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DATA EVALUATION RECORD

PYRIDATE

Mutagenicity--Salmonella/Mammalian-Microsome Mutagenicity Assay

STUDY IDENTIFICATION: Hoorn, A. J. W. Mutagenicity evaluation of pyridate technical in the Ames Salmonella/microsome reverse mutation assay. (Unpublished study No. E-9550 prepared by Hazleton Biotechnologies, Veenendaal, the Netherlands, for Chemie Linz, A.G., Linz, Austria; dated September 1986.) Accession No. 401077716-02.

APPROVED BY:

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Date: 6-19-87

1. CHEMICAL: Pyridate.

2. TEST MATERIAL: Pyridate technical, from batch No. 2556520 was described as a viscous brown oily liquid; the purity was not reported.

3. STUDY/ACTION TYPE: Mutagenicity--Salmonella/mammalian microsome mutagenicity assay.

4. STUDY IDENTIFICATION: Hoorn, A. J. W. Mutagenicity evaluation of pyridate technical in the Ames Salmonella/microsome reverse mutation assay. (Unpublished study No. E-9550 prepared by Hazleton Biotechnologies, Veenendaal, the Netherlands, for Chemie Linz, A.G., Linz, Austria; dated September 1986.) Accession No. 401071 6-02.

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7. CONCLUSIONS:

A. Eight doses of pyridate technical (1.0 to 10,000 µg/plate) were assayed in the absence and presence of S9 activation. Under the conditions of two independent assays, pyridate was not mutagenic in Salmonella typhimurium TA1535, TA1537, TA98, and TA100 at dose levels up to and exceeding the limit of solubility. Although cytotoxicity was not achieved in all strains, the test material was clearly demonstrated to be cytotoxic in TA1535 and TA1537. We, therefore, conclude that pyridate was adequately tested to the limit of solubility with some indication of cytotoxicity but no mutagenicity.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Pyridate technical, from batch No. 2556520, was described as a viscous brown oily liquid; the purity was not reported. The test material was stored at room temperature in the dark and was dissolved in dimethylsulfoxide (DMSO).

2. Test Organisms: S. typhimurium strains TA1535, TA100, TA1538, TA98, and TA1537 were obtained from B. N. Ames, University of California. Permanent frozen stocks were maintained for each strain and working cultures were held at 4°C on complete medium or on complete medium containing ampicillin (strains TA98 and TA100). Cells used in the assays were generated from the working cultures and were grown overnight at 37°C in oxoid nutrient broth No. 2.

3. S9 Activation: The S9 fraction used for metabolic activation was derived from the livers of Sprague-Dawley adult male rats induced with Aroclor 1254. The S9 mix contained 10% S9 fraction.

4. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was conducted in the absence of S9 activation with 14 doses of the test material ranging from 1.22 to 10,000 µg/plate. Single plates were used at each dose level; the solvent control (DMSO) was plated in duplicate. The preliminary cytotoxicity assay was performed as described below and was used to select doses for the mutation assay.

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[1]Only items appropriate to this DER have been included.

5. Mutation Assay:

a. Plating Procedures: Eight doses of the test material were assayed in the presence or absence of S9 activation with the five bacterial strains.

To tubes containing 2.0-mL volumes of molten top agar containing 50 µM biotin and 50 µM histidine, 0.05 to 0.1 mL of the appropriate test material solution, solvent, or positive control, 0.2 mL of an overnight broth culture of the appropriate tester strain, and 0.5 mL of 0.2 M phosphate buffer were added. For the S9-activated test, 0.5 mL of the S9 cofactor mix replaced the phosphate buffer. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for 2 days. All positive control, solvent control, and test material doses were plated in triplicate. At the end of incubation, plates were counted and the means and standard deviations were calculated.

b. Positive Controls: The following positive controls were used to determine the sensitivity of the test system for detecting mutagenicity:

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA1535 and TA100	-	Sodium azide	10.0
TA1537	-	Quinacrine mustard	5.0
TA1538 and TA98	-	2-Nitrofluorene	10.0
All strains	+	2-Anthramine	2.5

6. Evaluation Criteria: The test material was considered positive if a dose response was observed over three concentrations and the increase in revertants was ≥3-fold for strains TA1535, TA1537, and TA1538 and ≥2-fold for strains TA98 and TA100 over the appropriate solvent control value. An historical range of acceptable values for the solvent controls was included in the report.

B. Protocol: A protocol was not provided. 005972

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: Fourteen nonactivated doses, separated by 1:2 dilutions and covering a concentration range of 1.22 to 10,000 µg/plate, were assayed with strain TA100. Compound precipitation was reported at doses ≥39.06 µg/plate; the test material was not cytotoxic at any assayed dose.

B. Mutation Assay: Two independent trials were performed with eight doses ranging from 1.0 to 10,000 µg/plate in the presence and absence of S9 activation.

1. Trial One: In the absence of S9 activation, cytotoxicity (~50% or greater reduction in colonies) was observed in strains TA1535 and TA1537 at doses ≥10.0 µg/plate; only the lowest dose, 1 µg/plate, was essentially noncytotoxic with these strains. Reductions in mutant colonies were noted for strains TA1538, TA98, and TA100 over the majority of test material doses; however, no definitive cytotoxicity was demonstrated. The test material did not induce a mutagenic response in any tester strain. All strains responded to the mutagenic action of the appropriate nonactivated positive control.

The S9-activated test material was clearly cytotoxic in strains TA1537 at the four highest dose levels (1000 to 10,000 µg/plate) and in strain TA98 at 10,000 µg/plate. Slight, but not dose-related, decreases in revertant colonies of all strains were observed for the majority of test doses; however, no definitive evidence of cytotoxicity was revealed for strains TA1535, TA1538, or TA100. The S9-activated test material was not mutagenic. The S9-activated positive control, 2-anthramine, induced marked increases in reversion to histidine prototrophy of all tester strains.

Representative results from the first trial are presented in Table 1.

2. Trial Two: In the second trial, the nonactivated test material appeared to be less cytotoxic in strains TA1535 and TA1537 (lowest cytotoxic dose was 100 µg/plate) but caused nondose-related decreases in revertant colonies of TA100 (~40% decrease) at doses ≥100 µg/plate. For the remaining strains, slight but not dose-related decreases in revertant colonies were observed. The highest S9-activated dose (10,000 µg/plate) was cytotoxic for strains TA1535, TA1537, and TA98.

TABLE I. Representative Results of the First Salmonella typhimurium Mutagenicity Assay with Pyridate

Substance	Dose (µg/plate)	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethylsulfoxide	—	-	21.0± 8.9	5.0± 2.6	13.0± 3.6	23.3± 5.5	165.7± 4.2
	—	+	9.3± 4.5	9.0± 3.0	21.7± 7.1	28.7± 6.7	153.7±15.4
Positive Controls							
Sodium azide	10	-	1072.0±15.4	—	—	—	947.7±10.6
2-Nitrofluorene	10	-	—	—	1371.7±117.6	1093.0±51.9	—
Quinacrine mustard	5	-	—	1147.7±106.5	—	—	—
2-Anthramine	2.5	+	493.0±79.2	343.3± 27.1	2097.0±216.4	1996.7±74.2	2316.0±24.1
Test Material							
Pyridate	1.0[b]	-	16.3± 3.8	9.3± 2.5	15.3± 1.5	23.0± 2.6	150.7± 5.1
	10,000.0[c]	-	7.7± 0.6	0.0	11.7± 7.4	15.3± 2.5	125.7±13.6
	1.0[d]	+	7.0± 1.0	9.7± 1.5	21.7± 2.1	27.7±12.0	153.3±12.3
	10,000[c]	+	8.0± 2.0	3.0± 2.6	17.7± 5.5	14.0± 3.5	132.3± 9.0

[a] Mean and standard deviation of triplicate plates.

[b] Lowest assayed dose; intermediate doses (10, 100, 500, 1000, 2500, and 5000 µg/plate) were cytotoxic for strains TA1535 and TA1537. No increase in mutant colonies was observed for the remaining strains at these intermediate doses.

[c] Highest assayed dose; compound precipitation was observed at doses ≥100 µg/plate (information derived from results reported for a preliminary assay listing 39.06 µg/plate as the highest nonprecipitating dose).

[d] Lowest S9-activated dose; intermediate doses (10, 100, 500, 1000, 2500, and 5000 µg/plate) did not appreciably increase the frequency of mutant colonies. Doses ≥1000 µg/plate were cytotoxic for strain TA1537.

Minor inconsistencies in the cytotoxic effects of the test material were noted between the two independent trials. However, the overall findings of both assays are in agreement and indicate that exposure of the five tester strains to eight nonactivated and S9-activated doses of pyridate did not induce a mutagenic effect.

Representative results from the second trial are presented in Table 2.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded, "The test material, Pyridate technical, batch no. 2556520 did not exhibit genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic to Salmonella typhimurium indicator organisms under these test conditions according to our evaluation criteria."

B. A quality assurance statement was signed and dated September 16, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the author interpreted the data correctly. Although definitive cytotoxicity was not demonstrated for all strains, pyridate was clearly assayed beyond the limit of solubility with no indication of a mutagenic response in two independent trials.

The sensitivity of both assays to detect mutagenicity was adequately demonstrated by the response of all tester strains to the appropriate nonactivated or S9-activated positive control.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 6-14.

TABLE 2. Representative Results of the Second Salmonella typhimurium Mutagenicity Assay with Pyridate

Substance	Dose (µg/plate)	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]				
			TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethylsulfoxide	—	-	15.0± 1.7	6.7± 0.6	14.3± 3.5	25.0± 6.9	122.3± 0.6
	—	+	12.0± 5.3	7.3± 5.1	17.7± 3.8	24.7± 2.1	113.3± 7.0
Positive Controls							
Sodium azide	10	-	1038.0±60.6	—	—	—	1071.7±34.5
2-Nitrofluorene	10	-	—	—	1489.7±133.2	1268.3± 72.2	—
Quinacrine mustard	5	-	—	1155.0±88.4	—	—	—
2-anthramine	2.5	+	355.7±43.7	409.3±22.7	2118.0±240.4	1999.3±107.1	2369.7±71.6
Test Material							
Pyridate	1.0[b]	-	13.7± 3.2	7.3± 3.1	10.0± 3.0	19.0± 4.4	114.3± 6.7
	10,000[c]	-	4.0± 1.0	3.3± 1.5	8.0± 1.0	18.3± 1.5	68.7±14.5
	1.0[b]	+	9.3± 3.5	10.0± 6.1	20.7± 2.1	25.7± 3.1	129.0± 5.6
	10,000[c]	+	4.0± 2.6	3.3± 2.5	17.0± 5.6	14.3± 2.1	93.0±18.0

[a] Mean and standard deviation of triplicate plates.

[b] Lowest assayed dose; no appreciable increase in mutant colonies was observed for the intermediate doses (10, 100, 500, 1000, 2500, and 5000 µg/plate).

[c] Highest assayed dose; compound precipitation was observed at doses ≥100 µg/plate (information derived from results reported for a preliminary assay listing 39.06 µg/plate as the highest nonprecipitating dose).