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RPA 203328 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: 43904814

TEST MATERIAL (PURITY): RPA 203328 (99.7%)

SYNONYM(S): 2-Methanesuphonyl-4-trifluoromethylbenzoic acid (metabolite of isoxaflutole)

CITATION: Percy, A. (1994). RPA 203328 Salmonella typhimurium Reverse Mutation Assay (Ames Test); Rhône-Poulenc - Secteur Agro Centre de Recherche, Sophia Antipolis, France; Study Report No. SA 94057; Study completion date: October 13, 1994. Unpublished MRID No. 43904814.

SPONSOR: Rhône-Poulenc, Lyon, France

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 43904814), Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 were exposed to 100, 250, 500, 1000, 2500 or 5000 µg/plate RPA 203328 (99.7%) 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.

Cytotoxicity (i.e., generally observed as an extreme thinning of the background lawn of growth) was seen at levels ≥2500 µg/plate +/- S9. All strains responded to the mutagenic action of the appropriate positive control. **There was, however, no evidence that RPA 203328 induced a mutagenic response in either trial.**

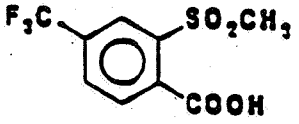
The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

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

A. MATERIALS:

1. Test Material: RPA 203328

Description: White powder
Lot/batch number: DA938
Purity: 99.7% (see section E., Study Deficiencies for comment on purity)
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
Other:		

Activation:

2-Aminoanthracene	2	µg/plate all strains including

3. Activation: S9 derived from Fischer 344 male

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

The rat liver homogenate (Batch No. FLI074) was supplied by Inveresk Research International, UK. 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: Six nonactivated and six S9-activated concentrations (100, 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.

B. TEST PERFORMANCE:

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

Preliminary Cytotoxicity/ Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and the mutation assays. To prepared tubes containing 2.5 mL of molten top agar, 0.1 mL of a 10-hour broth culture (1-10x10^9 cells/mL) of the appropriate tester strain and 0.1 mL of the appropriate test material dose, solvent, or positive control 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.

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 ≥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. No revertant colonies were seen at nonactivated 5000 µg/plate and minimal cytotoxicity (i.e., slight to moderate thinning of the background lawn of growth) was noted at 5000 µg/plate +S9 and at 2500 µg/plate -S9. Based on these findings, six test material doses ranging from 100 to 5000 µg/plate +/-S9 were selected for further investigation.

2. Mutation Assays: Representative results from the initial and confirmatory trials with RPA 203328 are presented in Tables 1 and 2, respectively. As shown, data from both trials were in good agreement and indicated that test material levels ≥2500 µg/plate with or without S9 activation were cytotoxic to all strains as indicated by effects on the background lawn of growth or by reductions in revertant colonies. There was, however, no indication of a mutagenic effect at any assayed concentration. 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 in both trials.

Based on the overall results, the study author concluded that RPA 203328 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 203328 was tested to the highest concentration recommended for microbial assays (5000 μg/plate) and was cytotoxic at doses ≥2500 μg/plate +/-S9 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 203328 was negative in this microbial gene mutation assay.

E. STUDY DEFICIENCIES: Test material purity was listed as 99.7% on Report Summary p. 9 and on the Certificate of Analysis, Study Report p. 44. We, therefore, assume that the purity listed on Study Report p. 11 (97.7%) was an error. This discrepancy did not affect the outcome of the study.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 203328

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]			
			TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 mL	16±5	16±7	32±6	106±12
	+	0.1 mL	15±2	14±3	36±6	103±10
Positive Control						
Sodium azide	-	1 µg	382±35	–	–	606±5
2-Nitrofluorene	-	1 µg	–	–	265±31	–
9-Aminoacridine	-	50 µg	–	295±98	–	–
2-Aminoanthracene	+	2 µg	197±19	189±67	1629±52	2208±149
Test Material						
RPA 203328	-	1000 µg[b]	18±4	17±10	32±5	99±13
	-	2500 µg[c]	14±3	7±4	22±7	78±8
	-	5000 µg[c]	–	–	–	–
	+	1000 µg[b]	16±3	13±4	37±2	104±14
	+	2500 µg[d]	8±3	16±4	21±6	80±7
	+	5000 µg[c]	10±0	11±3	30±6	78±9

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (100, 250 or 500 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Extreme thinning of the background lawn of growth for all strains was observed at this level.
[d]Slight to moderate thinning of the background lawn of growth was noted for the majority of strains at this dose.

Note: Data were extracted from the study report, Tables 3.1-3.6; pp. 27-29.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 203328

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a] TA1535	TA1537	TA98	TA100
Solvent Control						
Dimethyl sulfoxide	-	0.1 mL	18±4	13±5	30±6	114±12
	+	0.1 mL	12±4	14±4	30±10	100±9
Positive Control						
Sodium azide	-	1 µg	460±44	–	–	674±41
2-Nitrofluorene	-	1 µg	–	–	277±52	–
9-Aminoacridine	-	50 µg	–	403±130	–	–
2-Aminoanthracene	+	2 µg	281±15	298±24	2066±184	2635±271
Test Material						
RPA 203328	-	1000 µg[b]	14±5	18±7	32±6	114±20
	-	2500 µg[c]	15±8	9±3	22±3	100±5
	-	5000 µg[c]	–	–	–	–
	+	1000 µg[b]	13±2	11±4	38±7	92±3
	+	2500 µg[c]	8±2	7±1	27±3	82±12
	+	5000 µg[c]	5±3	–	21±4	78±10

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (100, 250 or 500 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Extreme thinning of the background lawn of growth was observed for the majority of strains at this level.

Note: Data were extracted from the study report, Tables 4.1-4.6; pp. 31-33.