GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

MUTAGENICITY STUDIES

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Date: 10/25/91
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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In Vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

MRID Number: 415614-03

TEST MATERIAL: R-25788

SYNONYMS: Dichlormid; EHC-0829-33

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: T-13182

TESTING FACILITY: ICI Americas Inc., Farmington, CT

TITLE OF REPORT: Mutagenicity evaluation in bone marrow micronucleus R-25788 T-13182.

AUTHOR: J. B. Majeska

REPORT ISSUED: December 31, 1987

CONCLUSIONS--EXECUTIVE SUMMARY: The single oral gavage administration of 1000, 1500, or 2000 mg/kg R-25788 to male mice or 500, 1000, or 1200 mg/kg to female mice did not cause a significant increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) in bone marrow cells harvested 24, 48, and 72 hours posttreatment. Deaths were observed in high-dose males and females, and a slight cytotoxic effect on the target organ (bone marrow cells) was seen in the high-dose groups at the 72-hour sacrifice. Based on these findings, we assess that R-25788 was adequately tested and found to be nonclastogenic in the mouse micronucleus assay. The study, therefore, satisfies Guideline requirements for genetic effects Category II, Structural Chromosomal Aberrations.

STUDY CLASSIFICATION: The study is acceptable. However, it is recommended that, in the future, slides should be coded prior to scoring and that this information should be included in the final report.

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: Corn oil
Other provided information: The test material was stored in the dark at room temperature (~20°C) and at ambient humidity. The report also indicated that analytical determinations were performed on the "test substance/vehicle mixtures" within 1 week of preparation and were found to be within 10% of the target concentrations.

2. Control Materials:

Negative/Route of administration: None

Vehicle/Final concentration/Route of administration: Corn oil (0.5 mL/animal) was administered by oral gavage.

Positive/Final concentration/Route of administration: Cyclophosphamide (CP) was dissolved in water and administered by an unspecified route at 100 and 150 mg/kg (males) and at 150 and 200 mg/kg (females).

Note: Only the 100-mg/kg and 150-mg/kg dose levels for males and females, respectively, were scored.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

(a) Range-finding assay: 1000, 1500, 2000, 2500, and 3000 mg/kg (5 males and 5 females per dose)

(b) Micronucleus assay: 1000, 1500, and 2000 mg/kg (males: 5/dose/sacrifice time)
500, 1000, and 1200 mg/kg (females: 5/dose/sacrifice time)

(c) Secondary group: Additional animals (up to 10/sex) were randomly selected to receive the solvent or the low, mid, or high dose of the test material. Animals in the secondary group were used only to replace animals that died in the respective primary groups.

4. Test Animals:

(a) Species mouse Strain CD1 Age 6-7 weeks
Weight range: At dosing 25.11 g (males), 21.29 g (females)

Source: Charles River Breeding Laboratories.

(b) No. animals used per dose:

(1) Range-finding assay: 5 males; 5 females
(2) Micronucleus assay: 15 males; 15 females

Note: Dosing was based on mean body weights (see Section 4a)

(c) Properly maintained? YES

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

(c) Positive control:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): _____ 24 hr x 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

x bone marrow N/A others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by cervical dislocation. Sacrifice time for the positive control group was 48 hours. Bone marrow cells were either aspirated or flushed from both tibiae, centrifuged, resuspended in fetal calf serum, and spread onto slides.

Prepared slides were fixed in absolute methanol, stained with 2% Giemsa solution, coverslipped, and scored. The report did not indicate whether slides were coded prior to scoring.

4. Statistical Methods: The data were evaluated for statistical significance at $p<0.01$ using the Kastenbaum-Bowman tables.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant ($p<0.01$) increase in micronucleated polychromatic erythrocytes (MPEs) compared to the solvent control was seen, and the response was dose- and/or time-related.

C. REPORTED RESULTS:

1. Range-Finding Assay: Groups of five male and five female mice received single oral gavage doses of 0, 1000, 1500, 2000, 2500, or 3000 mg/kg and were observed for signs of clinical toxicity and/or death for 3 days. Immediately after treatment, animals in all dose groups convulsed. Within 8 hours of treatment, one male and one female in the 2000-mg/kg group, three males and two females in the 2500-mg/kg group, and two males and three females in the 3000-mg/kg group died. Deaths observed at 24 hours were as follows: two females (1500 mg/kg); two males and one female (2500 mg/kg); and two males and two females (3000 mg/kg). At 48 hours, the remaining high-dose male died. Deaths reported in females at 48 hours were as follows: two at 1500 mg/kg, one at 2000 mg/kg, and one at 2500 mg/kg. One female administered 1500 mg/kg and another female administered 2000 mg/kg died at 72 hours. Overall, the results indicated that no males survived treatment with ≥2500 mg/kg and that 80% of the males receiving 2000 mg/kg survived. In females, ≥60% died within 3 days of administration of test material at levels of ≥1500 mg/kg; no deaths occurred in low-dose females. Based on these findings, treatment levels of 1000, 1500, and 2000 mg/kg (males) and 500, 1000, and 1200 mg/kg (females) were selected for the micronucleus assay.

2. Micronucleus Assay:

(a) Animal observations: The report stated that three high-dose males, one mid-dose female, and one high-dose female died prior to the scheduled sacrifice. Mice from the secondary group were used to replace the dead animals. No compound effects were reported for the low-dose group.

(b) Micronucleus assay: Representative results from the micronucleus assay conducted with R-25788 are presented in Table 1. At the 72-hour harvest, there was a decrease of ~54% and ~37% in the PCE/NCE ratio for high-dose males and females, respectively, compared to the corresponding vehicle control values. Although the PCE/NCE ratios for the control groups were relatively low at all harvest intervals, the values fell within the acceptable

TABLE 1. Representative Results of the Micronucleus Assay in Mice with R-25788

Substance	Dose	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group[b]	Number of MPEs per Group[b]	Average Percent MPEs per Group[b]	Average Number PCEs/1,000 NCEs per Group[b]	PCE/NCE[b] Ratio
Vehicle Control									
Corn oil	0.5 mL	24	M	5	5,000	1	0.02	125.4	0.13
			F	5	5,000	10	0.20	191.8	0.19
		48	M	5	5,000	2	0.04	240.6	0.24
			F	5	5,000	3	0.06	167.2	0.17
		72	M	5	5,000	4	0.08	219.0	0.22
			F	5	5,000	3	0.06	300.2	0.30
Positive Control									
Cyclophosphamide	100 mg/kg	48	M	5	5,000	37[c]	0.74	149.2	0.15
	150 mg/kg		F	5	1,592	31[c]	1.95	32.8	0.03
Test Material									
R-25788	2,000 mg/kg[d,e]	24	M	5	5,000	2	0.04	195.6	0.20
	1,200 mg/kg[d,e]		F	5	5,000	2	0.04	302.2	0.30
	2,000 mg/kg	48	M	5	5,000	2	0.04	195.2	0.20
	1,200 mg/kg		F	5	5,000	2	0.04	169.2	0.17
	2,000 mg/kg	72	M	5	5,000	4	0.08	99.6	0.10
	1,200 mg/kg		F	5	5,000	1	0.02	193.0	0.19

[a]Time after compound administration
[b]Values were calculated by our reviewers.
[c]Significantly higher than the negative control ($p<0.01$) by Kastenbaum-Bowman tables
[d]Results for the low-dose (1,000 mg/kg, males; 500 mg/kg, females) and mid-dose (1,500 mg/kg, males; 1,000 mg/kg, females) groups were not significantly different from the vehicle control.
[e]Three high-dose males, one high-dose female, and one mid-dose female died prior to the scheduled sacrificed. Animals in the secondary group were used to replace dead animals.

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

range ($\leq$0.1).[1] Since control group PCE/NCE ratios tended to increase with time, the reduced PCE/NCE ratios noted in high-dose animals at the 72-hour sacrifice suggest a slight effect on hematopoiesis. As further shown in Table 1, the evaluated levels of R-25788 (1000, 1500, or 2000 mg/kg males; 500, 1000, or 1200 mg/kg females) did not cause a significant increase in the frequency of MPEs at any harvest time. By contrast, MPEs were significantly ($p<0.01$) increased in male and female mice administered the positive control (CP at 100 mg/kg). From the overall findings, the study author concluded that R-25788 was negative in the mouse micronucleus assay.

3. REVIEWER'S DISCUSSION/CONCLUSIONS: Our assessment is in agreement with the study author that R-25788 was not clastogenic in this in vivo assay. The evidence of overt compound toxicity in conjunction with a slight adverse effect on bone marrow stem cells indicated that the high doses (2000 mg/kg for males and 1200 mg/kg for females) selected for the study adequately demonstrated that the maximum tolerated dose was achieved.

   Additionally, the sensitivity of the test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant results obtained with the positive control (100 mg/kg CP).

4. QUALITY ASSURANCE MEASURES: A signed quality assurance (QA) statement, indicating that the QA review of this study was completed on December 31, 1987, was present.

5. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-8; Appendix B, Protocol, CBI pp. 19-21.

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[1]Heddle, J. A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J. T., Newell, G. W., and Salamone, M. F. The Induction of Micronuclei as a Measure of Genotoxicity A report of the U.S. Environmental Protection Agency Gene-Tox Program, Mutat Res 123 (1983), 61-118

APPENDIX A

MATERIALS AND METHODS
CBI pp. 7-8