KBR 3023 Acute Neurotoxicity Screen (870.6_200)

EPA Reviewer: John E. Whalan 4-28-99
Registration Action Branch 2 (7509C)

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

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Neurotoxicity Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: §81-8

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

TEST MATERIAL (PURITY): KBR 3023 (≥97.4% a.i.)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester; 1-(1-Methyl propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine

CITATION: Sheets, L.P. and B.F. Hamilton. (1996) An acute dermal neurotoxicity screening study with technical grade KBR 3023 in Fischer 344 rats. Bayer Corporation, 17745 South Metcalf, Stilwell, Kansas, 66085-9104. Study No. 95-422-ET. October 14, 1996. MRID 44408715. Unpublished.

SPONSOR: Bayer AG, PF Zentrum Monheim 6100, 5090 Leverkusen, Bayerwerk, Germany.

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 44408715), KBR 3023 (≥97.4% a.i.) was applied to the shaved skin of young adult Fischer 344 CDF(F-344)/BR rats (12/sex/dose) for 24 hours at dose levels of 0, 200, 600 or 2000 mg/kg. The rats were evaluated for reactions in functional observations and motor activity measurements at 4 hours and 7 and 14 days posttreatment.

No neurological effects were observed at any treatment level. There were no treatment-related deaths during the study or treatment-related differences in the general appearance or behavior, body weights, absolute or relative brain weights, or gross or microscopic histology of the rats. **The neurotoxicological NOAEL is >2000 mg/kg; the NOEL is 2000 mg/kg.**

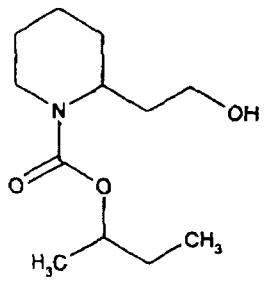
This study is classified **Acceptable** and satisfies the guideline requirement for an acute neurotoxicity study in rodents (870.6200).

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023 technical grade
   Description: Clear, colorless liquid
   Lot/Batch #: 030693
   Purity: ≥97.4% a.i.
   Stability of compound: It was stated that the concentration of active ingredient in the test substance under frozen storage was determined within 6 months of initiation of exposure and after terminal sacrifice. Data were not provided.
   CAS #: 119515-38-7
   Structure:



2. Vehicle and positive control: Positive control studies were referenced (MRID 42770301 and 43656301).

3. Test animals: Species: Rat
   Strain: Fischer 344 CDF(F-344)/BR
   Age and weight at study initiation: 9 weeks of age; male body weight 198-218 g; female body weight 144-160 g
   Source: Sasco Inc., Madison, Wisconsin
   Housing: Individually housed in suspended stainless steel wire-mesh cages
   Diet: Purina Mills Rodent Lab Chow 5001-4 in "etts" form, *ad libitum*, except was removed for approximately 2 hours prior to treatment
   Water: Tap water, *ad libitum*
   Environmental conditions:
      Temperature: 18.3-25.6 C
      Humidity: 40-70%
      Air Changes: Not reported
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: ≥6 Days

B. STUDY DESIGN

1. In life dates - Start: 6/12/95 End: 6/30/95

2. Animal assignment

Rats (48/sex) that had body weights within 20% of the mean weight for that sex were selected for use in the study. The selected rats were randomly assigned to the test groups in Table 1 in order that for each sex, groups had equivalent weights when treatment was initiated.

Table 1. Study design.[a]

Test Group	Dose to Animal (mg/kg)	Animals Assigned[b]	
		Male	Female
Control	0	12	12
Low	200	12	12
Mid	600	12	12
High	1200	12	12

[a] Dose levels were based on the results of an acute dermal toxicity study in which male and female rats were treated with a single dose of undiluted KBR 3023 at 0 or 2000 mg/kg body weight. The NOEL for that study was 2000 mg/kg. Since a peak effect time could not be established because no effects were evident at the limit dose, it was decided that neurobehavioral testing would be done 4-7 hours postdose.

[b] Neurobehavioral evaluation was performed on all rats in each treatment group. Neuropathological examination was conducted on tissues from six rats/sex/group.

3. Preparation and treatment of animal skin

Hair was clipped from the dorsal and lateral areas of the trunk of each rat. On the day of dose application, undiluted KBR 3023 was uniformly applied by pipette to a clipped area of the back. The treated area (30-39 cm^2) represented at least 10% of the body surface. All animals wore rodent jackets on the day of application that were removed approximately 24 hours following treatment. Control animals were untreated but were otherwise maintained in a similar fashion.

4. Statistics

Body weight and absolute and relative brain weight data for each treatment group were compared to those of the control group using one-way analysis of variance (ANOVA). If the variances were heterogeneous, the data were analyzed using Dunnett's test. Comparisons were conducted at the 5%, two-tailed level.

For motor and locomotor activity, data were analyzed using an ANOVA. Session activity data were analyzed using a repeated-measures ANOVA, followed by a one-way ANOVA and Dunnett's test if significance was observed. Interval data were analyzed using a two-way repeated-measures ANOVA , followed by a one-way ANOVA and Dunnett's test if significance was observed. Tests were conducted at the 5% level.

Continuous FOB data were analyzed using a repeated-measures ANOVA followed by a one-way ANOVA and Dunnett's test if significance was observed. Categorical data collected in the FOB were analyzed using General Linear Modeling and Categorical Modeling Procedures, with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts, respectively. Tests were conducted at the 5% level.

C. METHODS

1. Observations

Animals were examined at least once daily for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were conducted once daily. Bedding material in the cages was inspected to assess the consistency and relative amount of feces or unusual amount, color, and odor of urine.

2. Body weight

Animals were weighed prior to dosing, on the day of dosing, and on days 7 and 14 as part of the Functional Observational Battery.

3. Neurobehavioral Studies

Functional Observational Battery (FOB) and motor activity testing were performed on all animals during the week prior to the initiation of treatment, approximately 4 hours (minimum) after dosing, and at 7 and 14 days following treatment. Animals were evaluated individually in sets of eight over a 2-day period for each test week. The order of testing was semi-random so that the test groups were balanced across test times and test devices, and males and females were tested on separate days. Animals were acclimated to the testing rooms for at least 30 minutes prior to the initiation of testing.

Functional Observational Battery - Animals were evaluated using the FOB of tests described by Moser. The major groups of observations/measurements are listed below; detailed information about the FOB is presented in Attachment 1 to this DER. Comparative/historical control studies were conducted using acrylamide, carbaryl, and untreated rats; the data were not included in this submission.

HOME CAGE OBSERVATIONS	OPEN FIELD OBSERVATIONS
Posture	Piloerection
Piloerection	Respiratory abnormalities
Gait abnormalities	Posture
Involuntary motor movements	Involuntary motor movements
Clonic	Clonic
Tonic	Tonic
Vocalizations	Stereotypy
Other	Bizarre behavior
	Gait abnormalities
OBSERVATIONS DURING HANDLING	Vocalizations
	Arousal
Ease of removal from cage	Rearing
Reaction to handling	Defecation
Muscle tone	Urination
Palpebral closure	
Pupil size	**REFLEX/PHYSIOLOGIC OBSERVATIONS**
Pupil response	
Lacrimation	Approach response
Salivation	Touch response
Stains	Auditory response
Other	Tail pinch response
	Righting reflex
	Body temperature
	Grip strength
	Footsplay

Motor Activity - Motor activity testing was conducted in a sound-absorbing room with white noise to minimize acoustical variations during testing. Animals were tested for 90 minutes in one of eight figure-eight mazes. Each maze was equipped with eight infrared emitter/detector pairs; activity was measured each time a beam was interrupted. Motor and locomotor activity were reported for the entire 90-minute session and for each 10-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. To determine locomotor activity, consecutive interruptions of a given beam were not counted; ie., only one interruption of a given beam was counted until the rat relocated in the maze and interrupted another beam. Habituation was defined as a decrement in activity during the test session.

7. Sacrifice and Pathology

All test animals were sacrificed 15 days after treatment. Six rats/sex/group were anaesthetized using pentobarbital, perfused with sodium nitrite in phosphate buffer, and fixed *in situ* using a mixture of 4% glutaraldehyde and 4% formaldehyde in phosphate buffer. Brain weight was recorded upon removal from the skull prior to placement into fixative. Tissues were subjected to gross necropsy. Central and peripheral nervous system tissues were collected and processed for neuropathological examination. Only tissues from the control and 2000 mg/kg dose groups were examined microscopically. The following tissues were processed:

BRAIN Olfactory bulbs[a] Cerebral cortex[a] Caudate-putamen/globus pallidus[a] Hippocampus[a] Thalamus[a] Hypothalamus[a] Midbrain[a] Cerebellum[a] Medulla oblongata[a] SPINAL CORD Cervical[bc] Thoracic[bc] Lumbar[bc] Cauda equina[c] Grossly abnormal tissue	SPINAL NERVE ROOT FIBER AND GANGLION Cervical (bilateral) (dorsal and ventral) Lumbar (bilateral) (dorsal and ventral) Gasserian ganglion[c] Gastrocnemic muscle (unilateral)[c] PERIPHERAL NERVES Sciatic (bilateral)[bc] Tibial (bilateral)[b] Sural (bilateral)[b] Eyes[c] Optic nerves[c]

[a] Coronal sections of these tissues were evaluated.
[b] Cross sections of these tissues were evaluated.
[c] Longitudinal sections of these tissues were evaluated.

Tissues were embedded in paraffin wax, plastic (GMA) or epoxy resin, sectioned, and stained with H&E, LFB/CV or LFB, toluidine blue and/or Sevier-Munger stain.

The remaining 6 rats/sex/group were sacrificed by carbon dioxide asphyxiation without perfusion, and subjected to a complete gross necropsy examination. No tissues were dissected and/or fixed.

III. RESULTS

A. Observations

1. Mortality - No rats died during the study.

2. Clinical signs - No treatment-related differences in appearance or behavior were observed during the study.

B. Body weight and body weight gain

Body weights of rats in all treatment groups were similar to the corresponding control group throughout the study. At the end of the study, body weights of male test groups were 222-254 g, and female test groups were 151-173 g.

C. Functional Observational Battery

No treatment-related differences in FOB parameters were observed between the treatment and control groups.

D. Motor Activity Measurements

No treatment-related changes in motor and locomotor activity were observed between the treatment and control groups.

E. Sacrifice and Pathology

No treatment-related differences in absolute or relative brain and pituitary weights were observed between treated and control group rats. No treatment-related gross or microscopic postmortem differences were observed between rats in the treated and the control groups. All abnormalities appeared to occur randomly and sporadically in all study groups.

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that an acute limit dose of 2000 mg/kg, KBR 3023 produced no evidence of toxicity or neurotoxicity. The overall NOEL was concluded to be 2000 mg/kg for males and females.

B. Reviewer's Discussion

We agree with the study author that KBR 3023 did not cause toxic or neurotoxicological effects in treated rats. There were no differences in general behavior or appearance, body weights, FOB parameters, motor activity, absolute or relative brain weights, or gross histology. There was no evidence of treatment-related neuropathology in the 2000 mg/kg treatment group. Based on these findings, the neurotoxicological NOEL for KBR 3023 is 2000 mg/kg for male and female rats. The toxicological NOEL is also 2000 mg/kg for both sexes.

IV. STUDY DEFICIENCIES

None.