

US EPA ARCHIVE DOCUMENT

012255

RPA 202248

SALMONELLA [84-2]

Principal Reviewer: Nancy E. McCarroll  
Review Section III, Toxicology Branch  
II/HED (7509C)  
Secondary Reviewer: Sanjivani Diwan, Ph.D  
Review Section I,  
Toxicology Branch II/HED (7509C)

Signature: Nancy E. McCarroll  
Date: 8/27/96

Signature: Sanjivani Diwan  
Date: 8/22/96

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 Unpublished MRID No. 43904811.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  $\mu\text{g}/\text{plate}$  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  $\mu\text{g}/\text{plate}$  +S9 (plate incorporation  
method) or 5000  $\mu\text{g}/\text{plate}$  -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.

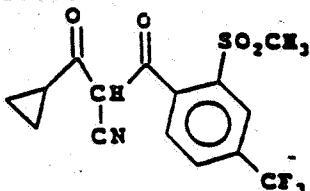
The study is classified as **Acceptable** and satisfies the guideline requirement for  
a microbial gene mutation assay (84-2).

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)

<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> hamster	<u>      </u> other
<u>      </u> other		<u>      </u> 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:

<u>Component</u>	<u>Concentration</u>
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose-6-phosphate	5 mM
NADP	4 mM
MgCl <sub>2</sub>	8 mM
KCl	33 mM
S9	10%

4. Test Organism Used: S. typhimurium strains  
 \_\_\_\_\_ TA97     TA98     TA100     TA102    \_\_\_\_\_ TA104  
 TA1535     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:     Standard plate test  
    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<sup>9</sup> 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  $\mu\text{g}/\text{plate}$  +/-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  $\mu\text{g}/\text{plate}$  + 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  $\mu\text{g}/\text{plate}$  +/-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  $\mu\text{g}/\text{plate}$  +S9 and for TA100 at 2500  $\mu\text{g}/\text{plate}$  +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  $\mu\text{g}/\text{plate}$  -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  $\mu\text{g}/\text{plate}$ ) 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 <sup>a</sup>				
			TA1535	TA1537	TA98	TA100	TA102
<u>Solvent Control</u>							
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
<u>Positive Control</u>							
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
<u>Test Material</u>							
RPA 202248	-	2500 µg <sup>b</sup>	11±3	13±1	44±5	134±21	306±13
	-	5000 µg	14±2	16±5	44±2	135±6	341±11
	+	2500 µg <sup>b</sup>	14±3	17±3	35±4	159±27 <sup>d</sup>	399±10
	+	5000 µg <sup>c</sup>	11±4	10±6	29±3	125±9	414±38

<sup>a</sup>Means and standard deviations of counts from triplicate plates.

<sup>b</sup>Results for lower doses (250, 500 or 1000 µg/plate +/-S9) were generally comparable to the corresponding negative control values.

<sup>c</sup>Thinning of the background lawn of growth noted at this level in all strains.

<sup>d</sup>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	Acti- vation	Dose per plate	Revertants per Plate of Bacterial Tester Strains <sup>a</sup>				
			TA1535	TA1537	TA98	TA100	TA102
<u>Solvent Control</u>							
Dimethyl sulfoxide	-	0.1 mL	13±2	19±5	38±5	146±20	362±19 <sup>b</sup>
	+	0.1 mL	14±1	15±7	41±3	147±9	419±11
<u>Positive Control</u>							
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 <sup>b</sup>
2-Aminoanthracene	+	2 µg	244±31	135±8	1139±104	1635±147	-
	+	5 µg	-	-	-	-	770±43
<u>Test Material</u>							
RPA 202248	-	2500 µg <sup>c</sup>	7±2	13±6 <sup>d</sup>	44±4	153±17	381±8 <sup>b</sup>
	-	5000 µg	9±3 <sup>d</sup>	6±3 <sup>d</sup>	36±6	114±5 <sup>d</sup>	391±24
	+	2500 µg <sup>c</sup>	14±1	11±7	40±3	140±19	416±7
	+	5000 µg	15±2	18±6	43±6	165±10	410±20

<sup>a</sup>Means and standard deviations of counts from triplicate plates.<sup>b</sup>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.<sup>c</sup>Results for lower doses (250, 500 or 1000 µg/plate +/-S9) were generally comparable to the corresponding negative control values.<sup>d</sup>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.