EPA Reviewer: John E. Whalan
Toxicology Branch 2 (7509C)

4-14-99

EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice
OPPTS Number: 870.5395 OPP Guideline Number: §84-2

DP BARCODE: D241232 SUBMISSION CODE: S534142
P.C. CODE: 070705 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): KBR 3023 (99.0% a.i.)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl ester

CITATION: Herbold, B. (1994) KBR 3023: Micronucleus Test on the Mouse. Bayer AG Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T 9055720, August 25, 1994. MRID 44408734. Unpublished

SPONSOR: Bayer AG, Friedrich-Ebert-Str. 217-333, D-5600 Wuppertal, Germany

EXECUTIVE SUMMARY:

In an *in vivo* mouse bone marrow micronucleus assay (MRID 44408734), groups of 20 male and female Hsd/Win: NMRI mice were dosed by a single intraperitoneal injection with KBR 3023 (99.0%, a.i.) in 0.5% aqueous Cremophor at 350 mg/kg body weight. Bone marrow cells were harvested at 16, 24, or 48 hours and scored for micronucleated polychromatic erythrocytes (MPCEs). Groups of 5 mice/sex were given a single intraperitoneal injection of 0.5% aqueous Cremophor (vehicle control) or cyclophosphamide as Endoxan (positive control) and bone marrow cells were harvested at 24 hours.

Clinical signs including apathy, roughened fur, lateral recumbency, spasm, extension and leaping spasm, twitching, difficulty in breathing and slitted eyes were observed in animals treated with KBR 3023 up to sacrifice; mortality was observed in 3/40 animals. No mortalities or clinical signs were observed in the control groups. A significant decrease ($p<0.01$) in the ratio of polychromatic to normochromatic erythrocytes was observed in animals sacrificed 48 hours after a 350 mg/kg dose of KBR 3023. The positive control induced significant increases in MPCEs. There was no significant increase in the frequency of MPCEs above controls after KBR 3023 treatment at any bone marrow sampling interval; therefore, the test article is considered negative in this micronucleus assay.

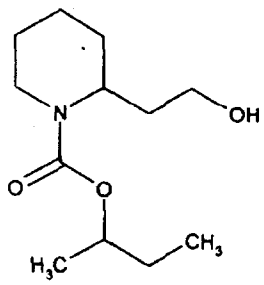
This study is classified as **acceptable**, and it satisfies the requirement for FIFRA Test Guideline **§84-2** for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023
   Description: Clear, colorless liquid
   Lot/Batch #: 010393
   Purity: 99.0 %
   Stability of compound: Reported to be stable for the duration of the study when stored at room temperature.
   CAS #: 119515-38-7
   Structure:



   Vehicle used: 0.5% aqueous Cremophor
   Comment: The test substance was stable in the vehicle for up to 24 hours at room temperature.

2. Control Materials:
   Vehicle/Final volume/Route of administration: 0.5% aqueous Cremophor/10 mL/kg body weight/intraperitoneal injection

   Positive/Final dose(s)/Route of administration: Cyclophosphamide in the form of Endoxan in deionized water/20 mg/kg body weight/intraperitoneal injection

3. Test compound administration:
   Volume of test substance administered: 10 mL/kg body weight

   Route of administration: intraperitoneal injection

   Dose levels used:

   Preliminary Toxicity Tests:
   First: 250, 350, and 500 mg/kg

   Micronucleus Assay:
   350 mg/kg

Rationale for dose selection: The dose level of 350 mg/kg used in the micronucleus assay was based on the results of the preliminary toxicity test. Clinical signs of toxicity were manifested at doses ≥250 mg/kg including the death of 3 of 5 animals in the 500 mg/kg dose group.

4. Test animals:
   a. Species: Mouse Strain: Hsd/Win: NMRI Age: approximately 6 -12 weeks

      Weight: approximately 27-49 grams on arrival
      Source: Harlan Winkelmann GmbH, Borchen, Germany

   b. No. animals used per dose:

      Toxicity Study: 5 animals including both sexes

      Micronucleus Assay: 5 males and 5 females per sampling interval and the vehicle control, and 5/sex for the positive control. An additional 5/sex were dosed at 350 mg/kg to replace any animals that might die.

   c. Properly maintained? **Yes**

B. TEST PERFORMANCE

1. Treatment and Sampling Times:
   a. Test compound:
      Dosing: _x_ once ___ twice (24 hr apart)
      ___ other (describe):

      Sampling (after last dose): ___ 6 hr _x_ 16 hr
      _x_ 24 hr _x_ 48 hr ___ 72 hr

   b. Vehicle and positive controls:
      Dosing: _x_ once ___ twice (24 hr apart)
      ___ other (describe):

      Sampling (after last dose): ___ 6 hr ___ 12 hr
      _x_ 24 hr ___ 48 hr ___ 72 hr

2. Tissues and Cells Examined:
   _x_ bone marrow ___ other (list):

   No. of polychromatic erythrocytes (PCEs) examined per animal: 1,000. The ratio of PCEs to normochromatic erythrocytes (NCE's) was calculated.

3. Details of slide preparation: At 16, 24, and 48 hours after dosing, 5 treated animals/sex were sacrificed (method not indicated). The vehicle and positive control groups were sacrificed 24 hours after dosing. The femoral bone marrow was removed and suspended in fetal calf serum. The marrow was then spread on glass slides and air-dried overnight. The slides were stained, rinsed in deionized water, air-dried, and mounted. The slides were coded prior to scoring.

4. Statistical methods: Non-parametric methods were used for analysis of the results. Wilcoxon's sum of ranks test was used to compare dosed groups with the highest mean to the concurrent negative control. Statistical significance was judged at the $p<0.05$ level. If the MPCE ratio was increased, the MNCE ratio was compared with the negative control group using the one-sided chi-square test. Statistical significance was judged at the $p<0.05$ level.

5. Evaluation Criteria: A positive response was a statistically significant ($p<0.05$) increase in MPCEs compared to the concurrent negative control for ≥ 1 sampling interval; historical control ranges should be exceeded. A negative response was observed when the values for MPCEs were not significantly greater than the concurrent negative control or they fell within the historical control range. An equivocal response was an increase in MPCEs compared to the negative control and above historical ranges, but not statistically significant, and the negative control was "not closely related to the data of the respective treatment group." This response would necessitate a repeat assay.

II. REPORTED RESULTS

A. Analytical Determinations: Data regarding the analysis of concentration of KBR 3023 in 0.5% Cremophor were not provided. Emulsion formulations of KBR 3023 in 0.5% aqueous Cremophor at 1.0 and 50.0 mg/mL were stable during storage for up to 24 hours at room temperature.

B. Toxicity Study: A preliminary study was performed in which groups of 5 mice of both sexes (not otherwise specified) were dosed at 250, 350 and 500 mg/kg, and examined daily for mortality and clinical signs of toxicity during the next 72 hours. Apathy, roughened fur, distended abdomen, spasm, extension and leaping spasm, twitching, difficulty in breathing, eyelids stuck together and salivation were observed in all dose groups. Deaths occurred in 3/5 animals in the 500 mg/kg group. Based on these results, 350 mg/kg was selected as the high dose for the micronucleus assay.

C. Micronucleus Assay:

1. Animal observations: Groups of mice (20/sex) were administered KBR 3023 by a single intraperitoneal injection at 350 mg/kg. Clinical signs similar to those manifested in the

preliminary trial were observed up to sacrifice, and deaths occurred in 3/40 treated animals. Mortality and symptoms of toxicity associated with the treated groups were absent in the control animals.

2. Micronucleus assay: The results of the micronucleus assay are presented as an attachment to this DER (study report Table 6, page 50). The recorded mean incidence of micronuclei per 1,000 polychromatic erythrocytes varied between 1.2 and 1.5 in the treated animals. KBR 3023 did not cause a significant increase in MPCEs compared to vehicle controls in bone marrow cells collected from male or female mice 16, 24, or 48 hours after dosing at 350 mg/kg. Decreases in the ratio of polychromatic to normochromatic erythrocytes, indicative of bone marrow cell depression, were observed at all sampling intervals with statistical significance ($p<0.01$) at 48 hours. The positive control, cyclophosphamide, induced significant ($p<0.01$) increases in MPCEs.

   The study author concluded that KBR 3023 was negative in this *in vivo* mouse micronucleus assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. We concur with the study author that KBR 3023 was negative in this *in vivo* micronucleus assay. We also agree with the study author that the decrease ($p<0.01$) in the ratio of polychromatic to normochromatic erythrocytes observed at 48 hours treatment at the 350 mg/kg dose level is indicative of bone marrow cell depression. The sensitivity of this test to detect a genotoxic response was demonstrated by the significant ($p<0.01$) increase in MPCEs induced by the positive control. We conclude that KBR 3023 was adequately tested and found non-genotoxic in this *in vivo* micronucleus assay.

B. STUDY DEFICIENCIES: There were no concurrent vehicle controls for the 16 and 48 hour sampling intervals. If a positive response had been observed in the treated groups at either of these intervals, an appropriate negative control would not have been available for comparison. In addition, dose preparations were not analyzed for the concentration of KBR 3023 in 0.5% aqueous Cremophor. However, cytotoxicity was observed. Therefore, these deficiencies would not be expected to alter the conclusions of the study.

DER# 44408734

Page 1 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
