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

JUN 19 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: ~~EPA Registration~~ Accession Nos. 233-(417-419) and 233-424
Mutagenicity Testing of [redacted] and Acarosan
Moist Powder (Active Ingredient: Benzyl Benzoate)
Proj. No. 9-0180 thru 9-0183
Tox Chem. No. 82

FROM: Brian Dementi, Ph.D., D.A.B.T. Brian Dementi 6/1/89
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Mike Mendelsohn
PM Team 17
Insecticide-Rodenticide Branch
Registration Division (H7505C)

THRU: Robert Zendzian, ~~Ph.D.~~, ~~Acting~~ Section Head 6/-/89 6/12/89
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Please find appended Data Evaluation Reports for the mutagenicity studies on [redacted] and Acarosan Moist Powder submitted by Fisons Corporation. The studies in question are the Escherichia coli Mammalian Microsome Mutagenicity Assay and the Salmonella typhimurium Mammalian Microsome Mutagenicity Assay, both of which were performed on both Acarosan products. The four studies were all found to be acceptable.

I - Escherichia coli Mammalian Microsome Mutagenicity Assays.

1) Acarosan - Moist Powder, study number 118506. The test material did not induce a cytotoxic or mutagenic effect in E. coli with or without metabolic activation at doses as high as 5000 ug/plate.

2) [redacted]

II - Salmonella typhimurium Mammalian Microsome Mutagenicity assays.

1) Acarosan - Moist Powder, study number 111508.
The test material did not induce a mutagenic effect in S. typhimurium with or without metabolic activation at doses up to a level of cytotoxicity which was the limit of solubility, i.e. 5000 ug/plate.

2) [redacted]

In accordance with your comments of April 25, 1989, it is TOX Branch's understanding that an assessment of the literature review on Acarosan, "Appraisal of the Safety of Acarosan Following Potential Human Exposure", will not be necessary. Furthermore, it is my understanding that we need only submit the mutagenicity DERs and that Registration Division will assemble the response, and in so doing address all pertinent issues.

Attachments (4)

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

DATA EVALUATION RECORD

ACAROSAN - MOIST POWDER

Mutagenicity--Escherichia coli
Mammalian Microsome Mutagenicity Assay

APPROVED BY:

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

Signature: [signature]

Date: 4/28/89

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

DATA EVALUATION RECORD

ACAROSAN- MOIST POWDER

MUTAGENICITY--Escherichia coli
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 J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

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

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

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

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

Signature: Acting
Date: 6/1/89

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DATA EVALUATION RECORD

CHEMICAL: Acarosan.

STUDY TYPE: Escherichia coli mammalian activation mutagenicity assay.

ACCESSION/MRID NUMBER: 408453-10.

TEST MATERIAL: Acarosan-Moist Powder.

SYNONYM(S)/CAS No.: Not listed.

SPONSOR: Gesellschaft fur Hausbiologishe Forschung, Mainz, F.R.G.

TESTING FACILITY: Cytotest Cell Research GmbH and Co., Darmstadt, F.R.G.

TITLE OF REPORT: Escherichia coli Reverse Mutation Assay with Acarosan-Moist Powder.

AUTHOR(S): Timm, A.

STUDY NUMBER(S): 118506.

REPORT ISSUED: November 26, 1987.

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CONCLUSIONS-Executive Summary:

Under the conditions of two independent Escherichia coli/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 cytotoxic or mutagenic effect in E. coli WP_2 uvrA either in the presence or absence of S9 activation. The highest assayed dose was not completely soluble and, therefore, represents the limit of solubility. It was concluded, therefore, that acarosan-moist powder was assayed to an appropriate concentration with no evidence of mutagenicity in this test system.

Study: Acceptable.

A. MATERIALS:

1. Test Material: Name: Acarosan-moist powder
   Description: White solid
   Lot No.: Prod. Mai 1987. purity: Listed as a mixture
   Contaminants: None reported
   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: Test material was not fully soluble at 50 mg/mL in DMSO.

2. Control Materials:
   Negative: Bacteria
   Solvent/final concentration: 100 μg/plate
   Positive: Nonactivation:
   Methyl methanesulfonate (MMS) 10 μL/plate

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

3. Activation: S9-derived from:

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

4. Test Organism Used: E. coli WP_2 uvrA.

   Test organisms were properly maintained. Yes.
   Checked for appropriate genetic markers (normal spontaneous mutation rate). Yes.

5. Test Compound Concentrations Used:

Preliminary cytotoxicity/mutagenicity assay: 1, 3, 10, 33, 100, 333, 1000, and 5000 μg/plate with or without S9 activation.

Repeat mutation assay: 10.0, 100.0, 333.3, 1000.0, and 5000.0 μg/plate with or without S9 activation.

B. TEST PERFORMANCE:

1. Type of E. coli Assay:

x	Standard plate test
_____	Pre-incubation (_) minutes
_____	"Prival" modification
_____	Spot test
_____	Other (describe).

2. Preliminary Assay: Cytotoxicity and mutagenicity of acarosan were assessed concurrently in E. coli WP_2 uvrA. Eight concentrations of the test material ranging from 1 to 5000 μg/plate, negative, solvent (DMSO), and the positive controls (MMS or 2-AA) were assayed in the presence and absence of S9 activation. Triplicate plates were prepared for each treatment and/or dose level. Compound precipitation was observed on plates containing 5000 μg/plate of the test material; the report stated that at 1000 μg/plate and lower, the test material was "completely dissolved." Although revertant colonies of E. coli WP_2 uvrA were lower than the concurrent control at the three highest doses with or without S9 activation, the counts were within the normal range of plating variability and, therefore, not considered to indicate a cytotoxic effect.

Similarly, no appreciable increase in reversion to tryptophan prototrophy occurred at any nonactivated or S9 activated dose of the test material. In contrast, increases in mutant colonies were seen following exposure to the positive controls (10 μL MMS/-S9 and 10 μg/plate 2AA +S9).

3. Repeat Mutation Assay: The repeat assay was conducted as described with five test material concentrations (10.0, 100.0, 333.3, 1000.0, and 5000.0 μg/plate in the presence or absence of S9 activation. Results of the second assay compared favorably with the initial finding indicating that acarosan-moist powder was neither cytotoxic nor mutagenic in E. coli WP_2 uvrA.

Representative results from both assays are presented in Table 1.

TABLE 1. Representative Results of the Escherichia coli Mutagenicity Assays with Acarosan-Moist Powder

Substance	S9 Activation	Dose/plate	Revertants per plate of E. coli WP_2 uvrA[a] Initial Assay	Repeat Assay
Negative Control				
Bacteria	-	--	33 ± 3.5	30 ± 0.6
	+	--	35 ± 1.0	35 ± 3.0
Solvent Control				
Dimethyl sulfoxide	-	--	32 ± 3.2	34 ± 2.6
	+	--	35 ± 4.5	34 ± 2.1
Positive Control				
Methylmethane-sulfonate	-	10 μL	520 ± 63.6	1425 ± 115.0
2-Aminoanthracene	+	10 μg	377 ± 62.2	447 ± 42.3
Test Material				
Acarosan-Moist powder	-	5000 μg[b]	22 ± 6.0	34 ± 4.0
	+	5000 μg	23 ± 4.5	28 ± 10.6

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

[b]Highest assayed dose and compound precipitation was observed at this level; results for lower doses (1, 3, 10, 33, 100, 333, and 1000 μg/plate +/-S9 in the initial assay and 10.0, 100.0, 333.3, and 1000.0 μg/plate +/-S9 in the repeat assay) were comparable to the solvent control values.

The author concluded that "acarosan-moist powder was not mutagenic in the Escherichia coli 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 was neither cytotoxic nor mutagenic when assayed up to the limit of solubility. The results further show that the sensitivity of the test system to detect a mutagenic effect was adequately demonstrated in both trials as indicated by the response of E. coli WP_2 uvrA to the direct-acting and promutagenic positive controls. 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 November 26, 1987.

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

APPENDIX A

Materials and Methods

Benzyl benzoate toxicology review

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___ Description of the product manufacturing process

___ Description of product 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

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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

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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.

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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.

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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.

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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).

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APPENDIX A

Materials and Methods

Ms. Caroline Gordon
Health Effects Division - CM2 - Room 816
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, Virginia 22202

Dear Ms. Gordon:

Enclosed is an initial draft for the following DER--ACAROSAN.

o Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay. Study No. 111508, Accession No. 408453-11. Dynamac No. 167-A and EPA No. 1-67A.

We have enclosed the confidential business information for the above report.

Sincerely,

Robert J. Weir, Ph.D.
Program Manager

APPENDIX A
Materials and Methods

Benzyl benzoate toxicology review

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___ The product confidential statement of formula

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X FIFRA registration data

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Benzyl benzoate toxicology review

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