012255

RPA 202248 **SALMONELLA [84-2]**

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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D224202 SUBMISSION NO.: S501233

PC CODE: 123000 TOX. CHEM. NO.: MRID NO: 43904811

TEST MATERIAL (PURITY): RPA 202248 (99.9%)

SYNONYM(S): 2-Cyano-3-cyclopropyl-4-(2-methylsulfonyl-4-trifluoro-methylphenyl) propan-1,3-dione (metabolite of isoxaflutole)

CITATION: Percy, A. (1995). RPA 202248 Salmonella typhimurium Reverse Mutation Assay (Ames Test); Rhône-Poulenc Agrochimie Centre de Recherche, Sophia Antipolis, France; Study Report No. SA 95360; Study completion date: November 10, 1995. Unpublished MRID No. 43904811.

SPONSOR: Rhône-Poulenc, Lyon, France

EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 43904811), Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 202248 (99.9%) in the absence or presence of S9 activation. The confirmatory assay was conducted using the preincubation modification to the standard plate incorporation test. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Minimal cytotoxicity was observed at 5000 µg/plate +S9 (plate incorporation method) or 5000 µg/plate -S9 (preincubation method). All strains responded to the mutagenic action of the appropriate positive control. **There was, however, no evidence that RPA 202248 induced a mutagenic response in either trial.**

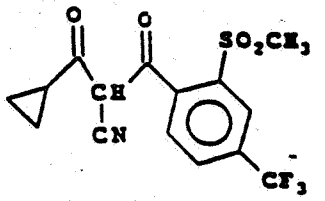
The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material: RPA 202248

Description: White powder
Lot/batch number: DJA16-R
Purity: 99.9%
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 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
Cumene hydroperoxide	200	µg/plate TA102
Other:		

Activation:

2-Aminoanthracene	2	µg/plate all strains except TA102
	5	µg/plate TA102

3. Activation: S9 derived from Fischer 344 male (222 g--average weight)

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

The rat liver homogenate (Lot No. FLI 078) was supplied by Inveresk Research International, UK. Prior to use, the S9 fraction was characterized for its ability to convert a set of standard mutagens to active forms in S. typhimurium TA1538. 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 __x__ 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 nonactivated and five S9-activated concentrations (250, 500, 1000, 2500 and 5000 µg/plate) were evaluated using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above for the initial mutation assay using the preincubation modification to the standard assay.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
__x__ Standard plate test
__x__ Pre-incubation (60) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (described).

(a) Preliminary Cytotoxicity/Plate Incorporation Mutation Assay: Similar procedures were used for the preliminary cytotoxicity and the initial mutation assay. To tubes containing 2.0 mL of molten top agar, 0.1 mL of a 10-hour broth culture (1-10x10^9 cells/mL) of the appropriate tester strain, 0.1 mL of the appropriate test material dose, solvent, or positive control and 0.5 mL of either sodium phosphate buffer (nonactivated series) or 0.5 mL of the S9-cofactor mix (S9-activated series) were added. The

contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈72 hours.

(b) Preincubation method: The preincubation modification to the standard assay was used for the confirmatory trial. The procedure was similar to the plate incorporation mutation assay with the exception that the bacteria, test or control solutions, buffer solution and S9 cofactor mix were preincubated at 37°C for 1 hour prior to the addition of top agar. The contents of each tube were overlaid onto plates of minimal glucose agar and plates were 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.

2. Evaluation criteria:

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 provided acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

Positive response: The test material was considered positive if it caused a reproducible and dose-related "biologically significant" 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 minimally cytotoxic (i.e., slight thinning of the background lawn of growth) at 5000 µg/plate + S9. S. typhimurium TA100 was unaffected by treatment with the nonactivated test material. Based on these findings, test material doses ranging from 250 to 5000 µg/plate +/-S9 were selected for further investigation.

2. Mutation Assays: In the initial trial, thinning of the background lawn of growth was apparent for all strains at 5000 µg/plate +S9 and for TA100 at 2500 µg/plate +S9. There was, however, no indication of a mutagenic response at any dose with or without S9 activation (Table 1). Summarized results from the confirmatory preincubation trial are presented in Table 2. As shown, thinning of the background lawn of growth and/or reduced his^+

colonies were observed in strains TA1535, TA1537 and TA100 at the highest nonactivated dose. Cytotoxic effects were also apparent in strain TA1537 at lower concentrations (1000 and 2500 μg/plate -S9). In contrast to the earlier findings, S9-activated RPA 202248 was not cytotoxic. Owing to the poor performance of the positive control used with strain TA102, this phase of testing was repeated. Data from the repeat test were in good agreement with the earlier findings and indicated that RPA 202248 was not mutagenic. 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.

Based on the overall results, the study author concluded that RPA 202248 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 202248 was tested to the highest concentration recommended for microbial assays (5000 μg/plate) and was minimally cytotoxic 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 RPA 202248 was negative in this microbial gene mutation assay.

E. STUDY DEFICIENCIES: NONE.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 202248--Plate Incorporation Method

Substance	Acti-vation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA1535	TA1537	TA98	TA100	TA102
Solvent Control							
Dimethyl sulfoxide	-	0.1 mL	12±1	11±3	37±2	148±13	314±10
	+	0.1 mL	13±2	16±3	35±5	206±15	395±27
Positive Control							
Sodium azide	-	1 μg	464±14	–	–	574±36	–
2-Nitrofluorene	-	1 μg	–	–	322±11	–	–
9-Aminoacridine	-	50 μg	–	289±140	–	–	–
Cumene hydroperoxide	-	200 μg	–	–	–	–	801±38
2-Aminoanthracene	+	2 μg	210±10	158±10	1530±76	1510±79	–
	+	5 μg	–	–	–	–	1007±69
Test Material							
RPA 202248	-	2500 μg[b]	11±3	13±1	44±5	134±21	306±13
	-	5000 μg	14±2	16±5	44±2	135±6	341±11
	+	2500 μg[b]	14±3	17±3	35±4	159±27[d]	399±10
	+	5000 μg[c]	11±4	10±6	29±3	125±9	414±38

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (250, 500 or 1000 μg/plate +/-S9) were generally comparable to the corresponding negative control values.
[c]Thinning of the background lawn of growth noted at this level in all strains.
[d]Thinning of the background lawn of growth noted at this level in the identified strain(s).

Note: Data were extracted from the study report Tables 3.1-3.6; pp. 30-32.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 202248--Preincubation Method

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a] TA1535	TA1537	TA98	TA100	TA102
Solvent Control							
Dimethyl sulfoxide	-	0.1 mL	13±2	19±5	38±5	146±20	362±19[b]
	+	0.1 mL	14±1	15±7	41±3	147±9	419±11
Positive Control							
Sodium azide	-	1 µg	457±51	–	–	520±53	–
2-Nitrofluorene	-	1 µg	–	–	354±33	–	–
9-Aminoacridine	-	50 µg	–	230±105	–	–	–
Cumene hydroperoxide	-	200 µg	–	–	–	–	974±143[b]
2-Aminoanthracene	+	2 µg	244±31	135±8	1139±104	1635±147	–
	+	5 µg	–	–	–	–	770±43
Test Material							
RPA 202248	-	2500 µg[c]	7±2	13±6[d]	44±4	153±17	381±8[b]
	-	5000 µg	9±3[d]	6±3[d]	36±6	114±5[d]	391±24
	+	2500 µg[c]	14±1	11±7	40±3	140±19	416±7
	+	5000 µg	15±2	18±6	43±6	165±10	410±20

[a]Means and standard deviations of counts from triplicate plates.
[b]The nonactivated phase of testing with this strain was repeated due to poor performance of the positive control. Presented results are from the repeat trial.
[c]Results for lower doses (250, 500 or 1000 µg/plate +/-S9) were generally comparable to the corresponding negative control values.
[d]Thinning of the background lawn of growth noted at this level in the identified strain(s).

Note: Data were extracted from the study report Tables 4.1-5.3; pp. 34-38.