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DATA EVALUATION RECORD

PYRIDATE

Mutagenicity--Micronucleus Assay in Mouse Bone Marrow

STUDY IDENTIFICATION: Taalman, R. D. F. M. and Hoorn, A. J. W. In vivo mouse micronucleus assay with pyridate technical. (Unpublished study No. 263.215.016 prepared by Hazleton Biotechnologies, Veenendaal, Netherlands, for Chemie Linz AG, Linz, Austria; dated December 10, 1986.) Accession No. 401164-01

APPROVED BY:

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Department Manager
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Signature: I. Cecil Felkner

Date: 6-19-87

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1. CHEMICAL: Pyridate technical.

2. TEST MATERIAL: Pyridate technical, batch No. 2556520, was described as a brown oily liquid. Its purity was not stated.

3. STUDY/ACTION TYPE: Mutagenicity--Micronucleus assay in mouse bone marrow.

4. STUDY IDENTIFICATION: Taalman, R. D. F. M. and Hoorn, A. J. W. In vivo mouse micronucleus assay with pyridate technical. (Unpublished study No. 263.215.016 prepared by Hazleton Biotechnologies, Veenendaal, Netherlands, for Chemie Linz AG, Linz, Austria; dated December 10, 1986.) Accession No. 401164-01

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7. CONCLUSIONS:

A. Under the conditions of the micronucleus assay, pyridate technical, assayed at three doses ranging from 0.4 to 4 g/kg, did not cause a clastogenic effect in mouse bone marrow. The results from cyclophosphamide (CP) treatment, the positive control, demonstrated the sensitivity of the assay to detect a positive response. Based on the mortality data, the dose range selected was adequate.

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, batch No. 2556520, was described as a brown oily liquid; its purity was not stated. The test material was mixed with corn oil, the vehicle control.

2. Test Animal: Adult male and female Swiss mice (random bred) were obtained from an unidentified dealer. Animals were group housed (five mice/cage) and quarantined for at least 5 days (environmental conditions were not reported). Food and water were available ad libitum. Animals were randomly assigned to dose groups and uniquely identified by ear tag. Dose groups were identified by cage card. Prior to study initiation, animals were weighed.

3. Dose Selection: In a range-finding study, the test material, at five doses ranging from 0.05 to 5 g/kg, was administered (route of administration was not specified) to three male and three female mice/dose group. The animals were observed twice daily for 7 days. Three animals in the high-dose (5 g/kg) groups died (one male, two females); therefore, the LD_{50} was estimated to be 5 g/kg. Based on the results of the toxicity study, the doses selected for the micronucleus assay were 0.4, 1.3, and 4 g/kg (high dose; 80% of the LD_{50}).

4. Positive Control: CP at 100 mg/kg was dissolved in saline and used as the positive control.

5. Dose Groups/Compound Administration: Thirty mice per dose group (15 males, 15 females) received an acute oral dose of the appropriate test material levels. Ten mice (five males,

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

five females) were administered an acute oral dose of the vehicle control or an intraperitoneal dose of the positive control. Dosing volumes were based on body weights.

6. Micronucleus Assay:

   a. Animal Sacrifice/Bone Marrow Harvest: Twenty-four, 48, and 72 hours after the test material was administered, or 24 hours after the vehicle and positive controls were administered, the animals were sacrificed by carbon dioxide asphyxiation. Bone marrow cells were harvested from both tibiae by aspiration into 3 mL of fetal calf serum. Samples were centrifuged and pellets were removed, resuspended in a drop of serum, spread onto slides, and air-dried. Prepared slides were fixed in methanol, stained in May-Gruenwald solution, coverslipped, mounted, and coded.

   b. Slide Analysis: One thousand polychromatic erythrocytes (PCE) per animal were scored for the number of micronucleated polychromatic erythrocytes (MPE). The frequency of PCE to erythrocytes was also determined by scoring the number of erythrocytes observed while scoring 1000 PCE for micronuclei.

7. Statistical Analysis: The data were analyzed by the one-tailed Student's t test and the tables of Kastenbaum and Bowman.

8. Evaluation Criteria: A test material was considered positive if it caused a significant ($p < 0.05$) dose-related increase in MPE or a reproducible significant response at one dose level.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Pyridate technical was administered orally at dose levels of 0.4, 1.3, and 4 g/kg to male and female mice. Prior to the 24-, 48-, and 72-hour sacrifices, death occurred in 4/10, 4/10, and 2/10 animals in the high-dose groups. In the low- and mid-dose groups, 1/10 mice died in each group prior to the 48-hour sacrifice. Female mice were more affected than the males.

The test material at the doses tested did not cause an increase in the frequency of MPE in the bone marrow of male or female mice.

The positive control, CP (100 mg/kg, ip), caused a significant ($p < 0.01$) increase in MPE, demonstrating the sensitivity of the test system to detect a clastogenic response.

Since there were no sex-related differences in the frequency of MPE, the results were combined (see Table 1).

TABLE 1. Representative Results from the Micronucleus Assay with Pyridate Technical

Substance	Dose	Harvest Interval (hour)	No. of Animals Analyzed	No. PCE[a] Scored per Group	No. of MPE per Group	Percent MPE per Group Mean ± SE	PCE/RBC Ratio
Vehicle Control							
Corn Oil	--	24	10[b]	10000	60	0.60±0.068	0.4
Positive Control							
Cyclophosphamide	100 mg/kg	24	10	10000	353	3.53±0.462**	0.3
Test Material							
Pyridate	4 g/kg[c]	24	6[d]	6000	28	0.47±0.102	0.3
		48	6[e]	6000	17	0.28±0.101	0.4
		72	8[f]	8000	25	0.31±0.111	0.5

[a]Abbreviations used:
PCE--Polychromatic erythrocytes
MPE--Micronucleated polychromatic erythrocytes
RBC--Red blood cells (mature erythrocytes).

[b]Five male and five female mice/group; results were combined by our reviewers.

[c]Highest dose assayed; results for lower doses (0.4 and 1.3 g/kg) were comparable to the vehicle control.

[d]Four animals died (three males, one female).

[e]Four animals died (one male, three females).

[f]Two animals died (two females).

**Significantly different from control value at $p < 0.01$.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that "The test article, Pyridate technical, was considered inactive in inducing micronuclei in polychromatic erythrocytes of the mouse under the conditions of this assay according to our evaluation criteria."

B. A quality assurance statement was signed and dated November 28, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study authors interpreted the data correctly and that pyridate technical did not cause an increase in MPE in the bone marrow of male and female mice. Based on the mortality data, the dose range selected was adequate. The positive control, CP, caused a significant positive response in both the male and female animals, demonstrating the sensitivity of the assay.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 8-10.

APPENDIX A

Materials and Methods