EPA Reviewer: Pamela Hurley, Ph.D. Pamela M Hurley 6/7/99
Registration Action Branch 2 (7509C)

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

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity (repeated dose dermal) [percutaneous] - Dogs

OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

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

TEST MATERIAL (PURITY): KBR 3023 (98.1% a.i.)

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

CITATION: Jones, R.D. and T.F. Hastings. (1995) Technical grade KBR 3023: A chronic percutaneous toxicity study in the Beagle dog. Bayer Corporation, 17745 South Metcalf, Stilwell, KS 66085-9104. Study No. 93-126-UK. December 1, 1995. MRID 44408718. Unpublished.

SPONSOR: Bayer AG, Fachbereich Toxikologie, Friedrich-Ebert-Strause 217-333, D-42096 Wuppertal, Germany.

EXECUTIVE SUMMARY:

In a chronic dermal toxicity study (MRID 44408718), KBR 3023 (98.1% a.i.) was applied to the clipped skin of Beagle dogs (4/sex/dose) at dose levels of 0, 50, 100 or 200 mg/kg/day, 5 days/week, for one year. Due to problems with the test substance (a liquid) flowing beyond the test site at higher dose levels, the high dose of 200 mg/kg had been previously agreed upon with the EPA.

No treatment-related dermal responses were observed on treated skin of dogs in any treatment group. No animals died during the study. There were no treatment-related differences in appearance, behavior, body weights, food consumption, ophthalmology, hematology, clinical blood chemistry or urine parameters, electrocardiography, blood pressure, clinical neurology, organ weights, or microscopic or gross histopathology between dogs in the treated and control groups. No neoplastic tissue was observed. **A LOAEL for systemic toxicity was not established; the NOAEL is 200 mg/kg/day. A LOAEL for dermal irritation was not**

established; the NOAEL is 200 mg/kg/day.

This chronic toxicity study in dogs is classified **Acceptable (§870.4100)** and satisfies the guideline requirement for a chronic toxicity study in non-rodents.

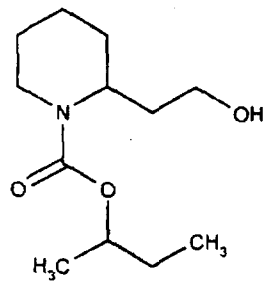
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023 technical grade
   Description: Clear, viscous liquid
   Lot/Batch #: PT030693
   Purity: 98.1% a.i.
   Stability of compound: It was stated that the stability of the test substance at room temperature and periodic active ingredient checks of the test substance were conducted. Since the test substance was applied undiluted, homogeneity and concentration determinations were not conducted. The periodic analytical measurements indicated that the purity of the test substance remained stable (ranging from 98.10 to 98.50%) over a period of a year.

   CAS #: 119515-38-7
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Dog
   Strain: Purebred beagle
   Age and weight at initial dosing (Day 0): Approximately 23-25 weeks of age; body weight range - males, 7.7-10.0 kg; females 5.9-8.2 kg
   Source: White Eagle Laboratories, Doylestown, PA.
   Housing: Individually housed in stainless steel cages
   Diet: Purina Dog Chow #5006-3, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
      Temperature: 17.8-28.9 C
      Humidity: 30-70%

Air Changes: Not reported
Photoperiod: 12-Hour light/dark cycle
Acclimation period: 18 Days

B. STUDY DESIGN:

1. In life dates - Start: 7/12/93 End: 7/14/94

2. Animal assignment

Healthy, vaccinated dogs (16/sex) were selected for use in the study, and were allocated to the test groups in Table 1 using a computerized, weight stratification based procedure.

Table 1. Study design.[a]

Test Group	Dose to Animal (mg/kg/day)	Animals Assigned	
		Male	Female
Control	0	4	4
Low	50	4	4
Mid	100	4	4
High	200	4	4

[a] The dose selection was based on the results of a subchronic rat study and a 28-day dermal pilot study using the Beagle dog; results were not reported in the present study. Also, due to problems with the test substance flowing beyond the clipped test site at higher dose levels, a high dose of 200 mg/kg/day was previously agreed upon with the EPA.

3. Preparation and treatment of animal skin

Hair on the nuchal and interscapular areas of each dog was depilated with clippers before the first dose, once weekly thereafter or as necessary depending on hair growth. Undiluted KBR 3023 was applied to the clipped area of each dog on each of five consecutive days/week for 13 weeks[1]. The same mechanical procedures were applied to the dermis of the control dogs. The site was not covered nor were the dogs fitted with Elizabethan collars. The dose site was not wiped between applications. On a few rare occasions, the dose site was gently blotted approximately 24 hours after application and prior to the next dose to remove visible residues that had migrated to inappropriate sites.

[1] The initial duration of the study was 90 days, however, in the absence of subchronic toxicity, the duration was extended to one year.

The dose volume and exposed surface area were calculated based on the individual weekly body weights. The formula for exposure site surface area was calculated:

$$(\text{Body weight})^{7}/0.10 = \text{area in cm}^2$$

The initial duration of the study was 90 days, however, in the absence of subchronic toxicity, the duration was extended to one year.

4. Statistics

Continuous data were analyzed using an ANOVA followed by a Student's t-test. Frequency data were analyzed by a Chi-Square followed by a Fisher's exact test. Significance was conducted at the 5%, two-sided level.

C. METHODS:

1. Observations

All animals were observed at least once daily for overt signs of toxicity, the ability to access feed and water, and the presence of feed wastage. Dermal dose site observations were recorded daily prior to the application of the next dose. Detailed physical examinations for clinical signs of toxicity were performed weekly on all animals.

2. Body weight

All animals were weighed once weekly during all study weeks and immediately prior to necropsy.

3. Food consumption

Food consumption by each animal was measured daily during the entire study.

4. Ophthalmoscopic examination

Ophthalmological examinations were performed on all animals following the period of quarantine/acclimation and prior to initiation of dosing. Ophthalmoscopic examinations were also conducted on all animals at 3, 6, and 9 months and just prior to study termination.

5. Blood

Blood was collected from all animals at least twice prior to administration of the test substance and after approximately 30, 60, 90, 180, 270, and 365 days of the study. Blood

was collected from each animal via venipuncture into EDTA or clot tubes. The animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count* (thombocytes)	X	Blood cell morphology
	Blood clotting measurements*	X	Reticulocyte count
	(Thromboplastin time)	X	Heinz bodies
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic toxicity studies.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium	X	Globulins
X	Sodium*	X	Glucose*
		X	Bilirubin
		X	Total serum protein (TP)*
	ENZYMES	X	Uric acid
		X	Triglycerides
X	Alkaline phosphatase (ALP)*	X	Thyroxine (T4)
	Cholinesterase (ChE)	X	Triiodothyronine (T3)
X	Creatine phosphokinase	X	Cytochrome P-450
X	Lactic acid dehydrogenase (LDH)*	X	0-demethylase
X	Serum alanine aminotransferase	X	N-demethylase
X	Serum aspartate aminotransferase*		
X	Gamma glutamyl transpeptidase		
	Glutamate dehydrogenase (GLDH)		

* Required for chronic toxicity studies.

6. Neurological Examinations

Neurological examinations of all animals were conducted prior to quarantine/acclimation and prior to initiation of dosing. Examinations included mental status/behavior, gait characteristics, postural status and reactions, spinal/cranial reflex tests, thoracic auscultation of the heart and lungs, and rectal body temperature measurements. Neurological examinations, thoracic auscultation, and rectal body temperature measurements were conducted on all animals at 3 months and just prior to study termination.

7. Electrocardiogram/Blood Pressure Assessments

Electrocardiograms and non-invasive blood pressure measurements of systolic, diastolic, and mean arterial pressure were performed on all animals once prior to administration of the test substance, at 3, 6, and 9 months, and just prior to study termination.

6. Urinalysis

Urinalysis was performed on all animals prior to dosing, following initiation of dosing, and after approximately 30, 60, 90, 180, 270, and 365 days of the study. The CHECKED (X) parameters were examined.

x	Appearance*	X	Glucose*
	Volume	X	Ketones*
X	Specific Gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Urobilinogen
		X	Leukocytes

* Required for chronic toxicity studies.

7. Sacrifice and Pathology

All test animals were sacrificed on schedule by intravenous injection of Fatal-Plus, and were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain* (cerebellum-midbrain, cerebellum, medulla/pons
X	Salivary glands*	XX	Heart*		
X	Esophagus*	X	Bone marrow*	X	Periph.nerve*
X	Stomach*	X	Lymph nodes*	X	Spinal cord
X	Duodenum*	XX	Spleen*		(3 levels)*
X	Jejunum*	X	Thymus*	XX	Pituitary*
X	Ileum*			X	Eyes (optic n.)*
X	Cecum*				
X	Colon*		UROGENITAL		
X	Rectum*				GLANDULAR
XX	Liver*+	XX	Kidneys*+		
X	Gall bladder	X	Urinary bladder*	XX	Adrenal gland*
X	Pancreas*	XX	Testes*+	X	Lacrimal gland
		X	Epididymides	X	Mammary gland
		X	Prostate	XX	Thyroids*
	RESPIRATORY	XX	Ovaries*+	X	Parathyroids*
		X	Uterus*	X	Third eyelid/lacrimal gland
X	Trachea*	X	Cervix		
XX	Lung*				OTHER
	Nasopharynx				
X	Larynx			X	Bone* (femur, rib/cc jct, sternum)
X	Nasal structures			X	Joint (femorotibial)
				X	Ears (internal structures)
				X	Muscle*
				X	Skin* (untreated + protocol)
				X	All gross lesions and masses*

* Required for chronic toxicity studies.
+ Organ weight required in chronic studies.
+ Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Mortality - No animals died during the study.

2. Clinical Signs - No treatment-related differences in clinical signs were observed in dogs from any treatment group. Typical dermal irritations included alopecia, rough coat, scab, redness, rash, sores, and raised zone. Scaly dermis observed in 4/8 dogs treated at 200 mg/kg/day was concluded to be exfoliated stratum corneum that accumulated at the surface layer of the compound; no associated dermal pathology, histopathological findings or dose-response relationship were observed.

B. Body weight and weight gain

No differences in body weights or body weight gains of dogs in any treatment group were considered treatment-related. For male treatment groups, final mean body weight gains were 28-44% higher than the control gain. For female treatment groups, final mean body weight gains did not follow a trend; the 50 and 200 mg/kg/day group female treatment groups had final mean body weight gains 25 and 12% lower, respectively, whereas for the 100 mg/kg/day female treatment group had a final body weight gain 6% higher than the control final mean body weight gain.

Mean Body Weights (g)								
	Males				Females			
Dose (mg/kg)	0	50	100	200	0	50	100	200
Day 0	8981.3	9010.0	8960.0	8652.8	7628.5	7438.3	7085.8	7019.8
Day 28	10089.0	10518.0	10008.5	9933.5	8661.0	7855.5	7922.8	7808.8
Day 91	11047.0	11721.3	11678.3	11176.5	9477.0	8691.3	8731.0	8508.3
Day 182	11686.8	12500.8	12302.0	11568.3	9942.0	9189.0	9248.0	9125.8
Day 357	11719.8	12942.5	12665.3	12137.8	10302.0	9433.8	9906.8	9402.8

C. Food consumption

1. Food consumption - No differences in food consumption by dogs in the treatment and control groups were considered treatment-related.

D. Ophthalmoscopic examination

No treatment-related ophthalmoscopic abnormalities were observed in any treatment group.

E. Blood work

1. Hematology - No treatment-related differences in hematology parameters were observed in any treatment group. Sporadic differences in the treatment groups were not concentration- and/or time-dependent although statistically significant ($p \leq 0.05$), and were not observed in both sexes.

2. Clinical Chemistry - No treatment-related differences in hematology parameters were observed. Sporadic differences between the treatment groups were not concentration- and/or time-dependent although statistically significant ($p \leq 0.05$), and were not observed in both sexes. Increased ($p \leq 0.05$) levels of total protein in all male treatment groups and globulin in the 100 and 200 mg/kg/day male treatment groups on Day 361 were not observed at other sampling intervals, and were not concentration- and/or time-dependent or observed in the corresponding females.

F. Urinalysis

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

G. Clinical Neurology

No compound-related ophthalmoscopic lesions were observed in the treatment groups.

H. Electrocardiography and Blood Pressure

Electrocardiograms for all treated and control dogs were normal. No differences in systolic, diastolic, mean arterial pressures or heart rate were considered treatment-related in the treatment groups.

I. Sacrifice and Pathology

1. Organ weight - No statistically significant differences in absolute or relative organ weights were observed between the treatment groups. Although mean absolute and relative spleen weights for the 200 mg/kg/day group males and females were higher than the corresponding control values, the increased values were not statistically significant and were associated with the high variation within the treated groups; therefore, these increases were not considered treatment-related.

Mean Organ Weights for Selected Tissues (g)								
Organ	Males				Females			
Dose (mg/kg)	0	50	100	200	0	50	100	200
Liver								
Absolute	395.0	399.9	377.0	395.9	322.2	330.3	295.9	325.0
Relative	3.4	3.1	3.1	3.3	0.5	0.6	0.4	0.4
Brain								
Absolute	81.7	77.9	72.9	78.7	83.9	77.3	75.9	76.4
Relative	0.7	0.6	0.6	0.7	0.8	0.8	0.8	0.8
Spleen								
Absolute	73.7	94.9	73.1	109.0	76.3	59.7	71.9	81.8
S.D.	13.5	45.2	73.1	43.4	23.1	7.6	25.1	25.5
Relative	0.6	0.8	0.6	0.9	0.7	0.6	0.8	0.9
S.D.	0.1	0.4	0.1	0.4	0.2	0.1	0.3	0.3
Kidney								
Absolute	70.5	65.6	72.8	74.8	52.7	52.4	49.2	53.6
Relative	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.6

2. Gross pathology - No differences in gross postmortem findings were observed between the treated and control dogs. All findings occurred randomly and sporadically in all test groups.

3. Microscopic pathology

a) Non-neoplastic - No treatment-related microscopic postmortem differences were observed between the treated and control dogs. All abnormalities appeared to occur randomly and sporadically in all study groups.

b) Neoplastic - No neoplastic tissue was observed in dogs from any test group.

Microscopic Pathology for Selected Tissues								
Organ & Lesion	Males				Females			
Dose (mg/kg)	0	50	100	200	0	50	100	200
Brain No abnormality detected	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Eyes No abnormality detected Cataract	4/4	4/4	3/4 1/4	4/4	4/4	4/4	4/4	4/4
Kidneys No abnormality detected	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Liver No abnormalities detected Inflammation, chronic active Microgranuloma	4/4	4/4	2/4 1/4[a] 1/4[a]	4/4	4/4	4/4	3/4 1/4[a]	4/4
Lungs No abnormality detected Congested Edema Inflammation, chronic active Microgranuloma	3/4 1/4[a]	3/4 1/4[a]	4/4	4/4	4/4	4/4	2/4 2/4[b] 1/4[b]	2/4 1/4[a] 1/4[a]
Skin (untreated) No abnormality detected Granuloma Skin (treated) No abnormality detected Hyperkeratosis Inflammation, chronic	4/4 3/4 1/4[a]	4/4 3/4 1/4[a]	4/4 4/4	4/4 4/4	3/4 1/4[b] 4/4	4/4 4/4	4/4 4/4	4/4 4/4
Spinal cord No abnormality detected	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

[a]Minimal severity: average severity of animals with lesion graded 1 (minimal) to 5 (severe)
[b] Mild severity (2 according to rating above)

III. DISCUSSION

A. Investigator's Conclusions

The study authors concluded that KBR 3023 was not toxic to dogs treated with repeated dermal applications of 50, 100 or 200 mg/kg/day for 1 year. There were no treatment-related effects on clinical observations, body weights, food consumption, ophthalmological examinations, organ weights, gross and microscopic evaluations. Electrocardiograms, blood pressure measurements, and clinical neurology testing results were similar between rats in treatment and control groups. The NOAEL was concluded to be 200 mg/kg/day.

B. Reviewer's Discussion

The purpose of this study was to determine the potential toxicity of KBR 3023 from repeated dermal exposures to the Beagle dog over a 1-year period.

We agree with the study authors that KBR 3023 did not produce treatment-related dermal or toxic effects in dogs from any treatment group. Abnormalities observed in treated dogs were also observed in control dogs, were not concentration- and/or time dependent, were observed in one sex only, and/or were considered normal background variation. Therefore, the NOAEL is 200 mg/kg/day and a LOAEL was not established.

Based on a meeting (1/31/90) between Bayer (then Miles Inc.) and the U.S. EPA, the dose level of 200 mg/kg was selected as the high dose for long-term studies [MRID 44408717, page 17]. This decision was based, in part, on the observation that the test substance spread considerably beyond the dose site when applied at concentrations of 500 mg/kg or higher.

IV. Study Deficiencies

No scientific or guideline deficiencies were noted in this study.