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Chemical:	**Fipronil**
PC Code:	**129121**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/15/95**
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 15 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Fipronil Metabolites - Review of Five Mutagenicity Studies

P.C. Code: 129121
DP Barcode: D214544
Submission: S480413

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer Virginia A Dobozy 8/31/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Rick Keigwin/Ann Sibold/PM 10
Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C) 9/11/95

and

Karl P. Baetcke, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C) 9/14/95

Registrant: Rhone-Poulenc

Action Requested: Review five mutatgenicity studies conducted with fipronil metabolites.

Recommendation: Toxicology Branch II has completed the reviews; all of the studies are classified as acceptable. There was no evidence of a mutagenic response in any of the studies.

Background

In the metabolism study in rats (MRID # 429186-55), of the metabolites tested in the following mutagenicity studies, only MB 45950 and RPA 200766 were isolated from the urine and/or feces.

DATA SUMMARIES

1) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-16

Material Tested: MB 45950 (98.9% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 10, 25, 50, 100 or 250 µg/plate MB 45950 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate +/-S9; the nonactivated test material was also cytotoxic toward the majority of strains at 100 µg/plate. **There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

2) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-17

Material Tested: RPA 200766 (>98% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 50, 100, 250, 500 or 1000 µg/plate or 50, 100, 250, 500, 1000 or 2500 µg/plate RPA 200766 in the absence or presence of S9 activation, respectively (initial trial) or 50-1000 µg/plate +/-S9 (confirmatory trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 1000 µg/plate -S9 and at ≥500 µg/plate +S9; cytotoxicity was not demonstrated at any dose with or without S9 activation. **There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

3) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-21

Material Tested: MB 46513 (98.6% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 10, 25, 59, 100 or 250 µg/plate MB 46513 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate with or without S9 activation; compound precipitation was also present at 100 µg/plate +/-S9. **There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

4) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-22

Material Tested: RPA 104615 (94.7% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 104615 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The test material was soluble and noncytotoxic at all levels. **There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

5) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 434011-02

Material Tested: RPA 105048 (98.6% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 105048 in the absence or

presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 5000 µg/plate +S9; cytotoxicity toward the majority of strains was also seen at the high dose with or without S9 activation. **There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

FIPRONIL SALMONELLA

EPA Reviewer: Nancy McCarroll
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EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy E. McCarroll
Date: 5/4/95
Signature: James N Rowe
Date: 5/4/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 432917-16

TEST MATERIAL: MB 45950

SYNONYM(S): Fipronil; 1H-Pyrazole-3-carbonitrile,5-amino-1-(2,6-dichloro-4-(trifluoromethylphenyl)-4-((trifluoromethyl)thio); $C_{12}H_4Cl_2F_6N_4S$

STUDY NUMBER(S): SA 93305

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: MB 45950 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: February 17, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-16), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 10, 25, 50, 100 or 250 µg/plate MB 45950 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate +/-S9; the nonactivated test material was also cytotoxic toward the majority of strains at 100 µg/plate. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: MB 45950

Description: Slightly yellow powder
Lot/ batch number: OP5502
Purity: 98.9% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: 120067-83-6
Structure: Not provided
Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

Positive: Nonactivation:
Sodium azide 1 µg/plate TA100, TA1535
2-Nitrofluorene 1 µg/plate TA98
9-Aminoacridine 50 µg/plate TA1537
Other:

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

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

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

The rat liver S9 homogenate (Lot no. 38) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

4. Test Organism Used: S. typhimurium strains

_____ TA97	x TA98	x TA100	_____ TA102	_____ TA104
x TA1535	x TA1537	_____ 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:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (10, 25, 50, 100 and 250 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition. A repeat test was conducted with strain TA1537 using comparable nonactivated levels of the test material.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

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

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈72 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation

was seen on plates containing ≥250 μg/plate +/-S9. The nonactivated test material was cytotoxic at insoluble levels; no cytotoxicity was apparent under S9-activated conditions. Based on these findings, the initial mutation assay was performed with test material doses of 10, 25, 50, 100 and 250 μg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that MB 45950 was insoluble and cytotoxic at the high dose (250 μg/plate +/- S9). Cytotoxicity, as indicated by a reduced background lawn of growth, was evident for the majority of strains at nonactivated 100 μg/plate. No evidence of a mutagenic effect was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that MB 45950 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. MB 45950 was tested to an insoluble and cytotoxic level (250 μg/plate) with no evidence of a mutagenic effect in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated February 17, 1994).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with MB 45950

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]			
			TA535	TA1537[b]	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 ml	19±2.5	17±3.9	32±5.8	135±6.8
	+	0.1 ml	17±3.3	15±2.9	41±5.8	126±13.0
Positive Control						
Sodium azide	-	1 µg	584±15.6	--	--	896±36.2
2-Nitrofluorene	-	1 µg	--	--	359±57.3	--
9-Aminoacridine	-	50 µg	--	457±102.4	--	--
2-Anthracene	+	2 µg	274±7.65	192±7.5	2213±124.0	2012±116.8
Test Material						
MB 45950	-	100 µg[c]	15±10.5[d]	12±3.6[d]	32±7.1	125±9.7[d]
	-	250 µg[c]	11±0.6[d]	--	24±7.2[d]	92±10.0[d]
	+	100 µg[c]	13±6.4[d]	14±1.7[d]	36±7.2	113±9.7
	+	250 µg[c]	12±3.0[d]	11±0.6[d]	31±1.7[d]	118±11.8[d]

[a]Means and standard deviations of counts from triplicate plates.
[b]Results from repeat nonactivated assay with this strain; initial assay was aborted due to the poor performance with the positive control.
[c]Results for lower doses (10, 25 or 50 µg/plate +/-S9) did not suggest a mutagenic effect.
[d]Thinning of the background lawn of growth was observed at this level.
[e]Highest assayed dose; compound precipitation reported on the majority of plates containing this concentration.

Note: Data were extracted from the study report, pp. 27-30.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with MB 45950

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]			
			TA535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 ml	20±5.2	14±4.2	36±3.5	144±9.4
	+	0.1 ml	18±2.8	16±2.7	37±9.5	121±7.3
Positive Control						
Sodium azide	-	1 µg	566±41.0	--	--	783±78.4
2-Nitrofluorene	-	1 µg	--	--	340±53.7	--
9-Aminoacridine	-	50 µg	--	364±125.9	--	--
2-Anthracene	+	2 µg	252±25.7	309±43.3	2465±125.9	2162±354.2
Test Material						
MB 45950	-	100 µg[b]	16±4.9[c]	8±3.1[c]	35±7.2	115±7.5[c]
	-	250 µg[d]	14±3.2[c]	--	25±2.9[c]	119±7.8[c]
	+	100 µg[b]	17±6.6	11±2.5	33±4.5	126±2.3
	+	250 µg[d]	10±0.6[c]	16±2.5[c]	36±2.0	132±27.0[c]

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (10, 25 or 50 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Thinning of the background lawn of growth was observed at this level.
[d]Highest assayed dose; compound precipitation reported on the majority of plates containing this concentration.

Note: Data were extracted from the study report, pp. 32-34.

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Signature: James N. Rowe
Date: 5/4/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 432917-17

TEST MATERIAL: RPA 200766

SYNONYM(S): Fipronil; 5-amino-3-carbamoyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole; $C_{12}H_6Cl_2F_6N_4O_2S$

STUDY NUMBER(S): SA 93174

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 200766 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: September 23, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-17), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 50, 100, 250, 500 or 1000 µg/plate or 50, 100, 250, 500, 1000 or 2500 µg/plate RPA 200766 in the absence or presence of S9 activation, respectively (initial trial) or 50-1000 µg/plate +/-S9 (confirmatory trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 1000 µg/plate -S9 and at ≥500 µg/plate +S9; cytotoxicity was not demonstrated at any dose with or without S9 activation. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

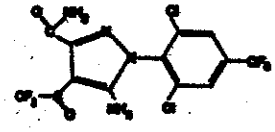
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RPA 200766

Description: White solid
Lot/ batch number: 57 TDS 62
Purity: >98% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: Not listed
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 4°C, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

Positive: Nonactivation:
Sodium azide 1 µg/plate TA100, TA1535
2-Nitrofluorene 1 µg/plate TA98, TA1538
9-Aminoacridine 50 µg/plate TA1537
Other:

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

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

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

The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

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:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five nonactivated doses (50, 100, 250, 500 and 1000 µg/plate) and six S9-activated doses (50, 100, 250, 500, 1000 and 2500 µg/plate) were evaluated using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above with the exception that 2500 µg/plate +S9 was not tested.

B. TEST PERFORMANCE:

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

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on plates containing ≥1000 µg/plate +/-S9; at 1000 µg/plate +S9,

compound precipitation was reported to be slight. No appreciable decrease in the number of his^+ revertant colonies was seen at any nonactivated or S9-activated level of the test material. Based on these findings, the initial mutation assay was performed with test material doses ranging from 50 to 1000 µg/plate -S9 and 50 to 2500 µg/plate +S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that RPA 200766 was insoluble at 1000 µg/plate -S9 and at ≥ 500 µg/plate + S9. The test material was also shown to be neither cytotoxic nor mutagenic with or without S9 activation. By contrast to the uniformly negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 200766 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 200766 was tested to insoluble levels (1000 µg/plate-S9; ≥ 500 µg/plate +S9) and failed to induce either a cytotoxic or mutagenic effect in a well-controlled study. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 23, 1993).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 200766

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	19±5	12±3	13±3	28±4	123±14
	+	0.1 ml	13±2	14±5	24±5	41±7	104±10
Positive Control							
Sodium azide	-	1 µg	509±69	--	--	--	694±45
2-Nitrofluorene	-	1 µg	--	--	405±12	394±8	--
9-Aminoacridine	-	50 µg	--	250±17	--	--	--
2-Anthracene	+	2 µg	291±14	236±47	1862±93	1495±236	1884±224
Test Material							
RPA 200766	-	500 µg[b]	20±6	12±1	14±4	33±4	112±9
	-	1000 µg[c]	14±4	9±3	21±5	36±7	112±8
	+	250 µg[b]	15±6	9±1	20±5	29±9	97±3
	+	2500 µg[c]	10±3	13±4	23±10	33±5	116±11

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest soluble level; results for lower doses (50, 100 or 250 µg/plate -S9 or 50 or 100 µg/plate +S9) did not suggest a mutagenic effect.
[c]Highest assayed concentration; compound precipitation noted at this dose. Results for intermediate S9-activated levels (500 or 1000 µg/plate) were negative.

Note: Data were extracted from the study report, pp. 26-28.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 200 766

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	20±8	14±3	16±5	26±3	122±10
	+	0.1 ml	16±3	9±4	18±4	35±9	104±8
Positive Control							
Sodium azide	-	1 µg	483±44	--	--	--	734±17
2-Nitrofluorene	-	1 µg	--	--	475±22	422±29	--
9-Aminoacridine	-	50 µg	--	379±51	--	--	--
2-Anthracene	+	2 µg	255±46	352±108	1700±148	1830±192	2183±163
Test Material							
RPA 200766	-	500 µg[b]	20±3	14±6	18±6	24±5	120±9
	-	1000 µg[c]	18±4	13±6	16±2	31±4	129±9
	+	250 µg[b]	14±5	18±3	26±4	35±6	107±14
	+	1000 µg[c]	16±1	15±4	20±2	32±8	98±5

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest soluble level; results for lower doses (50, 100 or 250 µg/plate -S9 or 50 or 100 µg/plate +S9) did not suggest a mutagenic effect.
[c]Highest assayed concentration; compound precipitation noted at this dose. Results for the intermediate S9-activated level (500 µg/plate) were negative.

Note: Data were extracted from the study report, pp. 30-32.

FIPRONIL SALMONELLA

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy L. McCarroll
Date: 5/4/95
Signature: James N. Rowe
Date: 5/4/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 432917-21

TEST MATERIAL: MB 46513

SYNONYM(S): Fipronil; 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole; $C_{12}H_4Cl_2F_6N_4$

STUDY NUMBER(S): SA 93135

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: MB 46513 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: August 24, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-21), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 10, 25, 59, 100 or 250 µg/plate MB 46513 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate with or without S9 activation; compound precipitation was also present at 100 µg/plate +/-S9. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

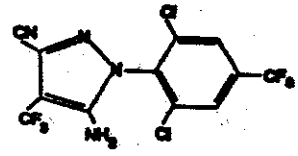
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: MB 46513

Description: Yellow solid
Lot/ batch number: 33 RJO 108
Purity: 98.6% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: Not listed
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 4°C, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

Positive: Nonactivation:

Sodium azide	1 µg/plate	TA100, TA1535
2-Nitrofluorene	1 µg/plate	TA 1538, TA98
9-Aminoacridine	50 µg/plate	TA1537
Other:		

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

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

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

The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

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:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (10, 25, 50, 100 and 250 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

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

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on plates containing ≥100 µg/plate +/-S9. Inhibition of the background lawn of growth was also noted at insoluble levels ≥250 µg/plate with or without S9 activation. Based on these findings, the initial

mutation assay was performed with test material doses of 10, 25, 50, 100 and 250 µg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that MB 46513 was insoluble and cytotoxic at the high dose (250 µg/plate +/- S9). Compound precipitation was also seen on plates containing 100 µg/plate of the test material under both conditions.Cytotoxicity, as indicated by a reduced background lawn of growth, was evident for all strains at 250 µg/plate +/-S9. No evidence of a mutagenic effect was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that MB 46513 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. MB 46513 was tested to an insoluble and cytotoxic level (250 µg/plate) with no evidence of a mutagenic effect in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 24, 1993).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with MB 46513

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	18±3	14±3	14±4	32±4	119±19
	+	0.1 ml	15±3	14±3	24±7	32±8	113±7
Positive Control							
Sodium azide	-	1 µg	489±11	--	--	--	699±53
2-Nitrofluorene	-	1 µg	--	--	355±49	314±55	--
9-Aminoacridine	-	50 µg	--	219±43	--	--	--
2-Anthracene	+	2 µg	311±68	228±28	1947±106	2228±149	2248±238
Test Material							
MB 46513	-	50[b]	22±8	10±4	13±2	30±7	136±12
	-	250[c]	19±7	12±3	17±3	30±4	129±13
	+	50[b]	18±5	11±2	27±9	32±7	110±6
	+	250[c]	18±0	16±3	26±2	29±6	120±16

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest soluble dose; results for lower doses (10 or 25 µg/plate +/-S9) were generally comparable to the corresponding negative control values.
[c]Highest assayed dose; compound precipitation and thinning of the background lawn of growth noted at this level. Compound precipitation was also seen at the intermediate level of 100 µg/plate +/-S9.

Note: Data were extracted from the study report pp.27-29.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with MB 46513

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	19±4	12±5	12±3	30±11	113±7
	+	0.1 ml	20±4	15±5	24±5	41±6	121±7
Positive Control							
Sodium azide	-	1 µg	444±14	--	--	--	641±29
2-Nitrofluorene	-	1 µg	--	--	419±37	404±21	--
9-Aminoacridine	-	50 µg	--	334±41	--	--	--
2-Anthracene	+	2 µg	236±14	237±28	1973±232	2104±243	2155±86
Test Material							
MB 46513	-	50[b]	16±2	14±3	16±8	32±6	126±13
	-	250[c]	21±2	13±6	16±0	27±1	95±2
	+	50[b]	18±5	15±4	25±9	46±7	112±12
	+	250[c]	16±7	15±3	21±7	39±8	121±9

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest soluble dose; results for lower doses (10 or 25 µg/plate +/-S9) were generally comparable to the corresponding negative control values.
[c]Highest assayed dose; compound precipitation and thinning of the background lawn of growth noted at this level. Compound precipitation was also seen at the intermediate level of 100 µg/plate +/-S9.

Note: Data were extracted from the study report pp. 31-33.

FIPRONIL

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EPA Section Head: James N. Rowe, Ph.D.
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Toxicology Branch II/HED 7509C

Signature: Nancy McCarroll
Date: 5/9/95
Signature: James N Rowe
Date: 5/18/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 432917-22

TEST MATERIAL: RPA 104615

SYNONYM(S): Fipronil; 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-pyrazole-4-sulfonic acid, potassium salt; $C_{11}H_4Cl_2F_3N_4O_3SK$

STUDY NUMBER(S): SA 93175

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 104615 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: October 12, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-22), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate, RPA 104615 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The test material was soluble and noncytotoxic at all levels. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

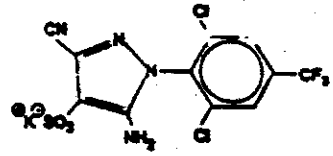
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RPA 104615

Description: White powdery solid
Lot/ batch number: 58 TDS 91
Purity: 94.7% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: Not listed
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 4°C, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

Positive: Nonactivation:

Sodium azide	1 µg/plate	TA100, TA1535
2-Nitrofluorene	1 µg/plate	TA98, TA1538
9-Aminoacridine	50 µg/plate	TA1537
Other:		

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

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

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

The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

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:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
__x__ Standard plate test
_____ Pre-incubation (____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (described).

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. The test material was soluble and noncytotoxic at all assayed levels with or without S9 activation. Based on these findings, the initial mutation assay was

performed with test material doses ranging from 250 to 5000 µg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement with the preliminary results and indicated that RPA 104615 was soluble at all concentrations and was neither cytotoxic nor mutagenic in the presence or absence of S9 activation. By contrast to the uniformly negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 104615 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 104615 was tested to the recommended high dose for a soluble noncytotoxic compound (5000 µg/plate +/-S9) and failed to induce a mutagenic effect in a well-controlled study. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated October 12, 1993).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 104615

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	19±3	13±3	14±3	35±7	128±5
	+	0.1 ml	16±4	20±4	22±5	37±7	135±20
Positive Control							
Sodium azide	-	1 µg	525±46	--	--	--	773±19
2-Nitrofluorene	-	1 µg	--	--	427±15	417±33	--
9-Aminoacridine	-	50 µg	--	228±67	--	--	--
2-Anthracene	+	2 µg	308±25	242±16	2044±67	2212±74	2928±123
Test Material							
RPA 104615	-	5000 µg[b]	18±6	16±4	19±7	37±6	125±2
	+	5000 µg[b]	15±6	17±7	27±4	41±3	108±14

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 104615

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethyl sulfoxide	-	0.1 ml	18±9	11±2	16±3	31±6	124±12
	+	0.1 ml	16±5	17±2	24±5	41±2	98±8
Positive Control							
Sodium azide	-	1 µg	514±57	--	--	--	765±29
2-Nitrofluorene	-	1 µg	--	--	452±24	363±34	--
9-Aminoacridine	-	50 µg	--	313±7	--	--	--
2-Anthracene	+	2 µg	289±43	335±22	2174±191	2566±364	1934±260
Test Material							
RPA 104615	-	5000 µg[b]	19±8	16±3	17±3	39±7	112±14
	+	5000 µg[b]	20±8	21±4	24±2	36±7	111±8

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest assayed level; results for lower doses (250, 500, 1000 or 2500 µg/plate +/-S9) did not suggest a mutagenic effect.

Note: Data from the initial trial were extracted from the study report, pp. 26-28.
Data form the confirmatory trial were extracted from the study report, pp. 30-32.

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EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy L. McCarroll
Date: 5-18-95
Signature: James N Rowe
Date: 5/18/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 434011-02

TEST MATERIAL: RPA 105048

SYNONYM(S): Fipronil; 1-(2,6-dichloro-4-trifluoromethylphenyl)-3-amido-5-amino-4-trifluoromethylpyrazole; $C_{12}H_4OCl_2F_6N_4$

STUDY NUMBER(S): SA 94009

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 105048 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: May 16, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 434011-02), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 105048 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 5000 µg/plate +S9; cytotoxicity toward the majority of strains was also seen at the high dose with or without S9 activation. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

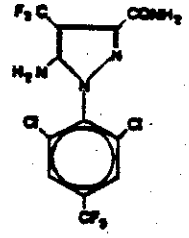
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RPA 105048

Description: White powder
Lot/ batch number: 57TDS134
Purity: 98.6% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: Not provided
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored with a desiccant at refrigerator temperatures, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

Positive: Nonactivation:

Sodium azide	1 µg/plate	TA100, TA1535
2-Nitrofluorene	1 µg/plate	TA98
9-Aminoacridine	50 µg/plate	TA1537

Other:

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

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

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

The rat liver S9 homogenate (Lot nos. 38 and 39) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 ____ 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:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

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

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈72 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on one of two plates containing the high nonactivated or S9-activated dose. There was no evidence of a cytotoxic effect at any level with or without S9 activation. Based on these findings, the initial mutation assay was performed with test material doses ranging from 250 to 5000 µg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that the high dose was insoluble under S9-activated conditions and induced a slight cytotoxic effect in the majority of tester strains both in the presence and absence of S9 activation. However, no evidence of a mutagenic response was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 105048 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 105048 was tested to a concentration that was insoluble in the presence of the S9-cofactor mix and slightly cytotoxic both with and without S9 activation (5000 µg/plate) but failed to induce a mutagenic response in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated May 16, 1994).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 105048

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a] TA535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 ml	19±5	10±2	28±5	115±10
	+	0.1 ml	18±5	15±5	43±11	108±6
Positive Control						
Sodium azide	-	1 µg	430±43	--	--	680±40
2-Nitrofluorene	-	1 µg	--	--	212±25	--
9-Aminoacridine	-	50 µg	--	232±67	--	--
2-Anthracene	+	2 µg	286±61	213±18	2107±83	1917±29
Test Material						
RPA 105048	-	5000 µg[b]	12±3[c]	10±4[c]	28±4	89±5[c]
	+	5000 µg[b,c]	10±1	7±3	35±5	85±6

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 105048

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a] TA535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 ml	19±4	18±4	33±8	122±13
	+	0.1 ml	12±2	11±3	34±6	115±12
Positive Control						
Sodium azide	-	1 µg	477±35	--	--	732±18
2-Nitrofluorene	-	1 µg	--	--	200±13	--
9-Aminoacridine	-	50 µg	--	416±83	--	--
2-Anthracene	+	2 µg	196±13	212±2	2105±172	2066±287
Test Material						
RPA 105048	-	5000 µg[b]	15±4[c]	8±2[c]	31±3	93±5[c]
	+	5000 µg[b]	12±6[c]	5±3[c]	31±7	71±6[c]

[a]Means and standard deviations of counts from triplicate plates.
[b]Highest assayed dose; compound precipitation seen at this level but only with S9 activation. Results for lower doses (250, 500, 1000 or 2500 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Thinning of the background lawn of growth observed at this dose.

Note: Data from the initial assay were extracted from the study report, pp. 27-29.
Data from the confirmatory assay were extracted from the study report, pp. 31-33.