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IPRODIONE MICRONUCLEUS

MUTAGENICITY STUDIES

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DATA EVALUATION REPORT

STUDY TYPE: In vivo micronucleus assay in mice

DP BARCODE: D214390

SUBMISSION NO.: S485489

PC CODE: 109801

MRID NUMBER: 435350-01

TEST MATERIAL: Iprodione

SYNONYM(S): 3-(3,5-Dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide

STUDY NUMBER(S): HRC Study Report Number RNP 442/941483

SPONSOR: Rhône-Poulenc Secteur Agro, Sophia Antipolis Cedex, France

TESTING FACILITY: Huntingdon Research Centre Ltd., Cambridgeshire, England

TITLE OF REPORT: Iprodione Mouse Micronucleus Test

AUTHOR(S): R.J. Proudlock and E.A. Elmore

REPORT ISSUED: August 17, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In an in vivo mouse micronucleus assay (MRID No. 435350-01), groups of five male and five female CD-1 mice received single oral gavage administrations of 750, 1500 or 3000 mg/kg. Bone marrow cells were collected 24, 48 or 72 hours after compound administration and were examined for micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the test animals as suspensions prepared in aqueous 1% methylcellulose.

One male and eight females in the high-dose group succumbed to treatment; other toxic signs at this concentration included piloerection, hunched posture, ptosis, lethargy and coma. Dose-related cytotoxic effects on the target tissue were also seen at 48 hours postdosing; the response was

significant ($p<0.01$) for the high-dose animals. There was, however, no evidence of a clastogenic or aneugenic effect at any dose or harvest time. The positive control induced the expected high yield of MPEs in both sexes.

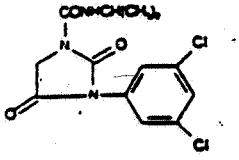
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a mouse micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: Iprodione

Description: White powder
Lot/ batch number: 9109801
Purity: 96.1%
Receipt date: March 2, 1994
Stability: Expiration date of May 21, 1994
CAS number: 36734-19-7
Structure:



Vehicle used: 1% Aqueous methylcellulose
Other provided information: The test material was stored at room temperature, protected from light. Dosing suspensions were prepared on the morning of use and samples of each dosing formulation used in the micronucleus assay were shipped frozen to ADME Bioanalysis, Mougins, France for analytical determinations.

2: Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: 1% Aqueous methylcellulose was administered once by oral gavage (dosing volume = 20 ml/kg).

Positive/final concentration/route of administration: Mitomycin C (MMC) was prepared in 0.9% saline and was administered once by oral gavage at a final dose of 12 mg/kg.

3. Test Compound:

Route of administration: Single oral gavage administration

Dose levels used:

(a) Preliminary toxicity test:

Phase I: 500, 1000, 2000 and 4000 mg/kg (2 males and 2 females per dose group)

Phase II: 1536, 1920, 2400 and 3000 mg/kg (2 males and 2 females per dose group)

(b) Micronucleus test: 750, 1500 and 3000 mg/kg

4. Test Animals:

(a) Species: Mouse Strain: CD-1 Age: ≈35 days(at receipt)
Weight range: 22-24 g (at receipt)
Source: Charles River, U.K. Ltd., Kent, England

(b) Number of animals used per dose:

Acute dose range-finding studies: 2 males and 2 females per group

Micronucleus assay:

- Treatment groups: 15 males 15 females
- Positive control: 5 males 5 females
- Vehicle control: 15 males 15 females

Note: A secondary group of 5 males and 5 females receiving the high dose was included for use in the event of unscheduled deaths in the primary group.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

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

(b) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr

2. Tissues and Cells Examined:

___x___ bone marrow _____ others (list):

Number of polychromatic erythrocytes (PCEs) examined per animal: 1000

Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000 ; NCEs were also examined to determine the number of micronucleated NCEs

3. Details of Slide Preparation: At 24, 48, and 72 hours post-administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by cervical dislocation. Animals in the positive control group were sacrificed at 24 hours. Bone marrow cells were recovered from both femurs and placed on slides containing one drop of fetal calf serum. Slides were fixed, dried, stained with 10% Giemsa, coded and scored.

4. Statistical Methods: The data were evaluated for statistical significance using the Wilcoxon's sum of ranks, Kruskal-Wallis' and Jonckheere's tests

5. Evaluation Criteria: The test material was considered positive if a "substantial", dose-related, and significant ($p<0.01$) increase in the frequency of micronucleated polychromatic erythrocytes (MPEs) compared to the concurrent vehicle control group value was observed. Bone marrow cytotoxicity was indicated by a "substantial", dose-related, and significant ($p<0.01$) decrease in the PCE:NCE ratio.

C. REPORTED RESULTS:

1. Preliminary Toxicity Tests: Animals were observed for mortality or other signs of clinical toxicity "regularly during the working day" for 72 hours postdosing in both phases of the preliminary studies. In Phase I, three of four animals (2/2 ♂; 1/2 ♀) administered the high dose (4000 mg/kg) died; no deaths occurred in the other treatment groups. Piloerection and hunched posture were noted in all treatment groups; lethargy, ptosis and increased respiratory rate was also observed in groups administered ≥1000 mg/kg. In addition, loss of the righting reflex and coma preceded death in the high-dose animals. From these findings, a second preliminary toxicity test was conducted with a narrower range of test material levels (1536, 1920, 2400 and 3000 mg/kg) administered to groups of two males and two females. Clinical signs (piloerection, lethargy, increased respiratory rate, and ptosis) were consistent with those observed in Phase I and were recorded for all treatment groups. Staggering gait and/or coma were also reported for all dose groups. Based on the overall results, levels of 750, 1500 and 3000 mg/kg were selected for evaluation in the micronucleus assay.

2. Micronucleus Assay:

(a) Analytical determinations: All dosing suspensions prepared for the micronucleus assay were found to be within ±10% of the intended concentrations.

(b) Animal observations: One of twenty males and eight of twenty females succumbed to treatment with the high dose. Three of the dead females were replaced with females from the secondary group. Clinical signs of compound toxicity also noted in the high-dose animals included piloerection, hunched posture, ptosis, lethargy and coma. Most of these signs were also seen in the mid-dose females up to 46 hours posttreatment. No deaths were reported in males or females receiving either 750 or 1500 mg/kg.

(c) Bone marrow observations: As the data presented in Study Report Table 1 (see attachment) indicate, exposure of male and female mice to the selected dose of Iprodione did not result in significant increases in the frequency of bone marrow cells with MPEs at 24, 48 or 72 hours postdosing. Although a nonsignificant elevation of MPEs was seen in the high-dose group at the 48-hour sampling time, the increase was well within the historical background range (0-2.5 MPEs/1000 PCEs) of the performing laboratory. A slight but dose-related decrease in the PCE:NCE ratios was, however, noted at the 48 hour sacrifice time; the response was significant ($p<0.01$) for the high-dose group. As stated by the study authors, the effect was transient and not clearly demonstrated at the subsequent sacrifice interval. By contrast, the positive control (12 mg/kg MMC) induced a marked and significant ($p<0.001$) cytotoxic and genotoxic response.

From the overall results, the study authors concluded that Iprodione did not cause chromosome damage in this study.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors correctly interpreted the data. Iprodione was evaluated over an adequate range of doses that included a high level (3000 mg/kg) that was overtly toxic to the test animals and slightly cytotoxic to the target tissue but failed to induce either a clastogenic or aneugenic effect in male or female mouse bone marrow cells. Additionally, the sensitivity of the test system to detect a positive response was clearly demonstrated by the significant ($p<0.001$) findings obtained with the positive control (12 mg/kg MMC). We conclude, therefore, that the study provided acceptable evidence that Iprodione is not genotoxic in this in vivo test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement indicating that the report was audited was signed and dated August 16, 1994. The actual study in progress, however, was not inspected.)

F. APPENDIX ATTACHED: Study Report Table 1, p.19