4-28-89

EPA No.: 68D80056
DYNAMAC No.: 167-A
TASK No.: 1-67A
April 28, 1989

# DATA EVALUATION RECORD

## ACAROSAN - MOIST POWDER

## Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4-28-89

EPA No.: 68D80056
DYNAMAC No.: 167-A
TASK No.: 1-67A
April 28, 1989

DATA EVALUATION RECORD

ACAROSAN- MOIST POWDER

Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

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

Signature: Nancy E. McCarroll
Date: 4-27-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-28-89

APPROVED BY:

Roman Pienta, Ph.D.
Department Manager

Signature: Roman J. Pienta
Date: 4-28-89

Brian Dementi, Ph.D.
EPA Reviewer, Section I
Toxicology Branch I (TS-769C)

Signature: Brian Dementi
Date: 5/5/89

Edwin Budd
EPA Section Head, Section I
Toxicology Branch I (TS-769C)

Signature: Acting
Date: 6/1/89

DATA EVALUATION RECORD

CHEMICAL: Acarosan.

STUDY TYPE: Salmonella/mammalian activation mutagenicity assay.

ACCESSION NUMBER: 408453-11.

TEST MATERIAL: Acarosan-Moist Powder.

SYNONYMS/CAS NO. Not listed.

SPONSOR: Gesellschaft fur Hausbiologische Forschung, Mainz, FRG.

TESTING FACILITY: Cytotest Cell Research GmbH and Co., Darmstadt, FRG.

TITLE OF REPORT: Salmonella typhimurium reverse mutation assay with Acarosan-Moist Powder.

AUTHOR(S): Timm, A.

STUDY NUMBER(S): 111508.

REPORT ISSUED: September 30, 1987.

CONCLUSION(S) - Executive Summary: Under the conditions of two independent Salmonella typhimurium/mammalian microsome plate incorporation assays, 10.0, 100.0, 333.3, 1000.0, and 5000.0 µg/plate acarosan-moist powder did not induce a mutagenic effect in S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 either in the presence or absence of S9 activation. Compound precipitation was noted at the highest assayed dose (5000 µg/plate) and a weak but reproducible cytotoxic effect was seen in strain TA100 at this level without S9 activation. It was concluded, therefore, that acarosan-moist powder was assayed up to an appropriate concentration with no evidence of mutagenicity.

Study: Acceptable.

A. MATERIALS:

1. Test Material: Name: Acarosan-Moist Powder
Description: White solid
Lot #: Prod. Mai 1987; purity: Listed as a mixture
Contaminants: None reported
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: The test material was not completely soluble at a concentration of 50 mg/mL in DMSO.

2. Control Materials:
Negative: Bacteria
Solvent/final concentration: 100 µL/plate
Positive: Nonactivation:
Sodium azide 10 µg/plate TA100, TA1535
4-Nitro-o-phenylene-diamine (4-NPA)
50 µg/plate TA98, TA1538, TA1537

Activation:
2-Aminoanthracene (2-AA) 10 µg/plate all strains.

3. Activation: S9 derived from

| | | | | | |
|---|---|---|---|---|---|
| x | Aroclor 1254 | x | induced | x | rat |
| | phenobarbital | | noninduced | | mouse |
| | none | | | | hamster |
| | other | | | | other |

| | |
|---|---|
| x | liver |
| | lung |
| | other |

If other, describe below. Describe S9 composition (if purchased, give details).

4. Test Organism Used: S. typhimurium strains

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TA97 | x | TA98 | x | TA100 | | TA102 | | TA104 |
| x | TA1535 | x | TA1537 | x | TA1538; list any others: | | | | |

Test organisms were properly maintained: Yes
Checked for appropriate genetic markers (rfa mutation, R factor): Yes

5. Test Compound Concentrations Used:

Preliminary Assay: 1, 3, 10, 33, 100, 333, 1000, and 5000 µg/plate with or without S9 activation with strains TA98 and TA100.

Mutation Assays: 10.0, 100.0, 333.3, 1000.0, and 5000.0 µg/plate with or without S9 activation in all strains.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x___ Standard plate test
_____ Pre-incubation (_) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

2. Preliminary Assay: Cytotoxicity and mutagenicity of the test material were assessed concurrently in strains TA98 and TA100. Eight concentrations of the test material ranging from 1 to 5000 µg/plate, negative, solvent (DMSO), and the appropriate positive controls (sodium azide, 4-NPA, or 2-AA) were assayed in the presence or absence of S9 activation using S. typhimurium TA98 and TA100. Triplicate plates were prepared for each treatment and/or dose level. Compound precipitation was noted at 5000 µg/plate; below this level, the test material was reported to be "completely dissolved." Lower than control colony counts for TA98 were noted at all nonactivated doses; an ≈50% reduction in revertant colonies of TA100 was seen at the highest nonactivated dose (5000 µg/plate). No cytotoxicity was observed under S9-activated conditions; no increase in revertants to histidine prototrophy ($His^+$) of either strain was seen following exposure to the eight test material doses with or without S9 activation (Table 1). In contrast, both strains responded to the mutagenic action of the appropriate positive controls.

3. Mutagenicity Assay: Two independent mutation assays were performed with five concentrations of the test material (10.0, 100.0, 333.3, 1000.0, and 5000.0 µg/plate) in the presence and absence of S9 activation. In the first trial, only strains TA1535, TA1537, and TA1538 were assayed. The data for TA98 and TA100 from the preliminary assay were considered by the study author to be part of the first mutation assay. As shown in Table 1, $His^+$ colonies of TA1535 and TA1538 were slightly reduced following exposure to nonactivated 5000 µg/plate. No appreciable increase in revertant colonies was seen at any dose level with or without S9 activation.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with Acarosan-Moist Powder

| Substance | S9 Activation | Dose (µg/plate) | Revertants per Plate of Bacterial Tester Strain[a] TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | | | | | | | |
| Bacteria | - | -- | 15 ± 0.6 | 10 ± 2.5 | 14 ± 0.0 | 28 ± 6.6 | 143 ± 11.1 |
| | + | -- | 11 ± 3.5 | 7 ± 2.6 | 23 ± 2.1 | 46 ± 6.7 | 144 ± 8.2 |
| Solvent Control | | | | | | | |
| Dimethyl-sulfoxide | - | -- | 13 ± 3.8 | 14 ± 5.0 | 18 ± 2.0 | 23 ± 7.0 | 133 ± 21.4 |
| | + | -- | 13 ± 5.5 | 7 ± 2.0 | 21 ± 3.1 | 37 ± 6.0 | 138 ± 1.7 |
| Positive Control | | | | | | | |
| Sodium azide | - | 10 | 883 ± 47.4 | -- | -- | -- | 797 ± 20.2 |
| 4-Nitro-o-phenylene-diamine | - | 50 | -- | 395 ± 35.2 | 1689 ± 125.7 | 2036 ± 209.0 | -- |
| 2-Aminoanthracene | + | 10 | 151 ± 29.7 | 252 ± 19.1 | 1390 ± 68.9 | 1237 ± 92.1 | 2265 ± 212.0 |
| Test Material | | | | | | | |
| Acarosan-moist powder | - | 5000[b] | 9 ± 3.1 | 11 ± 5.0 | 8 ± 1.2 | 13 ± 4.9 | 73 ± 12.7 |
| | + | 5000 | 13 ± 4.0 | 9 ± 3.1 | 24 ± 4.6 | 46 ± 9.6 | 120 ± 16.4 |

[a]Means ± standard deviations of the counts of triplicate plates.

[b]Highest assayed dose with or without S9 activation; results for lower doses (10.0, 100.0, 333.3, and 1000.0 µg/plate +/- S9 with all strains and additional doses of 1 and 3 µg/plate/+/-S9 with TA98 and TA100) did not indicate a mutagenic effect.

Representative results from the second assay are shown in Table 2. An ≈ ≤30% reduction in TA100 $His^+$ revertant colonies was seen after exposure to 1000 and 5000 µg/plate -S9. No cytotoxicity was observed for the other tester strains with or without S9 activation. The findings further indicated, in agreement with the results of the initial trial, that acarosan-moist powder was not mutagenic in this test system. All strains responded to the mutagenic action of the appropriate nonactivated and S9-activated positive controls.

The study author concluded that "acarosan-moist powder is considered to be nonmutagenic in this Salmonella typhimurium reverse mutation assay".

4. Reviewers' Discussion/Conclusions: We assess that the study was properly conducted and that the author's interpretation of the data was correct.

Acarosan-moist powder, assayed to the limit of solubility, induced a reproducible weak cytotoxic effect in TA100 under nonactivated conditions at the highest assayed dose (5000 µg/plate) but was not mutagenic in two independent assays either with or without S9 activation.

The sensitivity of the test system to detect direct-acting mutagens and promutagens was clearly demonstrated for all strains under nonactivated and S9-activated conditions. It was concluded, therefore, that the study provides acceptable evidence that acarosan-moist powder is not mutagenic in this test system.

5. Quality Assurance: A quality assurance statement was signed and dated September 30, 1987.

6. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-15.

TABLE 2. Representative Results of the Second Salmonella typhimurium Mutagenicity Assay with Acarosan-Moist Powder

| Substance | S9 Activation | Dose (µg/plate) | Revertants per Plate of Bacterial Tester Strain[a] TA1535 | TA1537 | TA1538 | TA98 | TA100 |
|---|---|---|---|---|---|---|---|
| Negative Control | | | | | | | |
| Bacteria | - | -- | 25 ± 6.4 | 13 ± 2.6 | 14 ± 3.1 | 26 ± 3.5 | 101 ± 10.8 |
| | + | -- | 13 ± 3.0 | 16 ± 3.1 | 30 ± 3.2 | 37 ± 1.2 | 115 ± 10.1 |
| Solvent Control | | | | | | | |
| Dimethyl-sulfoxide | - | -- | 25 ± 1.2 | 11 ± 1.5 | 14 ± 9.0 | 31 ± 3.5 | 102 ± 7.9 |
| | + | -- | 11 ± 2.5 | 19 ± 5.0 | 22 ± 4.0 | 24 ± 9.5 | 105 ± 8.1 |
| Positive Control | | | | | | | |
| Sodium azide | - | 10 | 1203 ± 32.0 | --- | --- | --- | 1243 ± 42.9 |
| 4-Nitro-o-phenylene-diamine | - | 50 | --- | 239 ± 28.8 | 1721 ± 195.4 | 2295 ± 162.8 | --- |
| 2-Aminoanthracene | + | 10 | 328 ± 22.8 | 325 ± 25.5 | 1314 ± 359.2 | 1381 ± 73.3 | 2215 ± 291.6 |
| Test Material | | | | | | | |
| Acarosan-Moist Powder | - | 5000[b] | 21 ± 3.8 | 11 ± 2.1 | 14 ± 5.3 | 24 ± 6.7 | 44[c] ± 6.7 |
| | + | 5000 | 16 ± 1.5 | 16 ± 8.5 | 30 ± 8.0 | 22 ± 2.1 | 93 ± 6.1 |

[a]Means ± standard deviations of the counts of triplicate plates.

[b]Highest assayed dose with or without S9 activation; results for lower doses (10.0, 100.0, 333.3, and 1000.0 µg/plate +/-S9) did not indicate a mutagenic effect.

[c]Revertant counts of TA100 were also reduced following exposure to 1000 µg/plate/-S9 (75 ± 10.4 revertants/plate).

# APPENDIX A
## Materials and Methods

Page_____ is not included in this copy.

Pages 10 through 16 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.