METHOMYL 11/7/1996 **MICRONUCLEUS**

EPA Reviewer: Nancy E. McCarroll Signature: _____
Review Section III, Toxicology Branch Date: _____
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EPA Section Head: James N. Rowe, Ph.D. Signature: _____
Review Section III,
Toxicology Branch II/HED (7509C) Date: _____

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mouse micronucleus assay; OPPTS 870.5395 [§84-2]

DP BARCODE: D227832 SUBMISSION NO.: S508273

PC CODE: 090301 TOX. CHEM. NO.: MRID NO: 44047703

TEST MATERIAL (PURITY): DPX-X1179-394 (98.35%)

COMPOSITION/SYNONYM(S): Ethanimidothioic acid, N-[[(methylamino)carbonyl]oxy]-methyl ester; S-methyl N-[(methylcarbamoyl)oxy]thioacetimidate; methomyl technical

CITATION: Bentley, K.S. (1995). Mouse Bone Marrow Micronucleus Assay of DPX-X1179-394; Haskell Laboratory, E.I. du Pont de Nemours and Co., Newark, DE; Medical Research Project No. 10210-001; Haskell Laboratory Report No. 413-95; Study Completion Date: October 19, 1995. (Unpublished) MRID NUMBER: 44047703

SPONSOR: Du Pont Agricultural Products, Wilmington, DE

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No: 44047703), groups of male and female CR1:CD®-1(ICR)BR mice received single oral gavage administrations of 3, 6 or 12 mg/kg DPX-X1179-394 (98.35%). High-dose groups consisted of six animals per sex per harvest time; mid- and low-dose groups contained five animals per sex per harvest time. Mice were sacrificed at 24, 48 and 72 hours postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the animals in sterile water.

Death occurred in one high-dose female; other signs of compound toxicity noted in high-dose males and females included lethargy and hyperactivity. There was no evidence of bone marrow cytotoxicity at any dose or harvest time. The positive control induced the expected high yield of MPEs in males and females. **There was, however, no evidence that DPX-X1179-394 induced a clastogenic or aneugenic effect in either sex at any dose or sacrifice time.**

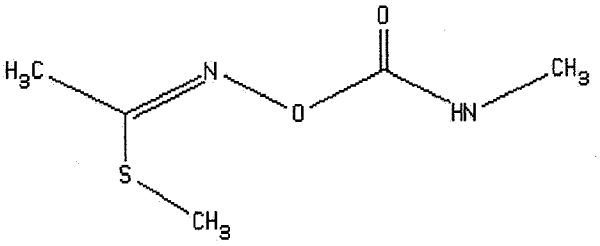
The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material: DPX-X1179-394

Description: White solid
Lot/batch number: DPX-X1179-394
Purity: 98.35%
Receipt date: Not reported
Stability: Assumed to be stable under the conditions of use.
CAS number: 16752-77-5
Structure:



Vehicle used: Sterile water
Other provided information: Storage conditions for the test material were not reported. Dosing solutions were prepared immediately prior to use. Analytical determinations were not performed to verify actual concentrations.

2. Control Materials:

Vehicle: Sterile water was administered by oral gavage at a dosing volume of 10 mL/kg.

Positive: Cyclophosphamide (CP) was prepared in sterile water and administered by oral gavage at a final dose of 40 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Volume of test substance administered: 10 mL/kg

Dose levels used:

(a) Acute dose range-finding study: 5, 10, 15, 20, 25, 30 and 40 mg/kg (males and females).

Note: Only females were administered 25 mg/kg.

(b) Micronucleus assay: 3, 6 and 12 mg/kg

4. Test Animals:

(a) Species: Mouse Strain: CR1:CD®-1(ICR)BR Age: ≈7 weeks (at dosing) Weight Range: 29.1-36.0 g (males); 21.5-27.3 g (females) Source: Charles River Breeding Laboratories, Inc., Raleigh, NC

(b) Number of animals used per dose:
Range finding-study: 3-5 (males); 2-7 (females)

Micronucleus assay (primary dose groups/sacrifice time):

- 6 males 6 females -- high-dose group/sacrifice time
- 5 males 5 females -- mid-and low-dose treatment groups and vehicle control group/sacrifice time
- 5 males 5 females -- positive control group--24 hour sacrifice only

(c) Animals were properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and sampling times:

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

(b) Positive control:
Dosing: x once _____ twice (24 hours apart)
Sampling (after last dose): x 24 hours ____ 48 hours
____ 72 hours

2. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 2000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000 erythrocytes (PCEs+NCEs)

4. Details of Cell Harvest and Slide Preparation: At 24, 48, and 72 hours after administration of the test material or vehicle control, the appropriate groups of animals were euthanized with CO_2. Animals in positive control group were sacrificed 24 hours postexposure. Bone marrow cells from both femurs of each animal were aspirated into prewarmed fetal bovine serum, centrifuged, resuspended in residual supernatant and spread onto slides using a Miniprep® automatic blood smearing instrument. Prepared slides were fixed in absolute methanol, stained with acridine orange (0.0125 mg/mL), coverslipped, coded and scored for PCEs, micronucleated PCEs (MPEs), and NCEs.

Note: When stained with acridine orange, MPEs appear as bright yellow-green fluorescent bodies within the reddish PCEs, and NCEs have a dark green appearance.

5. Statistical Methods: To determine whether the results from any dose or cell harvest time were significantly different ($p<0.05$) from vehicle control, the data were evaluated using an analysis of variance on transformed data (arcsine square-root function), Dunnett's, Kruskal-Wallis and/or Mann-Whitney U tests. Males and females were analyzed separately.

6. Evaluation Criteria: No criteria were provided to evaluate assay validity, a positive response, or the biological significance of the findings.

C. REPORTED RESULTS:

1. Dose Range-finding Study: Doses ranging from 5 to 40 mg/kg were evaluated in the preliminary study. All females administered ≥25 mg/kg of the test material and all males in the high-dose group died at unspecified times over the 3-day observation period. Other reported mortalities included: 4 of 5 males at 30 mg/kg, 3 of 5 males and 2 of 6 females at 20 mg/kg, and 1 of 4 males at 15 mg/kg. Toxic signs noted prior to death were: tremors, convulsions and/or gasping. Surviving animals in groups receiving ≥15 mg/kg were lethargic and/or displayed shallow breathing; these signs subsided within 1-3 hour of dosing. No deaths or clinical signs were seen in the 5- or 10-mg/kg test groups. Based on these findings, doses ranging from 3-12 mg/kg were selected for further study.

2. Micronucleus Assay:

   a. Animal observations: Two deaths (1 female in the high-dose group and 1 female in the vehicle control group) attributed to dosing error were reported. An additional high-dose female was found dead at 24 hours posttreatment; this death was considered to be compound related since there was no evidence of dosing trauma. Other clinical signs noted at 12 mg/kg included lethargy (2 animals) and hyperactivity (1 animal). Lethargy was also reported in 3 mice of the mid-dose group. The significant body weight change observed for the 48-hour sacrifice of the 12-mg/kg was not considered by our reviewers to be toxicologically relevant.

   b. Bone marrow analysis: Summarized results from the analysis of bone marrow cells harvested 24, 48, or 72 hours after exposure to DPX-X1179-394 are presented in Table 1. As shown, treatment with DPX-X1179-394 had no appreciable effect on erythropoiesis. Similarly, there was no evidence of a genotoxic response at any dose or harvest time. By contrast, the male and female mice in the positive control group responded to the genotoxic action of 40 mg/kg CP as indicated by the significant ($p<0.05$) induction of micronuclei in bone marrow cells recovered from both sexes.

   Based on the overall results, the study author concluded that DPX-X1179-394 was not genotoxic in this in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author's interpretation of the data was correct. DPX-X1179-394 was evaluated to a concentration (12 mg/kg) that caused mortality and other clinical signs of toxicity but failed to induce a clastogenic or aneugenic response. There was, however, no evidence that the test material was cytotoxic to the target tissue. The sensitivity of the test system to detect genotoxicity was demonstrated by the significant increases ($p<0.05$) in MPEs in both male and female mice exposed to the positive control (40 mg/kg CP).

   We conclude, therefore, that the study provided acceptable evidence that DPX-X1179-394 is negative in this in vivo micronucleus assay.

E. STUDY DEFICIENCIES: NONE.

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with DPX-X1179-394

Substance	Dose per kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Percent MPEs/PCEs per Group	PCE:NCE Ratio
Vehicle Control								
Water	10 mL	24	M	5	10,000	22	0.22	1.38
		48	M	5	10,000	14	0.14	0.90
		72	M	5	10,000	16	0.16	1.16
		24	F	5	10,000	14	0.14	1.35
		48	F	5	10,000	17	0.17	1.16
		72	F	4[b]	8,000	11	0.14	1.07
Positive Control								
Cyclophosphamide	40 mg	24	M	5	10,000	197	1.97*	1.07
		24	F	5	10,000	207	2.07*	1.10
Test Material								
DPX-X1179-394	12 mg[c]	24	M	6	12,000	24	0.20	0.84
		48	M	6	12,000	20	0.17	1.03
		72	M	6	12,000	10	0.13	0.77
		24	F	5[d]	10,000	21	0.21	1.14
		48	F	5[b]	10,000	18	0.18	1.14
		72	F	6	12,000	16	0.19	1.31

[a]Time after test material, vehicle or positive control administration by oral gavage.
[b]One female in this group died as a result of a dosing error.
[c]Death (one female) and other signs of compound toxicity [e.g., lethargy (2 mice) and hyperactivity (1 mouse)] were observed at this dose. Results for lower treatment groups (3 or 6 mg/kg) did not suggest a genotoxic effect.
[d]One female found dead prior to the scheduled sacrifice.
Abbreviations:
PCE = Polychromatic erythrocyte
MCE = Micronucleated polychromatic erythrocyte
NCE = Normochromatic erythrocyte

*Significantly different ($p \leq 0.05$) from the corresponding vehicle control group.

Note: Data were extracted from the Study Report, Tables 2 and 3 and Appendices A, B and C, pp. 14-22.

Sign-off date: 11/07/96
DP Barcode: D227832
HED DOC Number: 012098
Toxicology Branch: TB2