

US EPA ARCHIVE DOCUMENT

CONFIDENTIAL SUBJECT INFORMATION  
DOES NOT CONTAIN  
NATIONAL SECURITY INFORMATION (EO 12958)

4-28-89

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

DATA EVALUATION RECORD

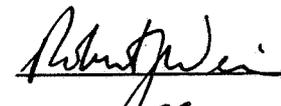
ACAROSAN - MOIST POWDER

Mutagenicity--Salmonella typhimurium/Mammalian Microsome  
Mutagenicity Assay

APPROVED BY:

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

Signature: \_\_\_\_\_



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 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 Pienta  
Date: 4-28-89

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

Signature: Brian Dementi *JM*  
Date: 5/5/89 *5/5/89*

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

Signature: Edwin Budd  
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  $\mu$ g/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  $\mu$ g/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: Acaroson-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

<u>x</u>	Aroclor 1254	<u>x</u>	induced	<u>x</u>	rat	<u>x</u>	liver
<u>      </u>	phenobarbital	<u>      </u>	noninduced	<u>      </u>	mouse	<u>      </u>	lung
<u>      </u>	none	<u>      </u>		<u>      </u>	hamster	<u>      </u>	other
<u>      </u>	other	<u>      </u>		<u>      </u>	other	<u>      </u>	

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

4. Test Organism Used: S. typhimurium strains

<u>      </u>	TA97	<u>x</u>	TA98	<u>x</u>	TA100	<u>      </u>	TA102	<u>      </u>	TA104
<u>x</u>	TA1535	<u>x</u>	TA1537	<u>x</u>	TA1538; list any others:	<u>      </u>		<u>      </u>	

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  $\mu\text{g}/\text{plate}$ , 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  $\mu\text{g}/\text{plate}$ ; 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  $\approx 50\%$  reduction in revertant colonies of TA100 was seen at the highest nonactivated dose (5000  $\mu\text{g}/\text{plate}$ ). No cytotoxicity was observed under S9-activated conditions; no increase in revertants to histidine prototrophy ( $\text{His}^+$ ) 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  $\mu\text{g}/\text{plate}$ ) 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,  $\text{His}^+$  colonies of TA1535 and TA1538 were slightly reduced following exposure to nonactivated 5000  $\mu\text{g}/\text{plate}$ . 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 Acaroson-Moist Powder

Substance	S9 Acti- vation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain <sup>a</sup>				
			TA1535	TA1537	TA1538	TA98	TA100
<u>Negative Control</u>							
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
<u>Solvent Control</u>							
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
<u>Positive Control</u>							
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
<u>Test Material</u>							
Acarosan- moist powder	-	5000 <sup>b</sup>	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

<sup>a</sup>Means ± standard deviations of the counts of triplicate plates.

<sup>b</sup>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  $\approx \leq 30\%$  reduction in TA100 His<sup>+</sup> revertant colonies was seen after exposure to 1000 and 5000  $\mu\text{g}/\text{plate}$  -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  $\mu\text{g}/\text{plate}$ ) 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 Acti- vation	Dose ( $\mu\text{g}/\text{plate}$ )	Revertants per Plate of Bacterial Tester Strain <sup>a</sup>				
			TA1535	TA1537	TA1538	TA98	TA100
<u>Negative Control</u>							
Bacteria	-	--	25 $\pm$ 6.4	13 $\pm$ 2.6	14 $\pm$ 3.1	26 $\pm$ 3.5	101 $\pm$ 10.8
	+	--	13 $\pm$ 3.0	16 $\pm$ 3.1	30 $\pm$ 3.2	37 $\pm$ 1.2	115 $\pm$ 10.1
<u>Solvent Control</u>							
Dimethyl- sulfoxide	-	--	25 $\pm$ 1.2	11 $\pm$ 1.5	14 $\pm$ 9.0	31 $\pm$ 3.5	102 $\pm$ 7.9
	+	--	11 $\pm$ 2.5	19 $\pm$ 5.0	22 $\pm$ 4.0	24 $\pm$ 9.5	105 $\pm$ 8.1
<u>Positive Control</u>							
Sodium azide	-	10	1203 $\pm$ 32.0	---	---	---	1243 $\pm$ 42.9
4-Nitro-o- phenylene-diamine	-	50	---	239 $\pm$ 28.8	1721 $\pm$ 195.4	2295 $\pm$ 162.8	---
2-Aminoanthracene	+	10	328 $\pm$ 22.8	325 $\pm$ 25.5	1314 $\pm$ 359.2	1381 $\pm$ 73.3	2215 $\pm$ 291.6
<u>Test Material</u>							
Acarosan- Moist Powder	-	5000 <sup>b</sup>	21 $\pm$ 3.8	11 $\pm$ 2.1	14 $\pm$ 5.3	24 $\pm$ 6.7	44 <sup>c</sup> $\pm$ 6.7
	+	5000	16 $\pm$ 1.5	16 $\pm$ 8.5	30 $\pm$ 8.0	22 $\pm$ 2.1	93 $\pm$ 6.1

<sup>a</sup>Means  $\pm$  standard deviations of the counts of triplicate plates.

<sup>b</sup>Highest assayed dose with or without S9 activation; results for lower doses (10.0, 100.0, 333.3, and 1000.0  $\mu\text{g}/\text{plate}$  +/-S9) did not indicate a mutagenic effect.

<sup>c</sup>Revertant counts of TA100 were also reduced following exposure to 1000  $\mu\text{g}/\text{plate}$ /-S9 (75  $\pm$  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.

---