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Registration Action Branch 2 (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Dermal
OPPTS Number: 870.4200

OPP Guideline Number: §83-2

DP BARCODE: D241232
P.C. CODE: 070705

SUBMISSION CODE: S534142
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): KBR 3023 Technical (97.4-98.5% a.i.)

SYNONYMS: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine

CITATION: Wahle, B.S., Christenson, W.R., (1996) Technical Grade KBR 3023: An Oncogenicity Dermal Toxicity Study in the Mouse. Bayer Corporation, Kansas City, MO., Laboratory Project Study ID# 93-221-TV, December 18, 1996. MRID 44408719. Unpublished.

SPONSOR: Bayer Corporation, Box 4913, Hawthorn Road, Kansas City, MO

EXECUTIVE SUMMARY:

In an oncogenicity study (MRID 44408719), KBR 3023 (technical, 97.4-98.5% a.i.) was administered dermally on the dorsal aspect of the trunk to 50 CD-1 mice/sex/dose at dose levels of 0, 50, 100, or 200 mg/kg/day on 5 consecutive days/week for 18 months. The administered dose volumes were based on the mean weekly body weight for each dose group. The exposure site was approximately 10% of the total body surface area.

Survival, body weights, food consumption and efficiency, and absolute and relative organ weights for both sexes at all doses were unaffected by treatment with KBR 3023. Clinical observations, hematological parameters, and gross and histopathological findings were also unaffected by treatment. At approximately 9 months, corneal lesions (ulceration, edema, vascularization, and opacity) were observed in all groups including the controls. It was concluded that the eye lesions were related to the use of the protective collars, which restricted normal grooming activities and decreased the ability of the animals to remove foreign matter from their eyes.

No increases in the incidences of any neoplasm were observed in dosed animals.

The chronic NOAEL was not observed. The chronic NOEL is 200 mg/kg/day.

Under the conditions of this study, there was no evidence of carcinogenic potential.

The mice could have tolerated higher dose levels. However, according to the study report and supporting minutes from meetings with Agency toxicologists, the highest dose level tested for all the chronic studies of all species was determined to be 200 mg/kg/day. This was verified by one of the Agency toxicologists. Two hundred mg/kg bodyweight/day was the highest dose level that could be tested without the chemical flowing off the back of the animal.

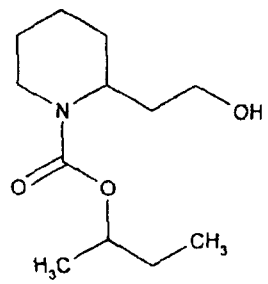
This study is classified as **acceptable (§83-2)** and satisfies the guideline requirements for a carcinogenicity dermal study in the mouse.

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
   Description: Clear liquid
   Lot/Batch #: 030693
   Purity: 97.4-98.5% a.i.
   Stability of compound: The compound is stable for 28 days when stored at room temperature and up to 18 months when stored frozen.
   CAS #: 119515-38-7
   Structure:



2. Vehicle and/or positive control: None

3. Test animals: Species: Mouse
Strain: CD-1 [ICR]/BR
Age and weight at study initiation: <8 weeks; 27.8-28.7g (males) and 24.4-25.4g (females)
Source: Charles River Breeders, Portage, MI
Housing: Suspended stainless steel wire-mesh cages 1 mouse/cage
Diet: Purina Mills Rodent Lab Chow 5001-4, ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 18-26 C
Humidity: 40-70%
Air changes: Not reported
Photoperiod: 12 hr dark/12 hr light
Acclimation period: 6 days

B. STUDY DESIGN:

1. In life dates - Start: 1/17/94 End: Not specified (80 weeks from start).

2. Animal assignment: Animals were assigned to treatment groups as indicated in Table 1 using a body weight dependent randomization process.

Table 1: Study design

Test Group	Dose to Animals M/F (mg/kg/day)	Number of Animals			
		Main Study 18 Months		Replacements [a]	
		Male	Female	Male	Female
Control	0	50	50	5	5
Low	50	50	50	5	5
Mid	100	50	50	5	5
High	200	50	50	5	5

a The additional mice/sex/dose were dosed for approximately the first month of treatment and were to serve as replacements for animals that died unexpectedly or developed non-compound related problems during that period of the study only. According to the information provided, one control male mouse died during weeks 1 through 14 and one control female mouse died during weeks 1 through 49.

3. Dose Selection: The rationale for dose selection was based on results from two

subchronic studies performed on mice and rats.

In the rat study, the animals were dosed dermally for 5 consecutive days/week with KBR 3023 at 0, 80, or 200, 500, or 1,000 mg/kg/day for 13 weeks. Acanthosis, hyperkeratosis, and/or hypertrophy of the sebaceous glands around the hair follicle of the dosing site were observed in all treated animals. After a 4 week recovery period, the skin changes were reversed. The changes in the treated skin were not dose related and were considered an adaptive response to chronic exposure to a liquid compound. Treatment related increases in liver and kidney weights as well as liver hypertrophy and hyaline degeneration of the kidney tubules were observed in the 500 and 1000 mg/kg/day animals. Based on the observed systemic toxicity, a 5 day/week dosing regime at 50, 100, or 200 mg/kg/day protocol was proposed for all further testing of KBR 3023.

In the mouse study, CD-1 mice (15/sex/dose) were dosed dermally for 5 consecutive days/week with KBR 3023 at 0, 80, or 200 mg/kg/day for 13 weeks. There were no treatment related effects on body weight, food consumption, clinical observations, mortality, hematology, organ weights, gross pathology, or histopathology parameters. The doses summarized in Table 1 above were selected for the 18-month mouse oncogenicity dermal study.

The protocol and dose selection for this study were discussed and approved by EPA prior to the start of the study. Copies of the memos reporting the meetings with EPA were submitted with the MRID (Appendix XII, pages 2790-2814).

4. Dosage Administration: Undiluted technical grade KBR 3023 was applied to a shaved area (4-5 cm^2) on the dorsal aspect of the trunk of each treated animal. The administered dose volumes were based on the mean weekly body weight for each dose group. Control animals were shaved, but not treated. The exposure site was approximately 10% of the total body surface area. All animals were fitted with Elizabethan collars (EJAY International, Glendora, CA) for the duration of the study.

5. Test Chemical Analysis: Undiluted technical grade KBR 3023 was stored frozen (-23 C). Approximately every two weeks, a fresh aliquot of the test compound "was made available for dosing". From the information provided, it was inferred that the aliquot was stored at room temperature. Prior to commencement of the study and again at 6, 14, and 20 months, the purity of the test chemical was assessed. In addition, stability was determined after 7, 14, 21, and 28 days of storage at ambient temperature (22 C).

Results:

Stability Analysis: It was stated that KBR 3023 is stable for up to 28 days of storage at room temperature. The data were not presented (Bayer Corporation unpublished report No. 107418 was cited).

Purity Analysis: The chemical purity of KBR 3023 was 97.4-98.5% throughout the study.

The information provided indicated that the test compound was stable for the duration of the study.

6. Statistics: Bartlett's test of equality or homogeneity of variance was applied to the hematology, organ weight data, and terminal body weight data. An analysis of variance (ANOVA) followed by Dunnett's test were applied to homogenous data. In the event of unequal variances, the data were subjected to a Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. The Fisher exact or chi-square and Fisher exact tests were applied to data indicating a trend.

C. METHODS:

1. Observations: Animals were inspected twice daily for signs of mortality/moribundity. Physical exams, including palpation for masses, were performed weekly. External surface areas, orifices, respiration, excretory products, behavior, and posture were also evaluated.

2. Body weight: Animals were weighed at initiation of dosing, at weekly intervals, and just prior to necropsy.

3. Food consumption: Food consumption for each animal was determined at weekly intervals.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not scheduled. However, an ocular examination was performed at 9 months because an eye lesion developed in all the dose groups, including the controls.

5. Blood Analyses: At 12 and 18 months, blood was collected (8-10 mice/group) for hematology and differential leukocyte analyses. The animals were not fasted prior to blood sampling. The following 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)
	Corrected leukocyte count (Cor WBC)	X	Mean corpusc. volume (MCV)
	Erythrocyte count (RBC)	X	Reticulocyte count
X	Platelet count	X	Erythrocyte morphology
X	Blood clotting measurements	X	Red cell distribution width
	(Thromboplastin time)	X	HGB distribution width
	(Clotting time)	X	Heinz bodies
	(Prothrombin time)		

6. Urinalysis: Data on urinalyses were not submitted. Based on Subdivision F guidelines, these data are not required for carcinogenicity studies.

7. Sacrifice and Pathology: All animals that died or were killed *in extremis* and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. Additionally, the (XX) organs were weighed. A complete complement of tissues was examined histologically for all animals.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (optic n.)
X	Jejunum	X	Thymus		GLANDULAR
X	Ileum		UROGENITAL	XX	Adrenal glands
X	Cecum	XX	Kidneys	X	Harderian gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
X	Liver	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate		OTHER
X	Pancreas	X	Seminal vesicles	X	Bone
	RESPIRATORY	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lungs	X	Vagina	X	All gross lesions and masses
	Nose	X	Cervix	X	Lacrimal/exorbital gland
	Pharynx	X	Preputial/clitoral gland	X	Zymbal's gland
X	Larynx				

II. RESULTS

A. Observations:

1. Toxicity - The general condition, behavior, and appearance of treated animals was considered unaffected by treatment. Rough coat and scabs were observed in all groups including the controls. These findings were related to the use of the protective collars that restricted normal grooming activities of the animals.

2. Mortality - No significant differences were observed in survival rates in either sex of the treated groups throughout the study when compared to the respective control group.

   At 52-54 weeks, the survival rate was excellent in males (98-100%) and in females (92-100%) among all treated groups.

   At 78 weeks, survival rates ranged from 86-94% in treated males and 76-86% in treated females, which exceeded the guideline requirement (not less than 25%) for this interval.

B. Body weight:

No treatment related differences were observed in body weight or body weight gains in either sex of the treated groups throughout the study when compared to the respective control group. There were statistically significant differences observed in the treated groups compared to the controls throughout the study. However, these differences were transient and not dose related, and therefore were not considered to be biologically significant.

Mean Body Weight and Monthly Body Weight Gain Data at Selected Time Periods (g)

Weeks	Males				Females			
Dose mg/kg/day	0	50	100	200	0	50	100	200
1	28.6	28.7	28.4	27.8	25.4	25.2	24.7	24.4*
5	32.0	32.3	32.1	31.6	28.4	28.1	27.9	27.8
Gain at 5	3.4	3.5	3.8	3.7	2.9	2.9	3.1	3.5
8	34.3	34.4	33.7	33.2*	30.1	29.7	29.3	29.4
Gain at 8	2.8	2.7	2.2*	2.5	2.5	2.2	2.1	2.3
13	35.1	35.6	35.2	35.3	30.7	30.8	30.7	30.6
Gain at 13	0.5	0.9	1.2*	1.7*	0.6	0.9	1.1	0.9
26	36.8	37.5	37.2	37.0	32.4	32.8	32.5	32.6

Mean Body Weight and Monthly Body Weight Gain Data at Selected Time Periods (g)								
Weeks	Males				Females			
Dose mg/kg/day	0	50	100	200	0	50	100	200
Gain at 26	0.5	0.5	0.5	0.3	0.7	0.4	0.6	0.4
52	37.2	37.6	36.9	36.4	33.3	33.7	33.0	33.0
Gain at 52	0.1	-0.2	-0.1	-0.3	-0.2	0.0	-0.1	-0.1
78	37.3	37.5	37.1	36.5	33.6	33.8	33.1	33.1
Gain at 78	0.6	0.8	-0.4*	-0.9*	0.8	0.5	-0.9*	-0.3

* $p \leq 0.05$

C. Food consumption:

1. Food consumption - There were no treatment-related differences in food consumption by the dosed groups compared to the concurrent controls throughout the study.

2. Food efficiency - There were no treatment-related differences in food conversion efficiencies (calculated as a grand mean for 78 weeks, g food/kg body weight/day) between the treated groups and the controls.

D. Ophthalmoscopic examination: No ophthalmoscopic examinations had been scheduled. However, at approximately 9 months an ulcerative-like condition of the cornea developed in all of the groups including the controls; this included corneal ulceration (8-24%), edema (2-16%), vascularization (8-24%), and corneal opacity (52-72%). It was concluded that the eye lesions were not related to exposure to KBR 3023, but were due to the protective Elizabethan collars worn by the animals resulting in restricting normal grooming activities and decreasing the ability of the animals to remove foreign matter from their eyes.

E. Blood analyses:

Hematology - No treatment related effects in hematology parameters were observed after dosing with KBR 3023 at 50, 100, or 200 mg/kg/day for up to 18 months. Several hematological parameters such as, white blood cell count, and hemoglobin in the low-dose females were significantly ($p \leq 0.05$) different from controls at the 12 month interval. However, these findings were transient and not dose related.

F. Sacrifice and Pathology:

1. Organ weights - There were no treatment related differences observed in absolute organ weights or organ weights relative to body weights. In the high-dose males, the absolute and relative spleen weights were increased (↑21-23%, not statistically significant [NS]) and in the high-dose females, the absolute and relative ovary weights were increased (↑155-157%, NS). However, there were no corroborative data to indicate that these findings were biologically significant.

2. Gross pathology - There were no treatment-related gross necropsy findings detected in the treated animals. Alopecia, edema and crusty zones of the head and neck, small thickened ears, and anasarca of the head musculature were detected in all groups, including the controls. It was concluded that these lesions were as a result of the use of the Elizabethan collars and the inability of the animals to perform grooming activities.

3. Microscopic pathology:

a) Non-neoplastic - There was a dose-dependent increase in the incidence of amyloid (rated minimal) of the treated skin of the low-dose (6/50, NS; vs 2/50 controls), the mid-dose (9/49, $p<0.05$), and the high-dose (13/50, $p\leq 0.05$) females. The increased incidence was restricted to the treated skin only and was judged to be not of toxicological concern. There were no other treatment related histopathological findings detected in the treated animals relative to controls.

b) Neoplastic - No significant increases in the incidences of any neoplasm were observed in the dosed animals. Alveolar/bronchiolar adenomas were detected in the high-dose animals (4-9/50 treated vs 2-5/50 controls). However, it was stated that the incidence of the finding fell within the laboratory control range of 1-14/50 mice.

Microscopic Examination of Selected Tissues (Incidences)								
Organ & Lesion	Males				Females			
Dose (mg/kg/day)	0	50	100	200	0	50	100	200
Liver								
Degeneration	-	-	1/50	-	-	-	-	-
Focus/area of cellular alteration	1/50	1/50	-	-	1/50	1/50	1/50	-
Hyperplasia, bile ducts	-	-	1/50	-	-	-	-	-
Hyperplasia, focal hepatocellular	2/50	-	-	2/50	-	-	-	-
Microgranuloma	21/50	28/50	27/50	21/50	33/50	30/50	27/50	33/50
Adenoma, hepatocellular	1/50	4/50	3/50	-	1/50	-	-	-
Carcinoma, hepatocellular	1/50	-	2/50	-	-	1/50	1/50	-
Hemangioma	-	-	1/50	-	-	-	-	1/50
Hemangiosarcoma	2/50	-	3/50	-	-	3/50	1/50	1/50
Malignant lymphoma	1/50	1/50	-	1/50	-	-	-	-
Kidney								
Glomerulonephritis	1/50	-	2/50	1/50	2/50	4/50	2/50	2/50
Necrosis	-	1/50	-	-	-	-	-	-
Hydronephrosis	3/50	4/50	3/50	-	1/50	-	1/50	1/50
Cyst	18/50	15/50	14/50	17/50	17/50	14/50	15/50	13/50
Lung								
Atelectasis	1/50	1/50	-	-	-	-	-	-
Hemorrhage	6/50	7/50	9/50	6/50	9/50	5/50	9/50	5/50
Fibrosis	1/50	-	-	1/50	-	-	-	1/50
Inflammation, chronic	4/50	5/50	7/50	11/50	16/50	10/50	13/50	16/50
Inflammation, lymphocytic	5/50	6/50	5/50	5/50	11/50	6/50	11/50	12/50
Macrophages, alveolar	2/50	1/50	1/50	1/50	3/50	4/50	-	3/50
Hyperplasia, alveolar/bronchiolar	1/50	6/50	3/50	6/50	-	5/50	4/50	3/50
Adenoma, alveolar/bronchiolar	5/50	6/50	3/50	9/50	2/50	1/50	2/50	4/50
Malignant lymphoma	-	2/50	-	1/50	-	1/50	-	-
Ovaries								
Cyst	-	-	-	-	34/50	36/50	29/50	38/50
Hyperplasia	-	-	-	-	-	1/50	1/50	1/50
Adenoma	-	-	-	-	-	1/50	-	-
Luteoma	-	-	-	-	-	-	1/50	-
Malignant lymphoma	-	-	-	-	-	-	-	1/50
Skin (treated)								
Acanthosis	4/48	4/50	9/49	4/50	2/50	3/50	3/49	3/50
Amyloid	1/48	8/50	9/49	7/50	2/50	6/50	9/49*	13/50*
Fibrosis	2/48	5/50	7/49	1/50	4/50	1/50	1/49	5/50
Ulcer	-	-	1/49	3/50	-	-	-	-

Microscopic Examination of Selected Tissues (Incidences)								
Organ & Lesion	Males				Females			
Dose (mg/kg/day)	0	50	100	200	0	50	100	200
Spleen								
Fibrosis	2/50	3/50	2/50	-	-	1/49	1/50	-
Hematopoiesis, extramedullary	8/50	6/50	4/50	6/50	9/50	10/49	5/50	9/50
Hyperplasia, lymphoid	-	1/50	-	-	-	-	1/50	2/50
Hemangiosarcoma	-	-	1/50	1/50	-	2/50	1/50	-
Leukemia, granulocytic	-	-	-	1/50	-	-	-	-
Malignant lymphoma	-	1/50	-	1/50	1/50	1/50	-	1/50

*Statistically significant from control ($p \leq 0.05$)

III. DISCUSSION

A. Investigators Conclusions - Body weight, food consumption, clinical signs, survival, hematology, organ weights, gross pathology, and histopathology parameters were unchanged following dermal exposure to KBR 3023 in mice. The chronic LOAEL was not observed. The chronic NOAEL is 200 mg/kg/day.

There was no evidence of a carcinogenic effect in mice after repeated dermal exposure to KBR 3023 for 18 months.

B. Reviewer's Discussion/Conclusions - Male and female mice were treated dermally with KBR 3023 at 0, 50, 100, or 200 mg/kg/day for 5 consecutive days/week for 18 months.

No significant differences were observed in survival rates in either sex of the treated groups throughout the study when compared to the respective control groups. At 78 weeks, survival rates ranged from 86-94% in treated males and 76-86% in treated females. Body weights, food consumption and efficiency, and absolute and relative organ weights for both sexes at all doses were unaffected by treatment with KBR 3023. Clinical observations, hematological parameters, and gross and histopathological findings were also unaffected by treatment.

At approximately 9 months, corneal ulceration (8-24%), corneal edema (2-16%), corneal vascularization (8-24%), and corneal opacity (52-72%) were observed in all groups including the controls. It was postulated that the condition was related to the use of the protective collars that restricted normal grooming activities and decreased the ability of the animals to remove foreign matter from their eyes and was not of toxicological concern.

No increases in the incidences of any neoplasm were observed in dosed animals.

The chronic LOAEL was not observed. The chronic NOAEL is 200 mg/kg/day. The mice could have tolerated higher dose levels. The results from the 90-day study in mice confirm this. However, the study report contains copies of minutes of several meetings with toxicologists from the Agency who confirmed that the highest dose level in all of the long term studies should be 200 mg/kg/day. This decision was based on weight of the evidence from both rat and mouse subchronic data and on problems with the test substance (a liquid) flowing beyond the test site at higher dose levels. Therefore, based on this decision, this study will be classified as acceptable.

In conclusion, no chronic toxicity was observed in mice dermally dosed with KBR 3023. However, the submitted study is classified as **acceptable (§83-2)** and satisfies the guideline requirements for a carcinogenicity study in mice because the Sponsor followed an EPA approved protocol.

C. Study deficiencies - Stability data for the test substance were not submitted.