed with the active ingredient only in accordance with established procedures.[1] Additionally, the study authors should furnish background data (spontaneous reversion rates and positive control results) on the tester strains to permit an independent assessment of strain verification and sensitivity. A QA/GLP statement of compliance should also be included with the final report.

Items 9 and 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Four samples of TX-1559 with the following descriptions were evaluated:

[1]De Serres, F. J. and Shelby, M. D., Recommendations on data production and analysis using the Salmonella microsomal mutagenicity assay, Mutat. Res. 64 (1979): 159-165.

[2]Only items appropriate to this DER have been included.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Lab No.	Sponsor's Identification	Description
TX-1559A	Dow Corning 5700 (lot No. BN126008)	42% 3-(trimethoxysilyl)-propyldimethyl-octadecyl ammonium chloride [redacted] 50% methanol.
TX-1559B	Dow Corning X9-5706	Prepared hydrolyzate of Dow Corning 5700 (Ref. E-2054-149).
TX-1559C	Artificial sweat extract	Dow Corning 5700 treated Gold Cup style 7953 (75% orlon/25% nylon); Burlington socks. Extracted with artificial sweat [Blood and Other Body Fluids, P. L. Altman and D. S. Dittmar, FASEB, 1961] for 24 hours on a New Brunswick rotary shaker at 125 ppm.
TX-1559D	Artificial sweat extract	Sock control. Same as (C) above without Dow Corning 5700 treatment.

Storage conditions were not reported; all samples were prepared in dimethyl sulfoxide (DMSO).

2. Test Organisms: Sixteen-hour broth cultures of S. typhimurium TA1535, TA1537, TA1538, TA98, and TA100 containing ~10^8 cells were used in the assay. Strain source, maintenance, and culture conditions were not reported.

3. S9 Activation: The S9 fraction used for metabolic activation was derived from the livers of Sprague-Dawley adult male rats induced with Aroclor 1254 and was obtained from Litton-Bionetics, Inc., Kensington, MD.

4. Mutation Assay

   a. Test Methods: Each test material was evaluated with the five bacterial strains in both nonactivated and S9-activated spot tests and plate incorporation assays. The number of doses assayed in the spot test was not specified; four concentrations of each test sample were tested in the plate incorporation assay. Details of the spot tests and plate incorporation assays are as follows:

1. Spot Test: Two milliliters of molten agar, supplemented with biotin and histidine, and containing 10^8 cells of the appropriate strain were poured over the surface of Davis Minimal Agar. For the S9-activated spot test, 0.5 mL of the S9 mix were added to the molten top agar immediately prior to use. Plates were allowed to harden and test materials, solvent, and positive controls were spotted in 10-µL volumes onto individual plates of each strain. Plates were incubated at 37°C for 48 hours.

2. Plate Incorporation Assay: The plate incorporation assays were conducted in a similar manner; however, prior to pouring top agar tubes, an unspecified volume of each test agent, solvent, or positive controls was added. The reaction ingredients were mixed and poured over mininal agar. After 48 hours of incubation at 37°C, the revertant colonies were counted. The number of replicates was not reported; however, based on reported data it was assumed that single plates/dose/strain/condition were used.

b. Positive Controls: The following positive controls were used in the spot and plate incorporation assays:

Strain	S9 Activation	Chemical	Concentration* (µg/plate)
TA1535 and TA100	-	Methylnitrosoguanidine (MNNG)	10
TA1537	-	9-Aminoacridine (9-AA)	100
TA1538 and TA98	-	2-Nitrofluorene (2-NF)	100
TA1535	+	2-Anthramine (2-AA)	100
TA1537	+	8-Aminoquinoline (8-AMQ)	100
TA1538, TA98 and TA100	+	2-Aminofluorene (2AF)	100

* Concentration for plate incorporation assay only; doses were not reported for spot tests.

5. Evaluation Criteria: The test material(s) was considered positive if the solvent control values were within the normal range and if there was a dose response over three concentrations, with the lowest increase in mutant colonies being greater than or equal to twice the control values.

6. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Spot Test: Data from the spot tests were not provided; the study author reported that all four test samples were negative and that the positive controls were active.

B. Plate Incorporation Assays: Four doses (50, 100, 250, and 500 µg/plate) of each test sample were assayed in the presence or absence of S9 activation. None of the test samples induced a definitive cytotoxic effect at any dose; compound precipitation, if any, was not reported. No appreciable increase in reversion to histidine prototrophy of any strain accompanied exposure to the four nonactivated and S9-activated doses of the four test samples.

Representative results of the mutation assays conducted with the four test agents are presented in Table 1.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded, "The test material, TX-1559, failed to exhibit mutagenic activity in both the activation and non-activation systems and is considered not to be mutagenic under the conditions employed."

B. A quality assurance statement was not presented.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study is unacceptable because none of the samples were assayed to a cytotoxic level and only single platings were performed at each dose. The lack of a cytotoxic effect induced by the formulated product, sample TX-1559A, is surprising when its intended use as an antimicrobial agent is considered; furthermore, the 50% methanol content should cause cytotoxicity.

Based on the reviewers' experience, the sensitivity of the tester strains to the positive controls is of equal concern. For example, strain TA1535 was more responsive to MMNG than the more sensitive plasmid-bearing TA100 strain. Similarly, the level of 2-AA (100 µg/plate) used in the S9-activated assay was high; in general, doses of S9-activated 2-AA in excess of 10 µg/plate are less mutagenic and more cytotoxic than lower concentrations. We assess, therefore, that the sensitivity of the test system to detect low level mutagenicity was questionable and requires confirmation by a repeat assay. Additionally, historical data on spontaneous reversion rates and responses to the positive controls should be included along with the repeat study. The inclusion of this information will allow an independent assessment of the test system's sensitivity under the existing conditions of the reporting laboratory.

TABLE I. Representative Results of the Salmonella typhimurium Mutagenicity Assays with TX-1559 A through D

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl-sulfoxide	-	—	18	9	22	46	111
	+	—	23	12	26	41	109
Positive Control							
Methylnitroso-guanidine	-	10	b	—	—	—	463
9-Amino-acridine	-	100	—	87	—	—	—
2-Nitrofluor-ene	-	100	—	—	b	b	—
2-Anthramine	+	100	b	—	—	—	—
8-Aminoquin-oline	+	100	—	63	—	—	—
2-Aminofluorene	+	100	—	—	b	b	477
Test Samples[c]							
TX-1559A	-	500	12	6	20	48	97
	+	500	16	6	19	34	75
TX-1559B	-	500	10	7	21	47	104
	+	500	23	12	26	46	96
TX-1559C	-	500	14	7	23	44	112
	+	500	20	11	24	37	102
TX-1559D	-	500	12	6	20	43	118
	+	500	30	10	25	40	112

[a] Results from single plates.

[b] $>10^3$ colonies.

[c] Highest dose assayed; results for all samples at the lower doses (50, 100, and 250 µg/plate) were comparable to the control values.

Item 15--see footnote 2.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 4-9.

APPENDIX A

Protocol

Page_____ is not included in this copy.

Pages 26 through 30 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.

__✓__ 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.

EPA: 68-02-4225
DYNAMAC No. 355-B
March 9, 1988

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DATA EVALUATION RECORD

DOW CORNING 5700 ANTIMICROBIAL AGENT

Mutagenicity--Transformation Assay Without Activation

STUDY IDENTIFICATION: Schechtman, L. M., Beard, S. F., and Sinsky, P. M. Activity of T 1482 in the in vitro mammalian cell transformation assay in the absence of exogenous metabolic activation. (Unpublished study No. T 1482 prepared by Microbiological Associates, Bethesda, MD, for Dow Corning Corp., Auburn, MI; dated March 5, 1979.) MRID No. 403852-12.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Ira Cecil Wilmington

Date: 3-9-88

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1. CHEMICAL: Dow Corning 5700 antimicrobial agent; T 1482 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride.

2. TEST MATERIAL: T 1482, Dow Corning 5700, from lot no. BN 126008, was described as a straw-colored liquid containing 50% methanol and 50% active ingredient (a.i.)

3. STUDY/ACTION TYPE: Mutagenicity--Transformation assay without activation.

4. STUDY IDENTIFICATION: Schechtman, L. M., Beard, S. F., and Sinsky, P. M. Activity of T 1482 in the in vitro mammalian cell transformation assay in the absence of exogenous metabolic activation. (Unpublished study No. T 1482 prepared by Microbiological Associates, Bethesda, MD, for Dow Corning Corp., Auburn, MI; dated March 5, 1979.) MRID No. 403852-12.

5. REVIEWED BY:

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6. APPROVED BY:

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Signature: I. Cecil Felkner
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Albin Kocialski, Ph.D.
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Signature: Henry P. Spencer 3/11/88
Albin B. Kocialski
Date: 3/14/88

7. CONCLUSIONS:

A. Dow Corning 5700 antimicrobial agent, T 1482, containing 50% a.i. and 50% methanol, was tested in a nonactivated BALB/3T3 transformation assay without exogenous metabolic activation. Due to limited solubility and homogeneity of the test material in the aqueous tissue culture medium, concentrations were expressed as dilutions rather than actual doses. Accordingly, four dilutions, ranging from $1:6 \times 10^4$ to $1:1.2 \times 10^5$, were assayed. Extreme cytotoxicity was seen at dilutions $\leq 1:10^4$; however, we were unable to determine if the cytotoxicity was caused by the 50% a.i. or the 50% methanol content of the formulated product. At both of the two lowest dilutions ($1:6 \times 10^4$ and $1:8 \times 10^4$), single type- III foci per 12 replicate cultures were seen; none were scored at the remaining levels.

Given the relative test material insolubility and solvent interference, we conclude that the a.i. was probably tested at the highest reasonable concentration. However, the acceptability of the study is questionable for the following reasons:

1. The inability to establish actual doses of the test material limits the usefulness of the data; the study provides no information that could be included in a toxicology profile or risk assessment of the test material.

2. The number of replicate platings at each level does not represent an adequate statistical sample; hence, the relevance, if any, of the single transformed foci at the two lowest dilutions cannot be assessed.

3. The exposure time (24 hours) was too short; the recommended treatment time for this assay is 3 days.[1]

4. A procarcinogen, rather than a direct-acting agent, is needed for demonstrating the intrinsic ability of the cell line to metabolize certain precursor carcinogens to an active state.

5. A quality assurance/good laboratory practice (QA/GLP) was not provided.

B. The study is unacceptable.

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[1]Heidelberger, C., Freedman, A. E., Pienta, R. J., Sivak, A., Bertram, J. S., Casto, B. C., Dunkel, V. C., Francis, M. W., Kakunaga, T., Little, J. B., and Schechtman, L. M. Cell transformation by chemical agents--a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res, 114(1983): 283-385.

8. RECOMMENDATIONS:

A. It is recommended that the assay be repeated using an established protocol.[2] Attempts should be made to find a more suitable solvent that would be compatible with the test system.

Additionally, the authors should report the statistical methods used to evaluate the data and provide a statement of compliance with QA/GLPs.

Items 9 and 10--see footnote 3.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: T 1482, Dow Corning 5700 antimicrobial agent from lot No. BN 126008, was described as a straw-colored liquid containing 50% methanol and 50% a.i. The test material was stored at room temperature and prepared as a 1:10 stock solution in dimethylsulfoxide (DMSO). The DMSO stock solution and all working dilutions were held at 4°C; further dilutions were prepared in growth medium.

2. Test Organism: Subconfluent stock cultures of BALB/3T3 (clone A31) cells were grown in Eagle's minimum essential medium, supplemented with 10% bovine serum, glutamine (2 mM), and antibiotics.

Cells were trypsinized, seeded at densities of 250 and 500 cells/dish for the cytotoxicity assays and 1 x 10^4 cells/dish for the transformation assay, and grown for 24 hours. Source, storage conditions, and cell maintenance were not reported.

3. Cytotoxicity Assay: Cytotoxic effects of the test material were measured by the reduction in the ability of the #3T3 cells to form colonies after treatment with five dilutions of the test material (1:10 to 1:1000) or the solvent control (DMSO). Triplicate flasks/dose, seeded at the appropriate cell density, were exposed for 24 hours to the selected test material dilutions. Exposed cells were washed, reincubated for 9 to 11 days, fixed, stained with Giemsa, and counted. Relative plating efficiency and/or relative colony-forming efficiency were calculated; an approximate LD_{50} was determined. Based on the results, doses for the transformation assay were selected to include at minimum, the LD_{50}, one dose above, and one dose below the LD_{50}.

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[2]Ibid.

[3]Only items appropriate to this DER have been included.

4. Transformation Assay: Prepared cultures, seeded with either 250 cells/dish for the parallel cytotoxicity assay, or 1 x 10^4 cells/dish for the transformation assays, were exposed to four selected dilutions of the test material, the solvent (DMSO), or the positive control, N-methyl-N'- nitro-N-nitrosoguanidine (MNNG) at 0.5 µg/mL. Triplicate plates were prepared for each treatment group in the cytotoxicity phase of the assay; 12 replicates per group were used in the transformation assay. At the conclusion of a 24-hour exposure period, the cells were washed and refed with growth medium. The cytotoxicity assay was conducted as described.

   Throughout the 4- to 6- week incubation period of the transformation assay, the cells were refed twice weekly with growth medium. Termination of the assay was accomplished by fixing and staining the monolayers and determining the number of foci present in each flask. Type-III foci, which the authors described as foci characterized by a loss of contact-inhibition that results in randomly oriented piling up of cells, were scored.

5. Evaluation Criteria:

   No criteria to establish the validity of the assay or the biological significance of the findings were presented.

6. Statistical Analysis:

   Statistical methods were not reported; however, the report indicated that data were evaluated for significance at $p < 0.05$.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Cytotoxicity Assay: The study authors reported that the test material formed a biphasic solution when introduced into tissue culture medium and required vigorous agitation to maintain a uniform suspension. Concentrations of the test material were, therefore, expressed as dilutions rather than actual doses of the test material in solution.

   The initial cytotoxicity assay was conducted with five dilutions of the test material (1:10, 1:100, 1:250, 1:500, and 1:1000). However, due to heavy compound precipitation and 100% cell death at these levels, a repeat cytotoxicity assay was performed. The second assay employed nine dilutions ranging from 1:1 x 10^9 to 1:1.6 x 10^5. Relative survival at dilutions $\leq$ 1:6 x 10^4 was zero; at these levels compound precipitation was observed. At the remaining high dilutions (~ 1:10^5), cell

survival increased in a dose-related manner and compound precipitation was reported to decrease with increasing dilutions. Based on these finding the study authors estimated that the LD_{50} of the test material ranged between the 1:1.8 x 10^4 and the 1:1.1 x 10^5 dilutions.

B. Transformation Assay: Four test dilutions (1:6 x 10^4, 1:8 x 10^4, 1:1 x 10^5, and 1:1.2 x 10^5) were evaluated in the transformation assay. As shown in Table 1, dose-related cytotoxicity (7.5% survival at 1:6 x 10^4 to 76.9% at 1:1.2 x 10^5) relative to the solvent control was observed. One type-III focus was scored at both of the lower two dilutions; transformation frequencies at 1:6 x 10^4 and 1:8 x 10^4 were 2.08 x 10^{-4}, and 0.32 x 10^{-4} respectively. However, the more meaningful parameter, average foci/dish, was calculated by our reviewers and found to be 0.08 at these dose levels. No transformed foci were seen at the two higher test material dilutions. Seven type-III foci were counted from cultures exposed to the positive control, MMNG, yielding an average foci/dish value of 0.6.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the test compound gave no statistically significant ($P > 0.05$) evidence of morphological transformation relative to the negative control condition. However, it should be noted that the agent did induce one morphologically transformed (type-III) focus at each of two different delivered doses, i.e., 1:6 x 10^4 and 1:8 x 10^4 dilutions.

B. A quality assurance statement was not presented.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We were unable to determine if the severe cytotoxicity observed in this study was associated with the a.i. or the 50% methanol content of the formulated product. The study authors should have assayed other solvents to determine whether something other than DMSO could have been used to solubilize the test material; however, the marked insolubility of the a.i. in aqueous medium probably precluded the use of other solvents. We assess, therefore, that the specific constraints placed on the assay by the test material's insolubility and solvent interference indicated that the highest attainable concentration was assayed. However, specific doses could not be determined and the data, therefore, provide no information that could be included in a toxicology profile of T 1482 or in risk assessment.

TABLE 1. Representative Results of the BALB/3T3 Nonactivated Transformation Assay with T 1482

Substance	Treatment Level	Percent[a] Plating Efficiency	% Relative Plating Efficiency	Total[b] Cells Exposed x 10^3	No. of Type-III Foci/ 12 Dishes	Average Foci/Dish[c]	Transformation Frequency[d] x 10^{-4}
Solvent Control							
Dimethylsulfoxide	0.25%	53.6	100	53.6(64.3)[e]	0	0	(<0.16)[e]
Positive Control							
N-methyl-N'-nitro-N-nitrosoguanidine	0.5 µg/mL	26.4	49.3	31.7	7	0.6	2.21
Test Material							
T 1482	1:6 x 10^4 [f]	4.0	7.5	4.8	1	0.08	2.08
	1:8 x 10^4	26.4	49.3	31.7	1	0.08	0.32
	1:1 x 10^5	30.4	56.7	36.5	0	0	<0.27
	1:1.2 x 10^5	41.2	76.9	49.4	0	0	<0.20

[a] $\frac{\text{Mean number of colonies/dish}}{\text{Number of cells plated}} \times 100.$

[b] Number of cells plated/dish (1 x 10^4) x % plating efficiency x 12 dishes.

[c] Calculated by our reviewers.

[d] $\frac{\text{Total number of type-III foci.}}{\text{Total number of cells exposed}}$

[e] Numbers in ( ) are calculated from the raw data by our reviewers.

[f] Dilutions of the test material.

The occurrence of type-III foci at the highest dilution (1:6 x 10^4) should be viewed with caution since pronounced cytotoxicity was seen at this level. However, the biological and/or statistical significance of the single type-III focus at the 1:8 x 10^4 dilution cannot be fully assessed because the number of replicates used in this assay (12) did not constitute an adequate sample size.[4] Since transformation is a rare event, the number of replicate platings at each dose level is of critical importance; in general, 15 to 20 dishes per dose are required to ensure a statistically significant sample size.

The study was further compromised because the 24-hour exposure of BALB/3T3 cells to the test material was shorter than the recommended treatment time (3 days).[5]

Although the assay was conducted without exogenous metabolic activation, prudence dictates that the investigators should have selected a procarcinogen, such a 3-methylcholanthrene, as the positive control to demonstrate the intrinsic ability of this cell line to metabolize and respond to procarcinogens. A chemical of this type rather than the direct-acting mutagen/carcinogen, MNNG, would have provided greater assurance that T 1482 was adequately tested. Based on these considerations, we conclude that the study is unacceptable and should be repeated with an appropriate solvent and positive control.

Item 15--see footnote 3.

16. CBI Appendix: Appendix A, Materials and Methods, CBI pp. 11-12; Appendix B, Protocol, CBI pp. 23-29.

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[4]Heidelberger et al. Mutat. Res. 114 (1983): 283-385

[5]Ibid.

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APPENDIX A

Materials and Methods

Page_____ is not included in this copy.

Pages 48 through 49 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.

✓ 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: Henry Spencer, Ph.D. 5/22/84
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Albin Kocialski, Ph.D. 5/22/84
Section VII, Toxicology Branch (TS-769C)

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DATA EVALUATION REPORT

Study Title: Activity of T1483 (Dow Corning® 5700 Antimicrobial Agent) in an In Vitro Mammalian Cell Transformation Assay in the Presence of Exogenous Metabolic Activation.

Laboratory: Microbiological Associates
5221 River Road
Bethesda, MD 20016

Date: May 11, 1979

Study No.: T1483

MRID No.: 403852-13

Material Tested: T1483 (Dow Corning 5700 Antimicrobial Agent) 50% ai in 50% w/v methanol. Lot No. BN126008 is 3-(trimethoxysilyl)propyldimethyloctadecyl ammonium chloride.

Authors: L.M. Schechtman and A. Isquith

Materials:

The antimicrobial, T1482 was supplied as a 50% ai in methanol. The test material was diluted 1:10 in DMSO to a stock solution and stored capped at 4 °C. The positive control used was Benzopyrene (BP) for exogenous activation systems. Solvent controls used DMSO.

Test Organism:

Stock cultures of BALB/3T3 (clone A31) cells were grown in Eagles' medium, supplemented with 10 percent fetal bovine serum, glutamine (2 mM) and antibiotics.

Methods:

Cytotoxicity of the test components and BALB/3T3 cells was carried out prior to transformation testing.

The cells were incubated for 2 hours at 37 °C with the test compound and activating suspension (see attachment). Following the treatment period, the exposed cells were washed, seeded and grown for 7 to 10 days for cytotoxicity assay. Following fixation and staining the viable cells were counted.

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Dilutions of exposure for cytotoxicity were:

Positive Control	12.5 ug/mL
Test Compound	Dilutions
with S-9	
	1:1 x 10^4
	1:2 x 10^4
	1:4 x 10^4
	1:8 x 10^4

Colony Transformation in Triplicate of Pooled Treatments

Test Materials	Concentrations
S-9 + DMSO (.25%)	--
Positive Control (S-9 + BP)	12.5 ug/mL
Test Material + S-9	Dilutions
	1:1 x 10^4
	1:2 x 10^4
	1:4 x 10^4
	1:8 x 10^4

Results: Cytotoxicity

Compound	Concentration	Relative Survival (%)
S-9 + DMSO	0	100
S-9 + BP	Dilution	35.5
S-9 + T1483	1:1 x 10^4	42.8
S-9 + T1483	1:2 x 10^4	55.0
S-9 + T1483	1:4 x 10^4	84.1
S-9 + T1483	1:8 x 10^4	83.2

Transformation in the Presence of Metabolic Activation

Compound	Concentration	(x 10^3) Cells at Risk	Number Type III Foci	(x 10^{-4}) T. Freq.
S-9 + DMSO	0	53.32	0	< .019
Positive Control				
S-9 + BP	12.5 ug/mL	18.60	3	1.61
	Dilution			
S-9 + T1483	1:1 x 10^4	22.44	0	< .45
S-9 + T1483	1:2 x 10^4	28.80	0	< .35
S-9 + T1483	1:4 x 10^4	44.04	0	< .23
S-9 + T1483	1:8 x 10^4	43.56	0	< .23

Conclusions:

Under the conditions of the test, metabolically activated transformation of A31 clone into Type III foci was not apparent.

However, this study was run under conditions not found in a standard protocol for this type assay, namely:

1. The exposure time was only 2 hours here while 3 days is the recommended time.

2. The number of replicate platings is from pooled treatments.

3. The actual doses should have been cited.

The study is unacceptable in its present form and should be repeated, taking into consideration the deficiencies cited above.

Attachment