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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

MRID Number: 415614-04

TEST MATERIAL: R-25788

SYNONYMS: Dichlormid; EHC-0829-33

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: T-13178

TESTING FACILITY: ICI Americas Inc., Farmington, CT

TITLE OF REPORT: Mutagenicity evaluation in Salmonella typhimurium R-25788 T-13178

AUTHOR: J. B. Majeska

REPORT ISSUED: December 31, 1987

CONCLUSIONS--EXECUTIVE SUMMARY: Under the conditions of two independently performed Salmonella typhimurium/mammalian microsome plate incorporation assays, at doses ranging from 0.188 to 3.00 mg/plate (188 to 3000 µg/plate), R-25788 did not induce a mutagenic effect in S. typhimurium strains TA1535, TA1537, TA98, or TA100 either in the absence or the presence of S9 activation derived from Aroclor 1254-induced rat or mouse livers. Higher concentrations (≥5000 µg/plate -/+ rat or mouse S9) were cytotoxic and insoluble. Based on these findings, it was concluded that R-25788 was tested over an appropriate range of concentrations with no evidence of a mutagenic effect. The study satisfies Guideline requirements for genetic effects Category I, Gene Mutations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: R-25788

Description: Amber liquid
Identification number: WRC 4921-35-11 GGD-0101
Purity: 97.2%
Receipt date: 9/30/87
Stability: Unspecified; expiration date: 5/90
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored in the dark at room temperature (~20°C) and at ambient humidity. The test material (50 µL) did not alter the pH or osmotic pressure of sodium phosphate buffer (2.5 mL) or sodium phosphate buffer (2.0 mL) containing 0.5 mL of an unspecified S9 mixture. Solutions of the test material were prepared immediately prior to use.

2. Control Materials:

Negative: Culture medium (Vogel-Bonner Minimal Medium supplemented with 2% glucose 0.5 mM biotin, and 0.5 mM histidine

Solvent/final concentration: DMSO/50 µL plate

Positive:

Nonactivation:

Sodium azide	10.0	µg/plate TA100, TA1535
2-Nitrofluorene	10.0	µg/plate TA98
ICR 191	5.0	µg/plate TA1537

Other:

Activation:

2-Aminoanthracene (2-AA)	4.0	µg/plate all strains with rat liver S9
	5.0	µg/plate all strains with mouse liver S9

Note: In the confirmatory test with strain TA1537, 5.0 µg/plate 2-AA was assayed with rat liver S9 activation.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat[a]	x liver
____ phenobarbital	____ noninduced	x mouse[b]	____ lung
____ none		____ hamster	____ other
____ other		____ other	

The rat liver S9 was prepared by the performing laboratory and was identified as Lot No. EHC-0475-26; the mouse liver S9 (Lot No. 0223) was purchased from Molecular Toxicology, College Park, MD.

S9 mix composition	Final concentration
Sodium phosphate buffer	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	34 mM
S9 (rat or mouse)	100 µL/mL

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: Storage condition not reported

Checked for appropriate genetic markers (rfa mutation, R factor): Yes

5. Test compound concentrations used:

(a) Preliminary cytotoxicity assay: Ten doses (20, 39, 78, 156, 313, 625, 1250, 2500, 5000, and 10,000 µg/plate) were evaluated with or without S9 activation in S. typhimurium strain TA100.

(b) Mutation assays:

(1) Initial: Five doses (188, 375, 750, 1500, and 3000 µg/plate) were evaluated in the absence and presence of S9 activation derived from rat and mouse liver microsomes; all tester strains were used.

(2) Confirmatory: As above

[a]Male Sprague-Dawley rats

[b]Neither the strain nor the sex of the mice used to prepare the S9 fraction were reported.

B. TEST PERFORMANCE:

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

2. Protocol:

(a) Preliminary cytotoxicity/mutation assays: Similar procedures were used for the preliminary cytotoxicity and the mutation assays.

Approximately 10^8 cells from 12- to 16-hour broth cultures of the appropriate tester strain, 0.5 mL of phosphate buffer, and 50 µL of the appropriate test material dose, solvent, or positive controls were added to tubes containing 2-mL volumes of molten top agar. For the S9-activated tests, 0.5 mL of the appropriate S9-cofactor mix replaced the phosphate buffer; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium, and incubated at 37°C for ≈48-72 hours. At the end of incubation, plates were scored for revertant colonies using an automatic colony counter. Means and standard deviations were determined from the counts of triplicate plates per strain, per dose, per condition.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (a) the medium, solvent, and positive control results were within the historical range of the reporting laboratory, (b) at least three doses were available for evaluation, and (c) the test material was assayed over a concentration range that included a cytotoxic level, a level that approached the solubility limit of the test material, or the maximum dose (from 3000 to 10,000 µg/plate) established by the performing laboratory for this assay system.

(2) Positive response: The test material was considered positive if it (a) caused a reproducible and dose-related (at least three concentrations) increase in mutant colonies of any tester strain and (b) at least one dose caused a 3-fold increase in mutant colonies of TA1535 or TA1537 or a 2.5-fold increase in mutant colonies of TA98 or TA100.

C. REPORTED RESULTS

1. Preliminary cytotoxicity assay: Ten doses of the test material ranging from 20 to 10,000 µg/plate were evaluated without and with rat liver S9 activation using strain TA100. Compound precipitation was apparent at 10,000 µg/plate +/- S9. The report stated that precipitation was also noted upon addition of the 5000-µg/plate concentration to the top agar. Mean revertant colony counts at ≥5000 µg/plate +/- S9 were reduced (≥42%) compared to the control. At lower concentrations, there was no clear indication of a cytotoxic effect. Based on these findings, the dose range selected for the nonactivated and S9-activated mutation assays was 188-3000 µg/plate.

2. Mutation assay: Representative results from the nonactivated and S9-activated initial and confirmatory mutation assays with R-25788 are presented in Tables 1 and 2, respectively. As shown, the slight reductions in revertant colonies of strains TA1535, TA1537, and/or TA100, which occurred under nonactivated or S9-activated (rat or mouse S9) conditions, were not reproducible or dose related. Our reviewers assume, therefore, that the reduced revertant colony counts probably resulted from normal plating variability. In general, revertant colony counts for strain TA98 were suppressed, compared to the control, at the majority of assayed levels, under all test conditions, and in both trials. At the highest assayed concentration (3000 µg/plate -/+ rat or mouse S9), there was a moderate reduction in mutant colonies of this strain in both the initial and repeat assays. However, R-25788 did not induce a mutagenic effect in any tester strain in either the absence or presence of rat or mouse liver S9 activation. In contrast, all strains responded to the appropriate nonactivated and S9-activated positive controls in both the initial and confirmatory assay. From the overall findings, the study author concluded that R-25788 was not mutagenic in this test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. Both in the absence and the presence of exogenous metabolic activation derived from rat and mouse liver microsomes, R-25788 was assayed to a subcytotoxic level but failed to induce a mutagenic effect. In addition, the response of all tester strains to the appropriate direct-acting or promutagenic positive control indicated that the assay had an adequate level of sensitivity to detect a mutagenic response. It was concluded, therefore, that R-25788 was not mutagenic in this microbial test system.

TABLE 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with R-25788

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]			
			TA1535	TA1537	TA98	TA100
Negative Control	--	-	17 ± 5	12 ± 4	40 ± 5	105 ± 29[d]
(Culture medium)	--	+[b]	21 ± 3	7 ± 5	56 ± 4[d]	108 ± 4[d]
	--	+[c]	15 ± 2	5 ± 4	37 ± 9	81 ± 12
Solvent Control						
(Dimethyl sulfoxide)	50 µL	-	15 ± 4	13 ± 6	28 ± 9	104 ± 19
	50 µL	+[b]	21 ± 3[d]	13 ± 5	46 ± 5	110 ± 9
	50 µL	+[c]	10 ± 1[d]	8 ± 2	37 ± 4	76 ± 3
Positive Controls						
Sodium azide	10 µg	-	1,012 ± 46	--	--	1,063 ± 43
ICR 191	5 µg	-	--	65 ± 20	--	--
2-Nitrofluorene	10 µg	-	--	--	916 ± 99	--
2-Aminoanthracene	4 µg	+[b]	272 ± 9	79 ± 3	2,368 ± 82	1,596 ± 436
	5 µg	+[c]	127 ± 15	278 ± 28	852 ± 200	1,148 ± 247
Test Material						
R-25788	1,500 µg[e]	-	23 ± 6	11 ± 3	22 ± 8	97 ± 18[d]
	3,000 µg	-	20 ± 2	11 ± 4	19 ± 5	105 ± 4
	1,500 µg[e]	+[b]	20 ± 4	7 ± 2	24 ± 6	112 ± 17
	3,000 µg	+[b]	20 ± 8	5 ± 3	23 ± 8	122 ± 7
	1,500 µg[e]	+[c]	25 ± 5	10 ± 5	17 ± 6	82 ± 7
	3,000 µg	+[c]	18 ± 2	4 ± 1	17 ± 2	70 ± 3

[a]Means and standard deviations of the counts from triplicate plates, except where indicated (see footnote d).
[b]Rat liver S9 activation
[c]Mouse liver S9 activation
[d]Means and standard deviations of the counts from duplicate plates; no explanation was provided for the reduction in replicates.
[e]Results for lower doses (188, 375, and 750 µg/plate -/+ rat or mouse S9) did not suggest a mutagenic effect.

NOTE: Phases of the assay (i.e., nonactivation, rat liver and/or mouse liver S9 activation) using various strains were conducted on different days.

TABLE 2: Representative Results of the Confirmatory *Salmonella typhimurium*/Mammalian Microsome Mutation Assay with R-25788

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]			
			TA1535	TA1537	TA98	TA100
Negative Control	--	-	20 ± 3	10 ± 1	25 ± 1[d]	130 ± 31
(Culture medium)	--	+[b]	17 ± 6	10 ± 5	29 ± 2[d]	108 ± 15
	--	+[c]	9 ± 2	6 ± 0	27 ± 6	115 ± 9
Solvent Control						
(Dimethyl sulfoxide)	50 µL	-	11 ± 4	9 ± 5[d]	25 ± 5	90 ± 11
	50 µL	+[b]	20 ± 2	10 ± 2	30 ± 5	105 ± 3
	50 µL	+[c]	10 ± 3	7 ± 1	22 ± 5	88 ± 7
Positive Controls						
Sodium azide	10 µg	-	852 ± 83	--	--	706 ± 64
ICR 191	5 µg	-	--	69 ± 11	--	--
2-Nitrofluorene	10 µg	-	--	--	473 ± 172	--
2-Aminoanthracene	4 µg	+[b]	189 ± 33	--	926 ± 102	807 ± 141
	5 µg	+[b]	--	43 ± 9	--	--
	5 µg	+[c]	42 ± 4	204 ± 40	1,658 ± 149	538 ± 174
Test Material						
R-25788	1,500 µg	-	14 ± 1[d]	6 ± 4	13 ± 3[d]	86 ± 15[d]
	3,000 µg	-	15 ± 2	4 ± 2	8 ± 4[d]	84 ± 4
	1,500 µg	+[b]	11 ± 4[d]	8 ± 5	15 ± 0[d]	110 ± 3[d]
	3,000 µg	+[b]	12 ± 3	5 ± 2	9 ± 2	74 ± 13
	1,500 µg	+[c]	13 ± 5	5 ± 1	14 ± 1	91 ± 18
	3,000 µg	+[c]	17 ± 3	5 ± 1	12 ± 5	83 ± 11

[a]Means and standard deviations of the counts from triplicate plates, except where indicated (see footnote d).
[b]Rat liver S9 activation
[c]Mouse liver S9 activation
[d]Means and standard deviations of the counts from duplicate plates, no explanation was provided for the reduction in replicates.
[e]Results for lower doses (188, 375, and 750 µg/plate -/+ rat or mouse S9) did not suggest a mutagenic effect.

NOTE: Phases of the assay (i.e., nonactivation, rat liver and/or mouse liver S9 activation) using various strains were conducted on different days.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes (A signed quality assurance statement, indicating that the quality assurance review of this study was completed on December 18, 1987, was present).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-8; Appendix B, Protocol, CBI pp. 27-31.