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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Date: **6-22-99**

Subject: KBR 3023 - Review of Toxicity Data
DP Barcode:D241258, PC Code:070705

To: Kevin Sweeney, PM Team 3
Registration Division (7505C)

From: John E. Whalan, Toxicologist
Health Effects Division/Registration Action Branch 2 (7509C)

John Whalan 6-22-99

Thru: Richard Loranger, Branch Senior Scientist
Health Effects Division/Registration Action Branch 2 (7509C)

R. Loranger

Introduction

The Bayer Corporation has requested the registration of KBR 3023 All Family Insect Repellent Spray and KBR 3023 All Family Insect Cream.which both contain 20% 1-piperidinecarboxylic acid. Both formulations are applied directly to the skin of adults and children. A complete data base was submitted in support of this registration. The only data gap is OPPTS Harmonized Test Guideline 870.5300. It is recommended that the CHO/HGPRT/Mammalian Activation Gene Mutation Assay be repeated.

The registrant worked in consultation with the Health Effects Division in designing protocols and selecting doses. HED requested that animal studies be performed by the dermal route to reflect the use pattern and to allow for accurate route-specific risk assessments. Bayer developed methodology that would allow laboratory animals to be dermally dosed for extended periods of time with minimal physical trauma. Because of the tendency for high doses to spread beyond the treatment sites. HED approved maximum doses of 200 mg/kg/day for the subchronic neurotoxicity, chronic toxicity, rabbit developmental toxicity, and reproduction studies; and 400 mg/kg/day for the rat developmental toxicity study.

Findings

Copies of the Data Evaluation Records (DERs) are attached to this memorandum. A summary of the toxicity of KBR 3023 can be found on the following pages.

Acute Toxicity: The data base adequately characterizes KBR 3023 as having low oral, dermal, and inhalation toxicity. It is Toxicity Category IV for acute inhalation toxicity, and primary dermal irritation; Toxicity Category III for acute oral and dermal toxicity (dermal limit test), and primary eye irritation; and it is not a dermal sensitizer. Tables 1 and 2 present the toxicity profile for KBR 3023.

Table 1. Acute Toxicity Profile of KBR 3023 Technical

OPPTS No. / Study Type	MRID	Results	Toxicity Category
870.1100 Acute Oral - Rat (w/ 2% Cremophor EL)	44408705 44408706	LD_{50} = 4743 mg/kg ♂ LD_{50} = 2236 mg/kg ♂	III
870.1200 Acute Dermal - Rat	44408707	LD_{50} >2000 mg/kg (limit test) NOEL = 2000 mg/kg NOAEL >2000 mg/kg	III
870.1300 Acute Inhalation - Rat	44408709	LC_{50} >4.364 mg/L NOAEL = 4.364 mg/L (slight body weight anomalies)	IV
870.2400 Primary Eye Irritation - Rabbit	44408710	Moderate ocular irritant	III
870.2500 Primary Skin Irritation - Rabbit	44408712	Not a dermal irritant	IV
870.2600 Dermal Sensitization - Guinea Pig	44408713 44408714 44408752 44408758	Not a dermal sensitizer*	--
870.6200 Acute Dermal Neurotoxicity	44408715	NOEL = 2000 mg/kg (HDT) NOAEL > 2000 mg/kg	--

* There is no evidence of dermal sensitization in a Buehler test of KBR 3023; this study was classified unacceptable because there was no positive control group (MRID 44408713). Two of five trace components of KBR 3023 tested in a maximization test were significant dermal sensitizers (MRID 44408714). No dermal sensitization was observed in Buehler tests of the 20% alcoholic and cream formulations (MRID 44408752 and 44408758).

Table 2. Toxicity Profile of KBR 3023 Technical

OPPTS No./Study Type	MRID	Results
870.3250 Dermal Subchronic - Rat	44408716	NOAEL (systemic) = 200 mg/kg/day LOAEL (systemic) = 500 mg/kg/day (diffuse liver hypertrophy, necrotic liver cells, slight hyaline degeneration in the kidneys, increased incidence of foci of tubular regeneration, and chronic kidney inflammation) NOAEL (dermal irritation) <80 mg/kg/day LOAEL (dermal irritation) = 80 mg/kg/day (scabs, red foci, and exfoliation at the dosing site) Complete reversal was seen after a 4-week recovery period.
870.3700 Dermal Developmental Toxicity - Rat	44408725	NOAEL (maternal) = 400 mg/kg/day (HDT; slight increases in absolute and relative liver weights; 9% and 5%, respectively) LOAEL (maternal) >400 mg/kg/day NOAEL (developmental) = 400 mg/kg/day (delayed ossification attributed to maternal stress due to the dermal dosing regimen) LOAEL (developmental) > 400 mg/kg/day
870.3700 Dermal Developmental Toxicity - Rabbit	44408721	NOAEL (systemic) >200 mg/kg/day (HDT) NOAEL (developmental) >200 mg/kg/day NOAEL (dermal irritation) <50 mg/kg/day LOAEL (dermal irritation) = 50 mg/kg/day (LDT)
870.3800 Dermal Reproductive Toxicity - Rat	44408727	NOEL (systemic) = 200 mg/kg/day (HDT) NOAEL (systemic) >200 mg/kg/day NOEL (reproductive) = 200 mg/kg/day NOAEL (reproductive) >200 mg/kg/day
870.4100 Dermal Chronic Toxicity - Dog	44408718	NOAEL (systemic) = 200 mg/kg/day (HDT) NOAEL (dermal irritation) = 200 mg/kg/day
870.4200 Dermal Carcinogenicity - Mouse (18 months)	44408719	NOEL = 200 mg/kg/day (HDT) NOAEL >200 mg/kg/day **There is no evidence of carcinogenicity.**
870.4300 Dermal Chronic Toxicity/Carcinogenicity- Rat	44408728	NOAEL = 200 mg/kg/day (HDT; liver cystic degeneration with no corroborating liver weight or clinical pathology anomalies) **There is no evidence of carcinogenicity.**

OPPTS No./Study Type	MRID	Results
870.5265 *Salmonella typhimurium* Mammalian Activation Gene Mutation Assay	44408729	Negative
870.5300 CHO/HGPRT/Mammalian Activation Gene Mutation Assay	44408731	Negative results in this assay cannot be considered a reliable indication of an absence of genotoxicity because the toxic response was not consistent at the dose levels tested, and dose concentration analyses were not performed.
870.5375 *In Vitro* Mammalian Chromosome Aberrations in CHO Cells	44408732	Clastogenic in CHO cells in the absence of metabolic activation.
870.5375 *In Vitro* Mammalian Chromosome Aberrations in CHO Cells	44408733	Clastogenic with metabolic activation for CHO cells; equivocal in the absence of metabolic activation.
870.5395 *In Vivo* Mammalian Cytogenetics - Micronucleus Assay in Mice	44408734	Negative
870.5550 Unscheduled DNA Synthesis in Rat Hepatocytes	44408730	Negative
870.6200 Subchronic Dermal Neurotoxicity - Rats	44408717	NOEL = 200 mg/kg/day NOAEL >200 mg/kg/day
870.6300 Dermal Developmental Neurotoxicity - Rat	–	Not required because there is no evidence of increased neurologic or developmental susceptibility.

OPPTS No./Study Type	MRID	Results
870.7485 Dermal and I.V. Metabolism - Rat	44408735	**Dermal:** Animals dosed dermally for 7 consecutive days absorbed 58-63% of the low-dose (20 mg/kg, 0.5 mg/cm^2) and 40-55% of the high-dose (200 mg/kg, 5 mg/cm^2) which suggests absorption saturation. Elimination was mostly in the urine. Approximately 19-33% of the dose was not recovered, most likely due to evaporation during the 7 day exposure. Radioactivity remaining in the carcass after 7 days was 0.4-0.5% in males and 0.2-0.4% in females. Absorption was rapid with half-lives of 0.8-3.4 hr. Maximum plasma concentrations were reached within 6-8 hours of dosing and were higher in females than males. Elimination of radioactivity from plasma of dermally dosed animals was dose- and sex-dependent. **I.V.:** Within 48 hours of I.V. dosing, urinary elimination was 74-82% in males and 87-90% in females; fecal elimination was 12-17% in males and 5-6% in females; and 0.4-0.5% remained in the body for both sexes. **Metabolism:** Nineteen metabolites were identified in urine and feces of both I.V. and dermally dosed rats. The metabolite profile and relative distribution of metabolites is similar in urine and feces, and between dose groups and sexes. The metabolism of KBR 3023 in rats primarily involves oxidation of the 2-hydroxyethyl group to an acid to form metabolite M16, coupled with hydroxylation of the 1-methylpropyl group to form metabolites M8, M9, and M10. The other minor phase I metabolites result from hydroxylation of the piperidine ring (M1-M4 and M7). Minor phase II metabolites result from conjugation of glucuronic acid with parent (M14 and M15) or phase I metabolites (M5, M6, and M11-M13).

OPPTS No./Study Type	MRID	Results
870.7485 Dermal Metabolism - Human [OPPTS guidelines do not require human data.]	44408736	Biotransformation of [^{14}C]KBR 3023 in humans primarily involves conjugation of KBR 3023 with glucuronic acid through the 2-hydroxyethyl moiety to form Metabolites M14 and M15, which together accounted for 43.1% of the radioactivity in urine. Other major metabolites in urine included: M5 (17.4%) another glucuronic acid conjugate which is hydroxylated in the 1-methyl-propyl moiety; M16 (8.5%) in which the 2-hydroxyethyl group is oxidized to an acid; M8 (6.2%) in which the 2-hydroxyethyl group is oxidized and the 1-methyl-propyl group is hydroxylated; and M11-M13 (6.9%) which are isomers in which the 2-hydroxyethyl group has been oxidized to an acid and conjugated with glucuronic acid. The remaining metabolites, M1-M4, M6, M7, M9, and M10 each accounted for ≤3.1% of the radioactivity in urine.
870.7600 Dermal Absorption - Rat	44408737	Rats were dermally dosed at nominal levels of 8, 40, or 200 mg/kg (0.133, 0.67, or 3.33 mg/cm^2) for 8 hours. Absorption after 8 hours was 14.4, 9.8, and 6.9% in males, and 27.4, 12.6, and 8.9% in females, respectively. Urinary excretion was the principle route of elimination. After 3-7 days, 1.5-3.0% of radioactivity remained in the body.
870.7600 Dermal Absorption - Human [OPPTS guidelines do not require human data.]	44408738	Humans were dermally dosed on the forearm with either neat KBR 3023 or 15% KBR 3023 in ethanol at a nominal dose of 15 mg/person (0.625 mg/cm^2). Absorption after 8 hours was 1.66% for neat KBR 3023, and 3.77% for 15% KBR 3023 in ethanol. Within 24 hours, 94% of the radioactivity was excreted, mostly in the urine.

Dermal Absorption and Elimination: Dermal absorption studies in humans and rats showed a marked species difference in absorption of neat KBR 3023 over a period of 8 hours. At equivalent doses (0.625 mg/cm^2 in humans; 0.67 mg/cm^2 in rats), dermal absorption was 1.66% in humans, 9.8% in male rats, and 12.6% in female rats. Thus, dermal absorption is 6-8 times greater in rats than in humans. Elimination in humans is rapid and primarily in the urine with 94% of the radiolabel eliminated within 24 hours. Elimination is slower in rats with 1.5-3% of radiolabel remaining in the body after 3-7 days. Thus, dermal absorption in humans is minimal, and that which is absorbed is rapidly eliminated. [Note: The registrant initiated and submitted the results of human absorption and metabolism studies. OPPTS guidelines do not require human data..]

Cumulative Toxicity: There is no marked cumulative toxicity. 013529

Dermal Irritation: Dermal irritation was seen in some studies, but missing from most. Dose-related scabs, red foci, and exfoliation were seen at the dosing sites in the subchronic toxicity study in rats (dermal irritation NOAEL <80 mg/kg/day). Dermal lesions in the reproductive toxicity and chronic rat studies were not compound-related. In the rabbit developmental toxicity study, there was no systemic toxicity, but the dosing sites had slight erythema, edema, and cracked skin (the dermal irritation NOAEL is <50 mg/kg/day). The sensitivity of the rabbits might be explained by the inherent permeability of their skin.

Subchronic Toxicity: The only repeated dose study with frank toxicity was the dermal subchronic study in rats. Other than the dermal lesions previously cited, liver and kidney lesions were seen at 500 mg/kg/day, including diffuse liver hypertrophy, necrotic liver cells, slight hyaline degeneration in the kidneys, increased incidence of foci of tubular regeneration, and chronic kidney inflammation. There were no corroborating clinical pathology anomalies, however. The systemic NOAEL in this study is 200 mg/kg/day. After a 4-week recovery period, the dosed and control groups were similar for all parameters.

Neurotoxicity: Two neurotoxicity studies were performed. There was no evidence of systemic toxicity or neurotoxicity at the highest doses tested in the acute (2000 mg/kg) and subchronic (200 mg/kg/day) neurotoxicity studies.

Developmental and Reproductive Toxicity: Dermal developmental toxicity studies were performed in the rat and rabbit. The rat maternal and developmental NOAELs were 400 mg/kg/day, the highest dose tested. At this dose, the dams had slight increases in liver weight (9% absolute, 5% relative) which was considered an adaptive response. The fetuses at this dose had delayed ossification attributed to maternal stress due to the dermal dosing regimen. No toxicity was seen in the rabbit does or fetuses at the maximum dose of 200 mg/kg/day, although dermal lesions were observed even at the low dose of 50 mg/kg/day (previously described). No systemic or reproductive toxicity was found in a dermal 2-generation reproductive toxicity study.

Chronic/Carcinogenicity and Mutagenicity Studies: No toxicity was observed in the dermal chronic toxicity study in dogs and the dermal carcinogenicity study in mice at 200 mg/kg/day in both species. In the dermal chronic toxicity/carcinogenicity study in rats, liver cystic degeneration was observed at the NOAEL of 200 mg/kg/day (highest dose tested), though with no corroborating liver weight or clinical pathology anomalies. There was no evidence of carcinogenicity in the mouse or rat. Mutagenicity studies indicate that KBR 3023 has clastogenic potential *in vitro* which is not expressed in whole animals. On April 22, 1999, the HIARC determined that KBR 3023 is **not likely** to be a carcinogen by the dermal route. There is no evidence of endocrine disruption.

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

4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS Number: 870.1100

OPP Guideline Number: §81-1

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. No.:

TEST MATERIAL (PURITY): KBR 3023 (99.1% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Krotlinger F. (1990) KBR 3023: study for acute oral toxicity to rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T9033201. July 16, 1990. MRID 44408705. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44408705), groups of five young adult male SPF-bred Wistar rats were given single oral doses of KBR 3023 (99.1% purity) at 100, 500, 2,000, 2,500, 3,150, 4,500, or 5,000 mg/kg (limit dose). The test substance was administered in a 2% aqueous Cremophor EL emulsion. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing.

LD_{50} (Males) = 4,743 mg/kg (estimated); (Females) = Not tested
LOAEL (Males) = 500 mg/kg (apathy, reduced motility, staggering gait, lassitude, labored breathing, salivation, temporary grooming movements, and soft stools)
NOAEL (Males) = Not applicable
NOEL (Males) = 100 mg//kg

KBR 3023 is classified as **TOXICITY CATEGORY III** based on the calculated LD_{50} value in male animals; data for females were not generated.

Mortality occurred in 2/5 males dosed at 4500 mg/kg and in 3/5 males dosed at 5000 mg/kg on the day of administration. No signs of toxicity were observed in males dosed at 100 mg/kg. In the higher dose groups, effects were similar between decedent and surviving animals and

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DATA EVALUATION RECORD

KBR 3023 Technical

Study Type: Acute Oral Toxicity (§81-1)

Work Assignment No. 3-53A (MRID 44408705)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 6/29/98

Disclaimer

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9

included apathy, labored breathing, temporary sporadic shaking movement, decreased motility, temporary convulsions, increased salivation, atonia, staggered gait, and temporary digging and/or cleaning gestures. Piloerection, spasmodic states, prostration, dyspnea, and bloody salivation were also observed in animals from the ≥2,500-mg/kg groups. Effects subsided from all surviving animals by day 1. No significant treatment- or dose-related effect on the body weight of surviving animals was observed.

Necropsy of decedent animals revealed patchy/dark red (2/5), fluid-containing (2/5), and/or distended (2/5) lungs; slightly dark (4/5) or patchy (1/5) livers; slightly pale (3/5) or patchy (1/5) spleens; patchy kidneys (1/5); a distended stomach (1/5); and a reddened (1/5) or yellow mucous contents-containing (1/5) small intestines. Necropsy of animals sacrificed after 14 days revealed a small testicle in a single male from the 2,000-mg/kg group and several grey stipples on the lung of a single male from the 2,500-mg/kg group.

The LD_{50} for males is well above the Toxicity Category III cutoff dose of 500 mg/kg. Although no females were dosed in this study, it is unlikely that females could be sufficiently sensitive to lower the Toxicity Category. Thus, this study satisfies the guideline requirements for an acute oral study in the rat and is classified **Acceptable (§870.1100)**.

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 #: 19009/89
   Purity: 99.1%
   CAS #: 119515-38-7

2. Vehicle: The test material was formulated into an aqueous emulsion <1 hour prior to dosing using demineralized water amended (2%, v:v) with Cremophor EL.

3. Test animals: Species: Rat (males only)
   Strain: Bor:WISW(SPF-Cpb)
   Age: Young adult (approximately 8 weeks)
   Weight: 167-188 g males
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*

10

Housing: Five/cage
Environmental conditions:
Temperature: 22 ± 2°C
Humidity: Approximately 50%
Air changes: ≥10/Hour
Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: August 1989

2. Animal assignment and treatment: Young adult male SPF-bred Wistar rats were randomly assigned to the groups listed in Table 1. Females were not included in this study. The non-fasted animals were then given a single oral dose of KBR 3023 by gavage. The test material was administered in a 2% aqueous Cremophor EL emulsion at a constant dosing volume of 10 mL/kg. The rats were observed for signs of toxicity and/or mortality "several times" on the day of (following) administration, and at least once daily thereafter for up to 14 days. Body weights were recorded on days 0 (prior to dosing), 3, 7, and 14. At 14 days, the surviving animals were sacrificed, and all animals (upon death) were necropsied and examined for gross pathological changes.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males
100	0/5
500	0/5
2,000	0/5
2,500	0/5
3,150	0/5
4,500	2/5
5,000[a]	3/5

[a]Limit dose.

3. Statistics: The acute oral LD_{50} value (without indication of gradient and confidence range) was estimated using a computer method described by Pauluhn. [Pauluhn, J., Uber die computergestutzte Abschatzung der LD50/LC50. Bayer AG Bericht-Nr.: 11835 (1983)].

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. Mortality occurred in 5/10 animals tested at ≥4,500 mg/kg on the day of administration.

Oral LD_{50} Males = 4,743 mg/kg (estimated)

B. Clinical observations: Clinical effects observed in decedent males included piloerection (5/5), apathy (5/5), decreased motility (5/5), prostration (5/5), atonia (5/5), labored breathing (5/5), temporary convulsions (5/5), staggered gait (4/5), increased salivation (4/5), temporary sporadic shaking movement (4/5), temporary spasmodic state (4/5), dyspnea (2/5), temporary cleaning gestures (2/5), temporary digging gestures (2/5), and bloody salivation (2/5).

No signs of toxicity were observed in males dosed at 100 mg/kg. Effects observed in survivors generally from all higher dose groups were similar to those observed in decedent animals and included apathy (23/25), labored breathing (22/25), temporary sporadic shaking movement (13/25), decreased motility (12/25), temporary convulsions (11/25), increased salivation (11/25), staggered gait (10/25), atonia (10/25), temporary digging gestures (8/25), temporary cleaning gestures (7/25), and soft feces (1/25; 500-mg/kg). Piloerection (12/15) and temporary or continuous spasmodic state (7/15) were also observed in survivors from the ≥2,500-mg/kg groups; a temporary tremor was observed in a single survivor from the 3,150-mg/kg group; and prostration was observed in a single survivor from the 5,000-mg/kg group. Effects subsided from all surviving animals by day 1; no delayed-onset effects were observed.

C. Body Weight: Upon comparison of the 0-, 7-, and 14-day data, no significant treatment- or dose-related effect on the body weight of surviving animals was observed. All animals gained weight during the study, with overall (0-14 days) average increases of 23-32% for all dose groups.

D. Necropsy: Necropsy of decedent animals revealed patchy/dark red (2/5), fluid-containing (2/5), and/or distended (2/5) lungs; slightly dark (4/5) or patchy (1/5) livers; slightly pale (3/5) or patchy (1/5) spleens; patchy kidneys (1/5); a distended (with aqueous food paste) stomach (1/5); and a reddened (1/5) or yellow mucous contents-containing (1/5) small intestines. Necropsy of animals sacrificed after 14 days revealed a small testicle in a single male from the 2,000-mg/kg group and several grey stipples on the lung of a single male from the 2,500-mg/kg group.

E. Deficiencies: The acute oral toxicity of females was not investigated in this study, and no explanation was provided. Preliminary studies using single animals/sex should have been conducted to define doses, to ascertain whether one sex is more sensitive, and to minimize the number of animals used in the definitive study.

12

12

DATA EVALUATION RECORD 013529

KBR 3023 Technical

Study Type: Acute Oral Toxicity (§81-1)

Work Assignment No. 3-53B (MRID 44408706)

Prepared for

Health Effects Division
Office of Pesticide Programs
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1921 Jefferson Davis Highway
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Prepared by

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Signature: Mary L. Menetrez
Date: 6/29/98

Disclaimer

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

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

4-14-99 JW

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat (Fasted)
OPPTS Number: 870.1100 OPP Guideline Number: §81-1

DP BARCODE: D241232 SUBMISSION CODE: S534142
P.C. CODE: 070705 TOX. CHEM. NO.:
EPA REG. NO.: 3125-LRE

TEST MATERIAL (PURITY): KBR 3023 (99.4% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Krotlinger F. (1993) KBR 3023: investigations of acute oral toxicity in rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T3029598. June 18, 1993 (in-life dates, May-June 1988). MRID 44408706. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44408706), groups of five young adult male SPF-bred Wistar rats were given single oral doses of KBR 3023 (99.4% purity) at 100, 500, 1,600, 2,000, or 2,500 mg/kg following an 18-hour fasting period. The test substance was administered in a 2% aqueous Cremophor EL emulsion. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing.

LD_{50} (Males) = 2,236 mg/kg (estimated); (Females) = Not tested
LOAEL (Males) = 500 mg/kg (languor, occasional spasms, apathy, aggravated breathing, and staggering gait)
NOAEL (Males) = Not applicable
NOEL (Males) = 100 mg//kg

KBR 3023 is classified as **TOXICITY CATEGORY III** based on the calculated LD_{50} value in fasted male animals; data for females were not generated.

Mortality occurred in 1/5 animals dosed at 2000 mg/kg and in 4/5 animals dosed at 2500 mg kg on the day of administration. No signs of toxicity were observed in males dosed at 100 mg/kg.

Effects observed in animals from the remaining groups included languor, temporary spasms, apathy, reduced motility, labored breathing, and staggered gait. Additional effects observed in animals from the ≥1,000-mg/kg groups included piloerection, soft stool, salivation, convulsions, tremors, shaking, prostration, increased urination, spastic gait, and/or difficulty in breathing. Effects subsided from surviving animals by day 4. No significant treatment- or dose-related effect on the body weight of surviving animals was observed.

Necropsy of the four decedent animals from the 2,500-mg/kg group revealed distended lungs (4/4); pale kidneys (3/4) and/or spleen (2/4); urine-filled bladder (1/4); reddened glandular stomach (1/4); slight lobulation of the liver (1/4); and a patchy liver (1/4). Data were not provided for the single decedent from the 2,000-mg/kg group. Necropsy of animals sacrificed after 14 days revealed no treatment-related internal abnormalities.

The LD_{50} for males is well above the Toxicity Category III cutoff dose of 500 mg/kg. Although no females were dosed in this study, it is unlikely that females could be sufficiently sensitive to lower the Toxicity Category. Thus, this study satisfies the guideline requirements for an acute oral study in the rat and is classified **Acceptable (§870.1100)**.

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 yellow liquid
   Lot/Batch #: 19001/87
   Purity: 99.4%
   CAS #: 119515-38-7

2. Vehicle: The test material was formulated into an aqueous emulsion immediately prior to dosing using demineralized water amended (2%, v:v) with Cremophor EL.

3. Test animals: Species: Rat (males only)
   Strain: Bor:WISW(SPF-Cpb)
   Age: Young adult (approximately 9 weeks)
   Weight: 167-179 g males
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin R 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*

Housing: Five/cage
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: Approximately 50%
Air changes: ≥10/Hour
Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: May-June 1988

2. Animal assignment and treatment: Young adult male SPF-bred Wistar rats were randomly assigned to the groups listed in Table 1. Females were not included in this study. Following an 18-hour fasting period, animals were given a single oral dose of KBR 3023 by gavage. The test material was administered in a 2% aqueous Cremophor EL emulsion at a constant dosing volume of 10 mL/kg. Food was returned 2 hours following administration. The rats were observed for signs of toxicity and/or mortality "several times" on the day of (following) administration, and at least once daily thereafter for up to 14 days. Body weights were recorded on days 0 (prior to dosing), 3, 7, and 14. At 14 days, the surviving animals were sacrificed, and all animals (upon death) were necropsied and examined for gross pathological changes.

TABLE 1. Doses, mortality/animals treated

Dose, mg/kg	Males
100	0/5
500	0/5
1,000	0/5
1,600	0/5
2,000	1/5
2,500	4/5

3. Statistics: The acute oral LD_{50} value (without indication of gradient and confidence range) was estimated by computer using a modified maximum-likelihood method [Bliss, C., J. Pharm. Pharmacol., 11:192-216 (1938); Pauluhn, J., On the computer-assisted estimation of LD50/LC50 (unpublished). Bayer AG Report No. 11835 (1983); Rosiello, A., et al., J. Tox. and Environ. Health, 3:797-809 (1977)].

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 1. Mortality occurred in 5/10 animals tested at ≥2,000 mg/kg on the day of administration.

Oral LD_{50} Males = 2,236 mg/kg (estimated)

B. Clinical observations: No signs of toxicity were observed in males dosed at 100 mg/kg. Effects observed in animals from the remaining groups included languor, temporary spasms, apathy, reduced motility, labored breathing, and staggered gait. Additional effects observed in animals from the ≥1,000-mg/kg groups included piloerection, soft stool, salivation, convulsions, tremors, and shaking. Prostration, increased urination, spastic gait, and/or difficulty in breathing were also observed in animals from the ≥1,600-mg/kg groups. Effects subsided from surviving animals by day 4. Individual data were not provided; therefore, the number of animals exhibiting effects/group could not be determined, and effects observed in decedent versus surviving animals could not be assessed.

C. Body Weight: Upon comparison of the 0-, 7-, and 14-day data, no significant treatment- or dose-related effect on the body weight of surviving animals was observed. All animals gained weight during the study, with overall (0-14 days) average increases of 40-58% for all dose groups.

D. Necropsy: Necropsy of the four decedent animals from the 2,500-mg/kg group revealed distended lungs (4/4); pale kidneys (3/4) and/or spleen (2/4); urine-filled bladder (1/4); reddened glandular stomach (1/4); slight lobulation of the liver (1/4); and a patchy liver (1/4). Data were not provided for the single decedent from the 2,000-mg/kg group. Necropsy of animals sacrificed after 14 days revealed no treatment-related internal abnormalities.

E. Deficiencies: The acute oral toxicity of females was not investigated in this study, and no explanation was provided. Preliminary studies using single animals/sex should have been conducted to define doses, to ascertain whether one sex is more sensitive, and to minimize the number of animals used in the definitive study.

DATA EVALUATION RECORD 013529

KBR 3023 Technical

Study Type: Acute Dermal Toxicity (§81-2)

Work Assignment No. 3-53E (MRID 44408707)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS Number: 870.1200

OPP Guideline Number: §81-2

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (98.5% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Sheets, L., and S. Phillips (1991) Acute dermal toxicity study with technical grade KBR 3023 in rats. Mobay Corporation, Stilwell, KS. Laboratory Study Number 90-022-GD. August 27, 1991. MRID 44408707. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44408707), five young adult Sprague Dawley rats/sex were dermally exposed to KBR 3023 (98.5% purity) at 2,000 mg/kg (limit dose) for 24 hours; the test substance was applied as received to a 16-cm^2 area (approximately 5% of the total body surface area). Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing.

LD_{50} >2,000 mg/kg (males and females)
NOAEL >2000 mg/kg (males and females)
NOEL = 2000 mg/kg (males and females)

KBR 3023 is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} values in both sexes.

All animals survived the 14-day observation period. Red nasal staining, urine staining, perianal staining, and red lacrimal staining were observed in up to 7/10 animals between days 0 and 2; the study author reported that these effects were a result of the wrapping procedure and not attributed to the test material. Dermal irritation, if present, was not reported. One female lost weight between 0 and 7 days, and exhibited an overall increase slightly less that the remaining four

females (4.9% compared to 6.7-15%). No treatment-related effect on body weight was observed in males, and necropsy after 14 days revealed no treatment-related gross abnormalities.

The study guidelines require that 10% of the total body surface be treated, but only 5% was treated in this study. This deficiency will be waived because a dermal dose of 5000 mg/kg in another study (MRID No. 44408708) was nontoxic to males. Considering both studies, this study satisfies the guideline requirement for an acute dermal study in the rat, and is classified **Acceptable (§870.1200)**.

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 viscous liquid
   Lot/Batch #: 19010/89
   Purity: 98.5%
   CAS #: 119515-38-7

2. Vehicle: None employed

3. Test animals: Species: Rat, albino
   Strain: Sprague Dawley
   Age: Young adult (8-10 weeks)
   Weight: 248-274 g males; 193-211 g females
   Source: Sasco, Inc., Houston, TX
   Acclimation period: ≥6 Days
   Diet: Purina Rodent Laboratory Chow (#5001), *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: One animal per cage
   Environmental conditions:
   Temperature: 18-26 °C
   Humidity: 40-70%
   Air changes: Not specified
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: May 29 - June 12, 1990

20

2. Animal assignment and treatment: Fur from the dorsal and lateral areas of five young adult Sprague Dawley rats/sex was clipped 1 day prior to dermal administration of KBR 3023 at 2,000 mg/kg (limit dose). The test substance was evenly applied as received to a 16-cm^2 (approximately 5% of the total body surface area) piece of gauze, which was then affixed to the clipped area and secured with tape and Vetrap adhesive bandage. Following a 24-hour exposure period, the coverings were removed and the test sites were gently wiped with water-moistened paper towels. The rats were observed for signs of toxicity and/or mortality at least once daily during the 14-day study. Body weights were recorded at 0 (prior to dosing), 7, and 14 days. At 14 days, surviving animals were sacrificed, necropsied, and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: All animals survived the 14-day observation period.

Dermal LD_{50} Males >2,000 mg/kg (males and females)

B. Clinical observations: Red nasal staining (7/10), urine staining (5/10), perianal staining (2/10), and red lacrimal staining (1/10) were observed between days 0 and 2. The study author reported that these effects were a result of the wrapping procedure and not attributed to the test material. In addition, salivation, red nasal staining, and clear lacrimal staining were observed in a single female on day 14. Again, these effects were not considered treatment-related. Dermal irritation, if present, was not reported.

C. Body Weight: No treatment-related effect on body weight was observed in males, who exhibited an overall (0-14 days) average increase of 25%. One female lost weight between 0 and 7 days, and exhibited an overall increase of 4.9%, compared to 6.7 to 15% increases for the remaining four females.

D. Necropsy: Necropsy of animals sacrificed after 14 days revealed no treatment-related gross abnormalities.

E. Deficiencies: The study guidelines require that 10% of the total body surface be treated, but only 5% was treated in this study. This deficiency will be waived because a dermal dose of 5000 mg/kg in another study (MRID No. 44408708) was nontoxic to males. Treatment-site dermal irritation should have been reported. This deficiency, however, has no significant effect on the results of the study and is therefore considered minor.

DATA EVALUATION RECORD

613529

KBR 3023 Technical

Study Type: Acute Dermal Toxicity (§81-2)

Work Assignment No. 3-53F (MRID 44408708)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Primary Reviewer:
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Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/29/98

Disclaimer

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22

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

4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat
OPPTS Number: 870.1200

OPP Guideline Number: §81-2

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (99.4% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Kroetlinger, F. (1993) KBR 3023: investigations of acute dermal toxicity in rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T7029600. June 18, 1993 (in-life dates, May 1988). MRID 44408708. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44408708), five young adult male SPF-bred Wistar rats were dermally exposed to KBR 3023 (99.4% purity) at 5,000 mg/kg (2.5X limit dose) for 24 hours; the test substance was mixed with cellulose powder and applied to >10% of the total body surface area. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postdosing.

LD_{50} (Males) >5,000 mg/kg (females not tested)
NOAEL (Males) >5000 mg/kg
NOEL (Males) = 5000 mg/kg

KBR 3023 is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} value in male animals; data for females were not generated.

All males survived and appeared normal during the 14-day observation period. No significant effect on body weight was observed, and necropsy after 14 days revealed no treatment-related gross abnormalities.

Since the acute dermal toxicity of females was not investigated, this study does not satisfy the guideline requirement for an acute dermal study in the rat and is classified **unacceptable (§81-2)**. This study may be upgraded to acceptable status if an adequate explanation as to why female animals were not included is provided.

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: Yellowish clear liquid
   Lot/Batch #: 19001/87
   Purity: 99.4%
   CAS #: 119515-38-7

2. Vehicle: To eliminate runoff, the test material was amended with cellulose powder, 300 mg/g, just prior (<1 hour) to application.

3. Test animals: Species: Rat (males only)
   Strain: Bor:WISW(SPF-Cpb)
   Age: Young adult (approximately 9 weeks)
   Weight: 213-227 g males
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin R 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Five/cage
   Environmental conditions:
   Temperature: 22 ± 3°C
   Humidity: Approximately 50%
   Air changes: ≥10/Hour
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: May 1988

2. Animal assignment and treatment: Fur from the back areas of five young adult male SPF-bred Wistar Dawley rats was clipped 1 day prior to dermal administration of

KBR 3023 at 5,000 mg/kg (2.5X limit dose). The test substance (a liquid) was mixed with cellulose powder on a 6.5- x 6.5-cm (42.25 cm^2; >10% of the total body surface area) piece of aluminum foil, and the foil was applied to the clipped skin (method of securing patch was not described). Following a 24-hour exposure period, the patches were removed and the test sites were gently cleaned with soap and water. The rats were observed for signs of toxicity, dermal irritation, and/or mortality "several times" on the day of (following) administration, and at least once daily thereafter for up to 14 days. Body weights were recorded at 0 (prior to dosing), 3, 7, and 14 days. At 14 days, surviving animals were sacrificed, necropsied, and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: All animals survived the 14-day observation period.

Dermal LD_{50} >5,000 mg/kg (males; females not tested)

B. Clinical observations: No treatment-related signs of toxicity nor dermal irritation were observed.

C. Body Weight: Upon comparison of the 0-, 7-, and 14-day data, no significant treatment-related effect body weight was observed. All males gained weight during the study, with an overall (0-14 days) average increase of 26%.

D. Necropsy: Necropsy of animals sacrificed after 14 days revealed no treatment-related gross abnormalities.

E. Deficiencies: The acute dermal toxicity of females was not investigated in this study, and no explanation was provided. As a result, this study does not fulfill guideline requirements and is deemed unacceptable. This study may be upgraded to acceptable status if an adequate explanation as to why female animals were not included is provided.

DATA EVALUATION RECORD

013529

KBR 3023 Technical

Study Type: Acute Inhalation Toxicity (§81-3)

Work Assignment No. 3-53I (MRID 44408709)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

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Primary Reviewer:
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Date: 6-27-98

Project Manager:
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Date: 6-29-98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat
OPPTS Number: 870.1300 OPP Guideline Number: §81-3

DP BARCODE: D241232 SUBMISSION CODE: S534142
P.C. CODE: 070705 TOX. CHEM. NO.:
EPA REG. NO.: 3125-LRE

TEST MATERIAL (PURITY): KBR 3023 (99.1% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Pauluhn, J. (1990) KBR 3023: study for acute inhalation toxicity in the rat to OECD Guideline No. 403. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T1033186. June 13, 1990. MRID 44408709. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 44408709), groups of five young adult SPF-bred Wistar rats/sex were dynamically exposed by nose-only inhalation to KBR 3023 (99.1% purity) aerosol at analytical concentrations of 2.153 or 4.364 mg/L for 4 hours. The test substance was formulated as a 75% concentration with polyethylene glycol 400:ethanol (1:1, v:v). Historical vehicle control data using ten animals/sex were provided. Animals were observed for clinical signs of toxicity and mortality for up to 14 days postexposure.

Inhalation LC_{50} >4.364 mg/L (males and females)
NOAEL = 4.364 mg/L (slight body weight anomalies)

KBR 3023 is classified as **TOXICITY CATEGORY IV** based on the observed LC_{50} values in both sexes.

All animals survived and appeared normal during the 14-day observation period. Overall, a slight dose-dependent relationship on body weight gain was apparent in both sexes. Males from the control, 2.153-, and 4.364-mg/L groups gained averages of 38, 29, and 26%, respectively, and

females from the three groups gained averages of 6.4, 5.9, and 4.4%, respectively. Necropsy after 14 days revealed no treatment-related gross abnormalities.

This study is classified **Acceptable (§870.1300)** and satisfies the guideline requirement for an acute inhalation study in the rat.

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 #: 19009/89
   Purity: 99.1%
   Density: 1.134 g/mL (temperature not specified)
   CAS #: 119515-38-7

2. Vehicle and/or positive control: The test substance was diluted to 75% in polyethylene glycol 400:ethanol (1:1, v:v) prior to use.

3. Test animals: Species: Rat
   Strain: Bor:WISW(SPF-Cpb)
   Age: Young adult (2-3 months)
   Weight: 171-193 g males; 171-191 g females (test and control groups)
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin R 1324 Diet for Rats and Mice, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Five/cage
   Environmental conditions:
   Temperature: 22 ± 2°C
   Humidity: Approximately 50%
   Air changes: Approximately 10/Hour
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: September 7-22, 1989

28

2. Exposure conditions: An cylindrical dynamic 20-L nose-only exposure chamber (Rhema Laboratories, Germany) constructed of PVC and equipped with radial animal ports was used in the study. During exposure, plexiglass tubes, each containing a single animal, were attached to the chamber with the nose portion of the cone protruding into the chamber. The exposure tubes (Rhema Labs) were designed so that the rat's tail was outside the tube, whereby preventing hyperthermic effects.

To generate test atmosphere, 75% KBR 3023 in polyethylene glycol 400:ethanol was delivered via a Braun infusion pump into a binary jet atomizer (Rhema Labs) using conditioned (water, dust and oil were removed) compressed air. The resultant aerosol was drawn through a 2-L baffling chamber prior to entering the top of the exposure chamber. During all exposures, the airflow through the chamber was maintained at approximately 10 L/min (equivalent to 27 chamber turnovers/hour), and the time required for 95% equilibration was about 6 minutes.

The nominal and analytical chamber concentrations are presented in Table 1. The actual test atmosphere concentration was determined analytically following equilibration, at mid-test, and towards the end of each exposure period. Samples (10 L) were collected from the animals' breathing zone using glass tubes filled with cotton wool. The cotton wool was extracted with carbon tetrachloride, and aliquots of the extracts were analyzed for KBR 3023 by gas chromatography in conjunction with flame-ionization detection (GC/FID). Results were adjusted for purity (99.1%).

TABLE 1. Exposure conditions

Nominal Conc. (mg/L)	Mean Analyt. Conc. (mg/L)	Mean Vehicle Conc. (ppm)	MMAD (µm)	GSD	Particles ≤5 µm
Control	0.0	20	N/A	N/A	N/A
15.0	2.153[a]	20	1.62	1.44	100
37.5	4.364[a]	50	1.80	1.49	100

[a]>Limit concentration

Particle size was apparently determined only once during each test exposure using a TSI-APS 3300 aerodynamic particle sizer equipped with a laser velocimeter. Each sample was collected for 30 seconds from the animals' breathing zone. The mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and percentages of particles ≤5 µm were calculated; values are reported in Table 1.

29

The temperature, recorded at 10-minute intervals, averaged 22 °C for both test exposures. The relative humidity, also recorded at 10-minute intervals, averaged 15.0% for the 2.153-mg/L exposure and 27.9% for the 4.364-mg/L exposure. Although not monitored, the turnover rate (27/hour) ensured an oxygen content of ≥19%.

3. Animal assignment and treatment: Animals were assigned to the test groups noted in Table 2. Rats were exposed to either 75% KBR 3023 or vehicle via nose-only inhalation for 4 hours. Animals were observed for signs of toxicity and/or mortality several times following exposure and twice daily thereafter for up to 14 days. Body weights were recorded at 0 (prior to exposure), 3, 7, and 14 days. At 14 days, all survivors were sacrificed, necropsied, and examined for gross pathological changes.

Table 2. Mortality/animals treated

Mean Analytical Conc. (mg/L)	Male	Female	Combined
Control[a]	0/10	0/10	0/20
2.153	0/5	0/5	0/10
4.364	0/5	0/5	0/10

[a]Vehicle control studies are conducted every 3 months.

4. Statistics: Body weight gains were evaluated by means of an analysis of variance. The homogeneity of the variances between the groups was checked with the Box test, and if a difference was noted, a pairwise *post hoc* comparison of the groups was made using Games and Howell's modification of the Tukey-Kramer significance test.

II. RESULTS AND DISCUSSION:

A. Mortality: Mortality data are presented in Table 2. All animals survived the 4-hour exposure and 14-day observation periods.

Inhalation LC_{50} >4.364 mg/L (males and females)

B. Clinical observations: No signs of toxicity were observed in test or control groups.

C. Body Weight: The mean percent change in body weights during the study is presented in Table 3. Upon comparison with the control group, statistically-significant ($p = 0.05$)

decreases were observed in males and females exposed at 2.153-mg/L between 3-7 days, and in males exposed at 4.364-mg/L between 0-3 and 3-7 days. Overall (0-14 days), a slight dose-dependent relationship was apparent in both sexes, whereas males from the control, 2.153-, and 4.364-mg/L groups gained averages of 38, 29, and 26%, respectively, and females from the three groups gained averages of 6.4, 5.9, and 4.4%, respectively.

Table 3. Body Weight Changes (%)

Mean Analytical Conc. (mg/L)	Interval (Days)			
	0-3	3-7	7-14	0-14
Male				
Control	6.4	15	13	38
2.153	5.1	4.0*	17	29
4.364	0.96*	8.2*	15	26
Female				
Control	0.83	1.4	4.0	6.4
2.153	2.0	-1.9*	5.8	5.9
4.364	-0.97	0.33	5.1	4.4

[a] Percentages derived from data on pages 39-51 in the study report.
* Significantly different from control group at 0.05 level.

D. Necropsy: Necropsy after 14 days revealed no treatment-related gross abnormalities.

E. Deficiencies: Particle size determination should have been conducted hourly during each exposure period. In this study, only one sampling/exposure was collected. It was apparent from the limited data that the aerosol was within the ideal MMAD range of 1-4 μm. Therefore, this deficiency is considered minor.

Although the humidity levels during both test exposures (averages of 15 and 28%) were less than the 40-60% limits set forth in Subdivision F guidelines, this deficiency should have no significant effect on the results of the study and is considered minor.

DATA EVALUATION RECORD 013529

KBR 3023 Technical

Study Type: Primary Eye Irritation (§81-4)

Work Assignment No. 3-53J (MRID 44408710)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

Pesticide Health Effects Group
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2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
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Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
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Signature: Mary L. Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

John Whalan 4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit
OPPTS Number: 870.2400

OPP Guideline Number: §81-4

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (96.7% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Wakefield, A. (1997) Primary eye irritation study in rabbits with technical grade KBR 3023. Corning Hazleton, Inc., Vienna, VA. Laboratory Study Number 18202-0-820. April 24, 1997. MRID 44408710. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44408710), 0.1 mL of KBR 3023 (96.7% purity) was instilled into the conjunctival sac of the right eye of three adult New Zealand White rabbits/sex. The animals were observed for up to 14 days following instillation, and eye irritation was scored by the Draize scale.

Although ocular irritation was evident in all treated eyes within 1 hour of instillation, it was most severe between 24 and 48 hours, with average irritation scores of 27.5 and 21.5, respectively. Between 24 and 48 hours, irritation included scattered or diffuse to easily discernible corneal opacity affecting up to 100% of the total area in 6/6 eyes, iridial effects in 3/6 eyes, slight to moderate conjunctival redness in 6/6 eyes, and very slight to slight conjunctival chemosis in 6/6 eyes. Positive conjunctival effects subsided by 96 hours, and corneal and iridial changes subsided by 7 days.

In this study, **KBR 3023 is a moderate ocular irritant** and is classified as **TOXICITY CATEGORY III** for primary eye irritation based on the corneal, iridial, and positive

conjunctival effects which subsided from all treated eyes by day 7.

This study is classified **Acceptable (§870.2400)** and satisfies the guideline requirement for a primary eye irritation study in the rabbit.

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 viscous liquid
   Lot/Batch #: 030693
   Purity: 96.7%
   pH: 8.61
   CAS #: 119515-38-7

2. Vehicle and/or positive control: None employed

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age: Adult
   Weight: 2.35-2.39 kg males; 2.27-3.33 kg females
   Source: Hazleton Research Products, Inc., Denver, PA
   Acclimation period: 7 Days
   Diet: PMI Feeds Certified Rabbit High Fiber Diet (#5325), *ad libitum* following a gradual increase
   Water: Tap water, *ad libitum*
   Housing: One animal per cage
   Environmental conditions:
   Temperature: 16-21 °C
   Humidity: 50 ± 10%
   Air changes: Not specified
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: December 17-31, 1996

2. Animal assignment and treatment: A 0.1 mL aliquot of KBR 3023 was instilled into

the lower conjunctival sac of the right eye of three adult New Zealand White rabbits/sex. The upper and lower lids were held together for 1 second before releasing to prevent loss of the material. The left eye of each animal served as an untreated control. The animals were observed for ocular irritation at 1, 24, 48, 72, and 96 hours and 7 and 14 days following instillation. Eye irritation was scored by the Draize Ocular Grading System. At the 24-hour and subsequent (when necessary) observation intervals, fluorescein dye was used to confirm the presence or absence of corneal ulceration. Following each fluorescein exam, the treated eyes were flushed with a 0.9% saline solution. The animals were also observed for signs of ill health at least once daily during the 14-day study.

II. RESULTS AND DISCUSSION:

A. Clinical observations: The incidence of positive ocular irritation is presented in Table 1. No abnormal clinical effects were reported.

Irritation was evident in all treated eyes within 1 hour of instillation and included iridial changes (scores of 1) in 2/6 eyes, and moderate conjunctival redness (scores of 2), slight to moderate conjunctival chemosis (scores of 2-3), and moderate to severe conjunctival discharge (scores of 2-3) in 6/6 eyes. Irritation was most severe between 24 and 48 hours, with average primary irritation scores of 27.5 and 21.5, respectively.

Between 24 and 48 hours, irritation included scattered or diffuse to easily discernible corneal opacity (scores of 1-2) affecting up to 100% of the total area (scores of 1-4) in 6/6 eyes, iridial effects (scores of 1) in 3/6 eyes, slight to moderate conjunctival redness (scores of 1-2) in 6/6 eyes, and very slight to slight conjunctival chemosis (scores of 1-2) in 6/6 eyes. Positive conjunctival effects subsided by 96 hours.

At 7 days, slight conjunctival redness (scores of 1) persisted in 4/6 eyes, though all corneal and iridial effects had subsided. No ocular irritation was evident after 14 days. In this study, KBR 3023 is a moderate ocular irritant.

TABLE 1. Incidence of Positive Ocular Effects

Observations	Number "Positive"/Number Tested						
	Hours					Days	
	1	24	48	72	96	7	14
Corneal Opacity	---	6/6	6/6	6/6	6/6	---	---
Iritis	2/6	3/6	3/6	1/6	1/6	---	---
Conjunctivae							
Redness	6/6	6/6	5/6	6/6	---	---	---
Chemosis	6/6	2/6	---	---	---	---	---
Discharge[a]	6/6	---	---	---	---	---	---

--- No positive observations.

[a] Discharge is not included in evaluating a positive reaction; however, scores of ≥2 are reported.

B. Deficiencies: There were no deficiencies that affected the results of the study.

DATA EVALUATION RECORD

013529

KBR 3023 Technical

Study Type: Primary Eye and Dermal Irritation (§81-4 and -5)

Work Assignment No. 3-53P (MRID 44408711)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C) John Whalan 4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit
OPPTS Number: 870.2400

OPP Guideline Number: §81-4

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (99.4% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Martins, T. (1994) KBR 3023: Study for irritant/corrosive potential for skin and eye (rabbit) to OECD Guideline No's. 404 and 405. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T5029680. May 5, 1994. MRID 44408711. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44408711), 0.1 mL of KBR 3023 (99.4% purity) was instilled into the conjunctival sac of one eye of three adult male New Zealand White rabbits. The animals were observed for up to 7 days following instillation, and eye irritation was scored by a modified Draize scale.

Effects observed in all treated eyes included scattered corneal opacity affecting up to 100% of the total area between 1 and 72 hours, slight to moderate conjunctival redness between 1 and 72 hours, very slight to slight conjunctival chemosis between 1 and 48 hours, and slight to moderate conjunctival discharge at 1 hour. No changes in the iris or aqueous humor were observed during the study. Positive conjunctival effects subsided by 48 hours, and corneal and all conjunctival irritation subsided by 7 days.

In this study, **KBR 3023 is a moderate ocular irritant**, and is classified as **TOXICITY CATEGORY III** for primary eye irritation based on the corneal and/or positive conjunctival effects which persisted in 3/3 eyes at 72 hours.

Since only three animals were used, this study does not satisfy the guideline requirement for a primary eye irritation study in the rabbit and is classified **Unacceptable (§870.2400)**. However, acceptable data have been submitted (MRID 44408710) and additional data are not required.

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 liquid
   Lot/Batch #: 19001/87
   Purity: 99.4%
   pH: Not determined
   CAS #: 119515-38-7

2. Vehicle and/or positive control: None employed

3. Test animals: Species: Rabbit, albino
   Strain: HC:NZW
   Age: Adult
   Weight: 2.9-3.1 kg (all male)
   Source: Interfauna UK Ltd.
   Acclimation period: ≥14 Days
   Diet: Ssniff K 4 Standard Diet, approximately 100-120 g/animal/day
   Water: Tap water, *ad libitum* between 7 AM and 7 PM
   Housing: One animal per cage
   Environmental conditions:
   Temperature: 20 ± 2 °C
   Humidity: Approximately 50%
   Air changes: 10/Hour
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: May 3-10, 1998

2. Animal assignment and treatment: A 0.1 mL aliquot of KBR 3023 was instilled into the lower conjunctival sac of one eye of three adult male New Zealand White rabbits.

The upper and lower lids were held together for 1 second before releasing to prevent loss of the material, and the contralateral eye of each animal served as an untreated control. The animals were observed for ocular irritation at 1, 24, 48, and 72 hours and 7 days following instillation. Eye irritation was scored by a modified Draize Ocular Grading System[1]. At the 24- and subsequent (when necessary) observation intervals, fluorescein dye was used to confirm the presence or absence of corneal ulceration. Following the 24-hour exam, the treated eyes were flushed with physiological saline solution.

II. RESULTS AND DISCUSSION:

A. Clinical observations: The incidence of positive ocular irritation is presented in Table 1. Irritation was evident in all treated eyes within 1 hour of instillation and included scattered corneal opacity (scores of 1) affecting up to 100% of the total area (scores of 4) in 2/3 eyes, and slight to moderate conjunctival redness (scores of 1-2), very slight to slight conjunctival chemosis (scores of 1-2), and slight to moderate conjunctival discharge (scores of 1-2) in 3/3 eyes. Fluorescein examination at 24 hours revealed scattered corneal opacity (scores of 1) affecting up to 100% of the total area (scores of 1 or 4) in 3/3 eyes; opacity persisted in all treated eyes through 72 hours, and was no longer evident at 7 days. No changes in the iris or aqueous humor were observed during the study. Positive conjunctival effects subsided by 48 hours, and all conjunctival irritation subsided by 7 days. In this study, KBR 3023 is a moderate ocular irritant. This is in disagreement with the study author, who reported that KBR 3023 is only slightly irritating.

B. Deficiencies: Subdivision F guidelines specify that six adult animals should be used for primary eye irritation studies. Since only three animals were used, this study does not satisfy guideline requirements and is deemed unacceptable. However, acceptable data have been submitted (MRID 44408710) and additional data are not required.

Aside from ocular irritation, the animals should have been observed at least once daily for general signs of health. This deficiency, however, has no significant effect on the results of the study and is considered minor.

[1]Fluorescein retention and aqueous humor (Tyndall effect) evaluations were included in the scale, and conjunctival discharge was described as "tear flow".

40

TABLE 1.Incidence of Positive Ocular Effects

Observations	Number "Positive"/Number Tested				
	Hours				Days
	1	24	48	72	7
Corneal Opacity	2/3	3/3	3/3	3/3	---
Iritis	---	---	---	---	---
Conjunctivae					
Redness	2/3	1/3	---	---	---
Chemosis	2/3	---	---	---	---
Discharge[a]	2/3	---	---	---	---

--- No positive observations.

[a] Discharge is not included in evaluating a positive reaction; however, scores of ≥2 are reported.

41

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

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS Number: 870.2500 OPP Guideline Number: §81-5

DP BARCODE: D241232 SUBMISSION CODE: S534142
P.C. CODE: 070705 TOX. CHEM. NO.:
EPA REG. NO.: 3125-LRE

TEST MATERIAL (PURITY): KBR 3023 (99.4% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Martins, T. (1994) KBR 3023: Study for irritant/corrosive potential for skin and eye (rabbit) to OECD Guideline No's. 404 and 405. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T5029680. May 5, 1994. MRID 44408711. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44408711), three adult female New Zealand White rabbits were dermally exposed to 0.5 mL of KBR 3023 (99.4% purity) for 4 hours. The test substance was applied as received to a single intact 6-cm^2 site per animal. Animals were observed for dermal irritation for up to 7 day following patch removal, and irritation was scored by the Draize scale.

Very slight erythema was observed at 1/3 sites 24 through 48 hours following patch removal. No other dermal irritation was observed during the 7-day study. The calculated Primary Irritation Index was 0.8. In this study, **KBR 3023 is not a significant dermal irritant**.

Since only three animals were used, this study does not satisfy the guideline requirement for a primary dermal irritation study in the rabbit and is classified **Unacceptable (§870.2500)**. Acceptable data have been submitted (MRID 44408712) so additional data are not required.

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 liquid
   Lot/Batch #: 19001/87
   Purity: 99.4%
   pH: Not determined
   CAS #: 119515-38-7

2. Vehicle: None employed

3. Test animals: Species: Rabbit, albino
   Strain: HC:NZW
   Age: Adult
   Weight: 3.0-3.1 kg (all female)
   Source: Interfauna UK Ltd.
   Acclimation period: ≥14 Days
   Diet: Ssniff K 4 Standard Diet, approximately 100-120 g/animal/day
   Water: Tap water, *ad libitum* between 7 AM and 7 PM
   Housing: One animal per cage
   Environmental conditions:
   Temperature: 20 ± 2 °C
   Humidity: Approximately 50%
   Air changes: 10/Hour
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: December 16-19, 1996

2. Animal assignment and treatment: Fur from the contralateral flank areas (approximately 36 cm^2 per side) of three adult New Zealand White rabbits/sex was clipped 1 day prior to dermal administration with 0.5 mL of KBR 3023. The test material was applied as received to a Hansamed Hypoallergen dressing (approximately 6-cm^2), which was placed on the clipped area. To serve as a control site, an additional dressing was moistened with "the binding agent" (not further characterized) and applied to the opposite flank area. The patches were secured with

elastic adhesive tape for a 4-hour exposure period. Following patch removal, the sites were gently washed with water. The rabbits were observed for dermal irritation 1, 24, 48, and 72 hours and 7 days following patch removal. Erythema and edema were scored separately using the Draize scale.

II. RESULTS AND DISCUSSION:

A. Clinical observations: Very slight erythema (score of 1) was observed at 1/3 sites 24 through 48 hours following patch removal. No dermal irritation was observed at the remaining two sites. The calculated Primary Irritation Index was 0.8. In this study, KBR 3023 is not a significant dermal irritant.

B. Deficiencies: Subdivision F guidelines specify that six adult animals should be used for primary dermal irritation studies. Since only three animals were used, this study does not satisfy guideline requirements and is deemed unacceptable. Acceptable data have been submitted (MRID 44408712) so additional data are not required.

Aside from dermal irritation, the animals should have been observed at least once daily for general signs of health. This deficiency, however, has no significant effect on the results of the study and is considered minor.

013529

DATA EVALUATION RECORD

KBR 3023 Technical

Study Type: Primary Dermal Irritation (§81-5)

Work Assignment No. 3-53M (MRID 44408712)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John E. Whalan
Registration Action Branch 1 (7509C)

4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit
OPPTS Number: 870.2500

OPP Guideline Number: §81-5

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (96.7% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Wakefield, A. (1997) Primary dermal irritation study in rabbits with technical grade KBR 3023. Corning Hazleton, Inc., Vienna, VA. Laboratory Study Number 18202-0-830. April 28, 1997 (Amendment date). MRID 44408712. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44408712), three adult New Zealand White rabbits/sex were dermally exposed to 0.5 mL of KBR 3023 (96.7% purity) for 4 hours. The test substance was applied as received to a single intact 6.25-cm^2 site per animal. Animals were observed for dermal irritation for up to 72 hours following patch removal, and irritation was scored by the Draize scale.

No dermal irritation was observed during the 72-hour observation period. The calculated Primary Irritation Index was 0.0. In this study, **KBR 3023 is not a dermal irritant**, and is classified as **TOXICITY CATEGORY IV**.

This study is classified **Acceptable (§870.2500)** and satisfies guideline requirements for a primary dermal irritation study in the rabbit.

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 viscous liquid
   Lot/Batch #: 030693
   Purity: 96.7%
   pH: 8.93
   CAS #: 119515-38-7

2. Vehicle: None employed

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age: Adult
   Weight: 2.137-2.268 g (combined sexes)
   Source: Hazleton Research products Inc., Denver, PA
   Acclimation period: 7 Days
   Diet: PMI Feeds Certified Rabbit High Fiber Diet (#5325), *ad libitum* following a gradual increase
   Water: Tap water, *ad libitum*
   Housing: One animal per cage
   Environmental conditions:
   Temperature: 16-21 °C
   Humidity: 50 ± 10%
   Air changes: Not specified
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: December 16-19, 1996

2. Animal assignment and treatment: Fur from the back and flank areas of three adult New Zealand White rabbits/sex was clipped 1 day prior to dermal administration with 0.5 mL of KBR 3023. The test material was applied as received to a single approximately 6.25-cm^2 site per animal beneath a gauze patch. The patches were secured with paper tape, and the trunk of each animal was loosely wrapped with Saran Wrap and secured with Elastoplast tape. Following a 4-hour exposure period, the coverings were removed and the sites were gently washed with tap water and paper towels. The rabbits were observed for dermal irritation 30 minutes and 24, 48, and 72 hours following patch removal. An untreated clipped area was used for comparison.

48

Erythema and edema were scored separately using the Draize scale. The animals were also observed once daily for signs of clinical toxicity or ill health.

II. RESULTS AND DISCUSSION:

A. Clinical observations: No dermal irritation was observed during the 72-hour observation period, and no abnormal clinical effects were reported. The calculated Primary Irritation Index was 0.0. In this study, KBR 3023 is not a dermal irritant.

B. Deficiencies: There were no deficiencies that affected the results of the study.

DATA EVALUATION RECORD 013529

KBR 3023 Technical

Study Type: Dermal Sensitization (§81-6)

Work Assignment No. 3-53Q (MRID 44408713)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

John Whalan 4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea pig
OPPTS Number: 870.2600

OPP Guideline Number: §81-6

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): KBR 3023 (99.4% purity)

SYNONYMS: 2-(2-Hydroxyethyl)-1-piperidinecarboxylic acid; 1-(1-methylpropoxycarbonyl)-2-(2-hydroxyethyl)piperidine; 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidine-carboxylate

CITATION: Dreist, M. (1991) KBR 3023: study for skin-sensitizing effect on guinea pigs (Buehler test). Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T7040355. August 30, 1991. MRID 44408713. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44408713) conducted with KBR 3023 (99.4% purity), 24 young adult male SPF-bred Bor:DHPW guinea pigs were tested using methods based on those derived by Buehler.

Very slight erythema was observed at 1/12 test sites and 1/12 control sites 48 and 72 hours following the single challenge exposure with KBR 3023 at 100%. Based on the results of this study, **KBR 3023 is not a dermal sensitizer**; however positive control data were not provided to validate the test methods and species employed.

Since positive control data were not provided, this study does not satisfy the guideline requirement for a dermal sensitization study in the guinea pig and is classified **Unacceptable (§870.2600)**. This study may be upgraded to acceptable status if additional positive control data obtained within 6 months of the definitive study using the Buehler method are provided.

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 #: 19009/89
   Purity: 99.4%
   CAS #: 119515-38-7

2. Vehicle and positive control: No test substance vehicle was employed, and positive control data were not generated or provided.

3. Test animals: Species: Guinea pig
   Strain: Bor:DHPW
   Age: Young adult (5-8 weeks)
   Weight: 319-401 g males (all groups)
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin 3020 Diet for Guinea Pigs, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Four/cage
   Environmental conditions:
   Temperature: 22 ± 2°C
   Humidity: Approximately 50%
   Air changes: Approximately 10/Hour
   Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: July 9 - August 9, 1991

2. Animal assignment and treatment: The study was conducted using methods derived by Buehler [Buehler, E., Arch. Dermatol., 91:171-175 (1965)]. Preliminary experiments were conducted with five male animals and 0.5 mL of either KBR 3023 as received (100%) or as 12, 25, or 50% dilutions in an unspecified solvent. Based on the results, the test material was administered at 100% for both phases of the definitive experiment.

   For the induction phase, fur on the back and flank areas of twelve young adult male animals was clipped one day prior to dermal administration with 0.5 mL of KBR 3023 (100%). A hypoallergenic dressing soaked in the test material was placed on the left flank of each animal and secured with Fermoflex adhesive tape. An additional 12

males were treated in the same manner using dry dressings. Following a 6-hour exposure period, the coverings were removed, and any excess test substance was removed from the skin by gently rinsing with physiological saline solution. Application of the test materials was repeated once weekly at 7-day intervals for 2 consecutive weeks (three total applications).

A single challenge exposure was conducted 2 weeks following the final induction treatment in the manner previously described. All test and control animals were challenged on the left flank with 0.5 mL of KBR 3023 (100%). An untreated dressing was also applied to the right flank and served as a control site. Following the 6-hour exposure, the treatment skin of each animal was depilated with Pilca cream.

The guinea pigs were observed for dermal irritation 24 hours following each induction exposure and 24, 48, and 72 hours following the challenge treatment. Skin reactions were scored according to the following scale:

0 - No reaction
0.5 - Slight redness in places
1 - Slight redness
2 - Moderate redness
3 - Severe redness

The animals were also observed for clinical effects at least once daily during the 31-day study, and body weights were recorded at the start and termination of the study.

II. RESULTS AND DISCUSSION:

A. Induction reactions and duration: No dermal irritation was observed 24 hours following each induction application.

B. Challenge reactions and duration: Forty-eight and 72 hours following the single challenge exposure with KBR 3023 at 100%, very slight erythema (score of 0.5) was observed at 1/12 sites. Similarly, 48 and 72 hours following challenge to control animals, very slight erythema was observed at 1/12 sites. Based on the results of this study, KBR 3023 is not a dermal sensitizer.

No signs of toxicity were observed during the study. In addition, no treatment-related effect on body weight was observed between animals from the test and control groups, with overall (0-31 days) average increases of 66 and 61%, respectively.

C. Positive control: No data were generated.

53

D. Deficiencies: Since positive control data were not generated or provided, the methods and species employed in this study were not validated. As a result, this study does not fulfill guideline requirements and is classified unacceptable. This study may be upgraded to acceptable status if positive control data obtained within 6 months of the definitive study using the Buehler method are provided.

013529

DATA EVALUATION RECORD

Five Trace Components of KBR 3023
(KBR 4230; KBR 4223; 2-(2-hydroxyethyl)piperidine;
chloroformic acid secondary butyl ester; and KBA 1542)

Study Type: Dermal Sensitization (§81-6)

Work Assignment No. 3-53T (MRID 44408714)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Crystal Mall II
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
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Primary Reviewer:
Christie E. Padova, B.S.

Signature: Christie E. Padova
Date: 6-27-98

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

55

KBR 3023

Dermal Sensitization Study (870.2600)

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

John Whalan 4-14-99

EPA Work Assignment Manager: S. Diwan, PhD
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea pig
OPPTS Number: 870.2600

OPP Guideline Number: §81-6

DP BARCODE: D241232
P.C. CODE: 070705
EPA REG. NO.: 3125-LRE

SUBMISSION CODE: S534142
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Five trace components of KBR 3023: KBR 4230 (purity not specified); KBR 4223 (purity not specified); 2-(2-hydroxyethyl)piperidine (purity not specified); chloroformic acid secondary butyl ester (purity not specified); and KBA 1542 (>99.5% purity)

SYNONYMS: None specified

CITATION: Diesing, L. (1991) Components of KBR 3023: KBR 4230, KBR 4223, 2-(2-hydroxyethyl)piperidine, chloroformic acid secondary butyl ester: study for skin-sensitizing effect on guinea pigs. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Numbers T3037030, T4037031, T8037224, T9037225, and T0037226. February 25, 1991. MRID 44408714. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, 17745 S. Metcalf, Stilwell, KS.

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44408714) conducted with KBR 4230 (purity not specified); KBR 4223 (purity not specified); 2-(2-hydroxyethyl)piperidine (purity not specified); chloroformic acid secondary butyl ester (purity not specified); and KBA 1542 (>99.5% purity), ten male guinea pigs/compound were tested using the maximization test method of Magnusson and Kligman. Two additional sets of five male animals/compound were used for adjuvant, vehicle, and challenge treatment-only controls. Positive control data were not provided.

No dermal irritation was observed upon challenge with 10 or 20% KBR 4230, 10% KBR 4223, or 6% 2-(2-hydroxyethyl)piperidine to previously-induced animals. In contrast, animals previously-induced with chloroformic acid secondary butyl ester exhibited 100% positive sensitization upon challenge with 25% chloroformic acid secondary butyl ester or 5% KBA 1542 (its anhydride). Similarly, animals previously-induced with KBA 1542 exhibited 100% positive sensitization upon challenge with 5% KBA 1542, 30% sensitization with 0.5% KBA 1542, and

70% sensitization with 5% chloroformic acid secondary butyl ester. Challenge with KBR 3023 did not elicit a positive response in either set of sensitized animals.

In this study, the three trace components **KBR 4230, KBR 4223, and 2-(2-hydroxyethyl)piperidine are not dermal sensitizers**; however the two trace components **chloroformic acid secondary butyl ester and KBA 1542 are significant dermal sensitizers**. Since positive sensitization was observed for two of the five compounds tested, data from this study are self-validating and positive control data are not required.

This study is classified as **Acceptable (§870.2600)** and satisfies the guideline requirement for a dermal sensitization study in the guinea pig.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Materials:

KBR 4230
Description: Clear liquid
Batch #: 19001/90
Purity: Not specified
CAS #: Not specified

KBR 4223
Description: Clear liquid
Batch #: 19001/90
Purity: Not specified
CAS #: Not specified

2-(2-Hydroxyethyl)piperidine (technical)
Description: White, fluffy and yellow solid lumps
Batch #: Not specified
Purity: Not specified
CAS #: Not specified

Chloroformic acid secondary butyl ester (technical)
Description: Clear liquid
Batch #: Not specified
Purity: Not specified
CAS #: Not specified

57

KBA 1542
Description: Clear liquid
Batch #: Not specified
Purity: >99.5%
CAS #: Not specified

KBR 3023[1]
Description: Clear colorless viscous liquid
Batch #: 19009/89
Purity: 99.1%
CAS #: 119515-38-7

2. Vehicle and positive control: Sterile physiological saline solution (w:v) amended with Cremophor EL (2% by volume) was used as the vehicle for all treatments. A positive control material was not used.

3. Test animals: Species: Guinea pig, SPF-bred
   Strain: Bor:DHPW
   Age: 4-9 Weeks
   Weight: 248-440 g (all male)
   Source: Winkelmann, Borchen, Kreis Paderborn
   Acclimation period: ≥7 Days
   Diet: Altromin 3020 Diet for Guinea Pigs, *ad libitum*
   Water: Tap water, *ad libitum*
   Housing: Five/cage
   Environmental conditions:
      Temperature: 22 ± 2°C
      Humidity: Approximately 50%
      Air changes: Approximately 10/Hour
      Photoperiod: 12-Hour light/dark cycle

B. STUDY DESIGN and METHODS:

1. In-life dates: May 8 - August 17, 1990 (definitive study)

2. Animal assignment and treatment: The concentrations of 2-(2-hydroxyethyl)piperidine and chloroformic acid secondary butyl ester used in the definitive experiment were selected based on the results of preliminary experiments [refer to pages 20-21 of the study]. Due to the limited initial quantity of the

[1] Used for challenge exposure in animals induced with chloroformic acid secondary butyl ester or KBA 1542.

remaining compounds, preliminary experiments were not conducted.

The study was conducted according to the guinea pig maximization test method of Magnusson and Kligman [J. Invest. Dermatol. 52:268-276 (1969); Contact Dermatitis 6:46-50 (1980)]. The concentrations used in the five definitive experiments are listed in Table 1. For each test substance, a test group consisting of ten male animals and two control groups consisting of five males were randomly assigned.

Table 1. Treatment Regimen.

Test Compound	Induction Concentration		Challenge Concentration		
	First	Second	First	Second	Third
KBR 4230	1%	10%	10%	20%	---
KBR 4223	1%	5%	10%	---	---
HEP[1]	2.5%	12%	6%	---	---
CSB[2]	2.5%	100%	25%	0.5%	5% KBA 1542
				100% KBR 3023	
KBA 1542[3]	1%	10%	5%	0.5%	5% CSB
				100% KBR 3023	

[1]2-(2-Hydroxyethyl)piperidine
[2]Chloroformic acid secondary butyl ester
[3]KBA 1542 is the anhydride of CSB

For the first induction treatment, fur on the backs and flanks of each animal was clipped 1 day prior to the following six 0.1-mL injections: two injections of Freund's complete adjuvant:physiological saline solution (1:1, v:v); two injections of test compound:Cremophor-amended saline; and two injections of test compound:adjuvant/Cremophor-amended saline (1:1, v:v). Injections were made in a row to the left and right of the backbone beginning at the cranial end. Animals in the control group received similar injections minus the test compound.

The second induction treatment was conducted 1 week following the intradermal treatment. A 0.5-mL aliquot of each test compound (refer to concentrations in Table 1) was applied to a 2 x 4-cm hypoallergenic dressing. The dressing was affixed

59

between or on the injection sites, covered with aluminum foil, and secured for a 48-hour exposure period with Fermoflex adhesive tape. Animals in the control group were treated with 0.5 mL of vehicle. On the day prior to the KBR 4230 and KBR 4223 treatments, the sites were pre-irritated with 10% sodium lauryl sulfate. At the end of the exposure period, residual test substance was removed with sterile physiological saline solution.

The first challenge treatment was conducted 2 weeks following the topical induction treatment. Additional challenge exposures were carried out 1 week later in each case. A 0.5-mL aliquot of the each test compound (refer to concentrations in Table 1) was applied to a hypoallergenic dressing. The dressing was affixed to the left flank of test and control group animals, and secured for a 24-hour exposure period with Fermoflex adhesive tape. For comparison, a dressing containing 0.5 mL of solvent was affixed to the right flank.

At the end of each exposure period, residual test substance was removed with sterile physiological saline solution. The first control group was used for the first challenge, and the second for the second challenge. If a third challenge was conducted, the relevant second control group was included in the treatment. Due to the skin irritation still remaining after the first challenge with chloroformic acid secondary butyl ester or KBA 1542, the right flank was used for the second and third challenges, and control dressings were not applied.

Dermal reactions were scored according to the following scale 48 and 72 hours following the start of each challenge exposure (24 and 48 hours following patch removal).

0 = No reaction
1 = Slight redness in places
2 = Confluent moderate redness
3 = Severe redness and/or swelling

Additional skin findings were descriptively recorded. The animals were observed at least once daily for clinical symptoms, and body weights were recorded prior to the start of the study, and on days 24, 31, and (if applicable) 38.

II. RESULTS AND DISCUSSION:

A. Induction reactions and duration: No data were collected.

B. Challenge reactions and duration:

KBR 4230: No dermal irritation was observed 48 to 72 hours following challenge first with 10% KBR 4230 then with 20% KBR 4230 to either previously-induced or control animals. Based on the results of this study, KBR 4230 is not a dermal sensitizer.

KBR 4223: No dermal irritation was observed 48 to 72 hours following challenge with 10% KBR 4223 to either previously-induced or control animals. Based on the results of this study, KBR 4223 is not a dermal sensitizer.

2-(2-Hydroxyethyl)piperidine: No dermal irritation was observed 48 to 72 hours following challenge with 6% 2-(2-hydroxyethyl)piperidine to either previously-induced or control animals. Based on the results of this study, 2-(2-hydroxyethyl)piperidine is not a dermal sensitizer.

Chloroformic acid secondary butyl ester: Forty-eight hours following the first challenge treatment with 25% chloroformic acid secondary butyl ester to previously-induced animals, slight to moderate erythema (scores of 1-2) and eschar formation were observed at 10/10 sites. After 72 hours, slight erythema persisted at 9/10 sites, eschar persisted at 10/10 sites, hardened skin was observed at 8/10 sites, and edema was observed at 6/10 sites. In comparison, no dermal irritation was observed 48 to 72 hours following challenge to control animals.

No dermal irritation was observed 48 to 72 hours following the second challenge procedure with either 0.5% chloroformic acid secondary butyl ester or 100% KBR 3023 to previously-induced or control animals.

Forty-eight to 72 hours following the third challenge treatment with 5% KBA 1542 to previously-induced animals, slight erythema (scores of 1) was observed at up to 9/10 sites and eschar formation was observed at 10/10 sites. In comparison, slight erythema was observed at 2/5 and 0/5 control sites 48 and 72 hours following application, respectively. Based on the results of this study, chloroformic acid secondary butyl ester is a significant dermal sensitizer, with 100% positive response following challenge with 25% chloroformic acid secondary butyl ester and 5% KBA 1542 (its anhydride). Challenge with KBR 3023 did not elicit a positive response.

KBA 1542: Forty-eight hours following the first challenge treatment with 5% KBA 1542 to previously-induced animals, slight to moderate erythema (scores of 1-2) and eschar formation were observed at 10/10 sites. After 72 hours, slight to moderate erythema persisted at 9/10 sites, eschar persisted at 10/10 sites, hardened skin was observed at 10/10 sites, and edema was observed at 8/10 sites. In comparison, no dermal irritation was observed 48 to 72 hours following challenge to control animals.

Forty-eight and 72 hours following the second challenge treatment with 0.5% KBA 1542 to previously-induced animals, slight erythema (scores of 1) was observed at 3/10 sites. Desquamation was also observed at 1/10 sites after 72 hours. In comparison, no dermal irritation was observed 48 to 72 hours following challenge to control animals. No dermal irritation was observed 48 to 72 hours following the second challenge procedure with 100% KBR 3023 to previously-induced or control animals.

Forty-eight to 72 hours following the third challenge treatment with 5% chloroformic acid secondary butyl ester to previously-induced animals, slight erythema (scores of 1) was observed at up to 5/10 sites and eschar formation was observed at 7/10 sites. In comparison, slight erythema was observed at 1/5 and 0/5 control sites 48 and 72 hours following application, respectively. Based on the results of this study, KBA 1542 is a significant dermal sensitizer, with 100% positive response following challenge with 5% KBA 1542, 30% positive with 0.5% KBA 1542, and 70% positive with 5% chloroformic acid secondary butyl ester. Challenge with KBR 3023 did not elicit a positive response.

No treatment-related signs of toxicity were observed during the study, and no effects on body weight were observed upon comparison of the test and control groups for the KBR 4230, KBR 4223, 2-(2-hydroxyethyl)piperidine, or KBA 1542 studies. A significant reduction in body weight gain was observed in test animals from the chloroformic acid secondary butyl ester study, with overall (0-38 days) increases of 87% for controls and 43% for test animals.

C. Positive control: No data were generated or provided.

D. Deficiencies: Positive control data were not generated or provided; however, since positive sensitization was observed for two of the five compounds tested, data from this study are self-validating.

62

DATA EVALUATION RECORD 013529

KBR 3023

Study Type: 81-8; Acute Dermal Neurotoxicity Screening Study in the Rat

Work Assignment No. 3-53U (MRID 44408715)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Reto Engler, Ph.D.

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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

63

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.**

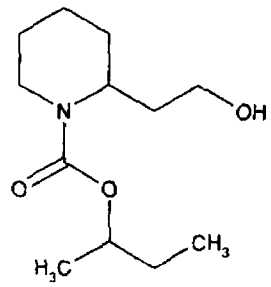
This study is classified **Acceptable** and satisfies the guideline requirement for an acute neurotoxicity study in rodents (870.6200).

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

05

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.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY.
SEE FILE COPY.

12

ATTACHMENT 1

PICARIDIN

Page _____ is not included in this copy.

Pages 74 through 80 are not included in this copy.

TXR 013529

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.

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_____ Internal deliberative information.

_____ Attorney-Client work product.

_____ Claimed Confidential by submitter upon submission to the Agency.

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DATA EVALUATION RECORD 013529

KBR 3023

Study Type: 82-3; 90-Day Dermal Toxicity Study in the Rat

Work Assignment No. 3-47A (MRID 44408716)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Date: 6/22/98

Project Manager
Mary L. Menetrez, Ph.D.

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Quality Assurance:
Reto Engler, Ph.D.

Signature:
Date:

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

Pamela M Hurley 2/12/99

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

DATA EVALUATION RECORD

STUDY TYPE: Dermal subchronic toxicity - 90-day rat
OPPTS Number: 870.3250 OPP Guideline Number: §82-3

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

TEST MATERIAL (PURITY): KBR 3023 (99.2% a.i.)

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

CITATION: Sheets, L.P. and S. G. Lake. (1995) A repeated dose 90-day dermal toxicity study with technical grade KBR 3023 in rats. Bayer Corporation, 17745 South Metcalf, Stilwell, Kansas, 66085-9104. Study No. 90-122-HC. November 1, 1995. MRID 44408716. Unpublished.

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

EXECUTIVE SUMMARY:

In a dermal subchronic toxicity study (MRID 44408716), KBR 3023 (99.2% a.i.) was applied to the shaved skin of young adult Sprague-Dawley (Sas:CD(SD)BR) rats (10-20/sex/dose) at dose levels of 0, 80, 200, 500 or 1000 mg/kg/day for 5 days/week, 5 hours/day, for 90 days. Following the 90 days of treatment, 10 rats/sex/dose were sacrificed. The remaining 10 rats/sex in the 0 and 1000 mg/kg/day groups were maintained without treatment for an additional 4 weeks to assess recovery potential.

Treatment-related lesions in rats from all treatment groups consisted of scabs, red foci, and exfoliation limited to the dose site. The incidence and frequency of scabs and red foci were concentration-dependent. Females in all treatment groups exhibited very slight erythema. KBR 3023 induced diffuse liver hypertrophy in both sexes treated at 1000, 500 or 200 mg/kg/day (statistically significant only in the 500 and 1000 mg/kg/day males). Individual liver cells were necrotic in the 1000 and 500 mg/kg/day group males (3 or 4/group), and in one 1000 mg/kg/day group female. Absolute and relative liver weights were increased in the 1000 (23-28%) and 500

mg/kg/day (8-14%) treatment groups. KBR 3023 was toxic to kidneys, causing minimal to slight hyaline degeneration in both sexes treated at 1000 or 500 mg/kg/day. The kidneys of the 1000 mg/kg/day group males exhibited an increased incidence of foci of tubular regeneration compared to the other test groups, chronic kidney inflammation (3/10 males), and increased absolute and relative weights (24-26%). The 500 mg/kg/day group males had increased relative kidney weights (14%). Urine pH and urobilinogen (males only) was decreased in the 1000 mg/kg/day group, and urine pH was decreased in the 500 mg/kg/day group compared to the controls. All compound-related changes returned to normal by the end of the 4-week recovery period. Other than dermal response on treated skin, no toxic response to KBR 3023 was noted in the 80 mg/kg/day treatment groups. No animals died during the study. There were no treatment-related differences in body weights or body weight gains, food consumption, ophthalmology, hematology parameters, clinical blood chemistry, or macroscopic organ morphology between rats in the treated and control groups. No neoplastic tissue was observed. **The LOAEL for systemic toxicity is 500 mg/kg/day, based on diffuse liver hypertrophy, individual necrotic liver cells, slight hyaline degeneration in the kidneys, an increased incidence of foci of tubular regeneration, and chronic kidney inflammation; the NOAEL is 200 mg/kg/day. The LOAEL for dermal irritation is 80 mg/kg/day based on the presence of scabs, red foci, and exfoliation at the dose site; the dermal irritation NOAEL is <80 mg/kg/day.**

This dermal subchronic toxicity study is classified **acceptable** and satisfies the guideline requirement for a repeated dose dermal toxicity study (§82-3).

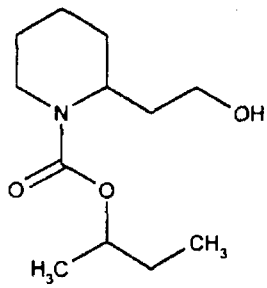
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging Statement was not provided.

83

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023 technical grade
   Description: Clear, viscous liquid
   Lot/Batch #: 19001/90
   Purity: 99.2% a.i.
   Stability of compound: Shown to be stable over the 90-day period between the start and termination of the study
   CAS #: 119515-38-7
   Structure:



2. Vehicle and positive control: None

3. Test animals: Species: Rat
   Strain: Sprague-Dawley Sas:CD(SD)BR
   Age and weight at study initiation: 8-10 weeks of age; male body weight 248-250 g; female body weight 211-214 g
   Source: Sasco Inc., St. Louis, Missouri
   Housing: Individually housed in suspended stainless steel wire-mesh cages
   Diet: Purina Mills Rodent Lab Chow 5001-4 in "etts" form, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   - Temperature: 18-26 C
   - Humidity: 40-70%
   - Air Changes: Not reported
   - Photoperiod: 12-Hour light/dark cycle

   Acclimation period: Approximately 3 weeks to laboratory conditions and 2 weeks to the collars

84

B. STUDY DESIGN

1. In life dates - Start: 9/24/90 End: 12/19/90

2. Animal assignment

Rats (70/sex) that had body weights within 20% of the mean weight for that sex and had normal ophthalmological examinations 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/day)	Animals Assigned[b]	
		Male	Female
1 Control	0	20	20
2 Low	80	10	10
3 Low-Mid	200	10	10
3 High-Mid	500	10	10
4 High	1000	20	20

[a] Dose levels were selected based on the results of a 3-week range-finding dermal toxicity study in which groups of 5 rats/sex/dose level were treated once daily with undiluted KBR 3023 at 0, 200, 500 or 1000 mg/kg/day for 5 days/week for 2 weeks and for 3 or 4 consecutive days during the third week. Liver weights were increased in females treated at 500 mg/kg/day (21%) and in both sexes treated at 1000 mg/kg/day (14 and 23%). There were no deaths or treatment-related clinical signs, effects on body weight, food consumption, serum chemistry, hematology or gross pathology. Based on these results, dose levels of 0, 80, 200, 500, and 1000 mg/kg/day were selected for the subchronic study.

[b] The control and 1000 mg/kg dose group consisted of 20 rats/sex; for each group, the additional 10 rats/sex were designated for a 4-week recovery period.

85

3. Preparation and treatment of animal skin

Hair was clipped from the dorsal and lateral areas of the trunk of each rat before the first dose was administered and at least once weekly thereafter, or as necessary, depending on hair growth. Undiluted test substance was applied as uniformly as possible to an approximate 16 cm^2 clipped area of the back, representing an area at least 10% of the body surface. The actual exposure area was determined to exceed 16 cm^2 at the two higher dose levels. At the highest dose level, that hair lateral to the dose site, on the sides and across the shoulders of the animals, appeared to be moistened with the test substance. This was still apparent after 24 hours when the next dose was to be applied. In addition, the dose spread to areas where the rats could ingest the dose through grooming, affording the opportunity for even greater exposure. **Therefore, it was anticipated by the investigators that exposure to dosages of 500 and 1000 mg/kg/day would exceed 10% of the body surface area and would include oral as well as dermal exposure.** All animals wore Elizabethan collars beginning 2 weeks prior to study initiation through study termination. The test substance was applied to each animal for 5 days/week for 90 days (13 weeks). Termination animals received an additional 2-4 applications during week 14.

Control animals were untreated but were otherwise handled in a similar fashion.

4. Statistics

Mean body weight, food consumption, hematology, blood clinical chemistry, immunoglobulins, urinalysis, and absolute and relative organ weights for each treatment group were compared to the corresponding control group. Data were initially analyzed using Bartlett's test for population homogeneity. Homogeneous data were evaluated by analysis of variance (ANOVA) followed by Dunnett's test if the difference was significant. Non-homogeneous data or comparisons involving two groups (recovery) were analyzed by non-parametric ANOVA of Kruskal-Wallis followed by a Mann-Whitney u-test for stepwise comparison. Gross and microscopic data were analyzed using the Chi-Square Test. If the differences were significant, a Fischer's Exact Test (one-tailed) was used to identify statistical significance between groups. Dunnett's test was conducted at the two-sided 0.1% confidence level; all other tests were conducted at the two-sided 5% confidence level.

C. METHODS

1. Observations

Animals were examined at least once daily for clinical signs. Dermal irritation was scored according to the Draize method beginning on Day 0.

2. Body weight

Animals were weighed on Day 0 prior to dosing and weekly during the treatment and recovery periods.

3. Food consumption

Food consumption for each animal was determined weekly during the treatment and recovery periods except for recovery animals during days 91-100. Mean dietary consumption was reported as g food/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on both eyes of all animals prior to randomization and during week 14. Examinations were conducted in normal ambient room lighting. The pupil reflex, conjunctiva, cornea and iris were examined prior to dilation with a mydriatic. Following mydriasis, the lens, vitreous humor, and retina were examined.

5. Blood

Blood was collected from the orbital sinus of each animal on the day following administration of the last dose. These evaluations were also conducted on all recovery animals on the day of sacrifice. All animals were fasted overnight prior to blood collection, except for 30 animals (3/sex/group) that were inadvertently not fasted. The CHECKED (X) parameters were examined in all samples analyzed.

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*	X	Erythrocyte morphology
	Blood clotting measurements*	X	Heinz Bodies
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

87

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	Total bilirubin
		X	Total serum protein (TP)*
	ENZYMES	X	Triglycerides
		X	A/G ratio
X	Alkaline phosphatase (ALK)	X	Uric acid
	Cholinesterase (ChE)	X	Thyroxine (T4)
X	Creatine phosphokinase	X	Liothyronine (T3)
X	Lactic acid dehydrogenase (LDH)	X	T3 Uptake
X	Serum alanine aminotransferase (also ALT, SGPT)*	X	Immunoglobulins G, A, and M
X	Serum aspartate aminotransferase (also AST, SGOT)*		
X	Gamma glutamyl transpeptidase (GGT)		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from all animals during treatment week 13 and from all recovery animals the week prior to sacrifice. The CHECKED (X) parameters were examined.

X	Appearance	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrite
X	Protein	X	Urobilinogen
X	Glucose		

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

7. Sacrifice and Pathology

All animals scheduled for sacrifice at the end of the treatment or recovery period were sacrificed at the termination of the study by CO_2 asphyxiation, and were subjected to gross pathological examination. The CHECKED (X) tissues were collected for

88

histological examination. Microscopic examination was conducted on all tissues from all treatment and recovery test groups. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain (3 levels)
X	Salivary glands	XX	Heart		
X	Esophagus	X	Bone marrow	X	Sciatic nerve
X	Stomach	XX	Lymph nodes	X	Spinal cord (3 levels)
X	Duodenum	XX	Spleen		
X	Jejunum	XX	Thymus	X	Pituitary
X	Ileum			X	Eyes (optic nerve)
X	Cecum				
X	Colon		UROGENITAL		
X	Rectum				GLANDULAR
XX	Liver*+	XX	Kidneys*+		
	Gall bladder	X	Urinary bladder	XX	Adrenal gland
X	Pancreas	XX	Testes*+	X	Harderian gland
		X	Epididymides	X	Lacrimal gland
		X	Prostate	X	Mammary gland
	RESPIRATORY	X	Seminal vesicle	X	Parathyroids
		XX	Ovaries	X	Thyroids
X	Trachea	X	Uterus		
XX	Lung*	X	Cervix		
	Nose				OTHER
	Pharynx				
X	Larynx			X	Bone* (femur/sternum/rib)
					Skeletal muscle*
				X	Skin* (treated
				X	and untreated)
					Skull (nasal cavity/ear)
				X	All gross lesions and masses*
				X	

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.
+ Organ weight required in repeated dose dermal toxicity studies.

II. RESULTS

A. Observations

1. Mortality - No animals died prior to scheduled sacrifice.

2. Clinical Signs - Rats in all treatment groups exhibited scabs and red foci at the dose site that were, in general, concentration-dependent (Table 2). Scabs were observed in 2/10 males in the 80 mg/kg/day, 5/10 males in the 200 mg/kg/day, 6/10 males in the 500 mg/kg/day, and 8/10 males in the 1000 mg/kg/day groups. Red foci were observed in

1/10 males in the 80 mg/kg/day, 3/10 males in the 200 mg/kg/day, 4/10 males in the 500 mg/kg/day, and 5/10 males in the 1000 mg/kg/day treatment groups. No control males exhibited scabs or red foci during the study. Scabs were observed in all male treatment groups throughout the treatment period, except were limited to days 54-74 only in the 80 mg/kg/day treatment group. Red foci were observed in the 80 mg/kg/day group males on day 25, the 200 mg/kg/day group males on days 17-25, the 500 mg/kg/day males on days 17-38, and the 1000 mg/kg/day group males on days 16-47. Scabs were observed in 1/10 females in the 80 mg/kg/day, 5/10 females in each of the 200 and 500 mg/kg/day, and 8/10 females in the 1000 mg/kg/day treatment groups. Red foci were observed in 1/10 females in the 80 mg/kg/day, 1/10 females in the 500 mg/kg/day, and 2/10 females in the 1000 mg/kg/day treatment groups; red foci were not observed in the 200 mg/kg/day group females. Individual control females exhibited scabs (day 69) or red foci (day 61). Scabs were observed in all female treatment groups throughout treatment (days 3-91), except were limited to day 61 in the 80 mg/kg/day group. Red foci were observed in the 1000 mg/kg/day female group on days 23-53, and on limited to day 24 or 61 in the 500 and 80 mg/kg/day female groups. Exfoliation at the dose site of all treated rats (10/sex) was observed beginning day 7 through the end of treatment, and was accompanied by an orange hue beginning on day 11. During the 4-week recovery period, exfoliation subsided in the 1000 mg/kg/day group males and females by day 12.

Table 2. Dermal irritation at the dose site of rats dermally treated with KBR 3023.

Clinical Sign	Treatment group (mg/kg/day)				
	0	80	200	500	1000
Males					
Scabs	0/10	2/10	5/10	6/10	8/10
Red Foci	0/10	1/10	3/10	4/10	5/10
Exfoliation	0/10	10/10	10/10	10/10	10/10
Females					
Scabs	1/10	1/10	5/10	5/10	8/10
Red Foci	1/10	1/10	0/10	1/10	2/10
Exfoliation	0/10	10/10	10/10	10/10	10/10

[a] Data obtained from page 22 and Table 1, pages 30-33 of the study report.

Females in all treatment groups exhibited very slight erythema (score of 1) during weeks 1-4; the incidence was concentration-dependent during week 2 (Table 3). In the 80 mg/kg/day

group, 2/10 females exhibited erythema on days 16 and 23. In the 200 mg/kg/day group, erythema was observed in 2/10 females on day 8 and 1/10 females on day 16. In the 500 mg/kg/day group, erythema was exhibited in 1/10 females on days 2-4, in 4/10 females on days 7-10, and in 1/10 females on day 16. In the 1000 mg/kg/day group, erythema was observed in 3/20 females on days 1-4 and in 8/20 females on days 7-10. Erythema was not observed in treated females after week 4. No treated males exhibited erythema.

Table 3. The incidence of very slight erythema during weeks 1-4 in female rats dermally treated with KBR 3023.[a]

Test Group (mg/kg/day)	Very slight erythema			
	Week 1	Week 2	Week 3	Week 4
0	0/10	0/10	0/10	0/10
80	0/10	0/10	2/10	2/10
200	0/10	2/10	1/10	0/10
500	1/10	4/10	1/10	0/10
1000	3/10	8/10	0/10	0/10

[a] Data obtained from Table 2, page 34 of the study report.

B. Body weight and weight gain

No treatment-related differences in body weights and body weight gains were observed in any treatment group compared to the controls. By the end of treatment (day 91), mean body weights of all male test groups were 421-434 g, and of all female test groups were 269-285 g.

C. Food consumption

No treatment-related differences in food consumption were observed in any treatment group during the 13-week treatment period.

D. Ophthalmoscopic examination

No treatment-related ophthalmoscopic abnormalities were observed at study termination.

91

E. Blood work

1. Hematology - No treatment-related differences were observed in hematology parameters of rats in any treatment group. Observed differences were not dose-dependent, occurred during the recovery period only, and/or lacked associated hematological changes that would suggest biological or toxicological significance.

2. Clinical Chemistry - No treatment-related differences were observed in clinical blood chemistry parameters of rats in any treatment group. Differences that were not considered treatment-related in the 1000 mg/kg/day treatment groups were increased cholesterol in males (25%) and increased T3B (9.6%) in females, both of which were the result of a very high value for a single rat. These increases were neither concentration-dependent nor considered biologically or toxicologically significant, although they were statistically significant ($p \leq 0.05$) compared to the controls.

   No differences in serum immunoglobulins G, M, and A levels were observed in any treatment group during the treatment or recovery periods.

F. Urinalysis

Both sexes in the 500 and 1000 mg/kg/day treatment groups had significantly ($p \leq 0.05$) decreased urine pH during the treatment period relative to the controls; the decreases were concentration-dependent (Table 4). The 1000 mg/kg/day group males also exhibited significantly ($p \leq 0.05$) decreased urobilinogen at the end of the treatment period. In the 1000 mg/kg/day treatment groups, urine pH and urobilinogen were similar to the control values at the end of the 4-week recovery period (Table 4). No other differences in urine parameters were considered treatment-related.

Table 4. Urine pH and urobilinogen in rats following 13 weeks of treatment with KBR 3023 and after 4-week recovery period.[a]

Test Group (mg/kg/day)	Treatment Period		Recovery period	
	pH	Urobilinogen (EU/dL)	pH	Urobilinogen (EU/dL)
Males				
0	8.4	1.0	8.6	0.9
80	8.2	1.0	--[b]	--
200	8.1	0.9	--	--
500	7.8*	1.0	--	--
1000	7.5*	0.6*	8.7	0.9
Females				
0	7.9	0.7	8.1	0.9
80	7.9	1.0	--	--
200	7.6	0.8	--	--
500	7.4*	1.0	--	--
1000	7.2*	0.9	8.1	0.9

[a] Data were obtained from Table UR1-SUM, pages 282-284, and Table UR2-SUM-REC, pages 592-606, of the study report.
[b] Not analyzed.
* Significantly different from control, $p \leq 0.05$.

G. Sacrifice and Pathology

1. Organ weight - Both sexes in the 1000 mg/kg/day treatment group exhibited significantly ($p \leq 0.05$) increased absolute and relative liver weights compared to the controls (Table 5). Females in the 500 mg/kg/day group also had significantly increased absolute and relative liver weights; the absolute and relative liver weights for both males and females in this dose group were 8-14% higher than the control weights. The 1000 mg/kg/day treatment groups had absolute and relative liver weights 23-28% higher relative to the control weights.

The 500 mg/kg/day group males had relative kidney weights 14% higher, and the 1000 mg/kg/group males had absolute and relative kidney weights 24-26% higher than the control weights ($p \leq 0.05$; Table 5). No other organ weight differences were considered treatment-related.

Following a 4-week recovery period, no differences in absolute or relative organ weights were observed in the 1000 mg/kg/day treatment groups compared to the controls (Table 5).

Table 5. Absolute and relative (organ weight/body weight) liver and kidney weights for rats following 13 weeks of treatment with KBR 3023 and after 4-week recovery period.[a]

Test Group (mg/kg/day)	Treatment Period				Recovery Period			
	Liver		Kidney		Liver		Kidney	
	Absolute (g)	Relative	Absolute (g)	Relative	Absolute (g)	Relative	Absolute (g)	Relative
Males								
0	15.123	3.809	3.342	0.845	14.885	3.542	3.477	0.818
80	15.488	3.844	3.363	0.838	--[b]	--	--	--
200	15.075	3.781	3.318	0.832	--	--	--	--
500	16.263	4.206	3.752	0.967*	--	--	--	--
1000	19.358*	4.827*	4.195*	1.045*	14.000*	3.556	3.340	0.847
Females								
0	8.333	3.338	2.038	0.816	7.866	3.107	1.916	0.757
80	8.513	3.347	2.030	0.800	--	--	--	--
200	8.755	3.439	1.950	0.767	--	--	--	--
500	9.466*	3.808*	2.089	0.841	--	--	--	--
1000	10.283*	4.173*	2.090	0.849	8.002	3.328	1.953	0.815*

[a] Data were obtained from Table OW1K-SUM, pages 492-502, and Table OW3K-SUM-REC, pages 700-710, of the study report.

[b] Not analyzed.

* Significantly different from controls, $p \leq 0.05$.

2. Gross pathology - No treatment-related gross postmortem differences were observed between rats in the treated and the control groups. Isolated abnormalities occurred randomly in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Diffuse liver hypertrophy and necrosis of individual liver cells were observed in males and/or females treated at 200, 500 or 1000 mg/kg/day only (Table 6). In the male treatment groups, liver hypertrophy was exhibited in 2/10 males treated at 200 mg/kg/day, 9/10 males treated at 500 mg/kg/day group, and 10/10 males treated at 1000 mg/kg/day; the incidence was significant ($p \leq 0.05$) for the 500 and 1000 mg/kg/day treatment groups compared to the controls. Individual liver cells were necrotic in 3/10 and 4/10 males in the 500 and 1000 mg/kg/day treatment groups, respectively. In the female treatment groups, liver hypertrophy was observed in 1/10 rats in the 200 mg/kg/day group, 2/10 rats in the 500 mg/kg/day group, and 4/10 rats in the 1000 mg/kg/day group. Individual liver cells were necrotic in one 1000 mg/kg/day female.

Kidneys of males in the 500 and 1000 mg/kg/day treatment groups exhibited an increased ($p \leq 0.05$) incidence of minimal to slight hyaline degeneration compared to the controls; 7/10 or 8/10 treated males were affected compared to 0/10 controls (Table 6). The kidneys of the 1000 mg/kg/day group males exhibited chronic inflammation (3/10), and a slightly increased incidence of foci of tubular regeneration (6/10) compared to the other treatment and control groups (3 or 4/10 per group).

All compound-related changes in rats from all treatment groups returned to within normal limits by the end of the 4-week recovery period (Table 6).

Table 6. Microscopic liver and kidney abnormalities in rats following 13 weeks of treatment with KBR 3023 and after a 4-week recovery period.

Microscopic Alteration	Treatment Period					Recovery Period				
	Treatment group (mg/kg/day)									
	0	80	200	500	1000	0	80	200	500	1000
Males										
Liver										
Diffuse hypertrophy	0/10	0/10	2/10	9/10*	10/10*	0/10	0/10	0/10	0/10	0/10
Necrosis, individual cell	0/10	0/10	0/10	3/10	4/10	0/10	0/10	0/10	0/10	3/10
Kidney										
Degeneration, hyaline	1/10	0/10	0/10	7/10*	8/10*	0/10	0/10	0/10	0/10	0/10
Inflammation, chronic	0/10	0/10	0/10	0/10	3/10	1/10	0/10	0/10	0/10	0/10
Regeneration, tubules	3/10	4/10	3/10	4/10	6/10	2/10	0/10	0/10	0/10	3/10
Females										
Liver										
Diffuse hypertrophy	0/10	0/10	1/10	2/10	4/10	0/10	0/10	0/10	0/10	0/10
Necrosis, individual cell	0/10	0/10	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10

Data obtained from Table MP1-SUM, pages 503-532 of the study report.

* Significantly different from control, $p \leq 0.05$.

No other microscopic findings were clearly attributable to treatment. Acanthosis and hyperkeratosis observed at the dose site of rats from all treatment groups were likely responses to the physical irritant properties of the test substance, rather than a toxic response to treatment. The severity of each condition was minimal to slight and did not increase with an increase in dose rate. The increased incidence of hypertrophy of the sebaceous glands observed in rats from all treatment groups compared to the controls may have resulted from the clogging effect of the undiluted test substance which could have prevented the normal release of glandular secretions.

b) Neoplastic - No neoplastic tissue was observed in rats in the treatment and control groups.

96

III. DISCUSSION

A. Investigator's Conclusions

The study author concluded that dermal application of KBR 3023 at doses of 80 to 1000 mg/kg/day produced clinical signs and microscopic lesions in the treated rats. Scabs, red foci, and exfoliation were observed in all treatment groups. Diffuse liver hypertrophy in the 500 and 1000 mg/kg/day group rats was accompanied by increased absolute and/or relative liver weights. Hyaline degeneration of kidney tubules in the 500 and 1000 mg/kg/day group rats was accompanied by increased absolute and/or relative kidney weights. Decreased urine pH in all rats in the 500 and 1000 mg/kg/day treatment groups, and decreased urobilinogen in the 1000 mg/kg/day group males were considered treatment-related. Incidences of acanthosis, hyperkeratosis, and/or sebaceous gland hypertrophy around hair follicles in treated skin from all treated animals were considered to be adaptive, reversible responses of the skin to an irritant and of no toxicological significance. The NOAEL for dermal irritation was established at <80 mg/kg/day for both sexes based on clinical signs and skin lesions at the dose site. The toxicological NOAEL was established at 200 mg/kg/day for both sexes based on liver and kidney alterations at the 500 and 1000 mg/kg/day dose levels.

B. Reviewer's Discussion

We agree with the study author that the NOAEL for dermal irritation of KBR 3023 is <80 mg/kg/day for both sexes, based on clinical signs and skin lesions at the dose site. Clinical signs included scabs, red foci, and exfoliation at the dose site. The incidence and frequency of scabs and red foci were, in general, concentration-dependent. All female treatment groups exhibited very slight erythema was observed.

We also agree with the study author that KBR 3023 adversely affected the livers of rats treated at 500 or 1000 mg/kg/day. Liver hypertrophy was observed in both sexes in the 200, 500, and 1000 mg/kg/day treatment groups, however, the hypertrophy observed at 200 mg/kg/day is considered to be an adaptive effect and not an adverse effect (it was also not statistically significantly increased in either sex). Individual liver cells were necrotic in the 500 and 1000 mg/kg/day group males and one 1000 mg/kg/day group female. Absolute and relative liver weights were increased in the 500 and 1000 mg/kg/day treatment groups.

KBR 3023 was also toxic to the kidneys of males treated at 500 or 1000 mg/kg/day, as evidenced by minimal to slight hyaline degeneration. The kidneys of the 1000 mg/kg/day group males had an increased incidence of foci of tubular regeneration compared to the other test groups (6/10 versus 3-4/10), and showed chronic inflammation in several males. Relative kidney weights were increased at both treatment levels, and absolute kidney weights were increased in the 1000 mg/kg/day group males compared to the controls. At

the end of treatment, decreases were observed in urine pH in the 500 and 1000 mg/kg/day group males and females, and in urobilinogen in the 1000 mg/kg/day group males.

All compound-related changes returned to normal by the end of the 4-week recovery period. No treatment-related differences in body weights or body weight gains, food consumption, ophthalmology, hematology parameters, clinical blood chemistry, or macroscopic organ morphology were observed between rats in the treated and control groups. No neoplastic tissue was observed. Following the 4-week recovery period, all toxic responses had disappeared.

The upper dose level of KBR 3023 that is recommended for chronic studies appears to be 1000 mg/kg/day, based on liver effects observed in rats treated at this dose level.

IV. **STUDY DEFICIENCIES**

No scientific deficiencies were noted in this study.

DATA EVALUATION RECORD 013529

KBR 3023

Study Type: 82-5; Subchronic Dermal Neurotoxicity Screening Study in the Rat

Work Assignment No. 3-53V (MRID 44408717)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

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4-28-99

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Dermal Neurotoxicity Study in Rats
OPPTS Number: 870.6200 OPP Guideline Number: §82-5

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) Subchronic dermal neurotoxicity screening study with technical grade KBR 3023 in Fischer 344 rats. Bayer Corporation, 17745 South Metcalf, Stilwell, Kansas, 66085-9104. Study No. 94-422-ET. Agricultural Division Report No. 107466. October 9, 1996. MRID 44408717. Unpublished.

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

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 44408717), 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) at dose levels of 0, 50, 100 or 200 mg/kg/day, 5 days/week for 13 weeks. The high dose of 200 mg/kg had been previously agreed upon with the EPA. The rats were evaluated for reactions in functional observations and motor activity testing at 4 hours and during weeks 4, 8 and 13 of treatment.

No treatment-related 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, food consumption, ophthalmology. absolute or relative brain weights, or gross or microscopic histology of the rats. **The neurotoxicological NOAEL is >200 mg/kg/day; the NOEL is 200 mg/kg/day for both sexes.**

This study is classified **Acceptable** and satisfies the guideline requirement for a subchronic dermal neurotoxicity study in rodents (870.6200).

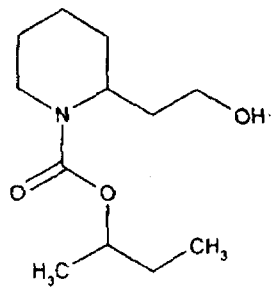
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

100

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 stability of the test substance for the duration of storage at room temperature has been established. 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: 8 weeks of age; male body weight 93.6-155.8 g; female body weight 94.4-139.3 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*
   Water: Tap water, *ad libitum*
   Environmental conditions:
      Temperature: 17.8-25.6 C
      Humidity: 40-70%
      Air Changes: Not reported
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: 3 Weeks (1 week to acclimate to ambient laboratory conditions; 2 weeks to acclimate to collars)

B. STUDY DESIGN

1. In life dates - Start: 11/21/94 End: 2/22/95

2. Animal assignment

Rats (48/sex) that had body weights within 20% of the mean weight for that sex and had normal ophthalmological examinations 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/day)	Animals Assigned[b]	
		Male	Female
Control	0	12	12
Low	50	12	12
Mid	100	12	12
High	200	12	12

[a] Dose levels were based on the results of a dermal toxicity study in which male and female rats were treated with undiluted KBR 3023 at 0, 80, 200, 500 or 1000 mg/kg/day for 5 days/week for 13 weeks. All dose levels produced mild micropathologic findings of the treated dermal area. The 500 and 1000 mg/kg/day dose levels caused increased liver and kidney weights, diffuse liver cell hypertrophy, individual hepatocyte degeneration/necrosis, and hyaline degeneration within the kidney tubules. The 1000 mg/kg/day dose also produced foci of renal tubular regeneration and chronic inflammation. At 500 and 1000 mg/kg/day, the dosages spread to an area exceeding 10% of the body surface area; mild microscopic dermal changes extending beyond the treated area and ulceration at the dose site were observed. For the present study, the dose level of 200 mg/kg/day was selected as the highest dose that does not produce ulceration or dosage spreading beyond the dose site. The dose level of 50 mg/kg/day was selected to produce no evidence of exposure, and the dose level of 100 mg/kg/day was selected as the intermediate dose.

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

102

3. Preparation and treatment of animal skin

Hair was clipped from the dorsal and lateral areas of the trunk of each rat. Undiluted KBR 3023 was uniformly applied to the clipped area of the back of each animal once daily, 5 days/week, for 13 weeks. The treated area (30-39 cm^2) represented at least 10% of the body surface. The dose was routinely applied at approximately the same time of day, during the morning hours except was applied in the afternoon hours during weeks 4, 8, and 13 so that neurobehavioral testing occurred before the daily exposure. All animals wore Elizabethan collars beginning approximately 2 weeks prior to the initiation of dosing except for at least 45 minutes prior to FOB and motor activity testing, and except for a replacement control female that wore a collar for 6 days only prior to initiation of treatment. The exposure site was not wiped during the study. Rats were kept in their cages during the exposure period. Control animals were untreated but were 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

All animals were examined twice daily (once daily on holidays and weekends) for mortality or clinical signs of moribundity and to determine whether the collars needed replacement. Detailed physical examinations for clinical signs of toxicity were performed once each week.

2. Body weight

Individual animals were weighed at study initiation and weekly during the treatment period.

3. Food consumption

Food consumption for each animal (g/animal) was measured weekly during the treatment period.

4. Ophthalmological examinations

Ophthalmological examinations were conducted on each animal prior to study initiation and during week 12. The pupillary reflex was tested using a penlight or transilluminator. A mydriatic agent was then applied to each eye to dilate the pupil. Following mydriasis, the eyelids, conjunctiva, cornea, aqueous humor, and lens were examined with a slit lamp microscope, and the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope with a condensing lens.

5. Neurobehavioral Studies

Functional observational battery (FOB) and motor activity testing were performed on all animals during the week prior to initiation of treatment and during weeks 4, 8, and 13 of treatment. Animals were evaluated individually in sets of eight over a 2-day period for each test week. The order of testing and assignment of animals to mazes were semi-random so that groups were balanced across test times and test devices and no animal was tested more than once in the same maze. Animals were acclimated to the testing rooms for at least 15 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 the 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. Approximately 1 to 2.5 hours after the last animal in the set had finished being tested in the FOB, all eight animals were placed individually in figure-eight mazes to measure motor activity. Each maze was equipped with eight infrared emitter/detector pairs; activity was measured each time a beam was interrupted. Animals were tested for 90 minutes. 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.

6. Sacrifice and Pathology

All test animals were sacrificed 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 200 mg/kg dose groups were examined microscopically. The following tissues were processed:

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

[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 treatment-related deaths occurred during the study. One 100 mg/kg/day group female died during the study, possibly as a result of an abdominal laceration noted 3 days earlier. The deaths of two control males and one control female during the study were considered incidental.

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 males in all treatment groups were higher than the control group throughout the study: the magnitude of the differences was due to steady increases in body weights for the treated males concurrent with unexplained body weight decreases in the control males. Body weights of females in all treatment groups were similar to the control group throughout the study. Mean final body weights of the male test groups were 207.9-223.7 g, and of the female test groups were 153.2-158.7 g.

C. Food consumption

No treatment-related differences in food consumption were observed between the treated and control groups. Mean food consumption values for male and female treatment groups was similar or somewhat higher (up to 14-15%) than values for the corresponding control group throughout the study.

D. Functional Observational Battery

No treatment-related differences in FOB parameters were observed between the treatment and control groups. Findings were attributed to the wearing of collars or were considered incidental in nature. Forelimb and hindlimb grip strength and landing foot splay were unaffected by treatment in all dose levels.

E. Motor Activity Measurements

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

F. Ophthalmoscopic examinations

No treatment-related ophthalmoscopic abnormalities were observed.

G. Sacrifice and Pathology

No treatment-related differences in absolute or relative brain 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 subchronic dermal exposure to KBR 3023 at dosages of 50 to 200 mg/kg/day produced no evidence of toxicity or neurotoxicity. The neurotoxicological NOEL was concluded to be 200 mg/kg/day for males and females.

B. Reviewer's Discussion

We agree with the study author that KBR 3023 was not toxic or neurotoxic to rats dermally treated at 50, 100 or 200 mg/kg/day for 13 weeks. There were no treatment-related differences in general behavior or appearance, body weights, food consumption, FOB and motor activity testing, absolute or relative brain weights or gross histology of the rats. There was no evidence of treatment-related neuropathology in the 200 mg/kg/day treatment group. Based on these findings, the neurotoxicological NOEL for KBR 3023 is 200 mg/kg/day for male and female rats. The toxicological NOEL is also 200 mg/kg/day for both sexes.

The high dose of 200 mg/kg was selected based on a meeting (1/31/90) in which Bayer (then Miles Inc.) and the U.S. EPA selected 200 mg/kg as the high dose for long-term (chronic toxicity/oncogenicity and reproduction) studies. 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

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY.
SEE FILE COPY.

ATTACHMENT 1

PICAMIDIN

Page _____ is not included in this copy.

Pages 111 through 117 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.

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013529

DATA EVALUATION RECORD

KBR 3023

Study Type: 83-1b; Chronic Toxicity (Repeated Dose Dermal) Study in Dogs

Work Assignment No. 3-53W (MRID 44408718)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
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Date: 6/22/98

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Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date:

Quality Assurance
Reto Engler, Ph.D.

Signature:
Date:

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Pamela Hurley, Ph.D. Pamela 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.

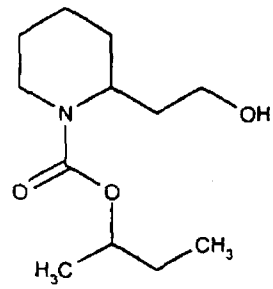
120

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.

122

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

123

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.

124

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.

125

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain* (cerebellum-midbrain,
X	Salivary glands*	XX	Heart*		cerebellum, medulla/pons
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.

126

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.

127

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)

130

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.

DP BARCODE: D239722

CASE: 289087 DATA PACKAGE RECORD DATE: 02/12/99
SUBMISSION: S529365 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: TOLERANCE PET ACTION: 240 G PET-TEMP TOLER
RANKING : 11 POINTS ()
CHEMICALS: 069089 Diethyl-2-(4-methylbenzyloxy)ethylamine %

ID#: 7G04891
COMPANY: GMJA SPECIALTIES
PRODUCT MANAGER: 22 CYNTHIA GILES-PARKER 703-305-7740 ROOM: CM2 249
PM TEAM REVIEWER: ROSE KEARNS 703-305-5611 ROOM: CM2 265
RECEIVED DATE: 07/22/97 DUE OUT DATE: 11/19/97

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 239722 EXPEDITE: N DATE SENT: 10/07/97 DATE RET.: / /
CHEMICAL: 069089 Diethyl-2-(4-methylbenzyloxy)ethylamine
DP TYPE: 001 Submission Related Data Package
CSF: Y LABEL: Y

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 12/16/97
DIV : HED	10/07/97	/ /	NEGOT DATE: / /
BRAN: RAB2	01/08/98	/ /	PROJ DATE: / /
SECT: IO	/ /	/ /	
REVR :	/ /	/ /	
CONTR: DYNAMAC	11/20/98	02/03/99	

* * * DATA REVIEW INSTRUCTIONS * * *

PRIORITY 11 - Appplication for temporary tolerance experimental use permit on oranges. New Chemical Screen. See attached transmittal list for MRID#s. (70880-EUP-R)

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
251165	RAB2/IO	11/27/98	/ /	Y	N	N
239749	IO/IO	10/07/97	12/16/97	Y	N	Y
248740	RAB2	08/24/98	11/02/98	Y	Y	Y
248744	RAB2	08/24/98	11/02/98	Y	Y	Y
248746	ERB4/IO	08/24/98	11/02/98	Y	Y	Y

152

013529

DATA EVALUATION RECORD

KBR 3023

Study Type: 83-2; 18-Month Oncogenicity Dermal Study in Mice

Work Assignment No. 3-47B (MRID 44408719)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
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Primary Reviewer:
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Signature: Mary L Menetrez
Date: 6/8/98

Secondary Reviewer:
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Date: 6/15/98

Project Manager:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 6/22/98

Quality Assurance:
Steve Brecher, Ph.D.
Signature: Steven Brecher
Date: 6/22/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

133

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

Pamela M Hurley 2/12/99

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

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Dermal
OPPTS Number: 870.4200 OPP Guideline Number: §83-2

DP BARCODE: D241232 SUBMISSION CODE: S534142
P.C. CODE: 070705 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.

134

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.

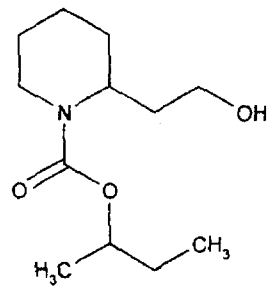
This study is classified as **acceptable (§83-2)** and satisfies the guideline requirements for a carcinogenicity dermal study in the mouse.

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:



135

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				

139

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.

145

DATA EVALUATION RECORD 013529

KBR 3023

Study Type: §83-3; Developmental Toxicity with Range finding in Rabbits

Work Assignment No. 3-47C (MRID's 44408721, 44408720)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 2 (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity with Range Finding in Rabbits
OPPTS Number: 870.3700 OPP Guideline Number: §83-3

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

TEST MATERIAL (PURITY): KBR 3023 technical (97.8% a.i.)

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

CITATION(s): Holzum, B. (1996) Developmental Toxicity Study in Rabbits After Dermal Application. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T0059079, March 15, 1996. MRID 44408721. Unpublished

Holzum, B. (1995) Range Finding Developmental Toxicity Study in Rabbits After Dermal Application. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Study Number T6059075. MRID 44408720. Unpublished

SPONSOR: Bayer AG, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44408721) KBR 3023 (97.8% a.i.) was dermally applied to 24 female CHBB:HM rabbits per dose level at 0, 50, 100, or 200 mg/kg body weight/day from days 0-28 of gestation.

Local dermal reactions at the dose site appeared in all treated animals. All treated females showed squamous skin beginning on gestation day 4-12, which continued until necropsy. A dose-related increase in slight erythema at the dose site was observed in all treated animals (low-dose, 11/24; mid-dose, 20/24; high-dose, 23/24 vs 1/24 controls). Very slight to slight edema was observed in the high-dose group (13/24 vs 0/24 controls). In addition, dermal toxicity was characterized by cracked skin at the dose site acting in a dose-dependent manner (low-dose, 2/24; mid-dose, 4/24; high-dose, 18/24 vs 0/24 controls).

There were no treatment-related effects noted in mortality, clinical signs, gross pathologic findings, or cesarean section parameters at any dose level. There were no treatment-related

147

effects on body weights or food consumption at dose levels of ≤200 mg/kg/day.

The maternal LOAEL for dermal irritation is 50 mg/kg/day.
The maternal NOAEL for dermal irritation is <50 mg/kg/day.

The maternal LOAEL for systemic toxicity was not established.
The maternal NOAEL for systemic toxicity is >200 mg/kg body weight/day.

There were no treatment-related effects on developmental parameters (pre- and post-implantation losses, number of fetuses per litter), fetal deaths, resorptions, altered growth, or malformations.

The developmental LOAEL was not established.
The developmental NOAEL is >≥200 mg/kg/day.

Dosing was considered adequate based on the results of the submitted range finding study (MRID 44408720) in which 3 pregnant female rabbits/dose were dosed at 0, 50, 200, 400, 700, or 1000 mg/kg body weight/day on gestation days 0-28. Maternal toxicity was observed at 1000 mg/kg body weight/day and was characterized by clinical signs of toxicity, gross pathology, severely decreased body weight gains, and decreased food consumption. The applied dose did not spread beyond the shaved area at the 50 mg/kg dose level. The area of humid and yellow stains increased in a dose-dependent manner for the 200 and 400 mg/kg groups. Severe lesions formed at the dose site for the higher dose groups of 700 and 1000 mg/kg. All treated animals showed erythema and squamous cells at the dose site. Edema and cracked skin were observed in animals dosed at ≥400 mg/kg.

This developmental toxicity study is classified **acceptable (§83-3[b]) and does satisfy the guideline requirements for a developmental toxicity study in the rabbit.**

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

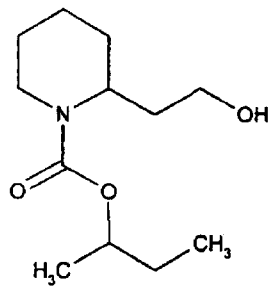
148

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023
   Description: Technical, clear, light yellow liquid
   Lot/Batch #: 030693
   Purity: 97.8% a.i.
   CAS #: 119515-38-7

   Structure:



2. Vehicle: None

3. Test animals: Species: rabbit
   Strain: CHBB:HM
   Age at mating: Not reported
   Weight at mating: 1892-2893 g
   Source: Dr. Karl Thomae GmbH, Biberach
   Housing: Individually in Macrolon cages with perforated cage sheets
   Diet: Ssniff® rabbit diet K-Z standard diet, Manufacturer (Ssniff Spezialdiäten GmbH,Soest), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22±2°C
   Humidity: 50-60%
   Air changes: At least 10/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): At least 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 8/21/95 end: 11/22/95.

2. Mating: Females were placed under observation (1 female:1 male) with stock males of the same strain. The day copulation was observed was designated day 0 of gestation.

3. Dose selection rationale: In a range finding study (MRID 4408720) included in the current submission, KBR 3023 (97.70% a.i.) was administered dermally (without occlusion) to 3 pregnant female rabbits/dose at dosages of 0, 50, 200, 400, 700, or 1000 mg/kg body weight/day on gestation days 0-28. Individual doses were based on the current body weights as determined daily before application.

Mortality, clinical observations, body weight, and food consumption were recorded. Dams were sacrificed on day 29 of gestation and gross necropsies were performed. The reproductive tract was removed and the number of corpora lutea in each ovary,. implantation sites (live and dead), individual weights and appearance of placentas, and gravid uterine weights were recorded. Fetuses were weighed and examined externally.

Maternal toxicity was observed at 1000 mg/kg body weight/day and was characterized by clinical signs of toxicity, gross pathology, severely decreased body weight gains, and decreased food consumption. Two 1000 mg/kg/day females showed severe weight loss and were killed for humane reasons on day 9 or 14 of gestation; these deaths left only one female in the high dose group. Clinical signs of toxicity noted in the high-dose group included lacrimation and reddish discoloration of the eyelid. Upon necropsy, one of the two sacrificed females of the high dose group displayed gastrointestinal changes consisting of whitish, granular stratifications in the gallbladder wall and hardened gastric contents.

The applied dose did not spread beyond the shaved area at the 50 mg/kg dose level. The area of humid and yellow stains increased in a dose-dependent manner for the 200 and 400 mg/kg groups, and spreading was evident after day 2 of gestation for the 200 mg/kg level and after day 1 of gestation for the 400 mg/kg level. Severe lesions formed at the dose site for the higher dose groups of 700 and 1000 mg/kg, and therefore, failed to indicate dose-dependent spreading since the compound was absorbed into the lesions. Movement beyond the dose site was apparent on day 0 of gestation for these higher dose levels. All treated animals showed erythema and squamous cells at the dose site. Edema and cracked skin were observed in animals dosed at ≥400 mg/kg.

The test compound had no affect on fertility rates at dose levels ≤700 mg/kg. The two high-dose females that were killed due to severe weight loss did not have implantations at necropsy, however, the one surviving female did have implantation sites. The numbers of corpora lutea and pre-implantation losses were unaffected by treatment at ≤1000 mg/kg/day. Post-implantation losses increased in the single high-dose female; this increase was due to an increased number of resorptions (2). The number of fetuses, fetal weight, external fetal findings, placental weights, and external placental findings were unaffected by treatment.

Based on the results of this range finding study, the doses summarized in Table 1 were selected for the full developmental toxicity study in rabbits.

150

Table 1. Animal assignment.[a]

Test Group	Dose level (mg/kg body weight/day)	Application volume (mL/kg body weight/day)	Number of Dams
Control[b]	0	0.20	24
Low	50	0.05	24
Mid	100	0.10	24
High	200	0.20	24

a Data extracted from study report, page 18.
b Control animals received only tap water.

4. Dosage preparation and analysis: Since analyses were performed on the same lot number, the following information was obtained from the KBR 3023 chronic oncogenicity study (MRID 44408728): undiluted technical grade KBR 3023 was stored frozen (-23°C) and approximately every two weeks, dosing aliquots were provided. From the information provided, it was inferred that the dosing aliquots were stored at room temperature. Prior to commencement of the study and approximately every 6 months, the stability of the test chemical stored at -23°C was assessed. In addition, stability analyses were performed on samples of KBR 3023 stored at room temperature (22°C) for 7, 14, 21, or 28 days.

   Results:
   Concentration/Stability Analysis: The chemical purity of KBR 3023 stored frozen was 96.7-98.5% throughout the study. It was stated that the compound is stable for 28 days when stored at room temperature.

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

5. Dosage administration: An area representing approximately 10% of the body surface area of each female was shaved at the beginning of the study and as needed thereafter. All doses were applied once daily, dermally without occlusion on gestation days 0-28. The compound was applied undiluted and dispersed uniformly using a spatula. Dosing was based on daily body weights. Dose sites of the control animals were treated with tap water. To prevent oral ingestion of the test substance, the animals were fitted with collars from day 0 (after mating) until necropsy. The animals had also worn these collars for at least 7 days prior to mating for acclimatization.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality and clinical signs twice daily. Body weights were recorded daily and food consumption was

recorded on gestation days 6, 11, 16, 21, 24, and 29. Dams were sacrificed on day 29 of gestation. At sacrifice, evaluations consisted of a gross examination of the brain, skeletal system, abdominal, pelvic, and thoracic organs. The reproductive tract was removed, examined, and the following were recorded:

- gravid uterine weight
- number of corpora lutea and implantations in each ovary
- number of fetuses (live and dead)
- weight and sex of all live fetuses
- individual weights and appearance of placentas

2. Fetal Evaluations - Each fetus was weighed, sexed, and examined for external, visceral, and skeletal abnormalities. Visceral exams were performed on the fetuses according to the STAPLES technique. For skeletal examinations, the cartilage was stained using alcian blue, fetuses were cleared with diluted potassium hydroxide, and were stained with alizarin red S.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine, appropriate statistical procedures.

2. Indices: The following indices were presented in the study report:

Fertility rate = # females with implantations/ # females mated x 100%
Gestation rate = # females with viable fetuses/ # females with implantations x 100%

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: There were no mortalities and no treatment-related effects noted in behavior or appearance. Following treatment, the number of females with soft feces increased (low-dose, 10/24; mid-dose, 9/24; high-dose, 18/24 vs 3/24 controls). These increases were not dose-dependent and therefore, not of toxicological significance and were considered to be related to the stress caused by local reactions at the dose site.

2. Body Weight: Absolute and corrected body weight gains in the dams were unaffected by treatment at levels ≤200 mg/kg body weight/day.

3. Food Consumption - Food consumption was unaffected by treatment up to and including 200 mg/kg body weight/day.

152

4. Gross Pathology - There were no treatment-related gross pathologic findings upon necropsy.

5. Dose Site Reactions - Local dermal reactions at the dose site were evident at all dose levels and acted in a dose-dependent manner. All treated females showed squamous skin beginning on gestation day 4-12 and continuing until necropsy. Slight erythema of the dose site, which appeared during the initial days of treatment and continued until necropsy, increased in a dose-dependent manner in all treated animals (low-dose, 11/24; mid-dose, 20/24; high-dose, 23/24 vs 1/24 controls). Very slight edema was observed in the high-dose group (10/24 vs 0/24 controls); slight edema also appeared at the dose site in the high-dose animals (3/24 vs 0/24 controls). Additionally, dermal toxicity was characterized by cracked skin at the dose site, increasing in a dose-related manner (low-dose, 2/24; mid-dose, 4/24; high-dose, 18/24 vs 0/24 controls). Table 3 summarizes findings at the dose site.

Table 3. Local reactions at the dose site.[a]

Observation	Dose(mg/kg/day)			
	0	50	100	200
Number Evaluated	24	24	24	24
Squamous skin	0	24	24	24
Erythema				
- very slight	10	13	4	0
- slight	1	11	20	23
- moderate	0	0	0	1
Edema				
- very slight	0	0	0	10
- slight	0	0	0	3
Cracked skin	0	2	4	18

a Data extracted from study report page 28.

6. Cesarean Section Data - Cesarean section observations are presented in Table 4. The fertility rates, numbers of corpora lutea, implantations, pre-implantation losses, and gestation rates were similar between control and treated groups. Two control females and one 200 mg/kg/day female aborted; one 200 mg/kg/day female resorbed all implantations. Although increases in individual fetal and placental weights were statistically significant in the 50 and 200 mg/kg/day groups, statistical significance was not achieved when calculated on a litter basis. In addition, all weights were within the range of historical

153

controls and changes in weight were not dose-dependent.

Table 4. Cesarean section observations [a]

Observation	Dose (mg/kg body weight/day)			
	0	50	100	200
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	24	22	22	24
Pregnancy Rate (%)	(100)	(92)	(92)	(100)
# Nonpregnant	0	2	2	0
# Total Dams Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted [b]	2	0	0	1
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	189	177	192	176
Corpora Lutea/Dam	8.6±2.1	8.0±1.1	8.7±1.6	7.7±1.9
Total # Implantations	174	161	178	165
Implantations/Dam	7.9±2.2	7.3±1.2	8.1±1.6	7.2±2.0
Total # Litters	22	22	22	23
Total # Live Fetuses	155	146	167	140
Live Fetuses/Dam	7.0±2.5	6.6±1.4	7.6±1.8	6.1±2.3
Total # Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Total # Resorptions	19	15	11	25
Early	0	0	0	2
Late	19	15	11	23
Resorptions/Dam	0.9±1.2	0.7±0.8	0.5±0.8	1.1±0.9
Early	0	0	0	0.1±0.4
Late	0.9±1.2	0.7±0.8	0.5±0.8	1.0±1.0
Litters with Total Resorptions	0	0	0	1
Mean Weight (g)				
Individual basis	35.32±5.98	37.12±5.40**	36.55±4.83	38.41±4.79**
Litter basis	36.45±4.89	37.22±4.46	37.10±3.32	38.75±3.16
Males (litter basis)	36.79±5.31	37.78±5.13	37.50±3.16	38.39±3.03
Females (litter basis)	35.72±5.18	36.63±4.88	36.67±3.98	39.36±3.78
Mean Placental Weight (g)				
Individual basis	3.78±0.69	4.06±0.69**	3.88±0.67	4.01±0.77*
Litter basis	3.89±0.52	4.09±0.46	3.94±0.46	4.08±0.56
Sex Ratio (% Male)	51	48	45	51
Pre-implantation Loss (%) [c]	8	9	7	6
Post-implantation Loss (%) [c]	11	9	6	15

a Data extracted from the study report, pages 136-230.
* $p < 0.05$, ** $p < 0.01$

154

b Aborted litters excluded from mean.
c Pre- and post-implantation loss percentages calculated by reviewers.
Pre-implantation loss index:
100- the % corpora lutea that are represented as implantation sites.
Post-implantation loss index:
100- the % implantations that are viable at the time of intrauterine inspection.

B. DEVELOPMENTAL TOXICITY: Fetal examinations included external, visceral, and skeletal observations at necropsy. Statistically significant increases in external, visceral, and skeletal malformation rates on a fetal basis were found in the low- and mid-dose groups (low-dose, 6 malformed fetuses; mid-dose, 9 malformed fetuses vs 0 malformed controls, $p<0.05$).

1. External Examination - Increased incidence of gross external malformations in the dose groups was not considered treatment-related since the findings were not dose-dependent. During external examination, two malformed fetuses were found in the high-dose group, both with arthrogryposis, but this incidence was not statistically significant. Six mid-dose and four low-dose fetuses were found to have arthrogryposis. Arthrogryposis was found in the historical control data at incidence levels similar to or higher than levels found in this study, therefore, increased levels in this study were considered unrelated to treatment. Data are shown in Table 5a.

Table 5a. External examinations [a]

Observations	Dose (mg/kg body weight/day)				
	0	50	100	200	Historical controls[b]
#Fetuses (#litters) examined	155 (22)	146 (22)	167 (22)	140 (22)	4040 (656)
Arthrogryposis[c]					
fetal incidence (litter incidence)	0 (0)	4 (4)	6 (3)	2 (2)	
%fetal incidence (% litter incidence)	0 (0)	3 (18)	4 (14)	1 (9)	0.00-5.56
% affected fetuses/litter	0	18	27	9	(0.00-23.08)

a Data extracted from the study report, Table 7, page 35.
b Calculated by reviewers using data on pages 375-382.
c % Affected fetuses/litter were calculated by reviewers.
* Statistically significant from control at $p<0.05$.

2. Visceral Examination - There were no treatment-related visceral variations or malformations observed at levels ≤200 mg/kg body weight/day. Upon visceral examination, two malformed fetuses in the mid-dose group were found to have a cardiac septal defect. Findings are presented in Table 5b.

155

Table 5b. Visceral examinations [a]

Observations	Dose (mg/kg body weight/day)			
	0	50	100	200
#Fetuses(#litters) examined	155 (22)	146 (22)	167 (22)	140 (22)
Cardiac septal defect [b]				
fetal incidence (litter incidence)	0 (0)	0 (0)	2 (2)	0 (0)
% fetal incidence (% litter incidence)	0 (0)	0 (0)	1 (9)	0 (0)
% affected fetuses/litter	0	0	9	0
#Deviations(#litters)	0 (22)	1 (22)	1 (22)	0 (22)

a Data extracted from the study report, Table 7, page 35 and page 85.
b % Affected fetuses/litter were calculated by reviewers.
* Statistically significant from control at $p<0.05$.

3. Skeletal Examination - The most common skeletal findings are presented in Table 5c. There were no treatment-related skeletal retardations or variations observed at any dose level when skeletal retardations and variations were calculated on a litter basis. At skeletal examination, one low-dose fetus was found with an anomaly of vertebrae and ribs; another low-dose fetus was found with fusion of the ribs. A mid-dose fetus was found to have fusion of caudal vertebral bodies. Skeletal variations were noted in the treatment groups, when compared to the controls, that indicate a decrease in incomplete ossification of the medial phalanx digit(s), medial phalanx toe(s), frontal bone, and parietal bone; however, this progressive effect on fetal ossification is not an adverse effect. The report stated that "the statistical significances when calculation was done on an individual basis reflect more progressed ossification when compared to the control group in all cases. This higher stage of ossification correlated with the incidentally higher fetal weights in all dose groups which also achieved statistical significance in the 50 mg and 200 mg/kg groups when calculated on an individual basis". The findings of skeletal variations were not statistically significant on a litter basis, were not dose-dependent, and were inside the historical control ranges; therefore, they do not indicate an adverse effect on fetal development.

156

Table 5c. Skeletal examinations [a]

Observations	Dose (mg/kg body weight/day)			
	0	50	100	200
#Fetuses (#litters) examined	155 (22)	146 (22)	167 (22)	140 (22)
Sternebra(e)				
Incomplete oss.-5th				
fetal incidence (litter incidence)	121 (22)	109 (22)	129 (21)	108 (22)
% fetal incidence (% litter incidence)	78.1 (100.0)	74.7 (100.0)	77.2 (95.5)	77.1 (100.0)
Medial phalanx digit(s)				
Incomple oss.- 5th right				
fetal incidence (litter incidence)	33 (11)	13* (6)	20 (8)	5** (4)
% fetal incidence (% litter incidence)	21.3 (50.0)	8.9 (27.3)	12.0 (36.4)	3.6 (18.2)
Incomplete oss.- 5th left				
fetal incidence (litter incidence)	36 (12)	16* (7)	19* (7)	5** (4)
% fetal incidence (% litter incidence)	23.2 (54.5)	11.0 (31.8)	11.4 (31.8)	3.6 (18.2)
Medial phalanx toe(s)				
Incomplete oss.- 5th right				
fetal incidence (litter incidence)	29 (10)	5** (3)	18 (7)	5** (4)
% fetal incidence (% litter incidence)	18.7 (45.5)	3.4 (13.6)	10.8 (31.8)	3.6 (18.2)
Incomplete oss.- 5th left				
fetal incidence (litter incidence)	29 (11)	7** (4)	20 (8)	5** (4)
% fetal incidence (% litter incidence)	18.7 (50.0)	4.8 (18.2)	12.0 (36.4)	3.6 (18.2)
Frontal bone				
Incomplete oss.- bilateral				
fetal incidence (litter incidence)	32 (13)	17 (7)	15* (7)	5** (5)
% fetal incidence (% litter incidence)	20.6 (59.1)	11.6 (31.8)	9.0 (31.8)	3.6 (22.7)
Parietal bone				
Incomplete oss.- bilateral				
fetal incidence (litter incidence)	30 (10)	13* (9)	13** (6)	5** (5)
% fetal incidence (% litter incidence)	19.4 (45.5)	8.9 (40.9)	7.8 (27.3)	3.6 (22.7)
Anomaly of vertebrae and ribs [b]				
fetal incidence (litter incidence)	0 (0)	1 (1)	0 (0)	0 (0)
% fetal incidence (% litter incidence)	0 (0)	0.68 (5)	0 (0)	0 (0)
% affected fetuses/litter	**0**	**5**	**0**	**0**
Fusion of ribs (cartilaginous part) [b]				
fetal incidence (litter incidence)	0 (0)	1 (1)	0 (0)	0 (0)
% fetal incidence (% litter incidence)	0 (0)	0.68 (5)	0 (0)	0 (0)
% affected fetuses/litter	**0**	**5**	**0**	**0**

157

Table 5c. Skeletal examinations [a]

Observations	Dose (mg/kg body weight/day)			
	0	50	100	200
#Fetuses (#litters) examined	155 (22)	146 (22)	167 (22)	140 (22)
Fusion of caudal vertebral bodies [b]				
fetal incidence (litter incidence)	0 (0)	0 (0)	1 (1)	0 (0)
% fetal incidence (% litter incidence)	0 (0)	0 (0)	0.60 (5)	0 (0)
% affected fetuses/litter	**0**	**0**	**5**	**0**

a Data extracted from the study report, Table 7, page 35, and pages 62-83.
b **% Affected fetuses/litter** were calculated by reviewers.
* $p<0.05$, ** $p<0.01$ Fisher's Exact

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study report concluded that dermal application of KBR 3023 (97.8% a.i.) at 0, 50, 100, or 200 mg/kg body weight/day to pregnant rabbits from gestation day 0-28 was associated with local reactions at the dose site at all dose levels. Slight erythema was evident at 200 mg/kg/day, squamous and cracked skin were apparent at the low-dose, and edema occurred at the high-dose level. Maternal behavior, mortality, food intakes, and body weight gains were unaffected by treatment. An increased incidence of females with soft feces at the high-dose level was attributed to stress caused by local reactions at the dose site. The maternal NOAEL is 100 mg/kg body weight/day. The maternal LOAEL is 200 mg/kg body weight/day.

Regarding intrauterine development, fertility rate, gestation rate, number of corpora lutea, pre-implantation loss, number of implantation sites, post-implantation loss, number of fetuses, fetal sex, fetal weight, and appearance and weight of placentas, all were unaffected by treatment at levels ≤200 mg/kg body weight/day. External, visceral, and skeletal examinations of the fetuses showed no effects of the test substance on fetal morphology at levels ≤200 mg/kg body weight/day. Teratogenic potential of KBR 3023 was not evident. The developmental NOAEL is 200 mg/kg body weight/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following dermal application of KBR 3023 (97.8% a.i.) at 0, 50, 100, or 200 mg/kg body weight/day to pregnant rabbits on days 0-28 of gestation, maternal toxicity was demonstrated by local reactions at the dose site. All treated females showed squamous skin beginning on gestation day 4-12 and continuing until necropsy. Slight erythema of the dose site, which appeared during the initial days of treatment and

158

treatment, the number of females with soft feces increased (low-dose, 10/24; mid-dose, 9/24; high-dose, 18/24 vs 3/24 controls). These increases were not dose-dependent and therefore, not of toxicological significance and were considered to be related to the stress caused by local reactions at the dose site.

There were no treatment-related effects noted in mortality, clinical signs, gross pathologic findings, or cesarean section parameters at any dose level. There were no treatment-related effects on body weights or food consumption at dose levels of ≤200 mg/kg/day.

The maternal LOAEL for dermal irritation is 50 mg/kg/day.
The maternal NOAEL for dermal irritation is <50 mg/kg/day.

The maternal LOAEL for systemic toxicity was not established.
The maternal NOAEL for systemic toxicity is ≥200 mg/kg body weight/day.

2. DEVELOPMENTAL TOXICITY: There were no treatment-related effects on developmental parameters (pre- and post-implantation losses, number of fetuses per litter), fetal deaths, resorptions, altered growth, or malformations. Skeletal retardations and variations noted in the treatment groups, when compared to the controls, indicate a decrease in incomplete ossification of the medial phalanx digit(s), medial phalanx toe(s), frontal bone, and parietal bone; however, this progressive effect on fetal ossification is not an adverse effect.

In the KBR 3023 rat developmental study (MRID 44408725), an increased incidence ($p \leq 0.05$) of incomplete ossification of the supraoccipital bone in the 400 mg/kg/day high-dose group was observed. This variation was considered to be treatment-related because (i) the fetal (43.6 % treated vs 30.3% controls) and litter (89% treated vs 75% controls) incidences were above the concurrent controls as well as the historical control ranges (fetal, 3.6-30.6, litter, 13.3-74.1) and (ii) supporting data were found in the submitted oral range finding study (MRID 44408722) in which an increased incidence of delayed bone ossification (70.8% treated fetuses vs 47.3% of controls, $p<0.01$) was observed in the 500 mg/kg/day group; litter incidences were not reported. While the increased incidence of incomplete ossification in rats is indeed an adverse effect, the decrease in incomplete ossification noted in rabbits is not an adverse effect.

No observations of developmental toxicity were noted in rabbits treated with KBR 3023:

a. Deaths/Resorptions: The numbers of resorptions/dam and viable fetuses/dam for the treatment groups were not significantly different from the concurrent controls.
b. Altered Growth: There were no treatment-related changes in fetal body weights at any dose level.
c. Developmental Variations: No significant, dose-related skeletal variations were noted which were outside the historical control range.
d. Malformations: There were no treatment-related developmental malformations noted at any dose level.

159

any dose level.

c. Developmental Variations: No significant, dose-related skeletal variations were noted which were outside the historical control range.
d. Malformations: There were no treatment-related developmental malformations noted at any dose level.

The developmental LOAEL was not established.
The developmental NOAEL is ≥200 mg/kg/day.

Dosing was considered adequate based on the results of the submitted range finding study (MRID 44408720) in which 3 pregnant female rabbits/dose were dosed at 0, 50, 200, 400, 700, or 1000 mg/kg body weight/day on gestation days 0-28. Maternal toxicity was observed at 1000 mg/kg body weight/day and was characterized by clinical signs of toxicity, gross pathology, severely decreased body weight gains, and decreased food consumption. The applied dose did not spread beyond the shaved area at the 50 mg/kg dose level. The area of humid and yellow stains increased in a dose-dependent manner for the 200 and 400 mg/kg groups. Severe lesions formed at the dose site for the higher dose groups of 700 and 1000 mg/kg. All treated animals showed erythema and squamous cells at the dose site. Edema and cracked skin were observed in animals dosed at ≥400 mg/kg.

This developmental toxicity study is classified **acceptable (§83-3[b]) and does satisfy the guideline requirements for a developmental toxicity study in the rabbit.**

C. STUDY DEFICIENCIES: None.

DATA EVALUATION RECORD

013529

KBR 3023

Study Type: 83-3a; A Developmental Toxicity Study of KBR 3023 in Rats

Work Assignment No. 3-53X (MRIDs 44408725 and 44408722 through 44408724)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-3a

DP BARCODEs:D241232, D241258, & D241261 SUBMISSION CODEs: S534142, S534200, & S534203

P.C. CODE: 070705 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): KBR 3023, Technical (97.4-97.7% a.i.)

SYNONYMS: 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester

CITATION: Astroff, A. B., (1996) A Developmental Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. Bayer Corporation, Stilwell, Kansas. Laboratory Study Number 95-622-DI, September 11, 1996. MRID 44408725. Unpublished

Holzum, B., (1990) Range-Finding Study for Embryotoxic Effects on Rats After Oral Administration. Bayer AG, Wuppertal, Germany. Laboratory Study Number T5033216/19645, August 28, 1990. MRID 44408722. Unpublished

Astroff, A. B., (1995) A Dose Range-Finding Developmental Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. Miles Inc., Stilwell, Kansas. Laboratory Study Number 94-612-ZK, March 10, 1995. MRID 44408723. Unpublished

Astroff, A. B., (1996) A Dose Range-Finding Developmental Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. Bayer Corporation, Stilwell, Kansas. Laboratory Study Number 95-622-DM, June 7, 1996. MRID 44408724. Unpublished

SPONSOR: Bayer AG, D-51368 Leverkusen, Bayerwerk, Bldg. 6210 Germany

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44408725), undiluted KBR 3023 (97.4-97.7% a.i.) was dermally administered to 30 Sprague-Dawley female rats/dose at dose levels of 0, 50, 200, or 400 mg/kg/day from days 0 through 20 of gestation.

162

Dermal effects were observed at the dose site of all treated groups. These findings were considered to be due to the Elizabethan collars (nasal stain) or topical application of the drug (scab formation or scaling and/or sloughing of skin). Slight maternal toxicity was noted as increases in absolute (↑9%, $p \leq 0.01$) and relative (↑5%, $p \leq 0.05$) liver weights in the high-dose group at final necropsy compared to controls. Histopathological data were not submitted. The liver was not a target organ in a previously submitted dermal oncogenicity study (MRID 44408719). Therefore, in the absence of corroborating histopathological data, the differences in liver weights are considered to be an adaptive response. The numbers of corpora lutea, implantation, viable fetuses, and the extent of pre-implantation losses were unaffected by treatment.

The maternal LOAEL is >400 mg/kg/day. The maternal NOAEL is 400 mg/kg/day.

There were no treatment-related external, visceral, or skeletal malformations or external and visceral variations noted at any dose level. Findings in all groups of incomplete ossification in the supraoccipital bone and increased incidences of rib ossification centers is attributed to maternal stress caused by the dermal dosing regimen.

The developmental LOAEL is >400 mg/kg/day. The developmental NOAEL is 400 mg/kg/day.

The developmental toxicity study in the rat is classified as **Acceptable/Guideline (870.3700)**.

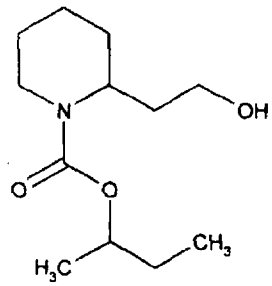
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

163

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023 technical
   Description: Clear, colorless liquid
   Lot/Batch #: 030693
   Purity: 97.4-97.7% a.i.
   CAS #: 119515-38-7
   Structure:



2. Vehicle: None

3. Test animals: Species: Rat
   Strain: Sprague-Dawley
   Age at mating: 12-15 weeks
   Weight at mating: 207.3-285.5 g (Day 0 gestation)
   Source: Sasco, Inc., Omaha, Nebraska
   Housing: Individually in suspended stainless steel wire-mesh cages
   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 hrs dark/12 hrs light

   Acclimation period (P): at least 6 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: June 26, 1995 end: August 10, 1995.
2. Mating: Rats were mated with a maximum of two females per male. Morning vaginal smears were taken and examined for the presence of sperm. The day on which sperm was identified in the vaginal smear was designated day 0 of gestation.

3. Animal Assignment: Animals were randomly assigned to dose groups as indicated in Table 1. Assignment was performed upon the identification of sperm in the vaginal smear.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
1 - Control	0	30
2 - Low (LDT)	50	30
3 - Mid (MDT)	200	30
4 - High (HDT)	400	30

4. Dose selection rationale: Dose selection for this study was based on two previous range-finding studies. In the first range-finding study (MRID 44408723), gravid Sprague Dawley rats were dermally administered KBR 3023 at 0, 50, 100, or 200 mg/kg body weight over gestation days 0 through 19. On gestation day 20, gross necropsies were performed on all dams and fetuses were examined externally. No compound-related maternal, embryo, or fetal effects were observed in any treatment groups. In view of these results, it was decided to perform another range-finding study with higher doses of KBR 3023 before proceeding to the definitive study. In the second range-finding study (MRID 44408724), gravid Sprague Dawley rats were dermally administered KBR 3023 at 0, 250, 500, 750, or 1000 mg/kg body weight/day over gestation days 0 through 20. On gestation day 20, gross necropsies were performed on all dams and fetuses were examined externally. The only compound-related effects were dermal scaling and sloughing of the dams. There were no significant necropsy findings or any effects on the reproductive, embryologic, or fetal parameters evaluated. Excessive spreading of the compound beyond the application area was noted at doses greater than 250 mg/kg/day. These results suggest that the physical limit of applications is less than 250 mg/kg/day. However, given the relatively short duration of a developmental toxicity study, a dose greater than 250 mg kg was used even though the spreading would be substantial.

Based on the results of these studies, 400 mg/kg/day was selected as the high dose for the subsequent dermal developmental toxicity study in rats. Low- and mid-dose levels chosen were 50 and 200 mg/kg/day, respectively.

An additional oral range-finding study (MRID 44408722) was submitted for review, but was not used in dose selection. In this range-finding study, groups of 25 inseminated Wistar rats were given KBR 3023 by stomach tube at doses of 0 and 500 mg/kg body weight/day, daily from day 6 to 16 of gestation. On day 20 of gestation, caesarean sections were carried out. Dams in the 500 mg/kg/day group showed diminished food and water intake and diminished body weight gain. The fetuses in the 500 mg/kg/day group exhibited an increased incidence of delayed bone ossification (70.8% treated

fetuses vs 47.3% of control fetuses, $p<0.01$), primarily in the vertebrae, skul, and hyoid. Litter incidences were not reported. All other reproductive or fetal parameters were comparable between groups or differences were not treatment-related.

5. Dosage preparation and analysis: KBR 3023 was used undiluted and stored at room temperature. The concentration of the test substance was determined by the Sponsor prior to use. Because the test substance was used undiluted, further testing of the concentration was not done. Dose homogeneity and stability analyses were also not performed by the study lab. According to the information provided by the Sponsor (page 14 of study report), the test material is stable for ≥6 months apparently under frozen conditions. In an accompanying study (MRID 44408719) submitted to EPA for review, the sponsor also cited a study (Bayer Corporation unpublished report No. 107418) in which the stability of KBR 3023 was determined after 7, 14, 21, and 28 days of storage at ambient temperature (22 C). The Bayer Corporation study concluded that KBR 3023 is stable for up to 28 days of storage at room temperature. The data from the Bayer study were not presented in MRID 44408719, however, the study was submitted to the Agency and reviewed separately.

6. Dosage administration: An area representing approximately 10% of the total surface area of each female was clipped at the beginning of the study and as needed thereafter. With two exceptions, all females wore Elizabethan collars for the duration of the study, beginning at least seven days prior to the initiation of dosing. The test formulation was administered by applying 0.0, 0.05, 0.20, and 0.40 ml/kg bodyweight of the undiluted solution (approximately 1 g/ml KBR 3023) to the animal's back. All doses were administered once daily on gestation days 0 through 20. Dosing was based on the daily body weight determination.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked twice daily for clinical signs. Body weights were recorded daily. Food consumption was recorded on gestation days 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20. The dose site was evaluated weekly and any spreading of the applied dose beyond the shaved dose site was noted. All dams were sacrificed following observations on day 20 of gestation. Aborting females were also sacrificed and subjected to full macroscopic examination. Examinations at sacrifice consisted of gross evaluations of the thoracic, abdominal, and pelvic cavities. The liver and thyroid were excised and weighed. The reproductive tract was removed and the following data were recorded:
   - pregnancy status
   - number of corpora lutea in each ovary
   - gravid uterine weight
   - numbers of live and dead fetuses
   - number of implantation sites
   - numbers of resorptions (early and late)
   - placental weight

166

2. Fetal Evaluations - Each fetus was weighed, sexed, and examined for external abnormalities. Approximately half of the live fetuses were given a gross visceral exam, then placed in Bouin's solution. Cranial examinations were performed on these fetuses according to the methods of Wilson. The other half of the fetuses were fixed in 70% denatured alcohol, eviscerated, processed with aqueous KOH and Alizarin Red S, and evaluated for general skeletal development.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: The following indices were calculated by the investigator:

Fertility Index= # pregnant/# sperm-positive x 100
Gestation Index= # with viable progeny/# pregnant x 100
Mating Index= # sperm-positive/# co-housed x 100

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations - All animals survived until the scheduled sacrifice. No treatment-related clinical signs of toxicity were noted. Clinical signs observed were considered to be due to the Elizabethan collars (nasal stain) or topical application of the drug (scab formation or scaling and/or sloughing of skin) observed in all dose groups. The investigators stated that these findings are "attributed to the adaptive response of the skin following the cumulative exposure to the test material".

2. Body Weight - Body weights and body weight gains were comparable between groups throughout the course of the study. Table 2 presents the results for body weight gain.

Table 2. Body Weight Gains (grams)[a]		
Group (mg/kg/day)	Entire Gestation Period[b]	Corrected Body Weight Gain[c]
Control	131.3 ± 5.63	59.5 ± 3.10
LDT	133.9 ± 3.67	60.3 ± 1.68
MDT	140.2 ± 3.20	55.8 ± 1.78
HDT	135.3 ± 4.80	57.2 ± 1.93

[a]Data extracted from (study number 95-622-DI and tables 3 and 8)
[b]The rats were dosed over the entire gestation period.
[c]Corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.

3. Food Consumption - Food consumption was not affected by treatment.

4. Gross Pathology - There were no treatment-related gross pathologic findings noted in any of the dams.

5. Organ Weights - Minor, but statistically significant increases in the absolute (↑9% $p \leq 0.01$) and relative (↑5%, $p \leq 0.05$) liver weights were noted in the high-dose group (Table 3). Thyroid weights were similar in the controls and dose groups.

Table 3. Mean absolute and relative liver weights at final necropsy.[a]

	Dose (mg/kg/day)			
	0	50	200	400
# Animals	24	29	27	28
Final Body Weight (g)	369	376	385	384
Absolute Liver Weight (g)[b]	15.27	15.60	16.16	16.68**
Relative Liver Weight[b]	4.14	4.15	4.19	4.35*

a Data extracted from the study report, Tables 6 and 7, pages 43 and 45.
b Absolute and relative liver weights were rounded to the nearest hundredth by the reviewers.
* and ** = $p \leq 0.05$ and $p \leq 0.01$, respectively.

6. Cesarean Section Data - Cesarean section observations are presented in Table 4. The numbers of corpora lutea, implantations, viable fetuses, the extent of resorptions/implantations and mean fetal weights were similar between control and

treated groups. There were no treatment-related differences observed in the high-dose group compared to controls, however the following were increased (not statistically significant) in the high-dose group (not including the one litter that was totally resorbed): (i) total resorptions (37 treated vs 22 controls); (ii) resorption/implantation ratio (9.20% treated vs 7.28% controls); (iii) early resorptions/dam (1.1 treated vs 0.8 controls); and (iv) post-implantation loss (9.5% treated vs 7.7% controls). The viable fetus/implantation ratio was decreased in the high-dose group (87.2% treated vs 92.3% controls). These differences were judged not to be treatment-related because (i) the absolute numbers from many of these parameters were higher in the high-dose group because more of the high-dose dams were pregnant than controls (28 treated vs 24 controls), (ii) the early resorptions/dam ratio was within historical control ranges (0.5-1.5); and (iii) percent post implantation loss was also within historical control ranges (3-14.4%). The decreased number of male offspring observed in the high-dose group (47% treated vs 57% controls) was considered an incidental occurrence.

Table 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	50	200	400
Animals Assigned (Mated)	30	30	30	30
Animals Pregnant	24	29	27	28
Pregnancy Rate (%)	(80)	(97)	(90)	(93)
Nonpregnant	6	1	3	2
Maternal Wastage				
Died	0	0	0	0
Died Pregnant	0	0	0	0
Died Nonpregnant	0	0	0	0
Aborted	0	0	0	0
Premature Delivery	0	0	0	0
Total Corpora Lutea(FTG)	345	428	423	418
Corpora Lutea/Dam	14.4±0.6[b]	14.8±0.5	15.7±0.4	15.5±0.3
Total Implantations(FTG)	302	379	395	402
Implantations/Dam	12.6±0.6	13.1±0.6	14.6±0.4	14.4±0.4
Total Litters	24	29	27	27
Total Live Fetuses	280	351	370	351
Live Fetuses/Dam	11.7±0.7	12.1±0.5	13.7±0.4	12.5±0.7
Total Dead Fetuses	0	1	0	0
Dead Fetuses/Dam	0	0.2±0.2	0	0
Total Resorptions	22	27	25	51 (37)[d]
Early	20	24	20	42 (31)[d]
Late	2	3	5	9
Resorptions/Dam	0.9±0.2	0.9±0.2	0.9±0.2	1.8±0.5 (1.3)[d]
Early	0.8±0.2	0.8±0.2	0.7±0.2	1.5±0.4 (1.1)[d]
Late	0.1±0.1	0.1±0.1	0.2±0.1	0.3±0.2
Litters with Total Resorptions	0	0	0	1
Mean Fetal Weight (g)	4.1±0.1	4.0±0.0	4.1±0.1	4.1±0.1
Males	4.2±0.1	4.1±0.0	4.2±0.1	4.2±0.1
Females	4.0±0.1	3.9±0.0	4.0±0.1	4.1±0.1
Sex Ratio (% Male)	57	51	49	47
Resorption/Implantation Ratio (%)	7.28[c]	7.12	6.33	12.69 (9.2)[d]
Viable Fetus/ Implantation Ratio (%)	92.3	93.4	93.7	87.2
Pre-implantation Loss (%)	11.8	11.7	6.2	7.2
Post-implantation Loss (%)	7.7	6.6	6.3	12.8 (9.5)[d]

[a]Data extracted from the study report, Tables 9 and 10; also Appendix IX, pages 160 through 163.
[b]Standard errors were rounded off to the nearest tenth by the reviewers.
FTG - Full term gestating females.
[c]Calculated by the reviewers.
[d] Number in parenthesis does not include litter totally resorbed.

B. DEVELOPMENTAL TOXICITY Fetal evaluations included external, visceral, and skeletal examinations. There were no treatment-related external, visceral, or skeletal malformations or external and visceral variations noted at any dose level. An increased incidence ($p \leq 0.05$) of incomplete ossification of the supraoccipital bone in the high-dose group was observed. This finding was considered to be treatment-related because (i) the fetal (43.6% treated vs 30.3% controls) and litter (89% treated vs 75% controls) incidences were above the concurrent controls as well as above the historical control ranges (fetal, 3.6-30.6; litter, 13.3-74.1) and (ii) supporting data were found in the submitted oral range-finding study (MRID 44408722) in which an increased incidence of delayed bone ossification (70.8% treated fetuses vs 47.3% of controls, $p<0.01$) was observed in the 500 mg/kg/day group; litter incidences were not reported. Also observed at the high-dose were increased fetal incidences of thoracic centra (↑34%, $p \leq 0.05$) and sternebrae #5 (↑17%, $p \leq 0.05$) and an increase in the number of rib ossification centers (↑113%, $p \leq 0.01$). However, the thoracic centra and sternebrae #5 findings were found at litter incidence rates within the historical control ranges (57.9-96.6% and 96.3-100%, respectively) and the increased incidences of rib ossification centers are considered to be not an adverse effect.

External, visceral, and skeletal fetal findings are presented in Tables 5a, 5b, 5c, and 5d below.

Table 5a. Fetal external examinations[a]

Observations [b]	Dose (mg/kg/day)			
	0	50	200	400
#Fetuses(litters) Examined	280(24)	351(29)	370(27)	351(27)
#Fetuses(#litters) with Malformations	1(1)	1(1)	1(1)	1(1)
#Fetuses(#litters) with Variations	0(0)	11(1)	1(1)	0(0)
Edema	0(0)	3.1**(3.4)	0(0)	0(0)
Pale	0(0)	0(0)	0.3 (3.7)	0(0)
Domed Head	0(0)	0(0)	0(0)	0.3(3.7)
Protruding Tongue	0(0)	0(0)	0(0)	0.3(3.7)
Torso Anasarca	0(0)	0.3(3.4)	0(0)	0(0)
Anal Imperforate	0(0)	0(0)	0.3(3.7)	0(0)
Acaudate Tail	0(0)	0(0)	0.3(3.7)	0(0)
Shortened Tail	0.4(4.2)	0(0)	0(0)	0(0)

a Data extracted from the study report, Table 12, pages 56, 57, and 58.
b Values for individual observations are as follows: %fetal(%litter).
** = $p \leq 0.01$.

Table 5b. Fetal visceral examinations.[a]

Observations [b]	Dose (mg/kg/day)			
	0	50	200	400
#Fetuses(litters) examined	135(24)	169(29)	178(27)	169(27)
#Fetuses(#litters) with Malformations	4(4)	1(1)	3(3)	2(2)
#Fetuses(#litters) with Variations	11(9)	3*(2*)	9(7)	20(13)
Left-sided Umbilical Artery	0.7(4.2)	0(0)	1.1(7.4)	1.2(7.4)
Enlarged Auricles	1.5(8.3)	0(0)	1.1(3.7)	1.8(11.1)
Reduced Heart Size	2.2(12.5)	0.6(3.4)	1.7(11.1)	0.6(3.7)
Hydroureter	5.9(25.0)	1.8(6.9)	2.8(14.8)	8.9(37.0)
Dilated Renal Pelvis	0.7(4.2)	0(0)	0.6(3.7)	0.6(3.7)
Malpositioned Kidney	0.7(4.2)	0(0)	0(0)	0(0)
Anophthalmia	0.7(4.2)	0(0)	0(0)	0(0)
Dilated Brain Ventricles	0(0)	0(0)	0(0)	0.6(3.7)
Cleft Palate	0(0)	0(0)	0(0)	0.6(3.7)

a Data extracted from the study report, Tables 13 and 14, pages 60 through 64.
b Values for individual observations are as follows: %fetal (%litter).
* = $p \leq 0.05$.

Table 5c. Fetal skeletal variations.[a]

Observations [b]	Dose (mg/kg/day) 0	50	200	400	Historical controls
#Fetuses (#litters) examined	145(24)	182(29)	192(27)	181(27)	847(116)
#Fetuses (#litters) with Variations	145(24)	182(29)	192(27)	181(27)	--
Incomplete ossifications:					
Supraoccipital	30.3 (75)	42.9 (90)	42.2 (82)	43.6* (89)	3.6-30.6 (13.3-74.1)
Thoracic Centra	42.8 (92)	45.6 (93)	50.5 (96)	57.5* (96)	15.7-68.7 (57.9-96.6)
Sternebrae #5	78.6 (100)	78.6 (100)	80.2 (100)	92.3* (100)	60.2-78.4 (96.3-100.0)
Abnormal ossifications:					
Rib ossification centers	11.7 (46)	8.2 (38)	20.3 (48)	24.9** (67)	0.5-20.0 (3.7-61.5)

a Data extracted from the study report, Tables 15 and 16, pages 66 through 85.
b Values for individual observations are as follows: %fetal (%litter). % litter rounded to whole number by the reviewers.
* = p≤0.05 and 0.01, respectively. --=information not provided.

Table 5d. Fetal skeletal malformations.[a]

Observations [b]	Dose (mg/kg/day)			
	0	50	200	400
#Fetuses (#litters) examined	145(24)	182(29)	192(27)	181(27)
#Fetuses (#litters) with Malformations	1(1)	1(1)	1(1)	0(0)
Missing Lumbar Arch	0.7(4)	0.5(3)	0.5(4)	0(0)
Missing Lumbar Centra	0.7(4)	0.5(3)	0.5(4)	0(0)

a Data extracted from the study report, Table 16, page 76.
b Values for individual observations are as follow: %fetal, (%litter). %litter rounded to whole number by the reviewers.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS The study author concluded that dermal administration of KBR 3023 at up to 400 mg/kg/day to pregnant rats during days 0-20 of gestation provoked no reproductive toxicity, embryo toxicity or teratogenic effects. The test article did cause cutaneous sloughing and scaling at the site of application. In addition, the high-dose group had significantly elevated absolute and relative liver weights. The maternal LOAEL is 400 mg/kg/day. A developmental LOAEL was not observed.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Following dermal administration of KBR 3023 (97.4-97.7% a.i.) at 50, 200, and 400 mg/kg/day to pregnant rats during days 0-20 of gestation, all treated groups experienced dermal sloughing and scaling in the area of administration. This finding was considered an adaptive response to prolonged exposure to the test material.

   The high-dose group had a marginal increase in absolute (↑9%, $p \leq 0.01$) and relative (↑5%, $p \leq 0.05$) liver weights at final necropsy. Histopathological data were not submitted. The liver was not a target organ in a previously submitted dermal oncogenicity study (MRID 44408719). Therefore, in the absence of corroborating histopathological data, the differences in liver weights are considered to be an adaptive response. The numbers of corpora lutea, implantations, viable fetuses, and the extent of pre-implantation losses were unaffected by treatment.

Maternal LOAEL >400 mg/kg/day
Maternal NOAEL = 400 mg/kg/day

2. DEVELOPMENTAL TOXICITY: A treatment-related increase in a skeletal variation was observed.

   a. Deaths/Resorptions: No effects on fetal viability were observed.
   b. Altered Growth: No effects on fetal growth were observed.
   c. Developmental Variations: An increased incidence ($p \leq 0.05$) of incomplete ossification of the supraoccipital bone in the high-dose group was observed. This finding is not considered to be treatment-related because (i) fetal and litter incidences in the controls were at the maximum historical levels, and (ii) there was no evidence of a dose response (all dosed groups had similar incidences), and (iii) it is inappropriate to compare incidence in a dermal study against oral historical values. Dermal historical data would likely have higher ranges for ossification variations. Thus, incomplete ossification is most likely a manifestation of maternal stress caused by the unusual dermal dosing regimen.

      Abnormal rib ossification centers in the high-dose fetuses (24.9% v 11.7%) are also not considered to be adverse because (i) fetal and litter incidences were marginally greater than historical values, (ii) it is inappropriate to compare incidence in a dermal study against oral historical values, (iii) "rib" ossification centers are frequently connected via cartilage to the vertebra transverse process (this can only be confirmed via dual staining techniques not used in this study), and (iv) an increase in rib ossification centers is associated with maternal stress. These conclusions regarding skeletal variations are based upon discussions with Sue Makris (HED) and the study director at Bayer AG on May 19, 1999.

   d. Malformations: No treatment-related malformations were observed.

Developmental Toxicity LOAEL > 400 mg/kg/day
Developmental Toxicity NOAEL = 400 mg/kg/day

The developmental toxicity study in the rat is classified as **Acceptable/Guideline (870.3700)** and satisfies the guideline requirement for a developmental toxicity study in the rat.

C. STUDY DEFICIENCIES: None.

176

013529

DATA EVALUATION RECORD

KBR 3023

Study Type: 83-4(a); A Two-Generation Reproduction Study and One-Generation Reproduction Pilot Study in Rats with KBR 3023.

Work Assignment No. 3-47E (MRIDs 44408727 and 44408726)

Prepared for

Health Effects Division
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Signature: _____
Date: 12/04/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

EPA Work Assignment Manager: Sanjivani Diwan, PhD
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4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Two Generation Reproduction Study - Rat
OPPTS Number: 870.3700 OPP Guideline Number: §83-4

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

TEST MATERIAL (PURITY): KBR 3023 Technical (96.7-97.7% a.i).

SYNONYMS: 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester

CITATION: Astroff, A.B., (1996). A Two Generation Reproductive Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. Bayer Corporation, Agricultural Division, Stilwell, Kansas. Laboratory Study Number 95-622-EH, December 18, 1996. MRID 44408727. Unpublished.

Astroff, A.B., (1995). A Pilot Reproductive Toxicity Study with KBR 3023 Technical in the Sprague-Dawley Rat. Bayer Corporation, Agricultural Division, Stilwell, Kansas. Laboratory Study Number 94-972-DA, September 8, 1995. MRID 44408726. Unpublished

SPONSOR: Bayer AG, D-51368 Leverkusen, Bayerwerk, Bldg. 6210, Germany

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 44408727), KBR 3023 (96.7-97.7% a.i) was administered to the shaved skin of Sprague-Dawley rats (30/sex/dose) at dose levels of 0, 50, 100, or 200 mg/kg/day for 5 days/week. P male and female exposure to KBR 3023 began at 8-9 weeks of age and lasted for 10 weeks prior to mating to produce F_1 pups. At 21 days of age, F_1 pups (30/sex/dose) were selected to become the parents of the F_2 generation and were treated with the same levels of KBR 3023 as their dam for 10 weeks prior to mating. P and F_1 females were continuously dosed throughout gestation and lactation.

No treatment-related parental systemic toxicity was observed. No treatment-related clinical findings, increases in mortality, differences in body weight gains or food consumption, or changes in reproductive performance were noted in the P females or males at any dose level. Dermal findings at the treatment site (acanthosis and hyperkeratosis) were considered to be due to the treatment methodology and not compound-related.

The systemic NOAEL is >200 mg/kg/day; the NOEL = 200 mg/kg/day.

There was no treatment-related reproductive toxicity. There were no clinical signs of toxicity or changes in pup weight, viability, or litter sizes noted in the pups at any dose level for the F_1 or F_2 generations. No treatment-related macroscopic findings in the F_1 or F_2 pups were observed at any dose level.

The reproductive NOAEL is >200 mg/kg/day; the NOEL = 200 mg/kg/day.

Dosing was considered adequate based on the results of a previously reviewed range finding study (MRID 44408716) in which KBR 3023 (99.2% a.i.) was applied to the shaved skin of young adult Sprague-Dawley rats (10-20/sex/dose) at dose levels of 0, 80, 200, 500, or 1000 mg/kg/day for 5 days/week, 5 hours/day, for 90 days. KBR 3023 elicited diffuse liver hypertrophy in both sexes treated at 1000, 500, or 200 mg/kg/day. The LOAEL for systemic toxicity is 500 mg/kg/day, based on liver and kidney effects, and the NOAEL is 200 mg/kg/day.

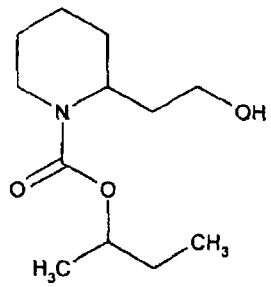
This reproductive toxicity study is classified **acceptable (§83-4|a|) and does satisfy the guideline requirement for a multi-generational reproductive toxicity (reproduction) study in rats.**

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, colorless liquid
   Lot/Batch #: 030693
   Purity: 96.7-97.7%
   CAS #: 119515-38-7
   Structure:



2. Vehicle: None

3. Test animals: Species: rat
   Strain: Sprague-Dawley
   Age at start of dosing: P: 8-9 weeks. F_1: 21 days (weaning).
   Weight at start of dosing:
   (P) Males: 197.2-275.5 g Females: 130.4-198.7 g
   (F_1) Males: 84.0-206.3 g Females: 72.5-162.1 g
   Source: Sasco Inc. Omaha, NE
   Housing: Suspended stainless steel cages during the pre-mating (1/cage) and mating (2/cage) period, in plastic cages during gestation (1 dam/cage) and lactation (1 dam and litter/cage) and for up to approximately one week post-weaning (1 litter/cage).
   Diet: Purina Mills Rodent Lab Chow 5001-4, *ad libitum*
   Water: Municipal tap water, *ad libitum*
   Environmental conditions:
   Temperature: 18-26°C
   Humidity: 40-70%
   Air changes: Not reported
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P): ≥6 days

B. PROCEDURES AND STUDY DESIGN

1. Study duration (in life dates): start - 5/2/95; end - 2/11/96.

2. Mating procedure: One male was caged with one female from the same test group until sperm (or a copulation plug) was observed in the vaginal tract. Cohabitation lasted no longer than 21 days. After successful mating, each pregnant female was individually placed into a cage with a solid bottom where it was kept throughout gestation and lactation.

3. Study schedule: Starting at approximately 8-9 weeks of age, P animals were administered the test compound dermally for 10 weeks before mating. After the mating period, P males were sacrificed and necropsied. P female dosing continued throughout gestation and lactation. Litters were culled to 8 pups/litter on day 4 of lactation and weaned at 21 days of age. P dams were sacrificed and necropsied after weaning. Upon weaning, F_1 animals were dosed dermally with the same dose of test compound as their dams for 10 weeks before they were mated to produce the F_2 generation. F_1 adults and F_2 litters were sacrificed and necropsied at weaning. Exposure of all animals to the test material was 5 days/week throughout the study.

4. Animal assignment: P animals were randomly assigned (stratified by weight) to test groups (Table 1).

Table 1. Animal assignment[a,b]

Test Group	Dose (mg/kg/day)	Animals/group	
		P Males	P Females
Control	0	30	30
Low-dose	50	30	30
Mid-dose	100	30	30
High-dose	200	30	30

a Test compound was administered 5 days/week from the beginning of the study until sacrifice.
b Data extracted from the study report page 20.

5. Dose selection rationale: Dose selection was based on the results of a previously reviewed 90-day dermal toxicity study (MRID 44408716) and a pilot reproductive toxicity study (MRID 44408726). In the 90-day dermal toxicity study, KBR 3023 (99.2% a.i.) was applied to the shaved skin of young adult Sprague-Dawley rats (10-20/sex/dose) at dose levels of 0, 80, 200, 500, or 1000 mg/kg/day for 5 days/week, 5 hours/day, for 90

days. Following the 90 days of treatment, 10 rats/sex/dose were sacrificed. The remaining 10 rats/sex in the 0 and 1000 mg/kg/day groups were maintained without treatment for an additional 4 weeks to assess recovery potential. Treatment-related lesions in rats from all treatment groups consisted of scabs, red foci, and exfoliation limited to the dose site. The incidence and frequency of scabs and red foci were concentration-dependent. Females in all treatment groups exhibited very slight erythema. KBR 3023 was hepatotoxic, as evidenced by diffuse liver hypertrophy in both sexes treated at 1000, 500, or 200 mg/kg/day. Individual liver cells were necrotic in the 1000 and 500 mg/kg/day group males (3-4/group), and in one 1000 mg/kg/day group female. Absolute and relative liver weights were increased in the 1000 (23-28%) and 500 mg/kg/day (8-14%) treatment groups. KBR 3023 was toxic to kidneys, causing minimal to slight hyaline degeneration in both sexes treated at 1000 or 500 mg/kg/day. The kidneys of the 1000 mg/kg/day group males exhibited an increased incidence of foci of tubular regeneration compared to the other test groups, chronic kidney inflammation (3/10 males), and increased absolute and relative weights (24-26%). The 500 mg/kg/day group males had increased relative kidney weights (14%). Urine pH and urobilinogen (males only) was decreased in the 1000 mg/kg/day group, and urine pH was decreased in the 500 mg/kg/day group compared to the controls. All compound-related changes returned to normal by the end of the 4-week recovery period. Other than dermal response on treated skin, no toxic response to KBR 3023 was noted in the 80 mg/kg/day treatment groups. No animals died during the study. There were no treatment-related differences in body weights or body weight gains, food consumption, ophthalmology, hematology parameters, clinical blood chemistry, or macroscopic organ morphology between rats in the treated and control groups. No neoplastic tissue was observed. The investigators determined that the LOEL for systemic toxicity was 200 mg/kg/day, based on diffuse liver hypertrophy in both sexes; and the NOEL was 80 mg/kg/day. The LOEL for dermal toxicity was determined to be 80 mg/kg/day based on the presence of scabs, red foci, and exfoliation at the dose site; a NOEL was not established.

In a pilot reproduction study (MRID 44408726), Sprague-Dawley rats were fitted with Elizabethan collars and dermally adminstered KBR 3023 at 0 or 200 mg/kg/day for 5 days/week throughout the study. Dosing started with a two-week pre-mating period and continued throughout mating, gestation and lactation. Litters were culled to 8 pups/litter on day 4 of lactation and weaned at 21 days of age. One pup/sex/litter from the F_1 generation was maintained beyond weaning and dermally treated with KBR 3023. There were no treatment-related clinical signs (including signs of dermal irritation) or effects on body weight or food consumption in the P generation and no treatment-related clinical signs or effects on body weight in the F_1 generation. There were no significant effects on any litter parameters or reproductive indices. No treatment-related necropsy findings were observed in either generation.

Based on the results of these studies, 200 mg/kg/day was chosen as the high-dose treatment level for the two-generation reproduction study. The low- and mid-dose levels chosen were 50 and 100 mg/kg/day, respectively.

6. Dosage preparation and analysis: KBR 3023 was applied neat to the animals' backs; therefore, no preparation and consequently, no analysis of homogeneity was required. The concentration of the batch of KBR 3023 used in this study was determined before, during, and after the study. Aliquots of the batch were maintained frozen prior to use. During use, an aliquot was thawed and maintained at room temperature. The compound has been reported to be stable at room temperature for up to four weeks. The test material was not adjusted for the percent of active ingredient.

   Results - Homogeneity Analysis: Not necessary.

   Stability Analysis: It was stated that neat KBR 3023 has been previously shown to be stable at room temperature for up to 4 weeks.

   Concentration Analysis: The percent active ingredient of KBR 3023 in batch # 030693 was 97.7% (prior to study start), 97.4% (during study), and 96.7% (after study termination).

   The information provided indicated that the test compound was stable for the duration of the study and that compound concentration was within acceptable limits.

7. Dosage administration: All P animals wore Elizabethan collars for the duration of the study, beginning at least seven days prior to the initiation of dosing. F_1 pups received their collars when placed into individual cages, approximately one week after weaning. Collars were removed during weighing. An area representing approximately 10% of the total surface area of each animal was clipped at the beginning of the study and as needed thereafter. The test formulation was administered by applying 0.0, 0.05, 0.10, and 0.20 ml/kg body weight of the undiluted solution (KBR 3023 density is approximately 1 g/ml) to the animal's back. All doses were administered once daily, 5 days/week (Monday-Friday). Dosing was based on the weekly body weight determination, except for F_1 pups, where body weight measurements were taken every three days from weaning until the start of the premating phase.

C. OBSERVATIONS

1. Parental animals: All parental animals were observed twice daily for clinical signs, morbidity and mortality, and once daily on weekends and holidays. In addition, a physical and clinical exam was performed once a week. Males were weighed pre-dose and weekly throughout the study. Females were weighed pre-dose, weekly throughout mating and pre-mating, on gestation days 0, 6, 13, and 20, and on lactation days 0, 4, 7, 14, and 21. Body weight gains, but not body weights, were compared statistically. Food consumption was measured daily for both sexes during the premating period. During gestation, maternal food consumption was recorded on days 0, 6, 13, and 20. During lactation, food consumption was recorded on days 0, 7, 14, and 21.

183

2. Litter observations: Litters were examined in detail once daily for clinical signs, morbidity, and mortality. Table 2 shows litter observations (X).

Table 2. F_1 and F_2 litter observations[a]

Observation	Time of observation (lactation day)					
	Day 0	Day4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
External alterations	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Sex of each pup	X	X	X	X	X	X

a Data extracted from the study report, page 22.
b Before standardization (culling).
c After standardization (culling).

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter with 4/sex/litter, as nearly as possible; excess pups were killed and necropsied.

3. Postmortem observations:

1) Parental animals: Sires were sacrificed after the mating period. Dams were sacrificed after the litters were weaned. These animals were subjected to postmortem examinations, including an examination of external surfaces and major organs. Uterine implant sites were also counted. In addition, the CHECKED (X) tissues were collected and examined histologically in all animals that died or were killed *in extremis* and those sacrificed on schedule. Additionally, the (XX) organs were weighed.

84

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

2) Offspring: Preweaning pups that died or were stillborn, day 4 culled pups, and weanling pups were examined macroscopically internally and externally. No tissues were collected from the pups.

D. DATA ANALYSIS

1. Statistical analyses: All collected data were subjected to routine, appropriate statistical procedures.

2. Indices:

Reproductive indices: The following reproductive indices as presented in the study report were calculated for the P and F_1 adults:

mating index = # of inseminated females/# of females paired x 100%
fertility index = # of females pregnant/# of inseminated females x 100%

Offspring viability indices: The following viability indices as presented in the study report were calculated for the F_1 and F_2 litters:

gestation index = # of females delivering a live litter/# of females pregnant x 100%
birth index = total # of pups/litter/ total # of implantation sites/litter x 100%
livebirth index = # of live pups at birth/litter/total # of pups born/litter x 100%
viability index = # of live pups/litter at day 4 (preculling)/# of live pups born/litter x 100%
lactation index = # of live pups/litter on day 21/# of live pups/litter at day 4 (postcull) x 100%
sex ratio = # of males/number of pups x 100%

3. Historical control data: No historical control data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs: No treatment-related mortality or clinical findings were noted in the P or F_1 males or females during premating, gestation, or lactation. In the P generation, one mid-dose female was found dead during the premating phase. In the F_1 generation, one control male and one high-dose male were found dead during the premating/mating phase. One high-dose male was sacrificed after exhibiting labored breathing, urine stain, rough coat, unthrifty appearance, and being cold to the touch. Clinical signs observed were lacrimation, scab formation, and lacerations. These signs were believed to be due to the wearing of Elizabethan collars and were not considered of toxicological significance.

   Scaling/sloughing of the skin at the treatment site was observed in the P generation in two high-dose males, two mid-dose females, and five high-dose females. In the F_1 adults, these signs were observed in one high-dose male and one mid-dose female. These signs were not believed due to compound biochemical toxicity, but rather due to physical effects of the compound on the skin. Similar effects have been seen when skin has been exposed to water or petrolatum. Therefore, these effects were not considered compound-related, but rather an adaptive response of the skin to exposure.

2. Body weight and food consumption: Sporadic, statistically significant ($p \leq 0.05$ or 0.01) decreases and increases in body weight gains occurred in P and F_1 adults during premating. Due to the sporadic nature and the occurrence of both increases and decreases, these differences were not considered treatment-related. Absolute body weights were not compared. However, the differences in body weights between controls and treatment groups in the P and F_1 generations at the end of premating were <7% (calculated by reviewers). No significant differences in body weight gains were noted in

186

P or F_1 females during gestation or lactation.

Sporadic, statistically significant ($p \leq 0.05$ or 0.01) decreases and increases in food consumption occurred in the P adults and F_1 females during premating and in the P and F_1 females during gestation and/or lactation. F_1 males did not show any significant differences between treated and control groups. Due to their sporadic nature and the presence of increases and decreases, these differences were not considered to be of toxicological concern.

3. Reproductive function:

   a. Estrous cycle length and periodicity: There were no significant differences in estrous cycle length and periodicity in the P and F_1 dams in this study. Precoital intervals were comparable to the controls (Table 3).
   b. Sperm measures: No sperm parameter observations were made in this study; however, there were no indications of treatment-related fertility abnormalities in P or F_1 male rats during this study.
   c. Sexual maturation (F_1): No observations were made pertaining to the sexual maturation rates of the F_1 or F_2 litters.

4. Reproductive performance: Reproductive performance results are presented in Table 3. There were no treatment-related effects noted in the reproductive performance of the P adults.

Table 3. Reproductive performance.[a]

Observation	Dose Group (mg/kg/day)			
	0	50	100	200
P Generation - Litter F_1				
Day to Insemination	3.5	3.5	3.8	3.4
Estrous Cycle Length (days)	4.6	4.2	4.2	4.5
Mating Index	100	100	96.7	100
Fertility Index	100	96.7	89.7	100
Gestation Index	100	96.6	100	100
Mean Gestation Interval (days)	22.5	22.5	22.0	22.5
Mean Implantations	12.6	12.8	12.9	12.1
Number of Litters	30	28	26	30
F_1 Generation - Litter F_2				
Day to Insemination	3.4	3.4	4.2	4.3
Estrous Cycle Length (days)	5.4	6.5	5.5	5.3
Mating Index	100	100	96.7	100
Fertility Index	96.7	90.0	89.7	96.7
Gestation Index	96.6	100	100	100
Mean Gestation Interval (days)	22.5	22.6	22.4	22.5
Mean Implantations	12.0	12.3	12.3	11.4
Number of Litters	28	27	26	29

a Data extracted from the study report Tables 4 and 18, pages 47 and 86.

5. Parental postmortem results

a) Organ weights: Compared to controls, absolute kidney and liver weights were decreased ($p \leq 0.05$) in the low- (kidneys - ↓7%; liver - ↓9%) and mid-dose P males (kidneys - ↓7%; liver - ↓10%), but not in the high-dose males (Table 4). The relative organ weights were not affected. Liver weights were decreased ($p \leq 0.05$) in the mid-

dose F_1 males (absolute ↓13% and relative ↓9%). Because there was no dose-dependent response, these differences were not considered of toxicological concern. There were no significant differences in the absolute and relative organ weights of the P and F_1 females.

Table 4. Absolute and relative organ weights in P and F_1 males at necropsy.[a]

Organ	Dose Group (mg/kg/day)			
	0	50	100	200
P Generation Males				
Absolute Organ Weights				
Kidneys	3.681	3.409*	3.434*	3.448
Liver	18.156	16.455*	16.428*	17.450
Testes	3.778	3.690	3.719	3.648
Relative Organ Weights				
Kidneys	0.923	0.885	0.891	0.914
Liver	4.530	4.271	4.242	4.617
Testes	0.953	0.960	0.967	0.970
F_1 Generation Males				
Absolute Organ Weights				
Kidneys	3.458	3.358	3.281	3.237
Liver	19.151	17.474	16.609*	17.684
Testes	3.828	3.652	3.718	3.595
Relative Organ Weights				
Kidneys	0.877	0.892	0.877	0.875
Liver	4.856	4.625	4.430*	4.759
Testes	0.973	0.974	0.999	0.985

a Data extracted from the study report P generation and F_1 generation Tables OW1K-SUM, pages 444 and 785.

* Significantly different from controls at $p \leq 0.05$.

b) Pathology: There were no significant necropsy findings in the P or F_1 adults. Dermal effects noted have been discussed (see Mortality and clinical signs) and were considered a result of the treatment methodology and not a direct effect of the the test compound itself.

B. OFFSPRING

1. Viability and clinical signs: There were no treatment-related clinical signs or treatment-related changes in mean litter size or viability indices in the F_1 or F_2 generation pups. Mean litter size and viability results from F_1 and F_2 litters during lactation are summarized in Tables 5a and b, respectively.

Table 5a. F_1 generation mean litter size and viability.[a]

Observation	Dose Group (mg/kg/day)			
	0	50	100	200
Mean litter size				
Day 0	12	11	12	11
Day 4[b]	12	11	11	11
Day 4[c]	8	8	8	8
Day 21	8	8	8	8
Number live pups[d]				
Day 0	349	318	305	325
Day 4[b]	345	312	296	316
Day 4[c]	227	221	206	231
Day 7	225	220	206	229
Day 14	225	220	206	229
Day 21	225	218	206	228
Number deaths[d]				
Days 0-4	4	6	9	9
Days 5-21	2	3	0	3
% Males (day 0)	50.2	44.8	47.1	45.4
Survival indices (%)				
Birth	94.0	90.8	92.2	89.3
Livebirth	97.5	98.1	98.4	98.1
Viability	98.8	98.3	96.8	97.5
Lactation	99.0	98.7	100	98.9

a Data extracted from the study report Tables 14 and Appendix IV, pages 71, 72 and 154 through 161.
b Before standardization (culling).
c After standardization (culling).
d Calculated by the reviewers.

Table 5b. F_2 generation mean litter size and viability.[a]

Observation	Dose Group (mg/kg/day)			
	0	50	100	200
Mean litter size				
Day 0	11	11	11	10
Day 4[b]	10	10	11	9
Day 4[c]	8	8	8	7
Day 21	8	8	8	7
Number live pups[d]				
Day 0	301	296	285	279
Day 4[b]	292	280	273	260
Day 4[c]	220	204	203	204
Day 7	220	204	202	204
Day 14	219	203	202	204
Day 21	219	203	202	203
Number deaths[d]				
Days 0-4	9	16	12	19
Days 5-21	1	1	1	1
% Males (day 0)	52.1	56.0	54.9	51.9
Survival indices (%)				
Birth	90.6	91.8	92.8	87.5
Livebirth	99.1	96.6	95.9	94.4
Viability	97.1	93.1	96.0	91.7
Lactation	99.6	99.5	99.5	99.5

a Data extracted from the study report Table 28 and Appendix XIX, pages 110, 111 and 293 through 300.
b Before standardization (culling).
c After standardization (culling).
d Calculated by the reviewers.

2. Body weight: There were no significant differences in pup weight or pup body weight gain for the F_1 or F_2 litters (Table 6).

Table 6. Mean pup weights for the F_1 and F_2 litters (g).[a]

Day of lactation	Dose Group (mg/kg/day)			
	0	50	100	200
F_1 generation				
Day 0	6.7	6.7	6.7	6.8
Day 4[b]	10.3	10.2	10.0	10.5
Day 4[c]	10.3	10.3	10.0	10.5
Day 7	16.1	15.7	15.6	15.9
Day 14	29.9	29.7	29.3	29.1
Day 21	48.0	48.3	46.8	47.5
F_2 generation				
Day 0	6.8	6.7	6.5	6.8
Day 4[b]	10.6	10.1	10.0	10.3
Day 4[c]	10.6	10.1	10.0	10.2
Day 7	16.0	15.4	15.2	15.4
Day 14	29.5	29.0	28.7	28.7
Day 21	46.5	46.9	44.4	45.7

a Data extracted from the study report Tables 12 and 26, pages 63 through 65 and 102 through 104.
b Before standardization (culling).
c After standardization (culling).

3. Offspring postmortem results:

a) Organ weights: Organs were not weighed for any of the pups in this study.

b) Pathology

1) Macroscopic examination: There were no treatment-related findings at necropsy in the F_1 or F_2 pups.

2) Microscopic examination: Histopathology was not performed on any offspring in this study.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Dermal administration of KBR 3023 at 50, 100, or 200 mg/kg/day did not cause any treatment-related clinical signs or effects on body weight or food consumption in adults or offspring throughout the study. There were no treatment-

related effects on any reproductive or litter parameters. Dermal findings (acanthosis and hyperkeratosis) were observed in both generations, but were considered to be due to the dermal application methodology, although the relationship between these findings and KBR 3023 cannot be excluded. No treatment-related necropsy findings were observed in the adults or pups, other than the aforementioned dermal findings. No treatment-related histopathologic findings were observed in the reproductive organs of either males or females.
The NOEL for reproductive toxicity was 200 mg/kg/day.

B. REVIEWER'S DISCUSSION: In this two-generation reproduction study, KBR 3023 was administered dermally to Sprague-Dawley rats at dose levels of 0, 50, 100, or 200 mg/kg/day (5 days/week). P male and female animal exposure to KBR 3023 (30/sex/dose) began at 8-9 weeks of age and lasted for 10 weeks prior to mating to produce the F_1 litters. At 21 days of age, F_1 pups (30/sex/dose) were selected to become the F_1 parents of the F_2 generation. F_1 parents were given the same levels of KBR 3023 as their dam for 10 weeks prior to mating.

The analytical data indicated that the actual concentration of KBR 3023 was within required limits. Because the compound was administered neat, no homogeneity data were required and previous studies demonstrating stability were acceptable.

1. Systemic Toxicity: No treatment-related parental systemic toxicity was observed. No treatment-related clinical findings, increases in mortality, differences in body weight gains or food consumption, or changes in reproductive performance were noted in the P females or males at any dose level. Dermal findings at the treatment site (acanthosis and hyperkeratosis) were considered to be due to the treatment methodology and were not considered compound-related.

The NOAEL for systemic toxicity was not established. The systemic NOEL is $\geq$200 mg/kg/day.

2. Reproductive Toxicity. There were no treatment-related clinical signs of toxicity or changes in pup weight, viability, or litter sizes noted in the pups at any dose level for the F_1 or F_2 generations. No treatment-related macroscopic findings were observed in the F_1 or F_2 pups at any dose level.

The NOAEL for reproductive toxicity was not established. The reproductive NOEL is $\geq$200 mg/kg/day.

No toxicity was seen in a pilot reproductive toxicity study in which Sprague-Dawley rats were dosed at 200 mg/kg/day (MRID 44408726). Nevertheless, dosing was considered adequate based on the results of a 90-day dermal toxicity study (MRID 44408716) in which KBR 3023 (99.2% a.i.) was applied to the shaved skin of young adult Sprague-Dawley rats (10-20/sex/dose) at dose levels of 0, 80, 200, 500, or 1000 mg/kg/day for 5 days/week, 5

hours/day, for 90 days. KBR 3023 elicited diffuse liver hypertrophy in both sexes treated at 1000, 500, or 200 mg/kg/day. The LOAEL for systemic toxicity was determined to be 500 mg/kg/day, based on liver and kidney effects, including diffuse liver hypertrophy, individual necrotic liver cells, slight hyaline degeneration in the kidneys, an increased incidence of foci of tubular regeneration, and chronic kidney inflammation; the NOAEL is 200 mg/kg/day. The LOAEL for dermal toxicity is 80 mg/kg/day based on the presence of scabs, red foci, and exfoliation at the dose site; a dermal NOAEL was not established.

This reproductive toxicity study is classified **acceptable (§83-4[a]) and does satisfy the guideline requirement for a multi-generational reproductive toxicity (reproduction) study in rats.**

C. STUDY DEFICIENCIES: There were no deficiencies noted in this study.

013529

DATA EVALUATION RECORD

KBR 3023

Study Type: 83-5; Combined Chronic/Oncogenicity Study - Rats

Work Assignment No. 3-47G (MRID 44408728)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Pamela M. Hurley, Ph.D. Pamela M Hurley 5/10/99
Registration Action Branch 2 (7509C)

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

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Oncogenicity
OPPTS Number: 870.4300 OPP Guideline Number: §83-5

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

TEST MATERIAL (PURITY): KBR 3023 Technical (96.7-98.5% a.i.)

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

CITATION: Wahle, B.S., and Christenson, W.R., (1996) Technical Grade KBR 3023: A Combined Chronic Toxicity/Oncogenicity Testing Study in the Rat. Bayer Corporation, Stilwell, KS, Laboratory Project Study ID# 92-222-OM, December 17, 1996. MRID 44408728. Unpublished.

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

EXECUTIVE SUMMARY:

In a combined chronic/oncogenicity study (MRID 44408728), undiluted KBR 3023 (technical. 96.7-98.5% a.i.) was administered dermally on the dorsal aspect of the trunk to 50 Sprague Dawley rats/sex/dose at dose levels of 0, 50, 100, or 200 mg/kg/day on 5 consecutive days/week for 24 months. In addition, 10-20 rats/sex/group were terminated at 12 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 parameters were also unaffected by treatment. In the 2-year males, there was a dose-dependent increase in the incidence of liver cystic degeneration (8/47, 11/46, 14/49, 20/47, in controls, low-, mid-, and high-dose groups, respectively; statistically significant [$p<0.05$] in the high-dose group only). These findings were not corroborated by changes in organ weight or in blood chemistry parameters.

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

The dose levels used in this study were from an EPA approved protocol.

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

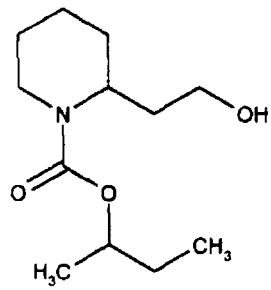
The submitted study is classified as **acceptable (§83-5)** and satisfies the guideline requirements for a combined chronic/oncogenicity study in the rat.

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: 96.7-98.5% 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:



   Other comments: The test material was stored under freezer conditions at all times. Dosing aliquots were removed every two weeks and stored under room temperature.

2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Sprague Dawley
   Age and weight at study initiation: Approximately 8 weeks; 180.2-182.8g (males) and 149.7-152.5g (females)
   Source: Charles River Breeders, Portage, MI
   Housing: Suspended stainless steel wire-mesh cages and polycarbonate cages; 1 rat/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: 10/4/93 End: Not specified

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 [a]			
		Interim Study (1-year sacrifice)		Main study (2-year sacrifice)	
		Males	Females	Males	Females
Control	0	20	20	50	50
Low	50	10	10	50	50
Mid	100	10	10	50	50
High	200	20	20	50	50

a The data were obtained from the study report, Table 2, page 33.

3. Dose Selection: The rationale for dose selection was based on results from a subchronic study in which rats 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 addition, it was determined that no more than 200 mg/kg/day could be administered to each animal because of run-off of the material and possible oral ingestion (personal communication, W. Phang 2/4/99).

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 X, pages 4199-4224).

4. Dosage Administration: On 5 consecutive days/week, undiluted technical grade KBR 3023 was applied to a shaved area 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) and approximately every two weeks, dosing aliquots were provided. From the information provided, it was inferred that the dosing aliquots were stored at room temperature. Prior to commencement of the study and approximately every 6 months, the stability of the test chemical stored at -23 °C was assessed. In addition, stability analyses were performed on samples of KBR 3023 stored at room temperature (22 °C) for 7, 14, 21, or 28 days.

   Results:

   Concentration/Stability Analysis: The chemical purity of KBR 3023 stored frozen was 96.7-98.5% throughout the study. It was stated that the compound is stable for 28 days when stored at room temperature.

   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 organ and terminal body weight and clinical pathology data. An analysis of variance (ANOVA) followed by Dunnett's test were applied to group means. In the event of unequal variances, the data were subjected to a Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test. The Chi-Square procedure was applied to the ophthalmology, necropsy, and micropathology data followed by the Fisher exact test if the data indicated a trend. For the Bartlett test, $p \leq 0.001$ was considered significant, $p \leq 0.05$ was considered significant for all other tests.

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 during the physical exam.

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 and calculated on a g/animal/day and g/kg body weight/day basis.

4. Ophthalmoscopic examination: The eyes of all the animals were examined prior to initiation of dosing. In addition, the eyes of all surviving 1-year (10-20 sex/dose) and 2-year animals (19-26 sex/dose) were re-examined prior to their scheduled sacrifice.

5. Blood Analyses: At 3, 6, 12, 18, and 24 months, blood was collected from the 2-year animals (14-20 rats/group) for hematology and differential leukocyte analyses. The animals were fasted overnight prior to blood sampling via the orbital sinus. 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)
X	Erythrocyte count (RBC)	X	Reticulocyte count
X	Platelet count	X	Erythrocyte morphology
	Blood clotting measurements		Red cell distribution width
	(Thromboplastin time)		HGB distribution width
	(Clotting time)	X	Heinz bodies
	(Prothrombin time)		

301

b. Clinical Chemistry:

	ELECTROLYTES
X	Calcium
X	Chloride
	Magnesium
X	Phosphorus
X	Potassium
X	Sodium
	ENZYMES
X	Alkaline phosphatase (AP)
	Plasma cholinesterase (PL-ChE)
	Erythrocyte cholinesterase (RBC-CHE)
	Brain cholinesterase (BR-CHE)
X	Creatine phosphokinase
X	Lactate dehydrogenase (LDH)
X	Serum alanine aminotransferase (ALT)
X	Serum aspartate aminotransferase (AST)
X	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

	OTHER
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Total Cholesterol
X	Globulins
X	Glucose (fasting)
X	Total & direct bilirubin
X	Total serum protein
X	Triglycerides
X	Uric acid

6. Urinalysis: Urinalyses were performed on the 2 year animals (18-20 sex/group) at 3, 6, 12, 18, and 24 months. The following CHECKED (X) parameters were examined. The animals were not fasted prior to collection of urine.

X	Clarity, appearance
X	Volume
X	Specific gravity
X	pH
X	Sediment (microscopic)
X	Protein
	Osmolality

X	Glucose
X	Ketones
X	Bilirubin
X	Blood
X	Color
X	Urobilinogen
X	Nitrite
	Creatinine
	Potassium
	Sodium
	Urea

7. Sacrifice and Pathology: All 1-year animals that were sacrificed on schedule and all 2-year animals that died or were killed *in extremis* during the second year 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. No necropsy or microscopic data were submitted on animals that died or were killed during the first year, which were 4 animals from the 1-year group and 11 animals from the 2-year group. A complete complement of tissues

was examined histologically for all other animals. Feet lesions from only 5 animals (male and female) and neck (collar) lesions from 5 animals (male and female) from the control and high-dose groups were examined microscopically.

	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
XX	Liver	X	Epididymis	X	Thyroids
	Gall bladder	X	Prostate		**OTHER**
X	Pancreas	X	Seminal vesicles	X	Bone/skull
	RESPIRATORY	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
XX	Lungs	X	Vagina	X	All gross lesions and masses
	Nose	X	Cervix	X	Lacrimal/exorbital gland
	Pharynx	X	Preputial/clitoral		
X	Larynx				

II. RESULTS

A. Observations:

1. Toxicity - All clinical observations were considered to be incidental and were not compound-related. It was stated that open wounds related to the use of the protective collars developed around the necks of approximately 20% of the male rats of each dose group, including the controls. After discussions with the Agency, the Sponsor removed the collars from all of the affected animals and stopped dosing for approximately 3 weeks; the data concerning the open wounds, stating the onset, the animals involved, and the dates when they were not dosed, were not summarized in the report. Corn oil was then applied to the collars until healing of the wounds occurred. Approximately half of the affected animals had a re-occurrence of the neck wounds. The animals from the main study (2-year) that continued to be affected (8, 9, 5, and 10 control, low-, mid-, and high-dose males, respectively and 0, 2, 1, 1 females, respectively) were sacrificed at the interim 1-year sacrifice in exchange for 1-year animals from the same dose group. These 1-year animals that were not sacrificed at the interim sacrifice continued on the study as part of the 2-year main study animals. It was not clear to the reviewers whether the interim 1-year animals that continued on the study were selected

203

randomly.

Lesions of the hind feet (including alopecia, raised zones, crusty zones, enlarged, and/or ulcers, severity grade, marked) developed in all male groups of the 1-year animals and in all male and female groups of the 2-year animals. The cause of the lesions was postulated to be related to body weight and the wire cage bottom; the incidences were not considered to be treatment-related since they were comparable in all groups. Panalog ointment was applied daily 5 days/week to the hind feet of all affected animals for up to two weeks. The 2-year animals from the main study that did not improve with the Panalog treatment were sacrificed at the interim sacrifice in exchange for 1-year animals from the same dose group; It was not clear to the reviewers how many of the 2-year animals were sacrificed during the interim sacrifice because of feet lesions.

Mortality - In the 1-year animals, two 200 mg/kg/day males and one 100 mg/kg/day female were sacrificed *in extremis* and one control female was found dead. All other 1-year animals survived to the scheduled sacrifice. Nineteen 2-year animals were either sacrificed *in extremis* or found dead during the first year . After 12 months, the survival rate was excellent in males (92-98%) and in females (94-96%) among all 2-year treated animals. Because of the neck and limb lesions discussed above, some animals originally scheduled to be sacrificed at the 1-year interim sacrifice were exchanged with animals originally scheduled to be sacrificed after 2 years of dosing . At 103 weeks, the survival rates in the 2-year animals were 46, 36, 46, and 36% in the controls, low-, mid-, and high-dose males, respectively, and 40, 50, 36, and 34% in the females, respectively. These survival rates exceeded the guideline requirement (not <25%) for this interval. The mortality data are summarized in Table 2 below.

Table 2. Mortality data [3]

Dosage			0	50	100	200
1 Year Animals						
Male		Total Animals	20	10	10	20
		Found Dead	0	0	0	0
		Euthanised	0	0	0	2
		Total Mortality	0	0	0	2
		% Survival	100	100	100	90
Female		Total Animals	20	10	10	20
		Found Dead	0	0	1	0
		Euthanised	1	0	0	0
		Total Mortality	1	0	1	0
		% Survival	95	100	90	100
2 Year Animals						
Male	Total Animals		50	50	50	50
	1st Year Mortality	Found Dead	2	2	0	2
		Euthanised	1	2	1	1
	2nd Year Mortality	Found Dead	10	15	14	16
		Euthanised	14	13	12	13
	Total Mortality		27	32	27	32
	% Survival		46	36	46	36
Female	Total Animals		50	50	50	50
	1st Year Mortality	Found Dead	0	2	1	0
		Euthanised	1	0	1	3
	2nd Year Mortality	Found Dead	5	3	5	5
		Euthanised	24	20	25	25
	Total Mortality		30	25	32	33
	% Survival		40	50	36	34

[3] Data extracted from study report, Tables CO-SUM and MORT1-SUM, pages 219, 227, and 295 through 298.

B. Body weight: No treatment-related differences were observed in body weight or body weight gains in either sex of the treated 1-year and 2-year groups throughout the study when compared to the respective control group.

The transient statistically significant differences observed in the treated groups compared to the controls throughout the study were considered to be not 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	248.3	244.9	241.1	241.4	188.3	182.9	186.7	183.0
5	388.5	380.7	377.4	373.1	246.5	239.7	249.1	244.9
Monthly Gain at 5	140.2	134.7	135.8	132.3	57.6	57.3	62.2	61.6
8	440.0	453.0	443.4	439.9	278.8	270.7	279.2	278.4
Monthly Gain at 8	70.5	92.4*	85.1*	87.1*	44.4	44.2	41.9	45.3
13	533.8	519.6	518.2	520.0	312.5	300.5	310.5	313.7
Monthly Gain at 13	61.7	47.4*	54.8	58.4	26.0	21.4*	24.6	25.1
26	601.4	603.2	598.2	592.0	356.2	350.9	351.0	361.0
Monthly Gain at 26	14.5	18.5	12.1	9.6	14.3	14.9	8.3	13.6
52	650.4	657.4	658.4	640.9	426.7	420.7	413.8	435.4
Monthly Gain at 52	0.8	0.1	1.8	3.8	7.4	9.5	4.2	7.5
101	538.8	547.3	568.9	550.7	416.3	430.4	431.0	447.5
Monthly Gain at 101	7.8	-7.5	-11.5	-12.1	-24.9	-1.5	1.7	5.1

* $p \leq 0.05$

226

C. Food consumption: There were no treatment-related differences in food consumption by the dosed groups compared to the concurrent controls throughout the study. The grand mean food consumption in the 2-year animals was approximately 31 g/male/day and 23-25 g/female/day in all dose groups including the controls. The intermittent statistically significant differences in food consumption by the treated animals compared to controls were considered to be not treatment-related.

D. Ophthalmoscopic examination: There were no treatment-related ophthalmoscopic findings in the 1- or 2-year animals.

E. Blood analyses:

1. Hematology and Differential Counts - No treatment-related effects in hematology parameters, including leucocyte differential counts, were observed after dosing with KBR 3023 at 50, 100, or 200 mg/kg/day for up to 24 months.

2. Clinical Chemistry - At the 3, 12, 18, and 24 month intervals, creatine kinase and lactate dehydrogenase were decreased (↓14-51% and 25-79%, respectively) in the 2-year high-dose males; these values were statistically significant ($p<0.05$) only at 3 and 12 months. The biological significance of these differences is not understood. Occasionally there were statistically significant differences in other clinical chemistry parameters; these transient differences were considered to be not treatment-related.

F. Urinalysis: There were no treatment-related differences observed in urinalysis parameters. At the 12, 18, and 24 month intervals, protein concentration in the high-dose females was consistently increased (↑54-150%). However, the protein values were not statistically significant and there were no indications of dose related and/or chronological trend.

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 1- or 2-year dose groups. In the 2-year males, the absolute adrenal weights were decreased (↓36-37%, not statistically significant [NS]) and the absolute heart weights were increased in the 50 and 100 mg/kg/day (↑10% each; $p<0.05$) male dose groups. These findings were considered to be incidental and not related to dosing with KBR 3023.

2. Gross pathology - All gross necropsy findings detected in the treated animals were considered to be incidental. Abscess, alopecia, crusty zones , ulcers, masses, discolored zones, and/or raised zones were noted in the neck region ("skin, other") across all of the dose groups of the 1- and 2-year animals, including the controls. It was concluded that these lesions were as a result of the use of the Elizabethan collars. Missing, crusty

zones, necrotic, ulcer, pitted zones, raised zones, and/or discolored zones of the tail were also noted across all dose groups; these lesions were considered to be associated to grooming problems related to the wearing of the collars. Alopecia, raised zones, crusty zones, edema, enlarged, masses, discolored zones, and/or ulcers of the hindlimbs were noted across all male groups of the 1-year animals and all male and female dose groups of the 2-year animals. The pathology report (pages 4162-4163 of study report) stated that these hindlimb findings were related to the combination of wire cage bottom and the heavy body weights of the rats. Body weights at sacrifice were 406-633g and 392-549g in the 1-year and the 2-year rats, respectively.

3. Microscopic pathology:

a) Non-neoplastic - Microscopic findings were limited to the liver and the treated skin . In the 2-year males, there was a dose-dependent increase in the incidence of liver cystic degeneration (8/47, 11/46, 14/49, 20/47, in controls, low-, mid-, and high-dose groups, respectively; statistically significant [$p<0.05$] in the high-dose group only). These are considered to be age-related lesions and it is considered to be a coincidence that they occurred in a dose-related matter (personal communication (1/26/99, Dr. Dawn Goodman, pathologist with Covance).

An increased incidence ($p<0.05$) of acanthosis was observed in the 1-year males. In the 2-year animals, the incidence of acanthosis and/or hyperkeratosis of the skin were increased ($p<0.05$) in the low- and mid- dose females and in the high-dose males and females. These increased incidences were restricted to the treated skin only and were judged to be not of toxicological concern. Other statistically significant ($p<0.05$) findings, such as an increased incidence of congestion of the adrenals in the 2-year high-dose males and increased incidence of microgranulomas in the lungs of the 2-year low- and high-dose females, were not dose-related and were considered to be not treatment-related.

b) Neoplastic - No significant increases in the incidences of any neoplasm were observed in the dosed animals.

Microscopic Examination of Selected Tissues (Incidences)								
Organ & Lesion	Males				Females			
Dose (mg/kg/day)	0	50	100	200	0	50	100	200
Liver								
Degeneration, cystic	8/47	11/46	14/49	20/47*	1/49	1/48	1/48	2/47
Focus/area of cellular alteration								
Hyperplasia/fibrosis, biliary	16/47	7/46	17/49	12/47	12/49	6/48	7/48	2/47
Adenoma, hepatocellular	-	-	-	-	-	1/48	-	-
Carcinoma, hepatocellular	1/47	2/46	1/49	2/47	-	-	-	1/47
Kidney								
Chronic nephropathy	37/47	41/46	44/49	42/47	21/49	16/48	17/48	20/47
Hyperplasia, epithelial cell	12/47	10/46	19/49	16/47	5/49	-	4/48	3/47
Lung								
Congestion	10/47	12/46	9/49	15/47	2/49	2/48	4/48	2/47
Microgranuloma	8/47	2/46	4/49	5/47	1/49	8/48*	4/48	10/47*
Mammary Gland								
Adenocarcinoma	-	-	-	-	6/48	4/48	6/48	4/47
Fibroadenoma	2/44	2/43	-	1/45	26/48	25/48	19/48	17/47
Ovaries								
Cyst	-	-	-	-	10/49	7/48	9/48	13/48
Pituitary								
Cyst	16/47	13/46	12/49	8/47	30/49	25/48	29/48	24/47
Adenoma	24/47	30/46	27/49	23/47	40/49	43/48	39/48	41/47
Skin (treated)								
Acanthosis	-	5/46*	4/49	16/47*	1/49	4/48	6/47*	12/46*
Hyperkeratosis	22/47	24/46	32/49	37/47*	3/49	10/48*	10/47*	22/46*
Brain								
Compression	3/47	7/46	5/49	8/47	26/49	23/48	22/48	24/47
Colon								
Parasitism	13/47	14/46	10/49	10/47	3/49	3/48	6/48	4/47
Heart								
Degeneration/Fibrosis/ Cardiomyopathy	40/47	37/46	44/49	40/47	28/49	32/48	27/48	30/47

Microscopic Examination of Selected Tissues (Incidences)								
Organ & Lesion	Males				Females			
Dose (mg/kg/day)	0	50	100	200	0	50	100	200
Adrenals								
Congestion	4/47	2/46	1/49	11/47*	1/49	-	1/48	2/47
Degeneration, cystic	4/47	3/46	10/49	5/47	43/49	41/47	44/48	34/47
Hemorrhage	6/47	3/46	9/49	3/47	40/39	40/47	41/48	26/47
Hyperplasia, cortical	10/47	2/46	7/49	11/47	5/39	5/47	4/48	4/47
Vacuolization	12/47	18/46	17/49	17/47	18/39	21/47	17/48	21/47
Pheochromocytoma	10/47	3/46	9/49	12/47	1/39	1/47	-	-

*Statistically significant from control ($p \leq 0.05$)
- means that none were observed in that group.

III. DISCUSSION

A. Investigators Conclusions - Survival, body weight, food consumption, clinical signs, hematology, organ weights, and gross pathology parameters were unchanged following dermal exposure to KBR 3023 in rats. Cystic degeneration of the liver in the 2-year high-dose males was observed, but no corroborating liver histopathological data were obtained. Acanthosis and/or hyperkeratosis of the treated skin across all doses in the 1- and 2-year animals was also observed. The chronic LOAEL was not observed. The chronic NOAEL is 200 mg/kg/day.

There was no evidence of a carcinogenic effect in rats after repeated dermal exposure to KBR 3023 for up to 24 months.

B. Reviewer's Discussion/Conclusions - Male and female rats (50/dose) were treated dermally with undiluted KBR 3023 (technical, 96.7-98.5% a.i.) at 0, 50, 100, or 200 mg/kg/day on 5 consecutive days/week for 24 months. In addition, 10-20 rats/sex/group were terminated at 12 months.

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 findings were also unaffected by treatment. In the 2-year males, there was a dose-dependent increase in the incidence of liver cystic degeneration (8/47, 11/46, 14/49, 20/47, in controls, low-, mid-, and high-dose groups, respectively; statistically significant [$p<0.05$] in the high-dose group only). These findings were not corroborated by changes in organ weight or in blood chemistry parameters.

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

The dose levels used in this study were from an EPA approved protocol.

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

The submitted study is classified as **acceptable (§83-5)** and satisfies the guideline requirements for a combined chronic/oncogenicity study in the rat.

C. Study deficiencies -Data pertaining to the neck and feet lesions should have been more adequately presented. The final in-life date was not specified. These deficiencies are minor and would not change the conclusions of this review.

DATA EVALUATION RECORD 013529

KBR 3023

Study Type: 84-2; Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)

Work Assignment No. 3-47H (MRID 44408729)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
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Date: 2/12/98

Project Manager:
Mary L. Menetrez, Ph.D.

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Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 2/13/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

212

EPA Reviewer: John E. Whalan
Toxicology Branch 1 (7509C)

John Whalan 4-14-99

EPA Secondary Reviewer: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella typhimurium*/mammalian activation gene mutation assay
OPPTS Number: 870.5265 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): KBR 3023 Technical (99.1% active ingredient, a.i.)

SYNONYMS: 1-(1-Methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidin

CITATION: Herbold, B.A., (1990) KBR 3023 *Salmonella*/Microsome Test, Bayer AG, Wuppertal, Germany. Lab Study Number T5033207. March 16, 1990. MRID 44408729. Unpublished.

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

EXECUTIVE SUMMARY:

In two independent reverse gene mutation assays in bacteria (MRID 44408729), strains TA98, TA100, TA1535, and TA1537 of *Salmonella typhimurium* were exposed to KBR 3023 technical (99.1% a.i.) in ethanol in the presence and absence of S9 mammalian metabolic activation. The four *S. typhimurium* strains were evaluated with KBR 3023 technical at concentrations of 8, 40, 200, 1000, and 5000 µg/plate. In a third assay, the same concentrations of KBR 3023 were tested in the presence of S9 at a reduced concentration.

KBR 3023 (99.1% a.i.) was tested up to the limit dose of 5000 µg/plate. Cytotoxicity described as a "weak" and "strain-specific" bacteriotoxicity was observed at 5000 µg/plate, but did not hinder adequate assessment of mutagenicity at this dosage. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline §84-2 for in vitro mutagenicity bacterial reverse gene mutation data.

213

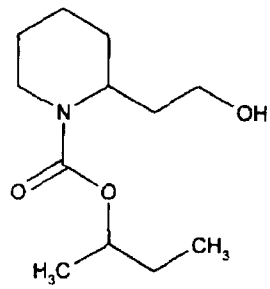
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

214

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023, technical
   Description: Clear, viscous liquid
   Lot/Batch #: 19009/89
   Purity: 99.1% a.i.
   Stability of compound: Reported to be stable for the duration of the study when stored under refrigeration.
   CAS #: 119515-38-7
   Structure:



   Solvent used: Ethanol (EtOH)
   Comment: The test substance was stable in the solvent for up to 4 hours at room temperature.

2. Control Materials:
   Negative: EtOH
   Solvent/final concentration: EtOH/0.1 mL per plate

   Positive:
   Nonactivation:
   Sodium azide 10 µg/plate TA1535
   2-Nitrofurantoin 0.2 µg/plate TA100
   4-Nitro-1,2-phenylene diamine
   0.5 and 10 µg/plate for TA98 and TA1537, respectively.
   Activation:
   2-aminoanthracine 3 µg/plate TA98, TA100, TA1535, and TA1537
   Dimethylsulfoxide (DMSO) served as the solvent for all positive controls.

3. Activation: S9 derived from

x	Aroclor 1254	x	induced	x	rat	x	liver
_	phenobarbital	_	non-induced	_	mouse	_	lung
_	none	_		_	hamster	_	other
_	other	_				_	other

A 30% (3:7, v/v) S9:cofactor mix was used for the first two dose/strain/condition tests conducted; a 10% (1:9, v/v) mix was used for the third test with 0.15 M KCl supplying the additional 20% volume. The cofactor stock solution (70 mL) contained: $MgCl_2$ x 6 H_2O (162.6 mg), NADP disodium salt (315.0 mg), glucose-6-phosphate disodium salt (179.1 mg), KCl (246.0 mg) and phosphate buffer (100.0 mM). The S-9 mix was prepared immediately prior to use, and 0.5mL were used per plate. The final S9 concentration in the cultures was approximately 2-6% (v/v).

4. Test organisms: *S. typhimurium* strains

_ TA97 x TA98 x TA100 _ TA102 _ TA104
x TA1535 x TA1537 _ TA1538

Properly maintained? **Yes**
Checked for appropriate genetic markers (rfa mutation, R factor)? **Yes**

5. Test compound concentrations used

Cytotoxicity and mutagenicity assay: *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were evaluated with KBR 3023 at concentrations of 8, 40, 200, 1000 and 5000 µg/plate in the presence and absence of S9 mammalian metabolic activation. Solvent and positive control groups were included.

B. TEST PERFORMANCE

1. Type of *Salmonella* assay:
   - x standard plate test
   - _ pre-incubation (_ minutes)
   - _ "Prival" modification *(i.e. azo-reduction method)*
   - _ spot test
   - _ other *(describe)*

2. Protocol:

Tester strains were inoculated into nutrient broth culture one day prior to dosing and incubated for approximately 17 hours. The test substance was diluted in ethanol and the

216

positive control substances were diluted in DMSO, each to specified concentrations. For the activation conditions, 0.1 mL of the appropriate tester strain culture (density not indicated), 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine . The mixture was poured over a minimal agar plate. For nonactivation conditions, 0.5 mL of phosphate buffer was substituted for the S9 mix. After incubation (37 C for approximately 2 days), the number of revertant colonies/plate were counted and recorded. The background lawn and/or the number of colonies and titers were examined to determine the toxicity of the test compound. Means for the mutation tests were determined from the counts of four plates per strain, per dose, per condition. Plates that were not counted immediately following incubation, were stored refrigerated until colony counting could be conducted.

3. Evaluation Criteria

(a) Assay validity: The following criteria were used to determine assay acceptability: (1) the mean control colony counts were within the ranges found in the laboratory historical controls and published data (2) positive controls induced appropriate responses, or (3) tester strain densities were determined "sufficient" by titer (4) any assay with a positive mutagenic response. Generated data had to confirmed by two additional independent assays.

(b) Positive response: A dose-related increase in the mean number of revertants- at least 2-fold higher than the solvent control for strains TA98, TA100 or TA1535, and 3-fold for the TA1537 strain- was considered a positive response. The result had to be reproducible.

II. REPORTED RESULTS

A. Cytotoxicity test and mutagenicity assay: The cytotoxicity test was run concurrently with the mutagenicity assay. It was reported that the test material was not cytotoxic to any of the strains at doses up to 1000 µg/plate. A weak, strain-specific bacteriotoxic effect was noted at the 5000 µg/plate dose level. *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 were evaluated with KBR 3023 at concentrations of 8 through 5000 µg/plate with and without metabolic activation. Four plates were used for each dose/strain/condition. Solvent and positive controls were included.

There were no significant increases in the mean number of revertant colonies in any of the tester strains at any dose level/condition. All positive controls induced an appropriate increase in the number of revertant colonies. The results of the mutagenicity assay are presented as an attachment to this DER (study report Table 5.2, pages 22-23).

217

Based on these findings, the study author concluded that KBR 3023 was not mutagenic under the conditions of this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study author's conclusion that KBR 3023 was not mutagenic under the conditions of the submitted microbial gene mutation assay. KBR 3023 was assayed over an appropriate dose range as it was tested to a cytotoxic concentration and the limit concentration with the *S. typhimurium* strains. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. This study is classified as acceptable.

218

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DATA EVALUATION RECORD 013529

KBR 3023

Study Type: 84-2; Unscheduled DNA Synthesis in Primary Rat Hepatocytes

Work Assignment No. 3-471 (MRID 44408730)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
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Date: 04/8/98

Project Manager:
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Date:

Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 4/10/98

Disclaimer

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222

EPA Reviewer: John E. Whalan
Registration Action Branch 2, (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

John Whalan 4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes

OPPTS Number: 870.5550 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): KBR 3023, 99.6% active ingredient (a.i.)

SYNONYMS: 1-(1-Methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidin

CITATION: Brendler, S. (1992) KBR 3023 Mutagenicity Study on Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures *In-Vitro*. Bayer AG, Department of Toxicology, Wuppertal, F. R. Germany. Study No. T3037076, April 29, 1992. MRID 44408730. Unpublished.

SPONSOR: Bayer AG, Friedrich-Ebert-Str. 217-333, D-5600 Wuppertal 1, F. R. Germany.

EXECUTIVE SUMMARY:

In two independent unscheduled DNA synthesis (UDS) assays (MRID 44408730), primary rat hepatocyte cultures were exposed to KBR 3023 (99.6% a.i.) in ethanol at concentrations ranging from 10 to 600 µg/mL for 18-24 hours.

Dose levels in the first UDS assay (50-600 µg/mL) were selected based on the results of a preliminary cytotoxicity assay. The results of the first UDS assay were not evaluated for UDS activity because only three dose levels were available due to toxicity at the three highest dose-levels (300-600 µg/mL) and a weak response was elicited by the positive control. The concentrations selected for the second UDS assay were 10, 25, 50, 100, 150, 200 and 250 µg/mL.

Insufficient cells were available for evaluation of nuclear grains at the 25 µg/mL and 250 µg/mL dose levels, with damaged and/or unattached cells preventing evaluation at these levels. However, five concentrations (10, 50, 100, 150, and 200 µg/mL) were available for evaluation of UDS activity. Relative cell survival ranged from 84.5% at 50 µg/mL to 64.3% at 200 µg/mL. There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced at any of these concentrations. The positive control induced the appropriate response.

223

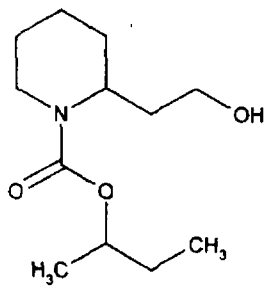
This study is classified as **Acceptable (§870.5550)** and satisfies the requirement for FIFRA Test Guideline for other genotoxic mutagenicity data.

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 fluid
   Lot/Batch #: 190012/89
   Purity: 99.6 active ingredient
   Stability of compound: Reported to be stable for the duration of the study.
   CAS #: 119515-38-7
   Structure:



   Solvent used: Ethanol (purity not specified)
   Other comments: The test substance was stored at room temperature. The test substance was diluted in ethanol to the appropriate concentrations immediately prior to use. Analysis of dosing solutions was not performed by the testing laboratory.

2. Control Materials:
   Negative: Ethanol
   Solvent/final concentration: Ethanol (1%, v/v)
   Positive (concentrations, solvent):
   2-acetylaminofluorene (2-AAF) dissolved in dimethylsulfoxide (DMSO) was used at a final concentration of 0.5 µg/mL.

3. Test Compound Concentrations Used:

   Preliminary Cytotoxicity assay: A preliminary cytotoxicity assay was performed using the following eight concentrations: 7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL.

224

UDS assay: Two trials were performed using the following concentrations:

Trial 1: 50, 100, 200, 300, 400, and 600 µg/mL.
Trial 2: 10, 25, 50, 100, 150, 200, and 250 µg/mL. A parallel cytotoxicity test was run with each trial using the same concentrations.

4. Media: Williams' Medium E containing L-glutamine, gentamycin sulfate, and 10% heat-inactivated fetal calf serum (WEC). Serum concentration was reduced to 1% during the treatment period.

5. Test Cells: Primary hepatocytes were obtained from young adult male Sprague-Dawley rats weighing 150-250 g, purchased from Lippische Versuchstierzucht, Extertal or Mus Rattus, Brunnthal. Rats were housed singly, and the animal room environment was maintained at 22°C, 95% relative humidity, and a 12 hour light/dark cycle.

6. Cell Preparation:

   a. Perfusion Technique: The liver from an anesthetized rat was perfused *in situ* with Hanks' balanced salt solution (without Ca^{+2}/Mg^{+2}) containing EGTA followed by a freshly prepared solution containing serum-free medium plus collagenase.

   b. Hepatocyte Harvest/Culture Preparation: The author indicated that hepatocytes were prepared according to the procedures described by Butterworth (Butterworth, et. al., *Mutation Res.*, 189:113-121, 1987) and seeded as follows: For the cytotoxicity assay, cells were seeded into two 60 mm culture dishes ($7.5x10^5$ viable cells per dish). Viability was determined by trypan blue exclusion. For the UDS assay, cells were seeded onto 25 mm round plastic coverslips in six-well culture dishes (3 wells/dose group and $3.75x10^5$ viable cells per well) containing WEC. Cultures were incubated at 37°C for 1.5-2.5 hours prior to treatment to allow for cell attachment.

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: After the attachment period, the medium in the culture dishes was replaced with medium (1% serum) containing the appropriate dose of test substance (7.8-1000 µg/mL) or ethanol (vehicle control). Two cultures were tested per concentration. Following an 18-24 hour treatment period, viable cell counts were determined by trypan blue exclusion. Cytotoxicity, expressed as relative percent survival, was obtained by comparing viable cell counts for treated groups with the results for the vehicle control.

2. UDS Assay:

225

a. Treatment: Triplicate cultures were used for each of the dose levels and the vehicle and positive controls for the UDS assay, and another set of duplicate cultures was used for the parallel cytotoxicity assay. After the attachment period, the culture medium was replaced with medium (1% serum) containing 10 μCi/mL ^{3}H-thymidine (14.5 Ci/mmole) and the test substance, positive, or vehicle control at the desired concentrations. After a treatment period of 20-24 hours, two cultures per treatment condition were evaluated for relative survival. After a treatment period of 21-21.5 hours, cell nuclei on coverslips from the UDS assay were swelled in 1% sodium citrate, fixed in acetic acid:ethanol (1:3), washed in distilled water, and air dried. The coverslips were mounted, cell side up, on microscope slides.

b. Preparation of Autoradiographs/Grain Development: The slides were dipped in Kodak NTB-2 emulsion (undiluted or emulsion:distilled water, 1:1; v:v) in the dark and stored for 4-10 days at 4°C in light-tight boxes containing dessicant. The photographic emulsion was then developed in Kodak D-19, and the slides were rinsed, fixed, air-dried, and stained in hematoxylin and eosin.

c. Grain Counting: The slides were read by microscopy with a 100x objective under oil immersion. The net nuclear grain count was determined for at least 50 cells on each slide (150 nuclei/treatment condition). Net nuclear grain count was determined by counting nuclear grains and subtracting the average number of grains in three nuclear-sized areas of cytoplasm adjacent to the nucleus. In addition, the number of nuclei in repair (net nuclear grain count ≥5) was determined. Nuclei in replicative DNA synthesis were excluded.

c. Data Presentation: The data were presented in tabular form. Means and standard deviations in the tables were calculated individually for each of the three (at least two) coverslips per dose. The following determinations were made:

Net Grains per Nucleus = Average number of net nuclear grain counts on triplicate coverslips (total of 150 cells).

Mean Cytoplasmic Grain Count = Average number of cytoplasmic grain counts (3 areas per cell) on triplicate coverslips.

% Nuclei with 5 or more grains = Number of cells with 5 or more net nuclear grain counts on triplicate coverslips/150) x 100.

Survival (%) = Number of viable cells relative to the vehicle control.

e. Evaluation Criteria:

Assay Validity: (1) The viability of the hepatocytes obtained by perfusion must be ≥50%. (2) The viability of attached cells for the UDS assay must be ≥80%. (3) The viability of cells in the vehicle controls must be ≥60% after 16-24 hours. (4) Nuclear grain count and cytoplasmic grain counts from 3 areas adjacent to each nucleus must be conducted for 50 cells from each slide. (5) Nuclei in repair are indicated by net nuclear grain count ≥5. (6) The average net nuclear grain counts (NG) for vehicle controls should range from -8 to 1, and no more than 10% of the cells should be in repair. (7) The NG for positive controls is expected to range from 10 to 25, with 70-100% of the nuclei with ≥5 net nuclear grains. (8) At least 2 replicate cultures and at least 50 cells per culture are necessary to evaluate grain count data from any treatment level. (9) A minimum of 4-5 dose levels will be analyzed for NG. (10) The highest analyzed dose must approach excessive toxicity (about 50% relative percent survival), result in test material insolubility, or reach the limit dose of 5 mg/mL. (11) Cells on fixed slides that are not viable or of abnormal morphology as well as isolated nuclei will not be counted; S-phase cells will be excluded but noted. (12) Vehicle control cytoplasmic grain counts >30 grains/adjacent nuclear-sized area indicate an invalid experiment.

Positive Response: A positive response was indicated by an average net nuclear grain count (population average or 150 cells) of ≥5 and by ≥20% of the cells responding (≥5 net nuclear grains/cell). A "marginal response," described as an average net nuclear grain count >1 but <5 might be considered a positive response if a dose-related increase in both average net nuclear grain count and percent cells in repair was observed. An additional assay may be required to properly define this response as positive.

f. Statistical Analysis: The percentage of cells in repair obtained from each dose group was compared to the vehicle control using a one-sided, 2 x 2, chi-square test corrected for continuity ($p = 0.05$). To assess the statistical significance of a result, the square root of the test statistic was compared to the upper 95% quantile ($p=0.05$) of the normal standard distribution.

II. **REPORTED RESULTS**: Analytical determinations of dose preparations were not performed. The test substance was reported as stable in the vehicle at concentrations of 0.08-50.0 mg/mL for 4 hours at room temperature.

A. Preliminary Cytotoxicity Assay: Eight concentrations of KBR 3023 ranging from 7.8 to 1000 µg/mL were tested to determine appropriate dose levels for the UDS assay. Cell viability at the initiation of the test was 78.8%. The test substance was reported to be soluble up to 100 mg/mL in the vehicle (ethanol) and freely soluble in the culture medium as a test substance/vehicle solution. After the 18-24 hour treatment period, the average viable cell count in the vehicle control was 64.6%. Relative survivals of 79.2%

227

and 35.1% were observed at 250 and 500 µg/mL, respectively; at 1000 µg/mL 0% relative survival was observed. Lower concentrations of the test substance (7.8-125 µg/mL) were essentially non-toxic to the cells. Based on these results, 600 µg/mL was selected as the highest concentration for the UDS assay.

B. UDS Assay: The first assay was performed at 50, 100, 200, 300, 400 and 600 µg/mL dose levels. Cell conditions were adequate for an UDS assay with initial cell monolayer viability of 79.9%, and a vehicle control average viable cell count of 78.8% after a 21 hour treatment period. In the parallel cytotoxicity assay, relative toxicities of 72.4% and 61.8% were observed at 100 and 200 µg/mL, respectively. However, at the 300-600 µg/mL dose levels excessive cytotoxicity was observed and insufficient cells were available for evaluation. The average net nuclear grain count (2.47 ±2.79) and average percent cells in repair (24.7%) for the positive control was considered a weak response based on the test evaluation criteria. In addition, there was a high number of cells in S-phase. For these reasons, the first assay was not used to evaluate the genotoxicity of the test substance.

Based on the results of the first UDS assay, the second assay was performed at dose concentrations of 10, 25, 50, 100, 150, 200 and 250 µg/mL. Cell viability of the attached cell monolayer was 82.2%. After the 21.5 hour treatment, the vehicle control average viable cell count was 78.6%. The background net nuclear grains for vehicle controls was reported in the normal range for the assay. Relative toxicity, determined by a parallel cytotoxicity assay, was 64.3% at the highest dose level accepted for evaluation (200 µg/mL). At 25 and 250 µg/mL relative toxicities of 76.1% and 80.6% were observed, but sufficient cells were not available for evaluation of nuclear grains. This was attributed to cell damage and/or unattached cells (25 µg/mL) and damaged cells due to test material concentration at the 250 µg/mL dose level. Therefore, 5 concentrations 10, 50, 100, 150, and 200 µg/mL were evaluated for UDS activity. Slides treated with the test substance or positive control were compared to the vehicle control. None of the test substance concentrations induced a significant increase in the mean net nuclear grain count. The positive control, 2-AAF at 0.5 µg/mL, produced a statistically significant increase in the average percent cells in repair (66.7%) and a marked increase in mean net nuclear grain count (6.77 ±1.23); both of these values were slightly below the historical ranges established by the laboratory for positive controls (78%-94% and 8.4-16.9, respectively) and the "expected values" stipulated in the study acceptance criteria.

The results of the two UDS assays and parallel cytotoxicity assay are presented as attachments to this DER (study report Tables 2 and 3, pages 30 and 31).

Based on these results, the study author concluded that, under the conditions of the study, KBR 3023 did not cause a significant increase in unscheduled DNA synthesis in the primary rat hepatocyte UDS assay.

228

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. KBR 3023 was tested to cytotoxic concentrations, and the vehicle control produced the appropriate response. The response of the positive control was lower than expected, thus reducing the sensitivity of the assay. However, it does not appear that a positive response with the test substance would have occurred, even with a better response from the positive control. It is concluded, therefore, that KBR 3023 was adequately tested and found not to be genotoxic under the conditions of this study.

It should also be noted that 95% relative humidity, reported by the author as standard husbandry conditions for the rats used for the study, is unsuitable for animal maintenance. It is assumed that this was reported erroneously, and does not represent the actual conditions.

B. STUDY DEFICIENCIES - The following deficiencies would not be expected to alter the conclusions of the study: (i) analysis of dose formulations were not performed. However, KBR 3023 was tested to cytotoxic concentrations. (ii) The purity of the vehicle control, ethanol, was not specified.

ATTACHMENTS

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013529

DATA EVALUATION RECORD

KBR 3023

Study Type: §84-2; CHO/HGPRT/Mammalian Activation Gene Mutation Assay

Work Assignment No. 3-47J (MRID 44408731)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Date: 6/9/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

233

EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster ovary (CHO) cells

OPPTS Number: 870.5300 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): KBR 3023 (99.6% active ingredient)

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

CITATION: Brendler, S. (1991) KBR 3023 Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay In-Vitro. Bayer AG, Department of Toxicology, Wuppertal, F. R. Germany. Study No. T7035540, November 13, 1991. MRID 44408731. Unpublished.

SPONSOR: Bayer AG, Friedrich-Ebert-Str. 217-333, D-5600 Wuppertal 1, F. R. Germany.

EXECUTIVE SUMMARY:

In two independently performed mammalian cell gene mutation assays at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus (MRID 44408731), Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to KBR 3023 (99.6% a.i.) in ethanol at doses ranging from 2.0 to 1500 µg/mL, with and without S9 activation. Cultures were exposed for 5 hours prior to plating for determination of cytotoxicity, expression, and selection of the mutant phenotype.

In a preliminary cytotoxicity assay, the highest dose level tested was 500 µg/mL, resulting in no toxicity with S9 activation and moderate toxicity (63% relative survival) without S9 activation. Consequently, dose levels up to 1500 µg/mL were used for the mutagenicity assays. At the highest dose level (-S9), extreme cytotoxicity resulted in both trials, whereas at 1000 µg/mL and lower, little or no toxicity occurred and, except for a non-reproducible positive response in one culture at 125 µg/mL, no mutagenicity was observed. In the first mutagenicity trial (+S9), the highest dose level (1500 µg/mL) again proved extremely toxic to the cells and, although relative survival was 10.9% at 1000 µg/mL, no toxicity was observed at 1250 µg/mL (95.2% relative

survival). Statistically significant increases in mutation frequencies were observed in one of two cultures each at 250 and 1000 µg/mL, but no increases were observed at 500 or 750 µg/mL. In the second trial, a dose-related toxicity was observed at 500-1500 µg/mL (88.5-36.5% relative survival), with no increase in mutation frequency; however, the response from the positive control was at the low end of the historical range, thereby reducing the sensitivity of the test.

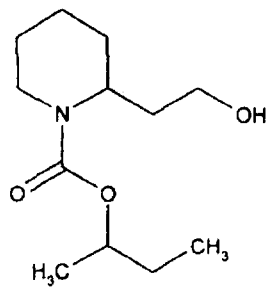
This study is classified **Unacceptable (§870.5300)** and not upgradable. The study failed to demonstrate a consistent toxic response at the dose levels tested, and the dose preparations were not analyzed for actual concentrations of KBR 3023. Therefore, the negative results obtained in these mutagenicity assays cannot be considered a reliable indication of an absence of genotoxicity of the test substance. The study does not satisfy the guideline requirement for *in vitro* mammalian forward gene mutation studies.

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 fluid
   Lot/Batch #: 190012/89
   Purity: 99.6 % active ingredient
   Stability of compound: The test article was stored at room temperature and was reported to be stable for the duration of the study.
   CAS #: 119515-38-7
   Structure:



   Vehicle used: Ethanol (EtOH)
   Comments: The purity of EtOH used as the vehicle was not specified.

2. Control Materials:
   Negative: Untreated and vehicle controls
   Vehicle/final concentration: EtOH/1% (v/v)

Positive:
Nonactivation: Ethyl methanesulfonate (EMS)/900 µg/mL
Activation: Dimethylbenzanthracene (DMBA)/20 µg/mL

Comment: The test substance was reported to be stable in the vehicle at 0.08-200 mg/mL for the duration of the treatment period and soluble in the vehicle up to a concentration of 100 mg/mL. The vehicles for the positive controls were not reported.

3. Activation: The commercially prepared S9 was derived from Aroclor 1254-induced male Wistar rat liver. The prepared S9 mixture contained 40% S9 (v/v) and included 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, and 1 mM NADP. The final S9 concentration in the cultures was 2% (v/v).

4. Test Cells: Chinese hamster ovary (CHO) cell line K_1-BH4 was the test system used. The cells were maintained in the growth media described below, and the cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 16-24 hours prior to treatment.

Properly maintained? **Yes**
Periodically checked for mycoplasma contamination? **Yes**
Periodically checked for karyotype stability? **Yes**
Periodically "cleansed" against high spontaneous background? **Yes**

Media: Ham's F12 supplemented with 10% heat-inactivated, fetal calf serum (FCS) 1mM L-glutamine, 50 units/mL penicillin, and 50 µg/mL streptomycin was used during the growth period. The growth media with heat-inactivated FCS reduced to 5% was used as the treatment medium. The selection medium was a hypoxanthine-free growth medium containing 6-thioguanine. The growth medium supplemented with 9 µg/mL thymidine, 10 µg/mL hypoxanthine, 22.5 µg/mL glycine, and 0.3 µg/mL methotrexate was used to reduce the frequency of spontaneous hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) mutants.

5. Locus Examined: HGPRT
Selection agent: 10 µg/mL 6-thioguanine (6-TG)

6. Test Compound Concentrations Used:

a. Preliminary Cytotoxicity Assays:
One rangefinding cytotoxicity experiment was conducted. Nine dose levels of KBR 3023 (2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125.0, 250.0, and 500.0 µg/mL) were evaluated in the presence and absence of metabolic activation (±S9).

b. Mutation assays:
Two trials each were conducted under nonactivated and activated metabolic conditions. For the two trials performed without S9, six dose levels of KBR 3023 (125, 250, 500, 750, 1000 and 1500 µg/mL) were tested. For the two trials evaluated with S9, six dose levels (250, 500, 750, 1000, 1250, and 1500 µg/mL) were tested.

B. TEST PERFORMANCE

1. Cell Treatment:
   a. Preliminary Cytotoxicity Assays:
   Exponentially growing CHO cells were plated in growth medium at 4 X 10^6/250 mL flask/dose level. After an attachment period of 16-24 hours, the cells in the treatment medium were exposed to the test compound or the vehicle alone for 5 hours (±S9). After exposure, the cell monolayers were washed with phosphate buffered saline (PBS), trypsinized, replated at 200 cells/plate, and then cultured for 7 days prior to cytotoxicity evaluation. The cell cultures were fixed with alcohol, stained with Giemsa, and the colonies (>50 cells) counted visually or by automatic counter. Cytotoxicity, expressed as "relative cloning efficiency," was determined by comparing colony counts from the cultures treated with test compound to those of the vehicle control. Three plates (60 mm petri dishes) for each dose level and vehicle control were evaluated.

   b.Mutagenicity Assays:
   Cells were plated in growth medium at 4 X 10^6/250 mL flask/dose level and positive, vehicle or negative control. After an attachment period of 16-24 hours, the cells in the treatment medium were exposed to the test compound, positive control, or vehicle alone for 5 hours (±S9). After exposure, the cell monolayers were washed with PBS, trypsinized, replated in growth medium at 1.5 x 10^6/250 mL flask with two flasks/treatment condition; cells for the parallel cytotoxicity assays were replated at 200 cells/plate in triplicate, cultured for 7 days, and evaluated for cytotoxicity ("survival to treatment") as described above.

   During the 6 day expression period, the cells plated for the mutation assay were subcultured (reseeded at 1.5 x 10^6/250 mL flask) on Days 3 and 6.

   After expression, cells from each culture flask were reseeded at 2 x 10^5 cells/100 mm dish (8 dishes/treatment condition) in selection medium. Concurrently, cells were seeded (200 cells/60 mm plate) in triplicate as in the cytotoxicity assays previously described. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 7 days, and then were fixed and stained with Giemsa. Colonies (>50 cells) were counted from the 100 mm dishes to determine mutant frequency (MF), and from the 60mm dishes to determine "absolute cloning efficiency." The assays with and without metabolic activation (±S9) were conducted independently.

2. Statistical Methods: A Poisson heterogeneity test (Armitage 1971) was used to evaluate MF. Statistical significance was determined at $p<0.05$.

3. Evaluation Criteria:

a.Assay validity: The assay was considered acceptable if the following criteria were met: (i) Each assay with and without metabolic activation had to be repeated in an independent second trial. (ii) The average cloning efficiency of the negative controls should be ≥50%; less than 50% was unacceptable. (iii) Relative survival should be 0-30% at the highest dose level, and should approximate the negative control at the lowest concentration. (iv) The background MF (average for negative controls) should be $\leq 25 \times 10^{-6}$, but if all other criteria are fulfilled, the test may be considered valid. (v) An observed MF is accepted only if absolute cloning efficiency is ≥10%, at least four dose levels are evaluated, ≥5 dishes per dose level are evaluated, and the positive control induces a MF ≥3x the MF of the negative control.

b.Positive response: The test article was considered positive if it produced the following: (i) a dose-dependent, reproducible increase in MF over ≥3 dose levels, and (ii) an increase in MF ≥2x the concurrent vehicle controls, or (iii) a reproducible increase in MF ≥2x the concurrent vehicle controls observed for one dose level "near the highest testable level."

c.Equivocal response: An equivocal response was observed when a significant and/or ≥2x increase in MF over concurrent vehicle controls was induced at ≥1 dose level in the absence of a dose-related increase.

II. REPORTED RESULTS

A. Analytical Determinations: Dose preparations were not analyzed for concentration of KBR 3023 in ethanol. The test substance was reported to be stable in the vehicle at 0.08-200 mg/mL for the duration of the treatment period. In addition, KBR 3023 was reported to be soluble for up to 100 mg/mL in the vehicle.

B. Cytotoxicity assay: In a preliminary trial, KBR 3023 was evaluated for cytotoxicity at nine concentrations ranging from 2.0 to 500.0 µg/mL (±S9). Cytotoxicity was estimated by a reduction in relative cloning efficiency (CE). At the highest three concentrations evaluated for cytotoxicity without S9 (125-500 µg/mL), relative CE was 63.3-64.1%; at all dose ranges with S9 activation, no cytotoxicity was observed. Based on these results, 125-1500 µg/mL (-S9) and 250-1500 µg/mL (+S9) were selected as the dose concentration ranges for the mutagenicity assay.

C. Mutagenicity Assay: Two trials of the mutagenicity assay were performed, and the results were as follows:

Nonactivation Conditions: KBR 3023 was evaluated for mutagenicity at six concentrations ranging from 125 to 1500 µg/mL. At the highest concentration evaluated, 1000 µg/mL, relative CE in the two trials was 105.1% and 261.2%, respectively, indicating that KBR 3023 was non-toxic at this dose level. At the highest concentration, 1500 µg/mL, the test article caused extreme cytotoxicity, preventing cell cloning and analysis; therefore, the remaining dose levels 125-1000 µg/mL were evaluated for MF in each trial. In the first trial, there was a large variation in the MF between the duplicate vehicle controls (12.8-42.4 mutants/10^6 cells). Therefore, the mean value was used for statistical evaluation, and there were no statistically significant increases in MF observed for the treated cultures at any dose level. In the second trial, a statistically significant increase in MF was observed in one of the two cultures at 125 µg/mL. However, this result was not observed in either culture from the first trial at this dose level. There were no statistically significant increases in MF observed for the treated cultures at any other dose level under nonactivation conditions. In contrast, the nonactivated positive control (900 µg/mL EMS) induced significant ($p<0.05$) increases in the mutation frequencies in both trials.

S9-Activation Conditions: KBR 3023 was evaluated for mutagenicity at six concentrations ranging from 250-1500 µg/mL. In the cytotoxicity phase of testing, the test article caused extreme cytotoxicity at the highest test concentration, 1500 µg/mL, preventing analysis at this level in the first trial. At the highest evaluated concentrations, 1000 and 1250 µg/mL, relative CE was 10.9% and 95.2%, respectively. In the second trial, at the two highest evaluated concentrations, 1250 and 1500 µg/mL, the relative CE was 25.4% and 36.5%, respectively. In the first mutagenicity assay, statistically significant increases in MF over the vehicle control were observed in one culture each at the 250 and 1000 µg/mL dose levels. These increases were not observed in the second trial at these dose levels. There were no statistically significant increases at any other dose level under activation conditions. However, in both trials, the activated positive control (20 µg/mL DMBA) induced significant ($p<0.05$) increases in the mutation frequencies.

The results of the mutagenicity assays (Study Report Tables 3-6, pages 29-32) are presented as an attachment to this DER.

III. DISCUSSION/CONCLUSIONS:

A. Investigator's Conclusions: Under the conditions of this study, the test article was negative for the induction of forward gene mutations at the HGPRT locus in CHO cells, both with and without metabolic activation.

B. Reviewer's Discussion: In the preliminary cytotoxicity assay, the highest dose level tested was 500 µg/mL, resulting in no toxicity with S9 activation and moderate toxicity (63% relative survival) without S9 activation (Study Report Tables 1 and 2, pages 27 and 28). Consequently, higher dose levels up to 1500 µg/mL were used for the mutagenicity assays.

At the highest dose level (-S9), extreme cytotoxicity resulted in both trials, whereas at 1000 µg/mL and lower, little or no toxicity occurred and, except for a non-reproducible effect in one culture at 125 µg/mL, no mutagenicity was observed. In the first mutagenicity trial (-S9), the highest dose level (1500 µg/mL) again proved extremely toxic to the cells and, although 1000 µg/mL resulted in 10.9% relative survival, 1250 µg/mL was not toxic (95.2% relative survival); individual cultures at 250 and 1000 µg/mL showed statistically significant increases in mutation frequency. In the second trial, a dose-related toxicity was observed from 500-1500 µg/mL (88.5-36.5% relative survival) without an increase in mutation frequency; however, the response from the positive control was at the low end of the historical range, thereby reducing the sensitivity of the test.

There are numerous inconsistencies in the data and the conduct of the study which lead the reviewer to classify the study as unacceptable. Generally, the study failed to demonstrate a consistency in toxic response to the dose levels tested, thus the biological significance of the results obtained in the mutagenicity assays cannot be determined. Because the dose preparations were not analyzed for actual concentrations of KBR 3023, the doses administered could not be verified. In addition, the mutagenic response occurred in individual cultures only, was not dose related, and not reproducible. Therefore, we disagree with the study author, and conclude that the genotoxicity of KBR 3023 in this CHO/HGPRT test remains undetermined.

C. Study deficiencies: Toxic responses and mutagenic responses were inconsistent. Although moderate toxicity was observed in the preliminary cytotoxicity assay at 125-500 µg/mL, in the mutagenicity assay without S9 activation, no toxicity or mutagenicity was observed over the same range and at doses up to 1000 µg/mL. Extreme toxicity was observed at 1500 µg/mL and KBR 3023 was not tested at dose levels between 1000 and 1500 µg/mL.

In the first mutagenicity trial with S9 activation, 1000 µg/mL and the 1500 µg/mL were highly toxic to extremely toxic, but no toxicity was observed at the 1250 µg/mL dose level. Although a good range of toxicity up to 1500 µg/mL was observed in the second trial +S9, the mutation frequencies of the positive control (29.7 and 49.6) were at the low end of the historical range (20.2-441.4) thus, reducing the sensitivity of the test system to detect mutagenesis. There were negative results at all levels with the exception of one culture each in the first trial (+S9) at the 250 µg/mL and 1000 µg/mL dose levels; the positive responses observed for mutation frequency were neither dose related nor reproducible.

Because the dose preparations were not analyzed for actual concentrations of KBR 3023, the doses administered could not be verified.

In addition to the deficiencies detailed above, an unexplained 2-month interval between the two mutagenicity trials without S9 activation may have contributed to the inconsistent results achieved in the study.

In the second mutagenicity trial (-S9), the mutation frequency for one vehicle control was unusually high (42.4×10^{-6}) hindering statistical analysis.

The author also reported historical vehicle control data for DMSO instead of ethanol, and the percent purity of the ethanol vehicle was not stated.

ATTACHMENT

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY

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013529

DATA EVALUATION RECORD

KBR 3023 Technical

Study Type: 84-2; *In vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells

Work Assignment No. 3-47K (MRID 44408732)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C) John Whalan 4-14-99

EPA Work Assignment Manager: Sanjivani Diwan, PhD
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations in Chinese hamster ovary cells
OPPTS NUMBER: 870.5375 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): KBR 3023 Technical (97.1% a.i.)

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

CITATION: Gudi, R. and Schadly, E. (1997) Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells. Microbiological Associates, Inc., 9630 Medical Center Drive, Rockville, Maryland. Laboratory Study No. G97AD67.330. August 4, 1997. MRID 44408732. Unpublished.

SPONSOR: Bayer AG, Business Group Consumer Products, Research and Development, D-51368 Leverkusen, Bayerwerk, Bldg. 6210, Germany.

EXECUTIVE SUMMARY:

In an *in vitro* mammalian cell chromosome aberration assay (MRID 44408732), Chinese hamster ovary (CHO) cell cultures were exposed to dimethylsulfoxide (DMSO) alone or to KBR 3023 (97.1% a.i.) in DMSO at concentrations of 63, 125, 250, 500, 1000, 2000, and 4000 µg/mL continuously without metabolic activation (-S9) until harvest at 20 hours and for 4 hours with metabolic activation (+S9) with harvest at 12 hours. Untreated cultures were included, as were cultures treated with Mitomycin C or cyclophosphamide, respectively, to serve as positive controls for the nonactivated and activated series.

KBR 3023 was extremely toxic at ≥2000 µg/mL, without S9 activation (≤3% cell viability) and at 4000 µg/mL with S9 activation (≤1% cell viability). Without S9 activation, clastogenic activity ($p \leq 0.01$) was observed at 500 and 1000 µg/mL, with an accompanying dose response ($p \leq 0.05$). With S9 activation, no clastogenic activity was observed at any dose level. Untreated and vehicle controls were within historical control ranges and positive controls induced the appropriate responses. Thus, KBR 3023 is considered a clastogen in Chinese hamster ovary cells

344

in the absence of metabolic activation.

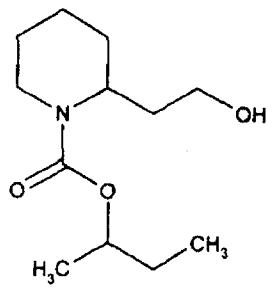
This positive study is classified as **Acceptable/Guideline** and satisfies the requirement for FIFRA Test Guideline **§870.5375** for *in vitro* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: KBR 3023 Technical
   Description: Clear, colorless liquid
   Lot/Batch #: 030693
   Purity: 97.1% a.i.
   Stability of compound: Stable frozen. The sponsor recommended storage at cool temperatures; the test substance was stored at room temperature, protected from light.
   CAS #: 119515-38-7
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
   Negative: Untreated and vehicle controls
   Solvent (final concentration): DMSO (1% in culture medium)
   Positive:
   Activation: Cyclophosphamide (10 and 20 µg/mL in distilled water)
   Nonactivation: Mitomycin C (0.08 and 0.15 µg/mL in distilled water)

3. Activation: Rat liver microsomal fraction (S9) was prepared from Aroclor 1254 induced male Sprague-Dawley rats. Immediately prior to use, frozen aliquots were thawed and added to a cofactor solution. The S9-cofactor mix contained 2 mM $MgCl_2$, 6 mM KCl, 1 mM glucose-6-phosphate, 1 mM NADP, and 20 µL S9 per mL serum-free McCoy's 5A

245

medium supplemented with streptomycin, penicillin, and L-glutamine. The final concentration of S9 in the culture medium was 2%.

4. Test Compound Concentrations Used:

a. Preliminary Cytotoxicity Assays:

Nine dose levels of KBR 3023 (0.5, 1.5, 5, 15, 50, 150, 500, 1500, and 5000 µg/mL) were tested by exposing cell monolayers for 6 hours in the absence of S9 activation or 4 hours in the presence of S9 activation and harvesting cells after 26 hours. Vehicle controls were included. Cell viability, growth index and growth inhibition, and average generation time were determined.

b. Mutagenicity Assays:

(1) Nonactivated conditions: Seven dose levels of KBR 3023 (63, 125, 250, 500, 1000, 2000, and 4000 µg/mL) were tested by exposing cell monolayers continuously until harvest after 20 hours of incubation.

(2) Activated conditions: Seven dose levels of KBR 3023 (63, 125, 250, 500, 1000, 2000, and 4000 µg/mL) were tested by exposing cell monolayers for 4 hours and harvesting after 12 hours of incubation.

Vehicle and untreated controls and the appropriate positive controls were included in the assays.

5. Test Cells: The Chinese hamster ovary CHO-K_1 cell line was the test system used. The cells were grown in 25 cm^2 tissue culture flasks containing 5 mL of McCoy's 5A medium with 10% fetal calf serum (FCS) and supplemented with L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 µg/mL). Cells were preincubated at 37°C, in a CO_2 incubator (5% CO_2) for 16-24 hours prior to treatment.

Properly maintained? **Yes**

Cell line or strain periodically checked for Mycoplasma contamination? **Yes**

Cell line or strain periodically checked for karyotype stability? **Only early passage (≤20) cells were used to assure stability**

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Single culture flasks were seeded with approximately 5×10^5 CHO cells on the day prior to treatment with KBR 3023. The culture medium was drained off at the start of treatment and replaced with 5 mL of fresh medium, to which

246

was added 50 µL DMSO alone or test substance in DMSO. For cultures requiring metabolic activation, 1.0 mL of 5X S9 mix was added to 4 mL of culture medium. Treatment times of 6 hours without S9 or 4 hours with S9 were selected. A 50 µL aliquot of 1 mM 5-bromo-2-deoxyuridine (BrdU) was added to each flask two hours after initiation of treatment. At completion of the treatment period the cells were washed, refed with medium containing 0.01 mM BrdU, and returned to the incubator for a total of 26 hours from the initiation of treatment. Two hours prior to cell harvest, Colcemid (0.1 µg/mL) was added to each flask. The cells were harvested by trypsinization, counted using a Coulter counter, and viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the vehicle control. Cells in metaphase were prepared for sister chromatid differentiation using a modified fluorescence-Giemsa technique. Slides were evaluated for the percentage of first (M_1), second (M_2), and third (M_3) division metaphase cells for estimation of the test substance effect on cell cycle kinetics. The average generation time (AGT) was calculated for each treatment condition[a]. The dose levels of KBR 3023 selected for the main study were based on cell growth inhibition and cell cycle delay observed in the preliminary cytotoxicity assay.

[a] AGT = (24 hrs x 100)/[(no. M_1 cells x 1) + (no. M_2 cells x 2) + (no. M_3 cells x 3)]

2. Cytogenetic Assay: The highest concentration selected for the evaluation of chromosome aberrations was based on ≤50% cell growth inhibition compared to the vehicle control in the preliminary cytotoxicity assay.

   a. Cell treatment: CHO cell monolayers were grown as described in section A.5 and treated as described in section A.4.b. Duplicate culture flasks were seeded with approximately $5x10^5$ cells on the day prior to treatment with KBR 3023. On the day of testing, the culture medium was drained off at the start of treatment and replaced with 5 mL of fresh medium, to which was added 50 µL of DMSO containing the appropriate concentration of KBR 3023. For cultures requiring metabolic activation, 1.0 mL of 5X S9 mix was added to 4 mL of culture medium. The appropriate untreated (medium alone or medium containing S9 mix), vehicle, and positive control cultures in duplicate were included. S9-activated cultures were exposed for 4 hours, the cells were rinsed, refed with fresh medium, and returned to the incubator for an additional 8 hours prior to harvesting. Non-activated cultures were exposed continuously up to cell harvest at 20 hours. A concurrent cytotoxicity test was conducted with both activated and non-activated studies. At cell harvest, aliquots of cell suspensions were counted using a Coulter counter, and cell viability was determined by trypan blue dye exclusion. The counts obtained were used to determine cell growth inhibition relative to the vehicle control.

   b. Spindle inhibition:
   Inhibitor used/concentration: Colcemid at 0.1 µg/mL of culture medium
   Administration time: 2 hours before cell harvest

c. Cell harvest: In cultures with or without metabolic activation, cells were harvested immediately after the two hour Colcemid treatment. The medium was poured off and cells were recovered from monolayers by trypsinization. Cells were centrifuged and the supernatant was removed. Hypotonic treatment with 0.075 M KCl solution at room temperature for 4-8 minutes was followed by centrifugation and fixation of the cells with two changes of Carnoy's fixative (methanol/glacial acetic acid, 3:1, v/v) and storage overnight in fixative at 2-6°C.

d. Details of slide preparation: Following fixation, the cells were centrifuged and resuspended in a small amount of fixative, then dropped onto microscope slides, air dried overnight, and stained with 5% Giemsa solution. Stained slides were air dried and coverslips were added.

e. Metaphase analysis:
No. of metaphases examined per dose: **200**
No. of metaphases examined in the vehicle control: **200**
No. of centromeres per metaphase figure: **20±2**
No. of metaphases examined in the cyclophosphamide positive control: **200**
No. of metaphases examined in the mitomycin C positive control: **200**
Scored for structural aberrations: **Yes**
Scored for numerical polyploidy: **No**
Coded prior to analysis: **Yes**

f. Evaluation criteria: The assay was considered acceptable if there was a significant increase ($p \leq 0.05$, Fisher's exact test) in the percentage of cells with chromosome aberrations induced by the positive control(s) and if the chromosome aberration rate for the untreated or vehicle controls was ≤6%.

A clastogenic response was claimed if the percentages of cells with aberrations were increased in a dose-responsive manner with one or more dose levels being statistically elevated relative to the vehicle control group ($p \leq 0.05$). A significant increase at the high dose only with no dose response was considered suspect. A significant increase at one dose level other than the high dose with no dose response was considered equivocal.

g. Statistical analysis: Statistical analysis of the percent aberrant cells was performed by pair-wise comparison of treated and positive control groups to respective vehicle controls using the Fisher's exact test. In the event of a positive Fisher's test at any test substance dose level, the Cochran-Armitage test was used to measure dose-responsiveness. Differences were considered significant at $p \leq 0.05$.

II. REPORTED RESULTS

KBR 3023 was soluble in DMSO at a concentration of 500 mg/mL, the maximum concentration tested. The dose preparations were not analyzed for actual concentration. It was reported that concentrations of KBR 3023 up to 5000 µg/mL did not alter the osmolality or significantly affect the pH in culture medium.

A. Preliminary Cytotoxicity Assay

In the preliminary cytotoxicity assay, CHO cells in culture were exposed to 9 KBR 3023 concentrations ranging of 0.5 to 5000 µg/mL for 6 hours in the absence of metabolic activation and for 4 hours in the presence of metabolic activation. Cultures were harvested 26 hours after initiation of treatment to determine the inhibition of growth and generation time. Cell growth inhibition was 100% at 5000 µg/mL ±S9. At the next highest dose, 1500 µg/mL, cell growth inhibition was 35% (-S9) and 28% (+S9). Lower dose levels were essentially non-toxic (±S9). At 1500 µg/mL, the highest scorable dose level, the average generation time determined by sister chromatid differentiation with BrdU was 12-13 hours (±S9). The results of the preliminary cytotoxicity assay were reported in Tables 1-4 of the Study Report. On the basis of this preliminary cytotoxicity assay, 7 dose levels ranging from 63 to 4000 µg/mL (±S9) were selected for the cytogenetic assay.

B. Cytogenetic Assay

In the cytogenetic assay, CHO cells in culture were exposed to 7 KBR 3023 concentrations ranging of 63 to 4000 µg/mL, continuously until harvest at 20 hours in the absence of metabolic activation, and for 4 hours in the presence of metabolic activation, with harvest at 12 hours. Without S9 activation, excessive toxicity was observed at dose levels of ≥2000 µg mL. At 1000 µg/mL, relative cell growth inhibition was 46%. Therefore, dose levels of 125, 250, 500, and 1000 µg/mL were analyzed for chromosome aberrations. With S9 activation, excessive toxicity was observed at 4000 µg/mL. At 2000 µg/mL, relative cell growth inhibition was 49%. Therefore, dose levels of 250, 500, 1000, and 2000 µg/mL were analyzed for chromosome aberrations. Results of the concurrent cytotoxicity assays were reported in study report Tables 5 and 7. At 500 and 1000 µg/mL (-S9), the mitotic indices were 74% of the vehicle control and relative cell growth inhibition was 32% and 46%, respectively. At these dose levels, the percentage of cells with structural aberrations was significantly increased ($p \leq 0.01$) and a dose response was evident ($p \leq 0.05$). In the S9-activated study, the percentage of cells with structural aberrations was not statistically increased above that of the vehicle control at any dose level. The accompanying positive controls (±S9) induced statistically significant increases in the percentages of aberrant metaphases. Results of the cytogenetic assays were reported in study report Tables 6 and 8 and summarized in Table 9. The summary results extracted from Table 9 are presented in Table 1 below.

Table 1. Summary of Clastogenic Effects of KBR 3023 in CHO Cells

Treatment	±S9	Treatment/Harvest Time (hrs)	Mitotic Index	Cells Scored	Aberrations Per Cell (Mean±SD)	Cells With Aberrations (%)
Untreated	-	20/20	5.7	200	0.050 ± 0.313	3.0
DMSO	-	20/20	5.7	200	0.020 ± 0.140	2.0
KBR 3023 (µg/mL)						
125	-	20/20	5.4	200	0.055 ± 0.350	3.0
250	-	20/20	4.7	200	0.045 ± 0.231	4.0
500	-	20/20	4.2	200	0.155 ± 0.492	11.5**
1000	-	20/20	4.2	200	0.205 ± 1.043	10.5**
Mitomycin C (0.08 µg/mL)	-	20/20	3.2	200	0.910 ± 1.404	48.0**
Untreated	+	4/12	6.4	200	0.015 ± 0.122	1.5
DMSO	+	4/12	6.0	200	0.045 ± 0.252	3.5
KBR 3023 (µg/mL)						
250	+	4/12	5.3	200	0.015 ± 0.122	1.5
500	+	4/12	5.3	200	0.025 ± 0.186	2.0
1000	+	4/12	6.0	200	0.040 ± 0.196	4.0
2000	+	4/12	0.2	31	0.290 ± 1.270	9.7
Cyclophosphamide (10 µg mL)	+	4/12	1.0	200	0.655 ± 1.294	36.5**

** $p \leq 0.01$

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that, in this *in vitro* cytogenetic test, KBR 3023 showed evidence of clastogenic activity at toxic dose levels in the absence of metabolic activation and no evidence of clastogenic activity at any dose level in the presence of metabolic activation.

250

B. Reviewer's Discussion

KBR 3023 was tested to toxic concentrations. A positive clastogenic response ($p \leq 0.01$) was observed at the two highest dose levels evaluated (500 and 1000 µg/mL) without metabolic activation. These dose levels were moderately toxic, with mitotic indices that were 74% of the vehicle control value. A positive dose response ($p \leq 0.05$) was also reported. At the highest dose level evaluated (2000 µg/mL) with metabolic activation, and the only cytotoxic dose (mitotic index 3% of vehicle control value), a 2.8X increase in the percent of cells with structural aberrations above the vehicle control value was observed. However, the response was not statistically significant, and it occurred in only one of the two culture flasks. There were no significant increases in clastogenic response above vehicle control values for any other dose level tested with metabolic activation. The untreated and vehicle control results were within the ranges of the reported historical controls. The sensitivity of the assay system to detect damage to chromosomes was adequately demonstrated by the results obtained with the positive controls (mitomycin C, -S9 and cyclophosphamide, +S9), which were also within the historical ranges reported for the laboratory. The reviewers agree with the study authors' conclusion that under the given experimental conditions, KBR 3023 was clastogenic without metabolic activation and non-clastogenic with metabolic activation for Chinese hamster ovary cells cultured *in vitro*.

C. Study deficiencies

Dose preparations were not analyzed for actual concentrations of test compound. However, this deficiency would not be expected to alter the study conclusions because KBR 3023 was tested to cytotoxic concentrations and a clastogenic response was observed in the absence of metabolic activation.

251

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DATA EVALUATION RECORD

KBR 3023

Study Type: 84-2; *In vitro* Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) Cells

Work Assignment No. 3-47L (MRID 44408733)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C) John Whalan 4-14-99

EPA Work Assignment Manager: Sanjivani Diwan, PhD
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations in Chinese hamster ovary cells
OPPTS NUMBER: 870.5375 OPP Guideline Number: §84-2

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

TEST MATERIAL (PURITY): KBR 3023 (99.5% a.i.)

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

CITATION: Gahlmann, R. (1996) KBR 3023: In Vitro Mammalian Chromosome Aberration Test With Chinese Hamster Ovary (CHO) Cells. Bayer AG, Fachbereich Toxicology, Friedrich-Ebert Straße 217-333, D-42096 Wuppertal, F.R.G. Study No. T 6034405. April 30, 1996. MRID 44408733. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, 8400 Hawthorn Road, Kansas City, Missouri.

EXECUTIVE SUMMARY:

In an *in vitro* mammalian cell chromosome aberration assay (MRID 44408733), Chinese hamster ovary (CHO) cell cultures were exposed to ethanol alone or to KBR 3023 (99.5% a.i.) in ethanol in two trials without metabolic activation (-S9) at concentrations of 4, 20, and 100 µg/mL continuously until harvest at 7, 18, or 27 hours (Trial 1); or at concentrations of 400, 800, and 1200 µg mL for 2 hours with harvest at 18 hours and an additional harvest at 27 hours for the 1200 µg mL dose level (Trial 2). Two trials were also conducted with metabolic activation (+S9) at concentrations of 100, 700, and 1400 µg/mL for 2 hours with harvests at 7, 18, or 27 hours (Trial 1); or at concentrations of 800, 1200, and 1600 µg/mL for 2 hours with harvest at 18 hours and an additional harvest at 27 hours for the 1600 µg/mL dose level (Trial 2). Untreated cultures were included, as were cultures treated with Mitomycin C or cyclophosphamide (as Endoxan) to serve as positive controls for the nonactivated and activated series, respectively.

KBR 3023 was tested to cytotoxic concentrations and the limit of solubility (2000 µg/mL). KBR 3023 was extremely toxic at ≥1600 µg/mL, without S9 activation (<1% cell viability) and at 2000 µg mL with S9 activation (<1% cell viability). With a 2-hour treatment in the presence of metabolic activation and cell harvest at 18 or 27 hours, significantly increased aberration

253

frequencies were obtained at 1200-1600 µg/mL. In the absence of metabolic activation, with treatment times of 7-27 hours, significant increases were obtained at 4-100 µg/mL; however, a dose response was not observed. With treatment time reduced to 2 hours (-S9), the aberration frequency was increased significantly only at the highest dose level (1200 µg/mL) harvested at 27 hours. The untreated and solvent controls gave the appropriate responses and the sensitivity of the assay to detect chromosome damage was adequately demonstrated by the results obtained with the positive controls.

Under the given experimental conditions, KBR 3023 was positive for clastogenicity with metabolic activation for Chinese hamster ovary cells cultured *in vitro*. The response for clastogenicity in the absence of metabolic activation was equivocal, because a significant increase in aberration frequency after a 2-hour treatment was observed only at the highest dose level (1200 µg/mL), and the observed increases in aberration frequencies at lower dose levels with treatment times of 7-27 hours were not dose related.

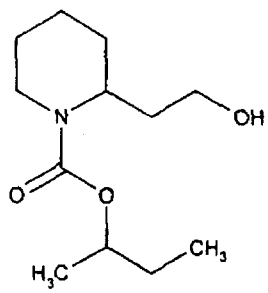
This positive study is classified as **Acceptable/Guideline** and satisfies the requirement for FIFRA Test Guideline **§870.5375** for *in vitro* cytogenetic mutagenicity data.

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, viscous liquid
   Lot/Batch #: 190012/89
   Purity: 99.5% a.i.
   Stability of compound: The test material was reported to have been analyzed prior to study initiation and approved for use for the duration of the treatment period. The test material was stored at room temperature.
   CAS #: 119515-38-7
   Structure:



   Solvent used: Ethanol (purity not reported)

2. Control Materials:
Negative: Untreated and solvent controls
Solvent (final concentration): Ethanol (≤1%)
Positive:
Activation: Cyclophosphamide (as Endoxan at 5, 10, 12.5, and 25 μg/mL in Hank's balanced salt solution)
Nonactivation: Mitomycin C (0.02, 0.08, 1, and 2 μg/mL in Hank's balanced salt solution)

3. Activation: Rat liver microsomal fraction (S9) prepared from Aroclor 1254 induced Wistar rats was obtained commercially. On the day of use, frozen aliquots were thawed and added to a cofactor solution. The S9-cofactor mix contained per 100 mL: $MgCl_2$ (162.2 mg), KCl (246 mg), glucose-6-phosphate (152 mg), NADP (78.8 mg), sodium phosphate buffer (60 mL), and S9 (40 mL). The final concentration of S9 in the culture medium was 2%.

4. Test Compound Concentrations Used:

a. Preliminary Cytotoxicity Assays:

Pre-test 1: Five dose levels of KBR 3023 (0.2, 2, 20, 200, and 2000 μg/mL) were tested (±S9); length of treatment was 7, 18, and 27 hours (-S9) or 2 hours (+S9)

Pre-test 2: Three dose levels of KBR 3023 (2, 10, and 50 μg/mL) were tested (-S9) with length of treatment 7, 18, and 27 hours; four dose levels of KBR 3023 (100, 200, 400, and 800 μg/mL) were tested (+S9) with length of treatment 2 hours

Pre-test 3: Five dose levels of KBR 3023 (400, 800, 1200, 1600, and 2000 μg/mL) were tested (-S9) with length of treatment 2 hours

b. Mutagenicity Assays:

(1) Nonactivated conditions:

First trial: Three dose levels of KBR 3023 (4, 20, and 100 μg/mL) were tested with length of treatment 7, 18, and 27 hours

Second trial: Three dose levels of KBR 3023 (400, 800, and 1200 μg/mL) were tested with length of treatment 2 hours

(2) Activated conditions:

First trial: Three dose levels of KBR 3023 (100, 700, and 1400 μg/mL) were tested with length of treatment 2 hours

255

Second trial: Three dose levels of KBR 3023 (800, 1200, and 1600 µg/mL) were tested with length of treatment 2 hours

5. Test Cells: The Chinese hamster ovary (CHO) WB-1 cell line was the test system used. The cells were grown in 75 cm^2 tissue culture flasks containing 20 mL of Ham's F12 medium with 5 or 10% fetal calf serum (FCS) and supplemented with L-glutamine (200 mM). penicillin (5000 units/mL), and streptomycin (5000 µg/mL). Cells were preincubated at 37°C, in a CO_2 incubator (5% CO_2) for 18-24 hours prior to treatment.

Properly maintained? **Yes**

Cell line or strain periodically checked for Mycoplasma contamination? **Yes**

Cell line or strain periodically checked for karyotype stability? **Yes**

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: Culture flasks were seeded with approximately $1x10^6$ CHO cells on the day prior to treatment with KBR 3023. The culture medium was drained off at the start of treatment and replaced with 20 mL of fresh medium, to which was added ethanol alone ("volume not documented") or 0.2 mL test substance in ethanol. For cultures requiring metabolic activation, 1 mL of S9 mix was added to 19 mL of culture medium. Treatment times of 7, 18, and 27 hours without S9 or 2 hours with S9 were selected. For the S9-activated cultures, cells were harvested 7, 18, or 27 hours after the beginning of treatment, respectively. In pre-test 3, cells were exposed to KBR 3023 for 2 hours (-S9) and harvested after 18 hours. Two hours prior to cell harvest, Colcemid (40 µg/mL water) was added to each flask. The cells were harvested by trypsinization, and survival was determined by counting cells using a hemocytometer. The mitotic index was determined for 1000 cells per culture. Single cultures were evaluated in pre-tests 1 and 2. while duplicate cultures were evaluated for pre-test 3. The dose levels of KBR 3023 selected for the first trial of the cytogenetic study were based on mitotic indices obtained from the three preliminary cytotoxicity tests.

2. Cytogenetic Assay: The highest concentration selected for the evaluation of chromosome aberrations was based on a 50% reduction in mitotic index compared to the solvent control in the preliminary cytotoxicity assay.

a. Cell treatment: CHO cell monolayers were grown as described in section A.5 and treated as described in section A.4.b. Duplicate culture flasks were seeded with approximately $1x10^6$ cells on the day prior to treatment with KBR 3023. On the day of testing, The culture medium was drained off at the start of treatment and replaced with 20 mL of fresh medium, to which was added ethanol alone or 0.2 mL test substance in ethanol. For cultures requiring metabolic activation, 1 mL of S9 mix was added to 19 mL of culture medium. The appropriate untreated (medium alone or

256

medium containing S9 mix), solvent, and positive control cultures in duplicate were included. Treatment times of 7, 18, and 27 hours without S9 or 2 hours with S9 were selected. In the first trial, cells were harvested 7, 18, or 27 hours after the beginning of treatment; in the second trial cells were harvested 18 or 27 hours after the beginning of treatment. Two hours prior to cell harvest, Colcemid (40 µg/mL water) was added to each flask. As a concurrent cytotoxicity test, the mitotic index was determined for 1000 cells per culture.

b. Spindle inhibition:
Inhibitor used/concentration: Colcemid at 40 µg/mL of water
Administration time: 2 hours before cell harvest

c. Cell harvest: In cultures with or without metabolic activation, cells were harvested immediately after the two hour Colcemid treatment. The medium was poured off and cells were recovered from monolayers by trypsinization. Cells were centrifuged and the supernatant was removed. Hypotonic treatment with 0.56% KCl solution at 37°C for approximately 4 minutes was followed by centrifugation and fixation of the cells with cold (4°C) ethanol/acetic acid, 3:1, v/v) with subsequent incubation in fixative for 20-30 minutes at room temperature.

d. Details of slide preparation: Following fixation, the cells were centrifuged and resuspended in a small amount of fixative, then dropped onto cooled microscope slides, air dried for at least 2 hours, and stained with 5% Giemsa solution. Stained slides were air dried and coverslips were added. Alternatively, fixed slides were submerged in methanol prior to staining with Giemsa.

e. Metaphase analysis:
No. of metaphases examined per dose: **200**
No. of metaphases examined in the solvent control: **200**
No. of centromeres per metaphase figure: **21**
No. of metaphases examined in the Endoxan positive control: **200**
No. of metaphases examined in the mitomycin C positive control: **200**
Scored for structural aberrations: **Yes**
Scored for numerical polyploidy: **No**
Coded prior to analysis: **Yes**

f. Evaluation criteria: The assay was considered acceptable if there was a significant increase ($p \leq 0.05$, Fisher's exact test) in number of metaphases with chromosome aberrations induced by the positive control(s) and if the chromosome aberration rate for the solvent control was within the historical range for the laboratory.

A positive test for clastogenicity was claimed if a statistically significant increase in aberration frequencies was observed that was also dose dependent. If either one of these criteria occurred alone, the test was considered equivocal.

257

g. Statistical analysis: Statistical analysis of the percent aberrant metaphases was performed by pair-wise comparison of treated and positive control groups to respective solvent controls using the Fisher's exact test. Differences were considered significant at $p \leq 0.05$. Significance at $p \leq 0.01$ was also indicated.

II. REPORTED RESULTS

KBR 3023 was demonstrated to be stable in ethanol at room temperature up to 4 hours at 0.08 mg/mL and up to 5 hours at 200 mg/mL. KBR 3023 in ethanol was soluble in culture medium up to a concentration of 2000 µg/mL. The dose preparations were not analyzed for actual concentration.

A. Preliminary Cytotoxicity Assay

In three independent preliminary cytotoxicity tests, CHO cells in culture were exposed to 3-5 KBR 3023 concentrations per test ranging from 0.2 to 2000 µg/mL for 2, 7, 18, and 27 hours in the absence of metabolic activation and harvested immediately after treatment (except that cells exposed for 2 hours were harvested after 18 hours), or for 2 hours in the presence of metabolic activation with cells harvested 7, 18, and 27 hours after initiation of treatment. The relative cell survival and mitotic index was <1% at 2000 µg/mL under all treatment conditions and harvest times. In pre-test 3 (-S9), a similar response was observed after a 2-hour treatment at 1600 µg/mL. At 0.2-1200 µg/mL responses ranging from nontoxic to highly toxic (5% relative survival and 12% relative mitotic index) were observed under a variety of treatment conditions and harvest times. Tabulated results of the preliminary cytotoxicity assay were reported on pages 20-23 of the Study Report. On the basis of this preliminary cytotoxicity assay, 3 dose levels ranging from 4 to 100 µg/mL (-S9) and 3 dose levels ranging from 100 to 1400 µg/mL were selected for the first trial of the cytogenetic assay.

B. Cytogenetic Assay

In the first trial, CHO cells in culture were exposed to KBR 3023 for 7, 18, or 27 hours without S9 activation at concentrations of 4, 20, or 100 µg/mL; cells were harvested immediately following treatment. With S9 activation, cells were exposed to KBR 3023 for 2 hours at concentrations of 100, 700, or 1400 µg/mL; cells were harvested 7, 18, or 27 hours after initiation of treatment. Without S9 activation, the mitotic indices relative to the solvent control for cells harvested after 27 hours of treatment at 20 and 100 µg/mL were reduced to 39.8% and 47.7%, respectively. No significant reduction (≥50%) in relative mitotic index was observed at any other dose level or treatment time. Statistically significant increases ($p \leq 0.05$ or $p \leq 0.01$) in aberration frequency were observed at all three harvest times, and the lowest dose level (4 µg/mL) had the highest aberration frequency (excluding gaps). With S9 activation, the relative mitotic indices at 1400 µg/mL for cells harvested 7 and 18 hours after initiation of treatment were reduced to 35.2% and 34.7%, respectively. No significant reduction in relative mitotic index was observed at any other dose level or harvest time.

Statistically significant increases ($p \leq 0.01$) in aberration frequency (including and excluding gaps) were observed at harvest times of 18 and 27 hours in cells treated with 1400 µg/mL KBR 3023. At the 7-hour harvest, the aberration frequency in cells treated with 1400 µg/mL was significantly increased ($p \leq 0.05$) only when gaps were included.

A second trial was conducted, because clastogenic activity was detected in the first trial, both with and without S9 activation. Because a dose response was not observed in the first trial without S9, higher dose levels were selected for the second trial and the treatment time was reduced to 2 hours. Higher dose levels were also selected for the second trial with S9 in order to assess aberration frequencies at cytotoxic concentrations.

CHO cells in culture were exposed to KBR 3023 for 2 hours without S9 activation at concentrations of 400, 800, or 1200 µg/mL. With S9 activation, cells were exposed to KBR 3023 for 2 hours at concentrations of 800, 1200, or 1600 µg/mL. Both with and without S9 activation, cells were harvested 18 hours after initiation of treatment. In addition, cultures at the highest dose levels (1200 µg/mL, -S9; 1600 µg/mL, +S9) and concurrent solvent controls were harvested at 27 hours.

Without S9 activation, the relative mitotic index at 1200 µg/mL for cells harvested after 27 hours of treatment was reduced to 67.1%. No reduction in relative mitotic index was observed at any dose level harvested at 18 hours. Statistically significant ($p \leq 0.01$) increases in aberration frequency (including and excluding gaps) and in the frequency of exchanges were observed at 1200 µg/mL harvested at 27 hours. A statistically significant ($p \leq 0.05$) increase in exchanges was also observed at 1200 µg/mL harvested at 18 hours. The aberration frequency (including and excluding gaps) was also increased (not statistically significant) at this dose level.

With S9 activation, the relative mitotic indices for cells treated with 1200 and 1600 µg/mL KBR 3023 and harvested 18 hours after initiation of treatment were reduced to 77.6% and 14.7%, respectively, and at 1600 µg/mL, to 47.1% after 27 hours. No significant reduction in relative mitotic index was observed at any other dose level or harvest time. Statistically significant increases ($p \leq 0.01$) in aberration frequency (including and excluding gaps) and the frequency of exchanges were observed at 1200 and 1600 µg/mL harvested at 18 hours. At 1600 µg/mL, a high degree of chromosome disintegration (defined as <50% of chromosomes with characteristic structural features within a given metaphase) was also observed. At the 27-hour harvest, the aberration frequency (including and excluding gaps) and frequency of exchanges at 1600 µg/mL were also increased significantly ($p \leq 0.01$). In both trials, the positive controls induced statistically significant ($p \leq 0.01$) increases in aberration frequencies.

Results of the cytogenetic assays were reported in Study Report Tables 2-7 (Trial 1) and 9-11 (Trial 2). Results of the concurrent cytotoxicity tests for Trials 1 and 2 were reported in Study Report Tables 1 and 8, respectively. The cytogenetic assay results are summarized in Tables 1 (Trial 1) and 2 (Trial 2) below.

Table 1. Summary of Clastogenic Effects of KBR 3023 in CHO Cells (Trial 1)[a]

Treatment (µg/mL)	±S9	Treatment/Harvest Time (hrs)	Mitotic Index (% of solvent control)	Cells With Aberrations (%)		
				+ Gaps	- Gaps	Exchanges
Ethanol	-	7/7	100	5.5	1.0	0.0
Untreated	-	7/7	89.8	3.5	2.5	0.5
KBR 3023 (4)	-	7/7	88.2	10.0	7.0**	0.5
KBR 3023 (20)	-	7/7	61.0	10.5*	6.5**	1.5
KBR 3023 (100)	-	7/7	75.9	9.0	5.0*	0.0
Mitomycin C (1)	-	7/7	43.9	20.0**	11.0**	2.0
Ethanol	+	2/7	100	5.5	3.5	0.0
Untreated	+	2/7	111.7	3.5	2.0	0.0
KBR 3023 (100)	+	2/7	73.2	7.5	6.5	1.0
KBR 3023 (700)	+	2/7	101.4	6.0	3.5	0.0
KBR 3023 (1400)	+	2/7	35.2	12.0*	7.0	0.5
Endoxan (25)	+	2/7	39.9	21.0**	15.0**	4.5**
Ethanol	-	18/18	100	4.0	0.5	0.0
Untreated	-	18/18	116.4	2.0	0.0	0.0
KBR 3023 (4)	-	18/18	90.6	7.0	5.0**	1.0
KBR 3023 (20)	-	18/18	102.9	4.5	1.5	0.0
KBR 3023 (100)	-	18/18	66.7	10.0*	4.5**	0.5
Mitomycin C (0.02)	-	18/18	39.2	14.0**	9.5**	1.0
Ethanol	+	2/18	100	4.0	2.0	0.0[b]
Untreated	+	2/18	105.3	3.0	0.5	0.0[b]
KBR 3023 (100)	+	2/18	73.5	4.0	2.5	0.5[b]
KBR 3023 (700)	+	2/18	61.8	5.5	4.0	0.0[b]
KBR 3023 (1400)	+	2/18	34.7	32.5**	28.5**	13.0[b]

Treatment (µg/mL)	±S9	Treatment/Harvest Time (hrs)	Mitotic Index (% of solvent control)	Cells With Aberrations (%)		
				+ Gaps	- Gaps	Exchanges
Endoxan (5)	+	2/18	38.2	20.0**	17.0**	5.5[b]
Ethanol	-	27/27	100	4.0	1.5	0.0
Untreated	-	27/27	135.2	4.5	3.0	0.0
KBR 3023 (4)	-	27/27	77.3	11.5**	7.5**	0.5
KBR 3023 (20)	-	27/27	39.8	5.0	2.0	0.0
KBR 3023 (100)	-	27/27	47.7	8.5*	4.5	0.0
Mitomycin C (0.08)	-	27/27	62.5	35.5**	30.0**	9.5**
Ethanol	+	2/27	100	4.0	2.0	0.5
Untreated	+	2/27	111.3	2.0	1.0	0.0
KBR 3023 (100)	+	2/27	83.5	3.5	3.5	0.0
KBR 3023 (700)	+	2/27	76.3	5.5	3.5	0.0
KBR 3023 (1400)	+	2/27	80.4	24.5**	22.0**	7.5
Endoxan (12.5)	+	2/27	121.6	27.0**	24.0**	4.5

a Data taken from Study Report Tales 1-7, pages 35-41
b Calculated by reviewer; statistical analysis not performed
* $p \leq 0.05$, ** $p \leq 0.01$

Table 2. Summary of Clastogenic Effects of KBR 3023 in CHO Cells (Trial 2)[a]

Treatment (µg/mL)	±S9	Treatment/Harvest Time (hrs)	Mitotic Index (% of solvent control)	Cells With Aberrations (%)		
				+Gaps	-Gaps	Exchanges
Ethanol	-	2/18	100	4.5	4.0	0.0
Untreated	-	2/18	105.6	4.0	3.0	1.0
KBR 3023 (400)	-	2/18	104.8	6.0	3.5	0.5
KBR 3023 (800)	-	2/18	88.0	5.5	4.5	1.5
KBR 3023 (1200)	-	2/18	103.2	9.0	7.5	3.0*
Mitomycin C (2)	-	2/18	17.6	87.5**	87.5**	69.0**
Ethanol	+	2/18	100	3.0	3.0	0.0
Untreated	+	2/18	115.5	3.0	2.0	0.0
KBR 3023 (800)	+	2/18	119.8	4.5	3.0	0.0
KBR 3023 (1200)	+	2/18	77.6	12.0**	9.0**	4.5**
KBR 3023 (1600)	+	2/18	14.7	51.9**	49.0**	22.1**
Endoxan (10)	+	2/18	69.0	52.0**	49.0**	30.0**
Ethanol	-	2/27	100	6.0	3.5	0.5
KBR 3023 (1200)	-	2/27	67.1	38.0**	38.0**	12.0**
Ethanol	+	2/27	100	6.0	5.0	0.0
KBR 3023 (1600)	+	2/27	47.1	44.0**	40.5**	13.5**

a Data taken from Study Report Tales 8-11, pages 42-45
* $p \leq 0.05$, ** $p \leq 0.01$

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study author concluded that in this *in vitro* cytogenetic test KBR 3023 showed evidence of clastogenic activity at cytotoxic dose levels (1200 µg/mL, -S9 and 1200-1600 µg/mL, +S9). In addition, statistically significant increases in the number of metaphases with aberrations were observed after continuous treatment at 4 and 100 µg/mL (-S9); however, the response was not dose dependent.

262

B. Reviewer's Discussion

KBR 3023 was tested to cytotoxic concentrations and the limit of solubility. With a 2-hour treatment period in the presence of metabolic activation, significant increases in aberration frequencies ($p \leq 0.01$) were obtained at 1200-1600 µg/mL when cells were harvested after 18 or 27 hours. At these dose levels, cytotoxicity was apparent after 18 hours and was less so or absent after 27 hours, suggesting recovery during the succeeding cell cycle. In the absence of metabolic activation, the results were less clear. Significant increases in aberration frequencies were obtained at the lowest dose level (4 µg/mL) after 7-27 hours of treatment ($p \leq 0.01$), at 20 µg/mL after 7 hours of treatment ($p \leq 0.01$), and at 100 µg/mL after treatment times of 7 and 18 hours ($p \leq 0.05$ or $p \leq 0.01$). Although the aberration frequency at all three dose levels was increased at 7 hours, a dose response was not obtained. In addition, these results were observed in the absence of cytotoxicity. When treatment was reduced to 2 hours, the response for clastogenicity at the highest dose level (1200 µg/mL) was negative after 18 hours, but positive after 27 hours. There were no significant increases in clastogenic activity above solvent control levels for any other dose level tested with metabolic activation. The untreated and solvent controls gave the appropriate responses. The sensitivity of the assay system to detect damage to chromosomes was adequately demonstrated by the results obtained with the positive controls (mitomycin C, -S9 and Endoxan, +S9).

The reviewers agree with the study author's conclusion that under the given experimental conditions, KBR 3023 was clastogenic with metabolic activation for Chinese hamster ovary cells cultured *in vitro*. However, the response was equivocal in the absence of metabolic activation, because a significant increase in aberration frequency was observed only at the highest dose level (1200 µg/mL) following a 2-hour treatment and the observed increases in aberration frequencies at lower dose levels with treatment times of 7-27 hours were not dose related.

C. Study deficiencies

The following deficiencies were noted: (i) the purity of ethanol used as solvent for the dose preparations was not indicated; (ii) the stability of the test substance in ethanol was demonstrated for up to 5 hours at room temperature, however, treatment times were as long as 27 hours at 37°C; (iii) dose preparations were not analyzed for actual concentrations of test compound; and (iv) historical negative control values used to determine the acceptability of the study were not reported. These deficiencies would not be expected to alter the study conclusions, because KBR 3023 was tested to cytotoxic concentrations and the test was positive for clastogenicity in the presence of metabolic activation.

DATA EVALUATION RECORD

013529

KBR 3023

Study Type: §84-2; *In vivo* Mammalian Cytogenetics - Micronucleus assay

Work Assignment No. 3-47M (MRID 44408734)

Prepared for

Health Effects Division
Office of Pesticide Programs
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

264

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.

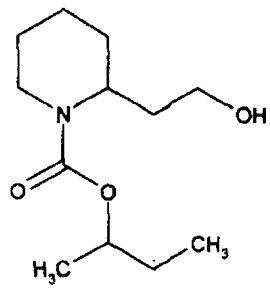
This study is classified as **acceptable**, and it satisfies the requirement for FIFRA Test Guideline **§84-2** for *in vivo* cytogenetic mutagenicity data.

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.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

Picaridin

Page 272 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.

_____ Internal deliberative information.

_____ Attorney-Client work product.

_____ Claimed Confidential by submitter upon submission to the Agency.

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.

013529

DATA EVALUATION RECORD

KBR 3023

Study Type: §85-1; Metabolism in Rats

Work Assignment No. 3-47N (MRID 44408735)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
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Date: 10/2/98

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Date: 10/2/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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

John Whalan 4-14-99

EPA Secondary Reviewer: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal and Intravenous Metabolism - Rat
OPPTS Number: 870.7485 OPP Guideline Number: §85-1

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

TEST MATERIAL (PURITY): KBR 3023 (≥98.3% a.i.)

SYNONYMS: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine;
1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropyl ester;
Propidine;

CITATION: Ecker, W. and Weber, H. (1997) [Hydroxyethyl-1-^{14}C]KBR 3023: Rat Metabolism Study After Intravenous Injection and After Dermal Application. Bayer AG, Institute for Metabolism Research and Residue Analysis, Leverkusen-Bayerwerk, Germany. Laboratory Project ID: M-182-0460-1. February 27, 1997. MRID 44408735. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division, Kansas City, MO

EXECUTIVE SUMMARY:

In a rat metabolism study (MRID 44408735), [2-(2-hydroxyethyl-2-^{14}C)] KBR 3023 (≥98.3% a.i.) was administered to five Sprague-Dawley, Crl:CD BR (SPF) strain rats/sex/dose group as a single intravenous dose at 20 mg/kg, a single dermal dose at 20 or 200 mg/kg (0.5 or 5 mg/cm^2), or a single dermal dose at 20 mg/kg following a 14-day pretreatment with unlabeled KBR 3023 at 20 mg kg. Intravenously (i.v.) dosed rats were terminated after 2 days. Dermally dosed rats were terminated after a single 7-day exposure period.

Within 48 hours of i.v. dosing, 91-97% of the administered dose was recovered from both sexes of each i.v. dose group. Expired air ($^{14}CO_2$) accounted for ≤0.02% of the dosed radioactivity in both sexes. Renal excretion was the primary route of elimination, with urinary excretion being higher in females (87-90% of dose) than males (74-82% of dose). Fecal excretion was a

274

secondary route of elimination and was higher in males (12-17% of dose) than females (5-6% of dose). Radioactivity remaining in the body accounted for 0.4-0.5% of the dose for both sexes.

[^{14}C]KBR 3023 was readily absorbed by rats following dermal dosing. Low-dose rats (with and without pretreatment) absorbed 58-63% of the applied dose by the end of the 7-day exposure period. Absorption was lower in high-dose animals (40-55% of dose), indicating possible saturation of the absorption process. Total recovery of radioactivity from dermally dosed rats following the 7-day exposure period was 73-81% of the administered dose for low-dose rats (with or without pretreatment) and 67-79% of the administered dose for the high-dose rats. Approximately 19-33% of the dose was not recovered from dermally treated rats, compared to 3-9% of the dose from i.v. dosed rats, most likely due to evaporation over the course of the 7-day exposure. As was observed with i.v. dose rats, renal excretion was the primary route of elimination. In low-dose rats (with or without pretreatment), renal excretion accounted for 55-56% of the dose for females and 43-46% of the dose for males, and fecal excretion accounted for 7% of the dose in females and 12-15% of the dose in males. Increasing the dose reduced the total levels of excretion further, but did not affect the pattern of excretion. In the high-dose groups, renal and fecal excretion respectively accounted for 26 and 7% of the dose in males and 36 and 4% of the dose in females. Radioactivity remaining in the carcass (excluding skin at treatment site) at sacrifice was low for all dermally dosed groups, accounting for 0.4-0.5% of the dose for males and 0.2-0.4% of the dose for females.

Excretion of radioactivity following i.v. or dermal dosing with [^{14}C]KBR 3023 was rapid. For intravenously dosed rats, 83-92% of the dose was excreted in the urine and feces within 24 hours of dosing. For dermally dosed rats (except high dose males), 32-50% of the dose was excreted within 48 hours dosing, equivalent to 66-81% of the total radioactivity excreted.

The general distribution of radioactivity in blood and tissues at the time of sacrifice was similar between sexes and dose groups, and the concentration of radioactivity in blood and tissues were comparable between males and females from the same dose group. The only consistent difference between sexes from all dose groups was that radioactivity in livers of males was 1.4-3.4x higher than in livers of females. In addition, high-dose males generally had higher concentrations of radioactivity in their blood and tissues than high-dose females. Besides skin from dermally dosed animals, concentrations of radioactivity were highest in liver, fat, kidneys, and lungs. The lowest concentrations of radioactivity were found in the brain.

With the exception of skin, concentrations of radioactivity in blood and tissues were higher for intravenously dosed rats than for dermally dosed rats at a comparable dose level. Pretreatment had little or no affect on the concentration of radioactivity in blood and tissues, but increasing the dermal dose from 20 to 200 mg/kg, proportionally increased the level of radioactivity in blood and tissues, although the increase was more pronounced for males than females.

Elimination of radioactivity from plasma of i.v. dosed rats was rapid and sex-dependent. Females had higher plasma concentrations and lower clearance values than males. For the dermally dosed groups, absorption was rapid with half-lives of 0.8-3.4 hr. Maximum plasma concentration were reached within 6-8 hours of dosing and were higher in females than males.

Elimination of radioactivity from plasma of dermally dosed animals was dose- and sex-dependent. Elimination of radioactivity from low-dose groups could be described using a single half-life, but elimination from the high-dose rats was biphasic. In each dermal dose group, females had greater areas under the plasma concentration curves (AUC), lower clearance (CL) values, and shorter mean residence times (MRTs) than males.

Nineteen metabolites were identified by MS and NMR in urine and feces of both i.v. and dermally dosed rats. Analysis of excreta from i.v. dosed rats identified 61-78% of the dose in urine and 6% of the dose in feces. For the dermally dosed groups, analysis of urine and feces identified 24-53% of the dose, or 75-85% of the radioactivity recovered in excreta.

With the exception of two metabolites M18 and M19 (each at <0.3% of dose) that were found only in feces, the metabolite profile and relative distribution of metabolites was the same in urine and feces, although actual metabolite levels were considerably lower in feces. There was also no qualitative difference in the metabolite profile between dose groups and sexes, and there was little effect of dose group on the relative distribution of metabolites in excreta. The only notable difference between dose groups was that minor amounts of parent (0.5-1.3% of dose) were detected in excreta of dermally dosed rats, whereas parent was not detected in excreta of i.v. dosed rats. While the overall metabolite pattern was similar between sexes, there were minor quantitative differences in the relative distribution of the principal metabolites between the sexes.

Regardless of dose group or sex, the principal metabolites identified in excreta of rats included: M8 (4.9-18.5% dose), M9 (5.7-21.1% dose), M10 (2.0-8.7% dose), and M16 (2.5-40.2% dose). Collectively these four metabolites accounted for 17.1-70.6% of the dose, or 67-91% of the identified metabolites. The remaining metabolites, M1-M7, M11-M15, M18, and M19 were minor components that individually accounted for <5.0% of the dose.

These data indicate that renal excretion is the principal pathway for elimination of KBR 3023 from rats following either intravenous or dermal dosing. The metabolism of KBR 3023 in rats primarily involves oxidation of the 2-hydroxyethyl group to an acid to form metabolite M16, coupled with hydroxylation of the 1-methylpropyl group to form metabolites M8, M9, and M10. The other minor phase I metabolites result from hydroxylation of the piperidine ring (M1-M4 and M7). Minor phase II metabolites result from conjugation of glucuronic acid with parent (M14 and M15) or phase I metabolites (M5, M6, and M11-M13).

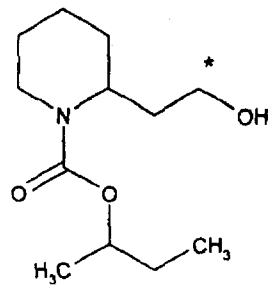
This study is classified **Acceptable**.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: KBR 3023
   Chemical Name: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine
   Description: Clear colorless liquid
   Lot/Batch Nos: 890814ELB01 and 921103ELB04
   Purities: 99.1% and 98.3% a.i.
   CAS #: 119515-38-7
   Structure:



*denotes position of ^{14}C-label

   [2-(2-hydroxyethyl-2-^{14}C)]KBR 3023
   Description: None
   Reference Nos.: KML 2061 (i.v. tests) and KML 2169 (dermal tests)
   Radiochemical purity: KML 2061, >99%, (by HPLC, TLC and GC)
   KML 2169, >98%, (by HPLC, TLC and GC)
   Specific activity: KML 2061, 106 µCi/mg; and KML 2169, 96 µCi/mg

2. Vehicle: The test material was dissolved in physiological saline for intravenous dosing, but was applied neat for the dermal applications.

3. Test animals: Species: Rat
   Strain: Sprague-Dawley, Crl:CD BR (SPF)
   Age: males, 9-10 weeks; females 18-21 weeks
   Weight at start of treatment: Males, 236-281 g; Females, 229-271 g
   Source: Lippische Versuchstierzucht, Hagemann GmbH, D-32699 Extertal, and Charles River Wiga GmBB, D-97633 Sulzfeld.
   Diet: Altromin 1324 certified standard feed for rats, Altrogge, D-32791 Lage/Lippe; 20 g/animal/day
   Water: Tap, *ad libitum*
   Housing: Individual Makrolon type III cages; and Makrolon metabolism cages following dosing with ^{14}C-labeled material.

Environmental conditions:
Temperature: 18-25 °C
Mean humidity: 46-83%
Air changes: 10-15/hour
Photoperiod: 12-Hour light/dark cycle
Acclimation period: ~1 week

B. STUDY DESIGN:

The study was designed to determine the absorption, metabolism, distribution and excretion of [^{14}C]KBR 3023, as a function of either a single intravenous injection or single and repeated dermal dosing of rats. Dermal dosing of rats was selected as the method of treatment for the main part of the study because dermal exposure would be the principal route of exposure in humans. The study was conducted from July 10, 1991 to March 29, 1994.

1. Group Arrangements

Animals were randomly assigned to each test group by lottery as they arrived at the testing facility. The dose groups used in this study are summarized in Table 1. Each dose group consisted of 5 rats/sex, except for the repeated dose group which also contained two reserve animals/sex.

The initial intravenous dosing group (test groups 1 and 2) was used to assess excretion of radioactivity over time in urine and feces and the extent of elimination of radioactivity as expired $^{14}CO_2$. The subsequent intravenous dose group (test groups 3 and 4) along with the dermal dosing groups (test groups 5-10), were used to assess excretion of radioactivity in the urine and feces, plasma pharmacokinetics, dermal absorption, distribution of residual radioactivity in tissues, and the metabolic profile in excreta. Rats dosed intravenously were sacrificed 2 days after dosing, and rats dosed dermally were sacrificed after a 7-day exposure period.

The 20 mg/kg dose level was selected for intravenous dosing as it was the highest concentration of KBR 3023 that could be dissolved in physiological saline and still form a stable solution at room temperature. For dermal dosing, the 200 mg/kg (high) dose, was selected as this level was equivalent to the maximum amount (50 µL) of neat KBR 3023 that could be applied to the 10 cm^2 treatment site without the test material flowing across the skin. The 20 mg/kg (low) dermal dose was selected as it represented a 10-fold reduction of the high dose.

Table 1. Dosing groups for [^{14}C]KBR 3023 studies.

Group No.[a]	Dose	Nominal dose (mg/kg)	Actual dose[b] (mg/kg)	Remarks
1	Single low i.v. dose given in tail vein	20	20.2	Examined excretion of radioactivity in urine, feces, and expired $^{14}CO_2$, and characterized metabolite profile in urine and feces. Animals were sacrificed after 2 days.
2			19.9	
3	Single low i.v. dose given in femoral vein	20	19.2	Examined excretion of radioactivity in urine and feces, and determined residual radioactivity in tissues at sacrifice (2 days post-dose). Examined plasma pharmacokinetics, and characterized metabolite profile in urine and feces. Urine and fecal extracts from Groups 1-4 were pooled and used for identification of metabolites.
4			20.3	
5	Single low dermal dose	20	15.7	Doses equivalent to 5 and 50 mg/rat or 0.5 and 5 mg/cm^2. Examined excretion of radioactivity in urine and feces, and radioactivity in plasma over time to characterize pharmacokinetics. Characterized metabolite profile in urine and feces. Determined residual radioactivity in blood and tissues at sacrifice following 7 day exposure period.
6			17.8	
7	Repeat low dermal dose	20	17.7	
8			17.1	
9	Single high dermal dose	200	195.9	
10			195.1	

a Odd and even numbered test groups were comprised of 5 males and 5 females/group, respectively. Test groups 7 and 8 also included two reserve animals each.

b Actual dose is the average of 5 rats /test group.

2. Dose preparation

For intravenous dosing, [^{14}C]KBR 3023 (106 µCi/mg) was diluted with non-labeled KBR 3023 (99.1% a.i.) to a final specific activity of 10.6 µCi/mg. The final test material was then diluted in physiological saline using an ultrasonic water bath at 30°C for 10 minutes to give a final concentration of 5 mg/mL. Fresh dosing solution was prepared for each test. Radioactivity in the dosing solutions were determined by liquid scintillation counting (LSC). TLC analyses of the dosing solutions indicated that the test compound was stable for at least 4 hours at room temperature.

For dermal dosing, [^{14}C]KBR 3023 (96 µCi/mg) was diluted with non-labeled KBR 3023 (98.3% a.i.) to a final specific activity of 9.6 µCi/mg for the low-dose applications and 0.96 µCi/mg for the high-dose applications. The test material was applied neat and radioactivity in dosing solutions was determined by LSC.

3. Dosing

For intravenous dosing, the test compound was injected into the tail vein for rats in test groups 1 and 2 and into the femoral vein for rats in test groups 3 and 4. Injection volumes were 4 mL/kg bw or ~1 mL/rat. Actual average doses were 19.2-20.3 mg/kg for each test group (Table 1).

The dermally dosed animals were dosed with a single 7-day exposure of the radiolabelled [^{14}C]KBR 3023. At least 24 hours before dermal dosing, the dorsal area on each animal was shaved and wiped with acetone, taking care not to abrade the skin. For animals that were pretreated for 14 days with non-labeled KBR 3023, the treatment area was marked and examined daily for hair growth. If necessary, the area was shaved again before the next application. For application of [^{14}C]KBR 3023, a rubber O-ring with an inner area of 10 cm^2 was glued to the shaved area on the back of each rat. While under slight anaesthesia, a topical application of neat [^{14}C]KBR 3023 was applied to the skin of each rat at 5 or 50 µL/rat for the low- and high-dose groups, respectively, using a calibrated syringe. The test material was evenly distributed with a glass rod, and a filter paper cover was glued to the rubber ring. The entire site was then covered with a protective tube-gauze dressing secured with adhesive tape. The average dose actually applied was 15.7-17.8 mg/kg for the single low-dose and repeated low-dose dermal groups, and 195.1-195.9 mg/kg for the single high-dose dermal group (Table 1).

Following dosing, rats were placed in individual metabolism cages suitable for the separate collection of urine and feces. Cages for intravenously dosed rats in test groups 1 and 2 were also fitted with traps suitable for the collection of expired $^{14}CO_2$.

4. Sample Collection

a. Intravenous dose groups. Urine was collected at 8, 24, 32, and 48 hours following dosing, and feces were collected at 24 and 48 hours. For animals in test groups 1 and 2, expired $^{14}CO_2$ was also collected at 8, 24, 32, and 48 hours post-dose using a trapping system containing ethanolamine:ethanol (1:1 v/v). Blood samples (50-100 µL) were collected from the tail vein of animals in test groups 3 and 4 pre-dose and at 0.17, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 24, 32, and 48 hours post-dose and separated into plasma and cell fractions by centrifugation. Animals were sacrificed 48 hours post-dose by exsanguination while under anaesthesia and any residual urine in the bladder was added to the final urine sample. The following tissues were collected from animals in test groups 3 and 4: skin, erythrocytes, plasma, bone (femur), brain, heart, liver, spleen, kidneys, fat (perirenal), muscle (femur), testes, uterus, ovaries, gastrointestinal (GI) tract, and residual carcass. Animals in test groups 1 and 2 were separated only into the GI tract and the residual carcass. Cages were washed by immersion in aqueous detergent.

b. Dermal dose groups. Urine and feces were collected at 24-hour intervals throughout the 7-day exposure period. Blood samples (50-100 µL) were collected from the tail

280

veins of each animal pre-dose, at 1, 1.5, 2, 3, 4, 6, 8, 24, 32, and 48 hours post-dose, and then at 24-hour intervals thereafter until sacrifice. Blood samples were centrifuged to obtain plasma. At the end of the exposure period, the tube-gauze and the O-ring were removed from the skin under anaesthesia, and the filter paper was separated from the O-ring. The skin at the treatment site was washed four times with a 1% detergent solution using cotton swabs. The tube-gauze, filter paper, O-ring and cotton swabs were all saved for radioassay. The animals were then sacrificed by exsanguination while under anaesthesia and any residual urine in the bladder was added to the final urine sample. The following tissues were collected from each animal: skin (application site), skin, erythrocytes, plasma, bone (femur), brain, heart, lungs, liver, spleen, kidneys, fat (perirenal), muscle (femur), testes, uterus, ovaries, GI tract, and residual carcass. Cages were washed by immersion in aqueous detergent.

c. Radioassay of samples. Samples of urine, cage wash, plasma, and $^{14}CO_2$ trapping solution were analyzed for total radioactivity directly by LSC. Fecal samples were lyophilized, weighed, homogenized and combusted prior to LSC. Glass rod applicators were rinsed with ethanol, and tube-gauzes and filter paper covers were rinsed and sonicated with ethanol. The cotton swabs used for skin washes were soaked and sonicated in water. Radioactivity in the resulting ethanol and water solutions was determined by LSC. Samples of skin, fat, uterus, and ovaries were solubilized for ~24 hours in tissue solubilizer prior to LSC. The remaining tissue samples were lyophilized and homogenized prior to combustion and LSC.

3. Metabolite Characterization

For quantitation of metabolites in urine, urine samples collected from 0-24 hours from rats in the i.v. dose groups (test groups 1-4) were pooled by dose group and sex, and urine samples collected from 0-96 hours from rats in the dermal dose groups (test groups 5-10) were also pooled by dose group and sex. Aliquots of pooled urine samples were lyophilized, reconstituted in 1/10 the original volume, and analyzed and quantified directly by reverse-phase HPLC.

For quantitation of metabolites in feces, lyophilized fecal samples from each collection interval from rats in the dermal dose groups were pooled by dose group and sex. For i.v. dosed rats, lyophilized fecal samples from each collection interval and each test group were pooled into a single sample for analysis. Pooled fecal samples were extracted repeatedly (5x), first with acetonitrile (ACN) and then with water. ACN extracts were analyzed and quantified directly by reverse-phase HPLC. Water extracts were concentrated to dryness, and reconstituted in methanol:water (4:1, v/v) prior to HPLC analysis.

Quantitative analyses of urine and fecal extracts utilized a reverse-phase HPLC system consisting of a C_{18} column using a mobile phase gradient of 1% acetic acid in water to 1% acetic acid in ACN, with a UV detector (210 nm) and an in-line radioactivity monitor.

For identification of metabolites M1-M17, a pooled urine sample from i.v. dose rats (test groups 1-4) was separated by preparative HPLC into four major metabolite fractions based upon metabolite polarity. Metabolites in each fraction were further purified and isolated by repetitive HPLC analyses. Selected metabolite fractions were silylated for GC/MS analysis using a mixture of N-methyl-N-trimethylsilyl-trifluoroacetamide with 5% trimethylsilyl-chloride. Isolated metabolite fractions were identified by MS and NMR analyses.

For identification of the fecal metabolites M18 and M19, ACN extracts from dermally dosed rats were pooled and subjected to repetitive HPLC analyses, collecting the late eluting fraction containing M18 and M19. Subsequent HPLC analyses isolated and purified these metabolites and their structures were determined by MS and NMR analyses.

4. Statistics

Radioactivity in terms of the % of the administered dose was reported for individual samples and as a total from each animal, along with means and coefficients of variation for each test group. Radioactivity in blood and tissues was also expressed in terms of concentration (μg equivalents/g). Plasma curve analyses were performed using the computer software program TOPFIT 2.0.

II. RESULTS

A. Absorption, Excretion, Distribution, and Recovery

1. Absorption

Although absorption was incomplete, [^{14}C]KBR 3023 was readily absorbed by rats following dermal dosing as indicated by the excretion of radioactivity in the urine and feces. Including radioactivity in the excreta, the body (with GI tract), and the skin at the application site, low-dose rats (with and without pretreatment) absorbed 58.2-63.0% of the applied dose by the end of the 7-day exposure period. Dermal absorption was dose dependent with lower levels of absorption occurring in the high-dose animals (40.0-54.6% of dose). Absorption was rapid given the time course of excretion; 44-79% of the total amount of radioactivity absorbed was recovered in excreta within the first 48 hours of exposure.

2. Excretion

Excretion of radioactivity following i.v. or dermal dosing with [^{14}C]KBR 3023 was rapid. For intravenously dosed rats, 83.4-92.1% of the dose was excreted in the urine and feces within 24 hours of dosing. Excretion of radioactivity in expired air ($^{14}CO_2$) was minor ($\leq$0.02% of dose) for both sexes. For dermally dosed rats (except high dose males), 32.0-

282

50.4% of the dose was excreted after 48 hours of exposure, equivalent to 66-80.5% of the total radioactivity excreted. Renal excretion (25.7-90.4% dose) was the primary route of elimination, with fecal excretion being a secondary pathway (3.6-16.5% of the dose). For each dose group, renal excretion was higher for females (35.7-90.4% of dose) than for males (25.7-86.8% of dose); whereas, levels of fecal excretion were higher for males (6.9-16.5% of dose) than females (3.6-7.1% of dose). Rats dosed intravenously had higher levels of urinary excretion (74.4-90.4% of dose) than dermally dosed rats (25.7-55.7% of dose), but similar levels of fecal excretion (i.v. dosed rats 4.81-16.5% dose; dermally dose rats, 3.6-14.9% dose).

Among the dermally dosed groups, cumulative excretion was similar for the single low-dose (58.8-62.8% of dose) and repeated low-dose groups (57.8-62.7% of dose), but was reduced in the high-dose group (39.7-54.2% of dose). However, the distribution of radioactivity between urine and feces was similar for each of the dermally dosed groups. The pattern of excretion over time was similar for the low- and high-dose groups. Urinary excretion was highest on Day 1 and declined steadily throughout the test period; fecal excretion was steady or increased slightly over the first two days of exposure and declined slowly thereafter. Pretreatment had a slight effect on the elimination of radioactivity over time. In rats pretreated for 14 days with KBR 3023 prior to dosing with [^{14}C]KBR 3023, there was a notable delay in excretion. Renal excretion was steady or increased during the first two days of exposure and declined steadily thereafter, and fecal excretion increased between Days 1 and 2 and then declined slowly thereafter.

a) Single i.v. low-dose: Following a single i.v. dose of [^{14}C]KBR 3023 (test groups 1-4), radioactivity was rapidly eliminated in the urine (Table 2). Within 24 hours of dosing, renally excreted radioactivity accounted for 71.0-79.8% of the dose for males and 84.9-87.3% of the dose for females. By the end of the test period (48 hours), total urinary excretion accounted for 74.4-82.3% of the dose for males and 86.8-90.4% of the dose for females. For both i.v. dosing regimes, levels of renal excretion were higher for females than males. Fecal excretion of radioactivity was a secondary route of elimination and was higher in males (12.3-16.5% dose) than in females (4.8-6.3% dose). Expired air ($^{14}CO_2$) accounted for only a minor fraction (≤0.02%) of the dosed radioactivity for both males and females. Cumulative urinary and fecal excretion was 90.9-94.6% of the dose for males and 91.6-96.6% of the dose for females.

Table 2. Recovery over time of radioactivity in expired air and excreta of rats following administration of a single i.v. dose of [^{14}C]KBR 3023 at 20 mg/kg.[a]

Sample	Percent of radioactive dose administered									
	Males[b] - Group 1					Females[b] - Group 2				
	8 hr	24 hr	32 hr	48 hr	Total	8 hr	24 hr	32 hr	48 hr	Total
Expire ($^{14}CO_2$)	0.012	0.001	0.001	0.002	0.016	0.015	0.005	0.001	0.001	0.02
Urine	53.33	17.66	2.16	1.24	74.39	69.94	17.39	1.69	1.33	90.35
Feces[c]	NS[d]	12.43	NS	4.08	16.51	NS	4.80	NS	1.47	6.27
Total	53.34	30.09	2.16	5.32	90.92	69.96	22.20	1.69	2.80	96.64
Sample	Males[e] - Group 3					Females[e] - Group 4				
	8 hr	24 hr	32 hr	48 hr	Total	8 hr	24 hr	32 hr	48 hr	Total
Urine	62.16	17.65	1.71	0.76	82.28	67.79	17.12	1.19	0.72	86.82
Feces[d]	NS	10.18	NS	2.16	12.34	NS	4.38	NS	0.43	4.81
Total	62.16	27.83	1.71	2.92	94.62	67.79	21.50	1.19	1.15	91.63

a Data are the mean of 5 animals/sex/group and are from Appendices A3, A5, A7, and A11 (pages 83, 85, 87 and 91) of the study report.
b Groups 1 and 2 were i.v. dosed via a tail vein and expired $^{14}CO_2$ was collected.
c Feces were sampled at 0-24 and 24-48 hour intervals.
d NS = Not sampled.
e Groups 3 and 4 were i.v. dosed via a femoral vein and expired $^{14}CO_2$ was not collected.

b) Single dermal low-dose: Following dermal application of a low dose of [^{14}C]KBR 3023, cumulative urinary and fecal excretion (including the cage wash) accounted for 58.8% and 62.8% of the dose for males and females, respectively (Table 3). As with the i.v. dose groups, excretion of radioactivity was primarily renal, and renal excretion was greater for females (55.1% dose) than males (46.1% dose). Males excreted higher levels of radioactivity (12.3% dose) in their feces than females (7.1% dose). For both males and females, renal excretion was highest on Day 1 at 20.2-31.3% of the dose and declined steadily to 0.39-0.59% of the dose on Day 7. Within 2 days of application, the 74-83% of the radioactivity recovered in the urine had been excreted. Fecal excretion was steady over the first two days of exposure (2.3-3.9% of dose/day) and declined slowly thereafter to 0.3-0.4% of dose by Day 7.

Table 3. Recovery over time of radioactivity in excreta of rats following dermal application of [^{14}C]KBR 3023 at 20 mg/kg[a].

Sample	Percent of radioactive dose administered							
	Males (Group 5)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	Total
Urine	20.22	14.01	6.04	2.95	1.31	0.96	0.59	46.08
Feces	3.88	3.74	2.06	1.11	0.64	0.55	0.29	12.27
Cage Wash	NS[b]	NS	NS	NS	NS	NS	0.49	0.49
Total	24.10	17.75	8.10	4.06	1.95	1.51	1.37	58.84
Sample	Females (Group 6)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	Total
Urine	31.26	14.36	5.89	1.84	0.89	0.47	0.39	55.10
Feces	2.25	2.51	1.23	0.38	0.22	0.07	0.43	7.09
Cage Wash	NS	NS	NS	NS	NS	NS	0.57	0.57
Total	33.51	16.87	7.12	2.22	1.11	0.54	1.39	62.76

a Data are the mean of 5 animals/sex at each sampling interval and are from Tables , and VI and Appendices A15 and A21 (pages 64, 95 and 105) of the study report.

b NS = Not sampled.

c) Repeated dermal low-dose: Pretreatment with KBR 3023 had a slight effect on the initial rate of excretion, but no effect on the cumulative excretion of radioactivity for males or females. Cumulative urinary and fecal excretion (including the cage wash) accounted for 57.8% and 62.7% of the dose for males and females, respectively (Table 4). As with the single low dermal and i.v. dose groups, excretion of radioactivity was primarily renal, and renal excretion was greater for females (55.7% dose) than males (42.6% dose). Males excreted higher levels of radioactivity (14.9% dose) in their feces than females (6.5% dose). Whereas renal excretion was highest for the single dermal low-dose groups on Day 1 and declined thereafter, renal excretion for repeated dose rats was steady or increased during the first two days of exposure and declined steadily thereafter. For males, urinary excretion accounted for 15.7% and 13.9% of dose on Days 1 and 2, respectively; and for females, urinary excretion accounted for 18.5% and 20.4% of the dose on Days 1 and 2, respectively. Although renal excretion was somewhat delayed, 70% of the radioactivity recovered in the urine was excreted within 2 days of application, as was noted in the single dermal dose groups. A more pronounced delay was noted in fecal excretion for both males and females, fecal excretion increased from Day 1 (1.0-2.8% dose) to Day 2 (2.9-5.6% dose) and then declined slowly to 0.08-0.32% of the dose on Day 7.

Table 4. Recovery over time of radioactivity in excreta of rats following dermal application of [^{14}C]KBR 3023 at 20 mg/kg after 14 consecutive daily doses of KBR 3023 at 20 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males (Group 7)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	Total
Urine	15.74	13.92	7.32	3.09	1.32	0.78	0.46	42.63
Feces	2.76	5.59	3.07	1.82	0.71	0.58	0.32	14.85
Cage Wash	NS[b]	NS	NS	NS	NS	NS	0.27	0.27
Total	18.50	19.51	10.39	4.91	2.03	1.36	1.05	57.75
Sample	Females (Group 8)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	Total
Urine	18.45	20.35	9.88	4.11	1.77	0.66	0.44	55.66
Feces	0.96	2.91	1.51	0.69	0.22	0.16	0.08	6.53
Cage Wash	NS	NS	NS	NS	NS	NS	0.49	0.49
Total	19.41	23.26	11.39	4.80	1.99	0.82	1.01	62.68

a Data are the mean of 5 animals/sex at each sampling interval and are from Tables V and VI and Appendices A15 and A21 (pages 64, 95 and 105) of the study report.
b NS = No sample.

c) Single high dermal dose: The cumulative excretion of radioactivity was reduced in both high-dose males (54.2% of dose; Table 5) and females (39.7% of dose), compared to the low-dose animals. However, the pattern and rate of excretion were similar for high- and low-dose dermally treated rats. As with the single low dermal and i.v. dose groups, excretion of radioactivity was primarily renal, and renal excretion was greater for females (35.7% dose) than males (25.7% dose). Males excreted higher levels of radioactivity (6.9% dose) in their feces than females (3.6% dose). For both males and females, renal excretion was highest on Day 1 at 13.0-20.5% of the dose and declined steadily to 0.35-0.69% of the dose on Day 7. Within 2 days of application, 75-83% of the total radioactivity recovered in urine had been excreted. For males, fecal excretion was steady over the first two days of exposure (2.1-2.5% of dose/day) and declined slowly thereafter to 0.3% of dose by Day 7. For females, fecal excretion increased from 0.8% of the dose on Day 1 to 1.5% of the dose on Day 2, and slowly declined thereafter to 0.27% of the dose on Day 7.

Table 5. Recovery over time of radioactivity in excreta of rats following dermal application of [^{14}C]KBR 3023 at 200 mg/kg.[a]

Sample	Percent of radioactive dose administered							
	Males (Group 9)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	168 hr	Total
Urine	13.02	6.33	2.62	1.40	0.85	0.78	0.69	25.69
Feces	2.50	2.13	0.93	0.50	0.30	0.23	0.33	6.92
Cage Wash	NS	NS	NS	NS	NS	NS	21.57	21.57
Total	15.52	8.46	3.55	1.90	1.15	1.01	22.59	54.18
Sample	Females (Group 10)							
	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr	68 hr	Total
Urine	20.47	9.23	3.00	1.32	0.86	0.49	0.35	35.72
Feces	0.75	1.51	0.60	0.22	0.17	0.08	0.27	3.60
Cage Wash	NS	NS	NS	NS	NS	NS	0.38	0.38
Total	21.22	10.74	3.60	1.54	1.03	0.57	1.00	39.70

a Data are the mean of 5 animals/sex at each sampling interval and are from Tables V and VI and Appendices A39 and A45 (pages 64, 35 and 145) of the study report.
b NS = No sample.

3. Tissue Distribution

Levels of radioactivity at sacrifice (2 days for i.v. dosing and 7 days for dermal dosing) in organs, tissues and blood from male and female rats from each dose group are summarized in Table 6. Generally, the pattern of distribution of radioactivity among tissues was similar between sexes and dose groups, and the concentration of radioactivity in blood and tissues were comparable between males and females from the same dose group. The only consistent difference between sexes from all dose groups was that radioactivity in livers of males was 1.4-3.4x higher than in livers of females. In addition, males from the high dose group generally had higher concentrations of radioactivity in their blood and tissues than females from the same dose group. Besides skin from dermally dosed animals, concentrations of radioactivity were highest in liver, fat, kidneys, and lungs. The lowest concentrations of radioactivity were found in the brain.

With the exception of skin, concentrations of radioactivity in blood and tissues were higher for intravenously dosed rats than for dermally dosed rats at a comparable dose level. Except for higher levels of radioactivity in skin from the application site of pretreated males, pretreatment with KBR 3023 had no effect on the level or distribution of radioactivity in rats. Increasing the dermal dose from 20 to 200 mg/kg, proportionally increased the level of radioactivity in blood and tissues, although the increase was more pronounced for males than females.

a) Single i.v. low-dose: Radioactivity was detectable in all blood and tissue samples from both male and female rats sacrificed 48 hours following a single i.v. dose of [^{14}C]KBR 3023 at 20 mg/kg. Concentrations of radioactivity in organs, tissues and blood were similar between males and females, except in liver (males, 0.208 µg/g; females 0.096 µg/g). For both sexes, the highest residue levels were found in liver (0.096-0.208 µg/g), kidneys (0.115-0.122 µg/g), and fat (0.095-0.111 µg/g). The lowest residue levels were found in brain (0.006-0.010 µg/g). The remaining samples had concentrations of radioactivity at 0.015-0.042 µg/g.

b) Single dermal low-dose: Radioactivity was detectable in all blood and tissue samples from both male and female rats sacrificed following 7 days of exposure to a single dermal dose of [^{14}C]KBR 3023 at 20 mg/kg. Concentrations of radioactivity in organs, tissues and blood were similar between males and females, except in liver (males, 0.042 µg/g; females 0.029 µg/g). Aside from skin at the application site (16.5-23.9 µg/g), the highest residue levels were found in skin (0.79-0.122 µg/g) and fat (0.065-0.086 µg/g), followed by kidneys, liver, lungs, uterus and ovaries (0.024-0.046 µg/g). The lowest residue levels were found in brain (0.002-0.003 µg/g). The remaining samples had concentrations of radioactivity at 0.005-0.013 µg/g. With the exception of skin, radioactivity in blood and tissues of rats dosed dermally at 20 mg/kg was lower than in the same samples from intravenously dosed rats.

c) Repeated dermal low-dose: Pretreatment with KBR 3023 for 14 days prior to a single dose of [^{14}C]KBR 3023 had only one notable effect on the distribution of radioactivity in tissues; radioactivity in skin at the application site was higher for males in the repeated dose group (110.5 µg/g) than males in the single dose group (16.5 µg/g). Concentrations of radioactivity in tissues and blood were similar between males and females, except in skin at the application site (males, 110.5 µg/g; females 19.7 µg/g), liver (males, 0.063 µg/g; females 0.036 µg/g) and fat (males, 0.066 µg/g; females 0.024 µg/g). Aside from skin at the application site, the highest residue levels were found in skin (0.079-0.109 µg/g) followed by fat, kidneys, liver, and lungs (0.024-0.066 µg/g). The lowest residue levels were found in brain (0.004-0.006 µg/g). The remaining samples had concentrations of radioactivity at 0.005-0.030 µg/g.

d) Single dermal high-dose: Increasing the dose of [^{14}C]KBR 3023 to 200 mg/kg increased the accumulation of radioactivity proportionally in blood and tissues, although the increase was more pronounced for males. With the exceptions of skin (application site), carcass, erythrocytes, brain, and lungs, concentrations of radioactivity in tissues were higher in males than females for the high dose group. However, the general pattern of distribution among blood and tissues was similar to the low dose group. Aside from skin at the application site (46.9-95.3 µg/g), the highest residue levels were found in skin, fat, liver, kidneys, ovaries, and lungs (0.181-0.717 µg/g). The lowest residue levels were found in the brain for males (0.034 µg/g) and bone for females (0.038 µg/g). The remaining samples had concentrations of radioactivity at 0.040-0.135 µg/g.

Table 6. Concentration of radioactivity in tissues and blood of rats dosed intravenously or dermally with [^{14}C]KBR 3023 at 20 or 200 mg/kg.[a]

Dose	µg/g (KBR 3023 equivalents)[b]							
	Single low i.v. dose (20 mg/kg)		Single low dermal dose (20 mg/kg)		Repeated low dermal dose (20 mg/kg)		Single high dermal dose (200 mg/kg)	
Tissue/organ	Male	Female	Male	Female	Male	Female	Male	Female
Skin (application site)	NA[c]	NA	16.49	23.86	110.50	19.66	46.91	95.25
Skin	0.027	0.034	0.122	0.079	0.079	0.109	0.583	0.264
Carcass	0.039	0.029	0.010	0.011	0.030	0.010	0.120	0.134
Erythrocytes	0.042	0.034	0.012	0.013	0.019	0.015	0.131	0.130
Plasma	0.020	0.018	0.008	0.005	0.010	0.005	0.131	0.060
Bone (femur)	0.020	0.015	0.009	0.007	0.014	0.008	0.105	0.038
Brain	0.010	0.006	0.003	0.002	0.006	0.004	0.034	0.086
Heart	0.032	0.023	0.010	0.010	0.013	0.010	0.135	0.086
Lung	NS[d]	NS	0.025	0.032	0.036	0.036	0.240	0.286
Liver	0.208	0.096	0.042	0.029	0.063	0.036	0.580	0.181
Spleen	0.031	0.032	0.009	0.008	0.016	0.009	0.102	0.058
Kidneys	0.122	0.115	0.037	0.046	0.051	0.054	0.477	0.291
Fat (perirenal)	0.095	0.111	0.065	0.086	0.066	0.024	0.717	0.503
Muscle (femur)	0.022	0.015	0.007	0.005	0.010	0.007	0.088	0.040
Testes	0.029	NA	0.005	NA	0.008	NA	0.071	NA
Uterus	NA	0.070	NA	0.024	NA	0.013	NA	0.122
Ovaries	NA	0.040	NA	0.039	NA	0.023	NA	0.325

a Animals dosed intravenously were sacrificed 2 days after dosing, and animals dosed dermally were sacrificed 7 days after initial dosing (the test material remained on the skin through the study period).

b Data are the mean of 5 animals/sex/dose group and were obtained from Tables XII, XIII and XIV (pages 70-72) of the study report.

c NA = Not applicable.

d NS = no sample.

4. Recovery of Radioactivity

Overall recovery of radioactivity was dependent on the method of dosing. Recovery the dosed material was higher for i.v. dosed rats (91.4-97.0% of dose) than for dermally dosed rats (67.0-81.2% of dose). For dermally dosed rats, a significant fractions of the dosed material was not recovered (18.9-33.1%). Recoveries were similar between males and females in the same dose group (Tables 7 and 8) with the exception of high-dose group (males, 79.1%; females, 67.0%).

The overall pattern of distribution among the different fractions was similar between dose groups. For both i.v. and dermally dosed rats, urine and feces together accounted for the largest fraction of the radioactivity (i.v., 90.9-96.6%; dermal 32.6-62.2%). In each dose group, renal excretion of radioactivity was higher in females (i.v., 86.8-90.4%; dermal, 35.7-55.7%) than males (i.v., 74.4-82.3%; dermal 25.7-46.1%), whereas fecal excretion was higher in males (all groups, 6.9-16.5%) than females (all groups, 3.6-7.1%). Excluding skin at the dermal treatment site, males also retained higher levels of radioactivity in their bodies (0.43-0.52%) than females (0.19-0.36%).

Comparison of the dermal dose groups indicated that pretreatment had no effect on the recovery or distribution of radioactivity, but increasing the dose reduced the % of the dose recovered in both urine and feces, while the amount of radioactivity associated with the non-biological material increased.

a) Single i.v. low-dose: Following a single i.v. dose of [^{14}C]KBR 3023 at 20 mg/kg, 91.4-95.1% and 91.9-97.0% of the administered dose was recovered from male and females, respectively, within 48 hours. For both males and females, expired $^{14}CO_2$ accounted from ≤0.02% of the dose and radioactivity in excreta accounted for 90.9-96.6% of the dose. Renal excretion of radioactivity was higher in females (86.8-90.4%) than males (74.4-82.3%), and fecal excretion was higher in males (12.3-16.5%) than in females (4.8-6.2%). Males also retained higher levels of radioactivity in their bodies (0.43-0.52%) than females (0.25-0.36%).

Table 7. Recovery of radioactivity from rats dosed intravenously with [^{14}C]KBR 3023 at 20 mg/kg.[a]

Dose	Percent of radioactive dose administered			
	Single low i.v. dose[b] (20 mg/kg)		Single low i.v. dose[b] (20 mg/kg)	
Sample	Male	Female	Male	Female
Expired $^{14}CO_2$	0.02	0.02	NS[c]	NS
Urine	74.39	90.36	82.28	86.82
Feces	16.51	6.23	12.34	4.80
GI tract	0.32	0.19	0.23	0.09
Remaining body	0.20	0.17	0.20	0.16
Total	91.44	96.97	95.05	91.87

a Data are the mean of 5 animals/sex/dose group and are from Table IV (page 63) of the study report).

b Animals in Groups 1 and 2 ($^{14}CO_2$ sampled) were dosed via a tail vein, whereas animals in Groups 3 and 4 were dosed via a femoral vein.

c NS = Not Sampled.

290

b) Single dermal low-dose: Following a 7-day dermal exposure to [^{14}C]KBR 3023 at 20 mg/kg, 73.4 and 74.1% of the administered dose was recovered from males and females, respectively. As was observed for i.v. dosed rats, renal excretion of radioactivity was higher in females (55.1%) than males (46.1%), and fecal excretion was higher in males (12.3%) than in females (7.1%). Males also retained higher levels of radioactivity in their bodies (0.46%) than females (0.20%).

c) Repeated dermal low-dose: Pretreatment with KBR 3023 for 14 days prior to dosing with [^{14}C]KBR 3023 had little or no effect on the recovery of radioactivity. After the 7-day exposure period, 77.4% and 81.2% of the administered dose was recovered from males and females, respectively. As in the other dose groups, renal excretion of radioactivity was higher in females (55.7%) than males (42.6%), and fecal excretion was higher in males (14.9%) than in females (6.5%). Males also retained higher levels of radioactivity in their bodies (0.47%) than females (0.23%). The only notable difference resulting from pretreatment was an increase in radioactivity associated with skin (7.9%) treatment site for males.

d) Single dermal high-dose: Following a 7-day dermal exposure to [^{14}C]KBR 3023 at 200 mg/kg, 79.1% and 67.0% of the administered dose was recovered from males and females, respectively. As in the other dose groups, renal excretion of radioactivity was higher in females (35.7%) than males (25.7%), and fecal excretion was higher in males (6.9%) than in females (3.6%). Males also retained higher levels of radioactivity in their bodies (0.44%) than females (0.19%). Increasing the dose level reduced the overall recovery for females, but not males. For both males and females, increasing the dose level reduced the % dose recovered in both urine and feces and increased the % dose recovered in the non-biological materials (which include skin wash). However, there was no effect on the % of dose retained in the body of either males or females.

In contrast to all other test groups, high-dose males had a large portion of the dose (21.6%) that was recovered in the cage wash. The study authors stated that this was probably due to incomplete rinsing of the urine collection funnel. If the cage wash is included with urine, then urinary excretion for high-dose males would be ~47% of the dose, which is comparable to the results from the dermal low-dose males, but higher than in the high-dose females.

Table 8. Recovery of radioactivity from rats dosed dermally with [^{14}C]KBR 3023 at 20 or 200 mg/kg.[a]

Dose	Percent of radioactive dose administered					
	Single low dermal dose (20 mg/kg)		Repeated low dermal dose (20 mg/kg)		Single high dermal dose (200 mg/kg)	
Sample	Male	Female	Male	Female	Male	Female
Non-biological materials[b]	12.92	9.35	11.27	16.79	24.18	26.47
Cage wash	0.49	0.57	0.27	0.49	21.57	0.38
Urine	46.09	55.10	42.63	55.65	25.69	35.73
Feces	12.27	7.09	14.85	6.52	6.90	3.59
Skin application site	1.20	1.75	7.88	1.55	0.29	0.60
GI tract	0.21	0.07	0.25	0.06	0.31	0.12
Remaining Carcass	0.25	0.13	0.22	0.17	0.13	0.07
Total	73.43	74.06	77.37	81.23	79.07	66.96

a Data are the mean of 5 animals/sex/dose group and are V and VI (page 64) of the study report.
b Includes radioactivity from gauze pad and filter paper cover from the application site, the rubber enclosure ring, and skin wash.

B. Plasma Kinetics

The study report presented data (Table 9) on the concentration of radioactivity in plasma of intravenously dosed rats from 0 to 48 hours and of dermally dosed rats from 0 to 168 hours following dosing with [^{14}C]KBR 3023 at 20 or 200 mg/kg. These data were used to calculate the kinetic parameters for the dermal absorption and elimination of radioactivity from blood, which are presented below in Table 10.

Elimination of radioactivity from plasma of i.v. dosed rats was rapid and sex-dependent, with plasma concentrations being higher in females than males and females having lower clearance values. For the dermally dosed groups, absorption was rapid with half-lives of 0.8-3.4 hr. Concentration of radioactivity in plasma reached a maximum within 6-8 hours of dosing and were higher in females than males. Elimination of radioactivity from plasma of dermally dosed animals was dose- and sex-dependent. Elimination of radioactivity from low-dose groups could be described using a single half-life, but elimination from the high-dose rats was biphasic. In each dermal dose group, females had greater AUC values, lower CL values, and shorter MRTs than males.

a) Single i.v. low-dose: Elimination of radioactivity from plasma of i.v. dosed rats was rapid and triphasic, with half-lives of 0.9, 5.2 and 45.5 hr for males and 0.7, 2.8, and 73.0 hr for females. With the exception of the final time point, plasma concentrations in females were

292

approximately twice as high in males. Accordingly, the AUC value was higher for females (101 μg/(mL*hr)) than males (60.2 μg/(mL*hr)) and clearance values were lower for females (3.2 mL/(min*kg)) than males (5.2 mL/(min*kg)). Although females renally excreted more of the dose than males, the time course of renal excretion was similar for both sexes, with 50% and 90% of renal excretion occurring by 5.1-5.3 and 17.7-18.8 hours post-dose, respectively.

b) Single dermal low-dose: Based upon plasma concentrations of radioactivity, dermal absorption of the dose was rapid, with an absorption half-life of 1.5 and 0.8 hr for males and females, respectively. Maximum concentrations in plasma were attained within 6 hours of dosing and were higher for females (1.6 μg/mL) than males (0.57 μg/mL). Elimination of radioactivity from plasma could be adequately described using a single half-life of 35.7 and 23.9 hr for males and females, respectively. As in the i.v. dosed groups, elimination radioactivity from plasma was sex-dependent with females having a higher AUC value and a lower clearance value than males. In addition, females had a shorter MRT than males and the times till 50% and 90% renal excretion were also shorter for females.

c) Repeated dermal low-dose: Dermal absorption was slightly delayed for both males and females dosed repeatedly compared to the single dose group. Maximum concentrations in plasma were attained within 8 hours. As in the single low-dose group, maximum concentrations in plasma were higher in females (0.8 μg/mL) than males (0.45 μg/mL), and elimination of radioactivity from plasma could be adequately described using a single half-life (males, 41.8 hr; females 28.9 hr). As in the other dose groups, elimination was sex-dependent with females having a higher AUC value, a lower clearance value, and a shorter MRT than males. However, times till 50% and 90% renal excretion were similar males and females.

d) Single dermal high-dose: As with the low-dose groups, absorption was rapid with a half life of 1.9 and 3.4 hr for males and females, respectively. Maximum concentrations in plasma were attained within 6-8 hours of dosing and were higher for females (11.7 μg/mL) than males (4.48 μg/mL). Elimination of radioactivity from plasma was biphasic with half-lives of 10.9 and 144 hr for males and 9.1 and 105 hr for females. As in the other dermal dose groups, females had a higher AUC value, a lower clearance value, and a shorter MRT than males. The times till 50% and 90% renal excretion were also shorter for females.

293

Table 9. Concentration of radioactivity in plasma over time in rats dosed intravenously or dermally with [^{14}C]KBR 3023 at 20 or 200 mg/kg.[a]

Dose	µg/mL (KBR 3023 equivalents)[b]							
	Single low i.v. dose (20 mg/kg)		Single low dermal dose (20 mg/kg)		Repeated low dermal dose (20 mg/kg)		Single high dermal dose (200 mg/kg)	
Time (hours)	Male	Female	Male	Female	Male	Female	Male	Female
0.17	23.081	45.853	--[c]	--	--	--	--	--
0.33	27.328	49.650	--	--	--	--	--	--
0.67	27.229	45.757	--	--	--	--	--	--
1.0	20.617	34.815	0.266	0.451	0.152	0.151	1.603	2.728
1.5	12.590	21.482	0.354	0.719	0.219	0.245	2.239	3.547
2	8.140	13.086	0.394	0.850	0.253	0.313	2.594	3.938
3	4.485	7.661	0.444	0.923	0.311	0.406	3.048	4.572
4	2.722	5.831	0.531	1.170	0.365	0.537	3.904	5.662
6	1.595	3.444	0.568	1.643	0.408	0.709	4.478	8.454
8	1.264	1.941	0.541	1.557	0.448	0.800	4.016	11.677
24	0.190	0.099	0.396	0.778	0.396	0.538	2.241	4.679
32	0.093	0.069	0.367	0.521	0.292	0.409	1.290	2.718
48	0.051	0.052	0.225	0.280	0.215	0.268	0.883	1.368
72	--	--	0.184	0.137	0.137	0.153	0.508	0.585
96	--	--	0.110	0.098	0.097	0.096	0.345	0.361
120	--	--	0.058	0.089	0.079	0.044	0.294	0.301
144	--	--	0.038	0.047	0.048	0.034	0.280	0.263
168	--	--	0.043	0.028	0.032	0.021	0.255	0.149

a Animals dosed intravenously were sacrificed 2 days after dosing, and animals dosed dermally were sacrificed 7 days after initial dosing (the test material remained on the skin through the study period).

b Data are the mean of 5 animals/sex/dose group and were obtained from Tables VII, VIII and IX (pages 65-67) of the study report.

c -- no sample.

294

Table 10. Kinetic parameters determined for whole blood following a single dose of [^{14}C]MB46513 at 1 or 10 mg/kg body wt.[a]

Pharmacokinectic parameters	Single low i.v. dose (20 mg/kg)		Single low dermal dose (20 mg/kg)		Repeated low dermal dose (20 mg/kg)		Single high dermal dose (200 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
Lag-time (t_{lag}) [hr]	--[b]	--	0.9	0.9	0.9	0.8	0.3	0.7
Absorption half-life ($t_{½a}$) [hr]	--	--	1.5	0.8	1.9	1.2	1.9	3.4
Time of maximum concentration (T_{max}) [hr]	--	--	6	6	8	8	6	8
Maximum concentration (C_{max}) [µg/mL]	--	--	0.57	1.6	0.45	0.8	4.48	11.7
1. Elimination half-life ($t_{½(1)}$) [hr]	0.9	0.7	35.7	23.9	41.8	28.9	10.9	9.1
2. Elimination half-life ($t_{½(2)}$) [hr]	5.2	2.8	--	--	--	--	144	105
3. Elimination half-life ($t_{½(3)}$) [hr]	45.5	73.0	--	--	--	--	--	--
Area under the curve (AUC) [µg/(mL*hr)]	60.2	101	32.3	58.1	29.2	37.1	198	360
Mean residence time (MRT) [hr]	10.1	11.7	53.7	36.6	63.1	44.2	122	56.5
Total clearance (CL)[c] [mL/(min*kg)]	5.2	3.2	5.1	3.2	6.4	5.0	8.8	3.7
Urine $T_{50\%}$ [hr][d]	5.3	5.1	28.8	21.2	33.6	35.1	23.7	20.9
Urine $T_{90\%}$ [hr][d]	18.8	17.7	81.8	64.2	82.8	80.3	91.7	67.6

a Data are the mean of 5 animals/sex/dose group and are extracted from Tables X and XI (pages 68 and 69) of the study report; pharmacokinetic parameters were calculated from plasma concentration curves using TOPIFT version 2.0 computer software.

b -- = not applicable.

c Clearances for dermally dosed animals were determined based upon the fraction of the dose actually absorbed.

c Times to 50% and 90% excretion of renal excretion radioactivity were determined by linear interpolation of renal excretion.

512

C. Metabolite characterization

Quantitative HPLC analyses isolated up to 19 radioactive components in excreta. The results of these analyses are summarized in Tables 11 and 12. The proposed pathway for biotransformation of KBR 3023 in rats is present in Appendix 1.

Metabolites M1-M4 and M6-M19 were isolated from urine and identified by MS and/or NMR, and the fecal metabolites M18 and M19 were isolated from feces and also identified by MS and NMR. Metabolite M5 was the only metabolite that was not conclusively identified; M5 was tentatively identified as an isomer of M6 based upon its chromatographic behavior.

Metabolites M1-M4 are related isomers in which the 1-methylpropyl and the piperidine ring have both been hydroxylated. Metabolites M5 and M6 are isomers in which the 1-methylpropyl group has been hydroxylated and the 2-hydroxyethyl group has been oxidized to an acid and conjugated with glucuronic acid. Metabolites M7-M10 are isomers in which the 2-hydroxyethyl group has been oxidized to an acid and the 1-methylpropyl group or piperidine ring has been hydroxylated. Metabolites M11-M13 are isomers that are glucuronic acid conjugates of metabolite M16, and metabolites M14 and M15 are isomers that are glucuronic acid conjugates of the parent compound. Metabolite M16 results from the oxidation of the 2-hydroxyethyl group to an acid, and metabolites M18 and M19 are fatty esters of the parent compound.

For the i.v. dose groups, analysis of urine identified 60.9-77.6% of the dose and analysis of the pooled fecal extract identified 6.2% of the dose. For the dermally dosed groups, analysis of urine and feces identified 24.0-52.8% of the dose, or 75-85% of the radioactivity recovered in excreta.

With the exception of metabolites M18 and M19, which were only found in feces, the same metabolites were identified in both urine and feces, and the relative distribution of these metabolites was the same in both matrices. There was also no qualitative difference in the metabolite profile between dose groups and sexes.

Although actual levels (% of dose) of metabolites varied between dose groups due to differences in the amount of radioactivity recovered in urine and feces, there was little effect of dose group on the relative distribution of metabolites in excreta. The only notable difference between dose groups was that minor amounts of parent (0.5-1.3% of dose) were detected in excreta of dermally dosed rats, whereas parent was not detected in excreta of i.v. dosed rats.

The metabolites are separated structurally and chromatographically into five major groups: M1-M4, M5-M10, M11-M15, M16 and M17 (parent), and M18 and M19. With the exception of test group 4 (i.v. dosed females), metabolites M1-M4 were minor components and individually accounted for 0.5-5.0% of the dose in each dose group. Collectively these metabolites accounted for 3.4-11.2% of the dose, or 10-22% of the identified metabolites.

Metabolites M5-M7 and M11-M15 were also minor components, each one accounting for ≤2.9% of the dose in each dose group. Regardless of dose group or sex, the principal metabolites identified in excreta of rats were M8 (4.9-18.5% dose), M9 (5.7-21.1% dose), M10 (2.0-8.7% dose), and M16 (2.5-40.2% dose). Collectively these four metabolites accounted for 17.1-70.6% of the dose, or 67-91% of the identified metabolites. Metabolites M18 and M19 were minor components, each accounting for <0.3% of the dose.

Although the overall metabolite pattern was similar between sexes, there were minor differences in the relative distribution of metabolites. Within each dose group, the levels of the isomers M8 and M9 were essentially the same for males, but levels of M9 were consistently 1.5 times higher than M8 in excreta of females. In addition, levels of M10 were generally 2 times greater in males than in females from the same dose group. However, levels of M16 were generally 3-5x higher in females than in males from the same dose group.

297

Table 11. Metabolite profile in excreta of rats dosed I.V.with [^{14}C]KBR 3023 at 20 mg/kg.[a]

Dose Group	Percent of administered dose				
	Analysis of Urine				Analysis of Pooled Fecal sample[b]
	Single low i.v. dose Groups 1 and 2 (20 mg/kg)		Single low i.v. dose Groups 3 and 4 (20 mg/kg)		
Compound/fraction	Male	Female	Male	Female	
KBR 3023 (M17)	--[c]	--	--	--	0.25
M1	0.96	1.37	1.08	--	0.07
M2	3.74	2.72	4.07	--	0.41
M3	1.41	1.94	1.38	--	0.34
M4	4.95	3.58	4.63	--	0.33
M5	2.60	1.39	--	0.58	-
M6	--	2.13	2.89	1.78	0.10
M7	--	1.74	--	1.03	0.08
M8	18.49	12.86	18.10	10.40	1.96
M9	17.51	21.12	19.22	15.99	1.37
M10	7.95	3.69	8.65	3.94	0.47
M13	--	1.45	--	--	-
M14	--	--	0.82	1.26	-
M15	--	2.75	1.29	2.36	-
M16	3.34	16.16	8.31	40.23	0.73
M18	-	-	-	-	0.07
M19	-	-	-	-	0.06
Total identified[d]	**60.94**	**72.91**	**70.44**	**77.56**	**6.22**
Unidentified HPLC peaks	0.43	--	0.67	--	0.10
Unresolved HPLC radioactivity[e]	9.54	11.22	10.38	5.07	1.20
Total unidentified	**9.97**	**11.22**	**11.05**	**5.07**	**1.30**
Unanalyzed fractions:					
Urine	3.40	3.04	2.47	1.91	NA[g]
Feces[f]	16.51	6.27	12.34	4.80	NA
Carcass + expired $^{14}CO_2$	0.55	0.38	0.42	0.24	NA
Total unanalyzed	**20.46**	**9.69**	**15.23**	**6.95**	NA
Total accounted for[h]	**91.37**	**93.82**	**96.72**	**89.58**	NA

a Quantitative metabolite data were obtained from HPLC analyses of urine (Table XXI, page 79) of study report.
b For analysis of feces, fecal samples from all i.v. dose groups were pooled for extraction; pooled sample averaged 9.98% of dose. Data were calculated by reviewer from data in Appendix B43 (page 199) of study report.
c -- = not detected.
d Metabolites were identified by MS and NMR analyses, except for M5 which is assumed to be an isomer of M6 based upon its chromatographic behavior.
e Unresolved HPLC radioactivity "Empties" is comprised of eluted radioactivity not associated with a peak.
f Total radioactivity in feces; fecal metabolites were identified and quantified in a pooled sample (groups 1-4).
g NA = not applicable.
h Total accounted for = (Total identified) + (Total unidentified) + (Total Unanalyzed).

298

Table 12. Metabolite profile in excreta of rats dosed dermally with [^{14}C]KBR 3023 at 20 or 200 mg/kg.[a]

Dose Group	Percent of administered dose					
	Single low dermal dose (20 mg/kg)		Repeated low dermal dose (20 mg/kg)		Single high dermal dose (200 mg/kg)	
Compound/fraction	Male	Female	Male	Female	Male	Female
KBR 3023 (M17)	1.30	1.02	0.80	1.17	0.48	0.71
M1	0.95	1.19	1.09	1.38	0.46	0.62
M2	2.62	2.16	3.52	2.51	1.57	1.09
M3	1.36	1.37	1.13	1.69	0.69	0.87
M4	2.60	1.91	4.21	2.04	1.57	0.80
M5	0.62	0.67	--[b]	0.88	--	--
M6	0.98	1.88	0.78	1.78	0.44	0.89
M7	1.23	1.38	0.96	1.18	0.71	0.77
M8	11.62	8.43	12.53	7.66	6.33	4.93
M9	11.53	13.46	10.75	12.52	5.68	7.54
M10	4.62	2.84	5.29	2.77	2.58	1.96
M12	--	0.76	--	--	--	1.77
M14	0.62	0.78	0.43	0.78	0.34	0.46
M15	0.89	1.46	0.44	1.31	0.54	0.94
M16	4.87	13.26	3.77	11.79	2.49	10.53
M18	0.26	0.14	0.09	0.14	0.06	0.10
M19	0.15	0.09	0.07	0.13	0.05	0.07
Total identified[c]	**46.26**	**52.81**	**45.89**	**49.73**	**23.97**	**33.46**
Unidentified HPLC peaks	0.55	0.56	0.80	0.93	0.30	0.18
Unresolved HPLC radioactivity[d]	5.85	5.78	6.49	6.00	4.41	3.68
Total unidentified	**6.41**	**6.35**	**7.28**	**6.93**	**4.71**	**3.87**
Unanalyzed fractions						
Urine	2.87	1.75	2.56	2.86	2.32	1.70
Fecal solids[e]	0.79	0.48	0.74	0.47	0.56	0.28
Carcass	1.66	1.95	8.35	1.78	0.73	0.79
Non-biological material[f]	13.41	9.92	11.54	17.28	45.75	26.85
Total unanalyzed	**15.86**	**12.35**	**20.63**	**19.53**	**47.04**	**27.92**
Total accounted for[g]	**68.53**	**71.51**	**73.80**	**76.19**	**75.72**	**65.25**

a Quantitative metabolite data were obtained from HPLC analyses of urine and fecal extracts (Table XXI, page 79).

b -- = not detected.

c All metabolites were identified by MS and/or NMR analyses, except for M5 which was tentatively identified as an isomer of M6.

d Unresolved HPLC radioactivity "Empties" is comprised of eluted radioactivity not associated with a peak

e Unanalyzed fecal solids consists of radioactivity in residual solids following extraction of fecal samples.

f Non-biological materials includes radioactivity associated with tube gauze cover, filter paper cover, O-ring, application site wash (cotton swabs), and cage wash.

g Total accounted for = (Total identified) + (Total unidentified) + (Total Unanalyzed).

299

III. DISCUSSION

A. Investigator's Conclusions

Following dermal dosing with [^{14}C]KBR 3023, 20-30% of the applied material was not recovered at the end of the test period (7 days). Based upon the insect repellent nature of the compound, the study authors concluded that the loss resulted from volatilization of the test material and estimated volatilization to be approximately 3% of the dose/day.

Dermal absorption of the applied material was incomplete and dose dependent. Rats in the low-dose groups (single and repeated) absorbed 60.5-66.1% of the dose and high-dose groups absorbed 40.5-54.9% of the dose. The majority of the absorbed radioactivity entered the body (39.9-62.9% of dose) and was not associated with the skin at the application site. Although incomplete, dermal absorption was rapid. Based upon analysis of residue concentration in plasma, 90% of the total absorbed radioactivity was absorbed within the first 24 hours and absorption half-lives varied from 50 minutes to 3 hour.

The percentage of excretion was sex- and dose-dependent. In all dermal dose groups the majority of excreted radioactivity was eliminated renally. Renal excretion was higher in females (55.1-55.7%) than in males (42.6-46.1%), whereas levels of fecal excretion were higher for males (12.3-14.9%) than females (6.5-7.1%). The total fraction of radioactivity excreted by the high-dose group was comparatively lower (feces, 3.6-6.9%; urine, 25.7-35.7%) for both males and females.

Renal excretion was rapid, 50% of the renally excreted radioactivity was excreted within 21-35 hours of dermal application for both sexes from each dose group, and 90% was excreted within 82-92 hours.

The plasma kinetics were sex- and dose-dependent. Plasma levels were higher in females from all dose groups, reflecting the higher level of renal elimination by females than males. For i.v. dosed animals, distribution and elimination kinetics were very fast, and the time course for plasma concentration was a strong indication of a very fast and thorough metabolism of the parent compound. For single and pretreated dermal low-dose groups, elimination from plasma was adequately described by half-lives varying from 24 to 42 hr, whereas the elimination process in high-dose animals was biphasic and characterized by two half-lives of 11 and 144 hr for males and 9 and 105 hr for females.

The metabolic profile indicates that the position of the ^{14}C-label within the molecule was stable with respect to possible degradation into C-1 fragments. Biotransformation in rats resulted primarily in the formation of phase I metabolites, in which 2-hydroxyethyl side chain was oxidized to the corresponding acid and the 1-methylpropyl side chain was mono-hydroxylated. The prominent metabolites in excreta, regardless of sex, dose level, and route of administration, were M8, M9, M10, and M16, which together accounted for 53.4-63.8% of the total radioactivity excreted by dermally dosed rats and 51.9-77% of the total radioactivity

300

excreted by i.v. dosed rats. Parent compound and its glucuronic acid conjugates as well as other phase II metabolites were minor components of the metabolic profile in rat excreta.

B. Reviewer's Discussion

Within 48 hours of i.v. dosing with [^{14}C]KBR 3023 at 20 mg/kg, 91-97% of the administered dose was recovered from male and female rats. For rats dosed dermally for 7 days with [^{14}C]KBR 3023, 73-81% of the dose was recovered from the 20 mg/kg dose groups (with or without pretreatment) and 67-79% of the dose was recovered from the 200 mg/kg dose group. Approximately 19-33% of the dose was not recovered from dermally treated rats, compared to 3-9% of the dose from i.v. dosed rats.

[^{14}C]KBR 3023 was readily absorbed by rats following dermal dosing as indicated by the levels of radioactivity in the urine and feces. Including radioactivity in excreta and the body, low-dose rats (with and without pretreatment) absorbed 58.2-63.0% of the applied dose by the end of the 7-day exposure period. Dermal absorption was dose dependent with lower levels of absorption occurring in the high-dose animals (40.0-54.6% of dose). Absorption was rapid given the time course of excretion; 44-79% of the total amount of radioactivity absorbed was recovered in excreta within the first 48 hours of exposure

For both i.v. and dermally dose rats, renal excretion was the primary route of elimination, with fecal excretion being a secondary route. Expiration of $^{14}CO_2$ accounted for ≤0.02% of the dose. For i.v. dosed rats, renal excretion was higher in females (86.8-90.4% of dose) than males (74.4-82.3%), and fecal excretion was higher in males (12.3-16.5%) than in females (4.8-6.2%). The same pattern was observed in dermally dosed animals, although total levels of excretion were lower. In low-dose rats (with or without pretreatment), renal excretion accounted for 55.1-55.7% of the dose for females and 42.6-46.1% of the dose for males, and fecal excretion accounted for 6.5-7.1% of the dose in females and 12.3-14.9% of the dose in males. Increasing the dose reduced the total levels of excretion further, but did not affect the pattern of excretion. In the high-dose groups, renal and fecal excretion respectively accounted for 25.7 and 6.9% of the dose in males and 35.7 and 3.6% of the dose in females. Radioactivity remaining in the carcass (excluding skin at treatment site) at sacrifice was low for all dose groups, accounting for 0.43-0.52% of the dose for males and 0.19-0.36% of the dose for females.

Excretion of radioactivity following i.v. or dermal dosing with [^{14}C]KBR 3023 was rapid. For intravenously dosed rats, 83.4-92.1% of the dose was excreted in the urine and feces within 24 hours of dosing. For dermally dosed rats (except high dose males), 32.0-50.4% of the dose was excreted within 48 hours dosing, equivalent to 66-80.5% of the total radioactivity excreted.

The general distribution of radioactivity in blood and tissues at the time of sacrifice was similar between sexes and dose groups, and the concentration of radioactivity in blood and tissues were comparable between males and females from the same dose group. The only consistent difference between sexes from all dose groups was that radioactivity in livers of

301

males was 1.4-3.4x higher than in livers of females. In addition, high-dose males generally had higher concentrations of radioactivity in their blood and tissues than high-dose females. Besides skin from dermally dosed animals, concentrations of radioactivity were highest in liver, fat, kidneys, and lungs. The lowest concentrations of radioactivity were found in the brain.

With the exception of skin, concentrations of radioactivity in blood and tissues were higher for intravenously dosed rats than for dermally dosed rats at a comparable dose level. Pretreatment had little or no affect on the concentration of radioactivity in blood and tissues, but increasing the dermal dose from 20 to 200 mg/kg, proportionally increased the level of radioactivity in blood and tissues, although the increase was more pronounced for males than females.

Elimination of radioactivity from plasma of i.v. dosed rats was rapid and sex-dependent, with plasma concentrations being higher in females than males and females having lower clearance values. For the dermally dosed groups, absorption was rapid with half-lives of 0.8-3.4 hr. Maximum plasma concentration were reached within 6-8 hours of dosing and were higher in females than males. Elimination of radioactivity from plasma of dermally dosed animals was dose- and sex-dependent. Elimination of radioactivity from low-dose groups could be described using a single half-life, but elimination from the high-dose rats was biphasic. In each dermal dose group, females had greater AUC values, lower CL values, and shorter MRTs than males.

Nineteen metabolites were identified by MS and NMR in urine and feces of both i.v. and dermally dosed rats. Analysis of excreta from i.v. dosed rats identified 60.9-77.6% of the dose in urine and 6.2% of the dose in feces. For the dermally dosed groups, analysis of urine and feces identified 24.0-52.8% of the dose, or 75-85% of the radioactivity recovered in excreta.

With the exception of metabolites M18 and M19 (fatty acid esters of the parent compound found only in feces, <0.3% dose), the same metabolites were identified in both urine and feces, and the relative distribution of these metabolites was the same in both matrices. There was also no qualitative difference in the metabolite profile between dose groups and sexes, and there was little effect of dose group on the relative distribution of metabolites in excreta. The only notable difference between dose groups was that minor amounts of parent (0.5-1.3% of dose) were detected in excreta of dermally dosed rats, whereas parent was not detected in excreta of i.v. dosed rats. While the overall metabolite pattern was similar between sexes, there were minor quantitative differences in the relative distribution of the principal metabolites between the sexes.

However, regardless of dose group or sex, the principal metabolites identified in excreta of rats included: M8 (4.9-18.5% dose), M9 (5.7-21.1% dose), M10 (2.0-8.7% dose), and M16 (2.5-40.2% dose). Collectively these four metabolites accounted for 17.1-70.6% of the dose, or 67-91% of the identified metabolites. The remaining metabolites, M1-M7, M11-M15, M18, and M19 were minor components that individually accounted for <5.0% of the dose.

In both i.v. and dermally dosed rats, metabolism of KBR 3023 primarily involves oxidation of the 2-hydroxyethyl group to an acid to form metabolite M16, coupled with hydroxylation of the 1-methylpropyl group to form metabolites M8, M9, and M10. Other minor phase I metabolites resulted from the hydroxylation of the piperidine ring (M1-M4 and M7), and minor phase II metabolites resulted from conjugation of glucuronic acid with parent (M14 and M15) or phase I metabolites (M5, M6, and M11-M13). The proposed pathway for biotransformation of KBR 3023 in rats is shown in Appendix 1.

IV. STUDY DEFICIENCIES

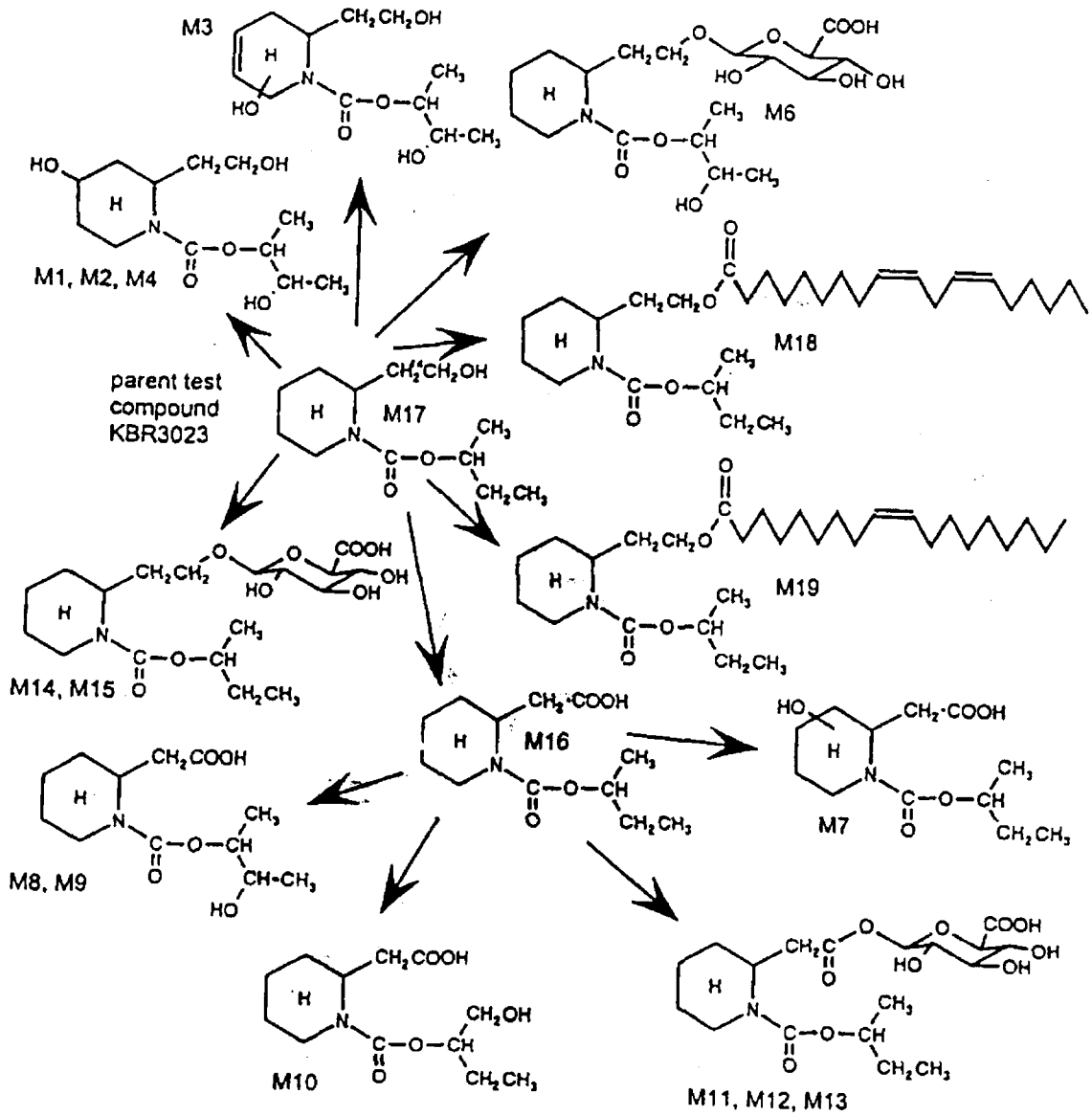
Only one major deficiency was noted in this rat metabolism study and it involved the low recovery of dosed radioactivity from dermally dosed animals. In each of the dermally dosed groups, 18.9-33.1% of the dosed radioactivity was not recovered, compared to a loss of 3-8.6% of the dose from the intravenously dosed groups. The study authors concluded that the material was lost by volatilization of the test compound from the treatment site at a rate of ~3% per day over the course of the 7-day exposure period. Although no information or data were provided to support this claim, it is feasible in light of the vapor pressure of the test article. The vapor pressure is 3.4×10^{-5} kPa at 20 °C, 5.9×10^{-5} kPa at 25 °C, and even greater at skin temperature. Thus, this study is classified **Acceptable**.

203

APPENDIX 1

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

Figure 8: Biotransformation scheme of KBR3023 in the rat



013529

DATA EVALUATION RECORD

KBR 3023

Study Type: Dermal Absorption and Metabolism Study - Humans

Work Assignment Nos. 3-47O and 3-47Q (MRIDs 44408736 and 44408738)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

306

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EPA Secondary Reviewer: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

4-14-99

DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption and Metabolism - Human
OPPTS Number: 870.7600 and 870.7485

DP BARCODE: D241232
P.C. CODE: 070705

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

TEST MATERIAL (PURITY): KBR 3023 (99.1% a.i.)

SYNONYMS: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine; 1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropyl ester

CITATIONS: Ecker, W. (1996) [Hydroxyethyl-1-^{14}C]KBR3023: Human Volunteer Metabolism Study After Dermal Application. Bayer AG, Institute for Metabolism Research and Residue Analysis, Leverkusen-Bayerwerk, Germany. November 27, 1996. MRID 44408736. Unpublished.

Selim, S. (1994) A Single Dose Open Label Study to Investigate the Absorption and Excretion of a ^{14}C-labelled Insect Repellent (KBR 3023) From Two Different Formulations After Dermal Application to Healthy Volunteers. Biological Test Center, Irvine, CA and Pharma Bio-Research Laboratories, Zuidlaren, Netherlands. Laboratory Study Numbers: P1092004/PBR-920310-2, June 20, 1994. MRID 44408738. Unpublished.

SPONSOR: Bayer Corporation, Stilwell, KS

EXECUTIVE SUMMARY: In a dermal absorption study (MRID 44408738), 6 healthy male volunteers/dose group were dosed dermally on a forearm with either neat [^{14}C]KBR 3023 (>98% a.i.) or [^{14}C]KBR 3023 in ethanol (15% w/w) at a nominal dose level of 15 mg/person (0.625 mg/cm^2). Both dose groups were exposed to the test material for 8 hours.

Dosed radioactivity was quantitatively recovered from both dose groups (97.0-98.1%). After 8 hours of exposure, dermal absorption of KBR 3023 was low (<4.0% of dose) for both dose groups and the majority of the dose was not absorbed (94.3-95.3%). Absorption of [^{14}C]KBR 3023 was slightly higher when applied as a 15% (w/w) solution in ethanol (3.77% dose) than when applied neat (1.66% dose). Only trace amounts of radioactivity were recovered in the feces (≤0.01% dose) and by stripping of the skin (≤0.03% dose) at the treatment site following

removal of the test substance. Nearly all the absorbed radioactivity was excreted in the urine (1.66-3.76%) for both dose groups, with 94% of the radioactivity in urine being excreted within 24 hours of initial dosing.

Concentrations of radioactivity in plasma drawn from the treated arm were similar between the two dose groups over time. Radioactivity in plasma increased to a maximum at 8 hours (73-97 dpm/500 µL), and then declined rapidly, returning to background levels by 8 hours after removal of the test substance.

In a related metabolism study (MRID 44408736), 4-12 hour urine samples from 3 of the 6 volunteers dosed with [^{14}C]KBR 3023 as a 15% solution in ethanol were pooled and analyzed by HPLC. Approximately 94% of the radioactivity in urine was identified. Biotransformation of [^{14}C]KBR 3023 in humans primarily involved conjugation of KBR 3023 with glucuronic acid through the 2-hydroxyethyl moiety to form Metabolites M14 and M15, which together accounted for 43.1% of the radioactivity in urine. Other major metabolites in urine included: M5 (17.4%) another glucuronic acid conjugate which is hydroxylated in the 1-methyl-propyl moiety; M16 (8.5%) in which the 2-hydroxyethyl group is oxidized to an acid; M8 (6.2%) in which the 2-hydroxyethyl group is oxidized and the 1-methyl-propyl group is hydroxylated; and M11-M13 (6.9%) which are isomers in which the 2-hydroxyethyl group has been oxidized to an acid and conjugated with glucuronic acid. The remaining metabolites, M1-M4, M6, M7, M9, and M10 each accounted for ≤3.1% of the radioactivity in urine.

In contrast to human urine, the principal metabolites in rat urine include M8, M9, M10, and M16 (MRID 44408735). In each of these metabolites, the 2-hydroxyethyl group has been oxidized to the corresponding acid, and in metabolites M8-M10, the 1-methylpropyl group has also been mono-hydroxylated. All of the glucuronide conjugated metabolites (M5, M6, and M11-M15) are minor components (<3.0% dose) of rat excreta.

The human dermal absorption and metabolism studies are classified as **Acceptable (guideline)** studies that adequately characterize dermal absorption in humans and identify metabolites in urine.

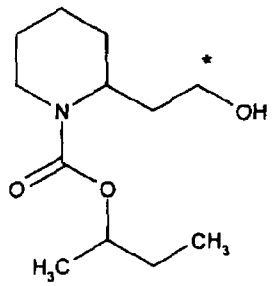
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

308

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: KBR 3023
   Chemical Name: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine
   Description: Yellowish liquid
   Lot/Batch #: 890814ELB01
   Purity: 99.1% a.i.
   CAS #: 119515-38-7
   Structure:



* denotes position of ^{14}C-label

Other Comments: Although the same material was used in the metabolism portion of the study, different information was presented for the test material. The non-labeled KBR 3023 was described as a clear colorless liquid that had a purity of 98.3% a.i. (Batch #921103ELB04).

[2-(2-hydroxyethyl-2-^{14}C]KBR 3023
Description: None
Reference #: KML 2159
Radiochemical purity: >98%, (determined by HPLC and GC)
Specific activity: 96 µCi/mg (final specific activity 2.5 µCi/mg)

2. Vehicle: The test substance was dissolved in ethanol for application to one test group. No vehicle was used for the other test group.

3. Test animals: Human males
   Age: 18-28 years
   Weight at start of treatment: 62.2-84.3 kg

4. Housing: All volunteers reported to the test facility (Pharma Bio-Research Clinical Research Center, Netherlands) one day prior to the start of the study and remained at the test facility throughout the 6-day study.

209

B. STUDY DESIGN and METHODS

The dermal absorption study was designed to examine absorption of neat [^{14}C]KBR 3023 in humans and the effect of a solvent carrier on dermal absorption. The study consisted of two groups of 6 healthy male volunteers/group dosed dermally at a nominal dose of 15 mg/individual equivalent to 0.625 mg/cm^2, based upon a 24 cm^2 treatment area. Individuals in one group were dosed dermally with 15 µL of neat [^{14}C]KBR 3023 (99.1% a.i.) and the second group was dosed dermally with 100 µL of [^{14}C]KBR 3023 in ethanol (15% a.i.). Actual application rates averaged 14.68 ± 0.10 mg/person (0.612 mg/cm^2) for the group dosed with neat KBR 3023 and 14.99 ± 0.02 mg/person (0.625 mg/cm^2) for the group dosed with 15% KBR 3023 in ethanol. All individuals in both dose groups were exposed for 8 hours.

The metabolism study was designed to examine the metabolites of [^{14}C]KBR 3023 in human urine following dermal dosing. As levels of radioactivity were higher in men dosed with 15% (w/w) [^{14}C]KBR 3023 in ethanol, only urine samples from this dose group were used for analysis. Fecal samples were not analyzed because of the low levels of radioactivity excreted in the feces.

1. Dose selection

The sponsor stated that the concentrations of test material selected for application were chosen because they are expected to encompass the range of KBR 3023 concentrations anticipated in commercial formulations. However, no rationale was provided as to why a 15 mg/individual (0.625 mg/cm^2) dose level was chosen.

2. Dose preparation

[^{14}C]KBR 3023 was diluted with unlabeled KBR 3023 to a specific activity of 2.5 µCi/mg. For application in ethanol, the test material was then diluted to ~15% w/w with ethanol. To verify the concentration of test substance and the homogeneity of the dosing solutions, triplicate aliquots of each dosing solution were taken and diluted to 10 mL. Each dilution was then subsampled in triplicate and radioactivity in the subsamples was determined by liquid scintillation counting (LSC).

For the neat [^{14}C]KBR 3023 dosing solution, the concentration of radioactivity was 39.42 ±0.67 µCi/dose volume of 15 uL. For the [^{14}C]KBR 3023 diluted in ethanol, the concentration of radioactivity was 38.49 ±0.39 µCi/dose volume of 100 µL, and the final concentration of KBR 3023 in solution was calculated to be 15.36% w/w.

3. Dosing

Six individuals were randomly assigned to each of the two test groups. In preparation for dosing, an application area on the volar aspect of the right or left forearm was outlined in ink and surrounded by an adhesive template (Duoderm®) from which a 4 x 6 cm rectangle had been removed. A 15 µL dose of neat [^{14}C]KBR 3023 or a 100 µL dose of 15% KBR

3023 in ethanol was spread evenly over the 24 cm^2 test site on each volunteer's forearm using calibrated glass micropipettes. Separate micropipettes were used for each volunteer. and each micropipette was retained for radioassay to determine the actual amount of KBR 3023 applied. The application site was then covered with a ventilated aluminum dome to prevent contact with the application site. The test material was applied in the morning (~8:00 am) on day-1 of the test period. During the subsequent 8-hour exposure period. the volunteers were under close supervision and were allowed to walk, sit or lay down.

4. Observations and sample collection

Individuals were weighed shortly before application and monitored clinically throughout the study. After the 8-hour exposure, the protective cover was removed and the treatment site was washed by wiping 12-16 times with cotton swabs soaked in isopropyl alcohol. The application site was then rinsed with a steady stream of isopropyl alcohol and covered with a gauze pad. All samples were saved for radioassay. To assess the amount of radioactivity remaining in or on the dermis, one-third sections of the treatment site were stripped using cellophane tape at 1, 23, and 45 hours posttreatment (9, 31, 53 hours after initial application). Moving distally, a different section was stripped at each time point. To surface strip each section, 12-16 pieces of tape were applied evenly and removed slowly; tape strips were then placed in scintillation vials for counting.

Blood samples were also collected simultaneously from the treated and non-treated arms prior to dosing and at 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 hours after application. Blood samples were then centrifuged and the plasma was frozen and saved for analysis. Urine was also collected from each volunteer pre-dose, at 4, 8, and 12 hours after application, and at 12-hour intervals thereafter until the end of the study at 128 hours after application. Fecal samples were collected individually throughout the study period. Urine and fecal samples were stored frozen until analysis.

The following samples were analyzed for radioactivity:

Micropipette applicators	Duoderm surrounding the application site
Protective cover	Wash swabs
Skin rinse	Gloves (used for application and removal)
Tape strips	Plasma
Urine	Feces

Aliquots of urine, skin rinsates, and plasma were radioassayed directly by LSC. along with isopropyl alcohol extracts of from applicator tips, protective covers, Duoderm. cotton swabs, and gloves. Tape strips were soaked in Insta-Gel and counted directly by LSC.

Fecal samples were homogenized in liquid nitrogen and triplicate aliquots were combusted prior to LSC.

5. Metabolite Characterization

For metabolite characterization and quantitation, the 4-12 hour urine samples from 3 of the 6 volunteers dosed with 15% (w/w) [^{14}C]KBR 3023 in ethanol were pooled for analysis. The pooled sample was concentrated and analyzed directly by reverse-phase HPLC. The HPLC system consisted of a C_{18} column, a variable wavelength detector, and an in-line radioactivity detector, and utilized a mobile phase gradient of 1% acetic acid in water to 1% acetic acid in acetontirile.

For identification of isolated metabolite fractions, HPLC peaks were compared to metabolite peaks isolated from an earlier rat metabolism study (MRID 44408735), in which metabolites were isolated using the same HPLC system and identified by NMR and GC anlayses. In addition, two peaks (M14 and M15) were isolated by HPLC and subjected to enzymatic hydrolysis using glucuronidase, and the resulting hydrolysate was analyzed by HPLC.

6. Data analysis

Means and standard deviations were calculated for samples from each dose group.

II. RESULTS:

A. Clinical observations

No adverse observations were noted from application of the test material.

B. Dermal absorption study

The average dose actually applied was 14.68 ±0.10 mg/person (0.612 mg/cm^2 or 0.207 mg/kg body weight) for the application of neat [^{14}C]KBR 3023 and 14.99 ± 0.02 mg/person (0.625 mg/cm^2 or 0.203 mg/kg body weight) for the application of 15% [^{14}C]KBR 3023 in ethanol.

The mean concentration (dpm/500 µL) of radioactivity in plasma obtained from the treated and non-treated arms of men in both dose groups is presented in Table 1. For both dose groups, radioactivity in plasma drawn from the non-treated arm was low; ≤2x background for the neat application and ≤3x background for the ethanol solution application. Concentrations of radioactivity in plasma from the treated arm for both dose groups were higher and were similar between dose groups over time. For both dose groups, the concentration radioactivity in plasma from the treated arm increased from 0-4 hours, declined at the 6 hour sampling, and then reached a maximum at 8 hours (73-97 dpm/500 µL). Following removal of the test

substances, radioactivity in plasma declined rapidly, returning to background levels by 8 hours post-treatment (16 hours after initial dosing).

The recovery of dosed radioactivity in urine, feces, and skin tape stripping over time is presented in Table 2 for both dose groups. For feces, radioactivity was detected (0.01% dose) only in Day-3 samples from one man in the neat dose group and three men in ethanol solution dose group. Nearly all the absorbed radioactivity was excreted in the urine. Although slightly more of the dose was excreted in urine of the ethanol solution dose group (3.76% dose) than in urine of the neat dose group (1.66% dose), the pattern and levels of excretion in urine were similar between dose groups. By 8 hours, 40-43% of the total radioactivity in urine had been excreted, with ~77% and ~94% of the total being excreted within 12 and 24 hours, respectively. After 60 hours (52 post-treatment), radioactivity in urine had returned to background levels for both dose groups.

Radioactivity remaining in or on the skin after removal of the test substances was also low, accounting for 0.01-0.02% of the dose at the initial stripping (1 hour post-treatment) and <0.01% of the dose at the two subsequent stripping.

The distribution of the dosed radioactivity among the various fractions is presented in Table 3 for both groups. The dosed radioactivity was quantitatively recovered from each dose group (97.0-98.1%) For each dose group, the majority of dosed radioactivity was not absorbed (94.3-95.3%) following the 8 hour exposure period. Dermal absorption of KBR 3023 was low (<4.0%) for both test groups, although absorption of [^{14}C]KBR 3023 was slightly higher when applied as a 15% (w/w) solution in ethanol (3.77% dose) than when applied neat (1.66% dose).

Table 1. Radioactivity in plasma drawn from the treated and non-treated arms following dermal dosing with [^{14}C]KBR 3023 at 15 mg/person.

Post-dose interval (hours)	dpm/500 µL[a]			
	Neat [^{14}C]KBR 3023 (>98%)		[^{14}C]KBR 3023 in ethanol (15%)	
	Treated arm	Non-treated arm	Treated arm	Non-treated arm
Pre-dose	1	4	3	5
2	14	4	21	6
4	65	6	44	8
6	32	8	18	12
8[b]	73	8	97	17
10	28	8	37	15
12	7	6	8	9
16	3	4	5	6
24	2	3	3	6
36	3	4	2	4
48	2	3	2	3
72	2	4	3	3
96	1	28[c]	2	6
120	1	4	2	14[d]

a Data are the average of 6 volunteers/dose group and were obtained from Tables 2, 3, 10 and 11 (pages 31, 32, 39 and 40) of the study report (MRID 44408738).

b Treatment site was washed after 8-hour dosing period.

c Values for 5 men were 1-7 dpm/500 µL (ave. 4 dpm/500 µL); one man's plasma sample was 150 dpm/500 µL.

d Values for 5 men were 2-6 dpm/500 µL (ave. 4 dpm/500 µL); one man's plasma sample was 64 dpm/500 µL.

Table 2. Radioactivity in urine, feces, and skin tape strippings from men dosed dermally with [^{14}C]KBR 3023 at 15 mg/person.

Post-dose intervals (hours)	Percentage of dose[a]					
	Neat [^{14}C]KBR 3023 (>98%)			[^{14}C]KBR 3023 in ethanol (15%)		
	Urine	Feces	Skin stripping	Urine	Feces	Skin stripping
Pre-dose	0.01	<0.01	--[b]	0.01	<0.01	--
0-4	0.11	<0.01	--	0.23	<0.01	--
4-8	0.54		--	1.37		--
8-12	0.63		0.01	1.30		0.02
12-24	0.26		--	0.64		--
24-36	0.06	<0.01	<0.01	0.10	<0.01	<0.01
36-48	0.02		--	0.03		--
48-60	0.01	<0.01	<0.01	0.02	0.01	<0.01
60-72	<0.01		--	0.01		--
72-84	0.01	<0.01	--	0.01	<0.01	--
84-96	<0.01		--	0.01		--
96-108	0.01	<0.01	--	0.01	<0.01	--
108-120	<0.01		--	0.03		--
120-128	<0.01		--	<0.01		--
Total	**1.66**	**<0.01**	**0.02**	**3.76**	**0.01**	**0.03**

a Data are the average of 6 volunteers/dose group and were obtained from Tables 4, 5, 6, 12, 13 and 14 (pages 33-35 and 41-43) of the study report (MRID 44408738).

b -- indicates that no sample was taken.

Table 3. Distribution of radioactivity following a single 8-hour dermal exposure of human males to [^{14}C]KBR 3023 at 15 mg/person (0.62 mg/cm^2).

Fraction	Percent Dose	
	Neat [^{14}C]KBR 3023 (>98% a.i.)	[^{14}C]KBR 3023 in ethanol (15.36% a.i.)
Absorbed		
Urine	1.66	3.76
Feces	<0.01	0.01
Total absorbed	**1.66**	**3.77**
Absorbable		
Tape strippings	0.02	0.03
Total potential absorption	**1.68**	**3.80**
Not Absorbed		
Swabs	79.37	74.77
Skin rinsate	1.04	0.78
Enclosure	3.85	6.34
Duoderm	10.98	12.27
Gloves	0.08	0.16
Total not absorbed	95.32	94.32
Total recovered	**97.00**	**98.12**

a Data are the average of 6 volunteers/dose group and were obtained from Tables 7, 8, 15 and 16 (pages 36, 37, 44, and 45) of the study report (MRID 44408738).

C. Metabolite Characterization

Urine samples from the three individuals who had the highest levels of urinary excretion were used for metabolite analysis. All three individuals were in the 15% [^{14}C]KBR 3023 ethanol solution dose group and excreted totals of 3.17%, 4.88%, or 7.0% of the dose in urine. Average total urinary excretion was 5.02% of the dose for these individuals, compared to 3.76% of the dose for the entire group. The pooled 4-12 hour urine sample from these three individuals averaged 3.62% of the dose, or 72% of all the radioactivity excreted in their urine.

HPLC analysis isolated eleven regions of radioactivity from human urine (Table 4), which fell into four general regions. The most polar group (M1-M4) was poorly defined, eluting between 18-22 minutes and accounting for 3.1% of the radioactivity in the sample. The next region contained distinct peaks (M5-M10) each accounting for 0.9-17.4% of the sample radioactivity and eluting from 28-36 minutes. The third region contained a small broad peak

96

(M11-M13, 6.9%) and two large distinct peaks (M14, 15.8% and M15, 27.3%) eluting between 40-44 minutes. The last region contained one peak (M16) eluting at ~49 minutes and accounting for 8.5% of the sample radioactivity. These peaks of radioactivity had been previously isolated from rat urine.

Table 4. Metabolite profile in urine of male volunteers dosed dermally for 8 hours with a 15% (w/w) [^{14}C]KBR 3023 in ethanol at 15 mg/person (0.625 mg/cm^2 or 0.203 mg/kg body weight)[a].

Metabolite/fraction	% of radioactivity in urine sample[b]	% of dose[c]
M1 - M4	3.1	0.11
M5	17.4	0.63
M6	2.3	0.08
M7	0.9	0.03
M8	6.2	0.22
M9	2.6	0.09
M10	2.4	0.09
M11 - M13	6.9	0.25
M14	15.8	0.57
M15	27.3	1.00
M16	8.5	0.31
Parent	ND[d]	ND
Total identified	**93.5**	**3.38**
Unknowns R1	5.6	0.20
R2	0.9	0.03
Total Recovered	**100**	**3.61**

a Pooled urine sample used for analysis was obtained from 3 volunteers.
b Data were obtained from Table III (page18) of the study report (MRID 44408736).
c Calculated by reviewer based upon the average % of dose in urine for analyzed fraction (3.62% dose).
d ND = not detected.

To confirm the identity of the major peaks of radioactivity, human urine was separately fortified with metabolites M14, M15, and M16, which had been isolated from rat urine and identified by NMR and GC. HPLC analyses of the spiked urine showed that these rat metabolites co-chromatographed with their respective peaks in human urine. In addition, enzymatic hydrolysis of peaks M14 and M15 with glucuronidase released the parent compound, confirming that these metabolites were glucuronic acid conjugates of the parent as in the rat metabolism study. No unchanged [^{14}C]KBR 3023 was detected in human urine.

III. DISCUSSION

Investigator's Conclusions - In the dermal absorption study, the author concluded that dermal absorption of KBR 3023 in human males was low (<4% of the dose) over the 8-hour exposure period when applied either neat (>98% a.i.) or as a 15% (w/w) solution in ethanol. KBR 3023 which was absorbed was rapidly excreted in the urine. No evidence was found to indicate that dermally applied KBR 3023 accumulated in the skin.

In the metabolism study, the author concluded that the position of the ^{14}C-label within the molecule was stable. As in an earlier rat metabolism study (MRID 44408735), no degradation to C-1 fragments was observed.

Conjugation of parent with glucuronic acid formed the prominent metabolites (M14 and M15) of KBR 3023 in man; together these metabolites accounted for 43.1% of the radioactivity in urine. Other significant metabolites in human urine included M16 (8.5%) and M5 (17.4%). Metabolite M16 results from oxidation of the hydroxyethyl side-chain, and M5, which was tentatively identified as an isomer of M6, results from hydroxylation of the 1-methylpropyl side chain and conjugation with glucuronic through the 2-hydroxyethyl side chain.

Metabolites M7-M10, which were the principal metabolites identified in rat excreta, were minor components in human urine, each accounting for 0.9-6.2% of the radioactivity. These metabolites result from oxidation of the hydroxyethyl side chain accompanied by hydroxylation of the 1-methyl-propyl side chain or oxidation of the piperidine moiety.

Metabolites M1-M4, which result from the hydroxylation of the methylpropyl side chain with accompanying oxidation of the piperidine moiety, were also minor components in human urine, together accounting for 3.1% of the radioactivity.

Including metabolite M5, which was tentatively identified, a total of 94% of the radioactivity in urine was identified.

Reviewer's Conclusions - Healthy male volunteers were dosed dermally on the volar aspect of a forearm with [^{14}C]KBR 3023 either neat (>98% a.i.) or as a 15% (w/w) solution in ethanol at a nominal dose level of 15 mg/person (0.625 mg/cm^2). Both dose groups were exposed to the test material for 8 hours.

Dosed radioactivity was quantitatively recovered from both dose groups (97.0-98.1%). After 8 hours of exposure, dermal absorption of KBR 3023 was low (<4.0% of dose). For both dose groups, the majority of the dose was not absorbed (94.3-95.3%). Absorption of [^{14}C]KBR 3023 was slightly higher when applied as a 15% (w/w) solution in ethanol (3.77% dose) than when applied neat (1.66% dose). Only trace amounts of radioactivity were recovered in the feces (≤0.01% dose) and by stripping of the skin (≤0.03% dose) at the treatment site following removal of the test substances. Nearly all the absorbed radioactivity was excreted in the urine (1.66-3.76%) for both dose groups, with 94% of the radioactivity in urine being excreted within 24 hours of initial dosing.

Concentrations of radioactivity in plasma drawn from the treated arm were similar between the two dose groups over time. Radioactivity in plasma increased to a maximum at 8 hours (73-97 dpm/500 µL), and then decline rapidly, returning to background levels by 8 hours after removal of the test substances.

HPLC Analyses of urine samples from 3 volunteers dose with 15% (w/w) [^{14}C]KBR 3023 in ethanol identified ~94% of the radioactivity in urine. Biotransformation of [^{14}C]KBR 3023 in humans primarily involved conjugation of KBR 3023 with glucuronic acid through the 2-hydroxyethyl moiety to form Metabolites M14 and M15, together accounting for 43.1% of the radioactivity in urine. Other major metabolites in urine included: M5 (17.4%) another glucuronic acid conjugate which is hydroxylated in the 1-methyl-propyl moiety; M16 (8.5%) in which the 2-hydroxyethyl group is oxidized to an acid; M8 (6.2%) in which the 2-hydroxyethyl group is oxidized and the 1-methyl-propyl group is hydroxylated; and M11-M13 (6.9%) which are isomers in which the 2-hydroxyethyl group has been oxidized to an acid and conjugated to glucuronic acid. The remaining metabolites, M1-M4, M6, M7, M9, and M10 each accounted for ≤3.1% of the radioactivity in urine. The proposed pathway for biotransformation of KBR 3023 in man is shown in Appendix 1.

In contrast to human urine, the principal metabolites in rat urine include M8, M9, M10, and M16. In each of these metabolites, the 2-hydroxyethyl group has been oxidized to the corresponding acid, and in metabolites M8-M10, the 1-methyl-propyl group has also been mono-hydroxylated. All of the glucuronide conjugated metabolites (M5, M6, and M11-M15) are minor components (<3.0% dose) of rat excreta.

IV. STUDY DEFICIENCIES

No deficiencies were noted in these human dermal absorption and metabolism studies. Guideline 870.7600 (dermal penetration) requires use of the laboratory rat as the test system. Guideline 870.7485 (metabolism and pharmacokinetics) recommends using whatever species is most relevant to humans. Because of the way this test article will be used, these studies are classified as **Acceptable (guideline)** studies that adequately characterize dermal absorption in human male volunteers and identify metabolites excreted in urine.

APPENDIX 1

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

013529

DATA EVALUATION RECORD

KBR 3023

Study Type: Nonguideline Dermal Absorption Study - Rats

Work Assignment No. 3-47P (MRID 44408737)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
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Primary Reviewer:
Jay D. Early, M.S.

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Date: 9/14/98

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Signature: _____
Date: 9/21/98

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: _____
Date: 9/30/98

Quality Assurance:
Steven Brecher, Ph.D.

Signature: _____
Date: 9/29/98

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C) John Whalan 4-14-99

EPA Secondary Reviewer: Sanjivani Diwan, Ph.D.
Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption - Rat
OPPTS Number: 870.7600 OPP Guideline Number: §85-3

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

TEST MATERIAL (PURITY): KBR 3023 (98.2% a.i.)

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

CITATION: Warren, D.L. and Sturdivant, D.W. (1997) Dermal Absorption of Technical KBR 3023 in Rats. Bayer Corporation, Stilwell, KS. Laboratory Study Numbers: 96-722-IC/96-929-IM, January 28, 1997. MRID 44408737. Unpublished.

SPONSOR: Bayer AG, Research and Development, D-51368 Leverkusen, Bayerwerk, Bldg.. 6210 Germany

EXECUTIVE SUMMARY: In a dermal absorption study (MRID 44408737), 12 Sprague-Dawley rats/sex/dose group were dosed dermally with neat [^{14}C]KBR 3023 (98.2% a.i.) at nominal dose levels of 8, 40, or 200 mg/kg body weight (0.133, 0.67, or 3.33 mg/cm^2) for 8 hours. Four rats/sex/dose were assessed for dermal absorption at the end of the 8-hour exposure period and at 24 hours after the start of exposure. The final four rats/sex/dose group were sacrificed when radioactivity in their urine declined to background levels (3-7 days after start of exposure). Dosed radioactivity was quantitatively recovered from each dose group (85.6-111.7%).

Regardless of dose level and sampling interval, radioactivity absorbed and remaining at the treatment site was 9.8-20.4% of the dose for males and 17.4-32.2% of the dose for females, indicating a difference in absorption between sexes. Dose level also initially had an effect on the percent dose absorbed and the percent dose remaining in or on the skin at the treatment site. Immediately following the 8-hour exposure, the percent of the dose absorbed was 14.4, 9.8, and 6.9% in males and 27.4, 12.6, and 8.9% in females at the low-, mid-, and high-dose levels, respectively. For skin at the treatment site, the situation was reversed with radioactivity remaining in or on the skin at the 8-hour interval being highest for the high-dose animals (9.2-13.7% of dose) and lowest for the low-dose animals (4.8-5.5% of dose). At subsequent sampling intervals, the percent dose absorbed increased while radioactivity remaining at the treatment site

322

declined. By the final sampling interval, dose level essentially had no effect on the percent of the dose absorbed or remaining on the skin.

Although there were differences between the sexes in the percent dose absorbed, the distribution and excretion of the absorbed radioactivity was similar between the sexes and dose groups. At each dose level for both sexes, the majority of the absorbed dose remained in the body (including blood) and treatment site skin (males, 14.2-14.5% dose; females, 15.6-20.2% dose) immediately following the 8-hour exposure period. At subsequent sampling intervals, radioactivity in the body and in the treatment site skin declined steadily while the percentage of the dose excreted increased. By the final sampling interval (3-7 days post-treatment), 86-93% of the absorbed radioactivity had been excreted (12.0-24.1% dose), and radioactivity remaining in the body and/or treatment site skin accounted for 1.5-3.0% of the dose. Urinary excretion (including cage wash) was the principle route of elimination of the absorbed radioactivity, accounting for 75-98% of the excreted radioactivity.

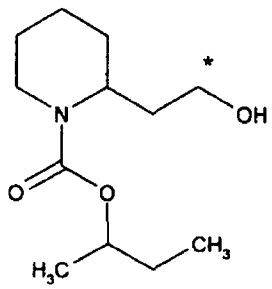
This study is classified as **Acceptable (guideline)** for characterizing dermal absorption in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: KBR 3023
   Chemical Name: 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine
   Description: Clear liquid
   Lot/Batch #: PT.030693
   Purity: 98.2% a.i.
   CAS #: 119515-38-7
   Structure:



* denotes position of ^{14}C-label

   [2-^{14}C-2-hydroxyethyl]KBR 3023
   Description: None
   Reference #: KML 2169
   Radiochemical purity: >99%, (method of determination unspecified)
   Specific activity: 96 µCi/mg

2. Vehicle: None.

3. Test animals: Species: Rat
   Strain: Sprague-Dawley
   Age: Unspecified
   Weight at start of treatment: Males, 209-250 g; Females, 202-245 g
   Source: SASCO, Inc., St. Louis, MO
   Diet: Purina Rodent Chow 5001-4 (etts form), *ad libitum*
   Water: Tap, *ad libitum*
   Housing: Individual stainless steel cages
   Environmental conditions:
      Temperature: 18-26 °C
      Mean humidity: 40-70%
      Air changes: Not specified
      Photoperiod: 12-Hour light/dark cycle
   Acclimation period: ≥6 days

B. STUDY DESIGN and METHODS

Prior to conducting the dermal absorption study in rats, a preliminary *in vitro* study was conducted to assess potential volatilization of the test material from the treatment site. Glass plates were covered with synthetic skin (DuoDERM CGF), and a protective rubber ring was glued to the DuoDERM on each plate. Using a microcapillary pipet, neat [^{14}C]KBR 3023 was applied in triplicate to the synthetic skin on prewarmed (36 °C) plates in volumes of 2 or 50 µL, (equivalent to the low and high dose volumes used in the animal study). The enclosures were then covered with a nonocclusive laminated filter, which consisted of an inner PTFE layer and an outer carbon-impregnated layer. The plates were maintained at 36 °C on a warming tray for 8 hours and then separated into their components. Radioactivity on each component was determined either directly or by extraction with methanol or acetone. The degree of volatilization was considered to be the amount of radioactivity associated with the filter cover.

The main study was designed to examine dermal absorption of [^{14}C]KBR 3023 in rats under conditions comparable to a previously conducted dermal absorption study in humans (MRID 44408738) and to toxicity studies in rats which have utilized dermal dosing. Accordingly, the following modifications were made to the Subdivision F §85-3 dermal absorption study: (I) the study included female rats; (ii) all animals were dosed for 8 hours; (iii) absorption was assessed at different intervals following the 8-hour dosing period; and (iv) the dose levels used were comparable to the previous study in humans or the toxicity studies in rats.

The study consisted of three groups of 12 rats/sex/group dosed dermally at target doses of 2, 10, or 50 mg/animal, equivalent to 0.133, 0.67, 3.33 mg/cm^2 based upon a 15 cm^2 treatment area. Actual application rates averaged 1.84, 9.25, and 48.5 mg/rat for males and 1.84, 9.59, and 48.3 mg/rat for females. All rats in each dose group were exposed for 8 hours. Four rats per dose group were sampled (i) at the end of the 8-hour exposure, (ii) 24 hours after the beginning of exposure (16 hours post-treatment), and (iii) when radioactivity levels in urine dropped to background levels (3-7 days after beginning of exposure period).

1. Dose selection

The high dose of 50 mg/rat was selected to be equivalent to 200 mg/kg (based on 250 g rat), which was the high dose used in previous toxicity studies in which test animals were dosed dermally over ~10% of their body area. The middle dosage of 10 mg/rat (0.67 mg/cm^2), or about 40 mg/kg, was selected to approximate, on a mg/cm^2 basis, the dosage used in the human dermal absorption study (0.615 mg/cm^2). The low target dose of 2 mg/rat, or about 8 mg/kg, was chosen because this level reflects estimated human exposure patterns and was the minimal application volume (2 µL) that could be accurately applied.

2. Dose preparation

For application, [^{14}C]KBR 3023 was diluted with unlabeled KBR 3023 to a specific activity of 3.65 x 10^5 dpm/mg for the high dose, 1.82 or 2.47 x 10^6 dpm/mg for the middle dose, and 1.1 x 10^7 dpm/mg for the low dose. The [^{14}C]KBR 3023 solutions were applied neat to the treatment site, and each dosing solution was analyzed to determined the radiochemical purity and chemical purity. The stability and homogeneity of the dosing solutions could be not assessed as details of these analyses were not provided.

3. Animal preparation and dosing

Twelve animals/sex were randomly assigned to each of the three dose groups. At least 24 hours before dosing, an area of appropriate size on the back of each animal was shaved and wiped with acetone, taking care not to abrade the skin. A rubber ring enclosure (approximately 15 cm^2) was glued to the shaved area on the back of each rat. A topical application of neat [^{14}C]KBR 3023 was applied to the skin at 2, 20 (10+10), or 50 µL using a glass microcapillary pipet. Applicator tips were saved and radioassayed to determine the amount actually applied to each animal. A nonocclusive cover, consisting of an inner PTFE layer and an outer carbon-impregnated layer, was then glued onto the rubber ring, and an Elizabethan collar was placed on each animal's neck. Rats were then returned to their cages and stainless steel pans with mesh screens were placed under each cage for the collection of urine and feces.

4. Animal observations and sample collection

Animals were weighed shortly before application and clinical observations were made daily until sacrifice. All animals were exposed to the test material for 8 hours. After exposure, the protective cover was removed and the treatment site was washed by wiping twice with pads moistened with a 5% aqueous detergent solution, then wiping twice with pads moistened with distilled water, and finally wiping with a dry pad. All samples were saved for radioassay. For animals that were held longer than the initial 8-hour exposure, a new protective cover was placed over the treatment site. Urine and feces were collected at sacrifice for animals sampled at 8 and 24 hours and on a daily basis for animals held up to 7 days. Urine samples were stored at room temperature until radioassayed, and fecal samples were stored frozen.

Four rats/sex/dose group were sacrificed immediately following the 8-hour exposure period and another four rats/sex/dose group were sacrificed 16 hours later (24-hours). The final 4 rats/sex/dose group were sacrificed individually when radioactivity in urine reached background levels, which occurred up to 7 days after the beginning of the exposure period. For sacrifice, rats were anesthetized with halothane and the diaphragm was cut and a blood sample was collected by cardiac puncture. Residual urine in the bladder was collected and added to the urine sample from the animal. The protective cover and rubber ring were removed and saved, and skin at the treatment site was excised along with the underlying

subcutaneous muscle layer. The cages were rinsed with methanol. All samples were retained for analysis.

The following samples were analyzed for radioactivity:

Applicator tip	Rubber enclosure
Protective cover	Gauze pads (Skin wash)
Skin at treatment site	Carcass
Blood	Urine
Feces	Cage wash

Samples of urine, cage washes, and applicator tips were radioassayed directly by liquid scintillation counting (LSC) , along with extracts or rinses of the rubber rings, protective covers, and pads used for skin washes. Homogenized feces and blood were radioassayed by LSC following combustion. Skin and carcass samples were digested with 4N KOH in methanol for a minimum of 2 weeks, neutralized, and radioassayed directly by LSC.

3. Data analysis

Means were calculated for samples from each dose group, and the standard deviation was also calculated for treatments from the preliminary volatilization study.

II. RESULTS:

1. Preliminary volatilization study

Following an 8-hour exposure period on synthetic skin held at 36-41 °C, 113.4% (±31.3%) of the radioactivity applied to the low dose (2 µL) plates was recovered, with 1.64% (±0.19%) of the dose being associated with the covering filters. For the high dose (50 µL) applications, 89.7% (±1.8%) of the applied radioactivity was recovered, of which 0.39% (±0.18%) was associated with the covering filters.

2. Dermal absorption study

The average dosages (mg/rat and mg/cm^2) of [^{14}C]KBR 3023 actually applied to each dose group are presented in Table 1. Actual doses were 86-92% of the target dose for the low-dose group, 88-93% for the mid-dose group, and 92-93% for the high-dose group. The dosed radioactivity was quantitatively recovered from each dose group. Recoveries were similar between the sexes and at each sampling interval. With regards to dose level, recoveries were similar for the low dose (86.6-98.0%) and high dose (85.6-92.9%) groups, but were generally higher for the mid-dose groups (96.5-111.7%).

Following the 8-hour exposure period, 40.4-80.5% of the applied material was recovered in the skin wash and 3.2-30.6% was associated with the protective cover (Table 1) from all animals. Together with the radioactivity recovered from the rubber ring enclosure (1.1-4.8%), these fractions represent the percentage of the dose which was not absorbed (59.2-94.3%; Table 2). Dose level and the post-treatment sampling interval had no apparent effect on the percentage of the dose which was not absorbed (low-dose, 59.2-77.0%; high-dose, 65.7-81.2%). There was a difference in absorption between the sexes, however. Regardless of dose level and sampling interval, 66.8-94.3% of the dose (average of 80.3%) was not absorbed by males, compared to 59.2-85.2% (average of 70.8%) for females. The difference between sexes was also observed in the percentage of the dose absorbed. Including skin at the treatment site, 9.8-20.4% of the dose was absorbed by the males and 17.4-32.2% of the dose was absorbed by the females. Dose level also had an effect on the percent absorbed. At the 8-hour interval, the low, mid, and high dose males had absorbed 14.4, 9.8, and 6.9% of the dose, respectively, and the low, mid, and high dose females had absorbed 27.4, 12.6, and 8.9% of the dose, respectively. However, differences in the percent absorbed between dose levels decreased at the 24-hour interval and there was no effect from dose level at the final sampling interval.

At each dose level for both sexes, the majority of the absorbed dose remained in the body (including blood) and treatment site skin (males, 14.2-14.5% dose; females, 15.6-20.2% dose) immediately following the 8-hour exposure period, with excreted radioactivity accounting for 1.9-13.5% of the dose. At subsequent sampling intervals, radioactivity in the body and in the treatment site skin declined steadily while the percentage of the dose excreted increased. At the 24-hour sampling interval, excreted radioactivity accounted for 4.1-19.4% of dose and radioactivity remaining in the body accounted for 4.3-8.5% of the dose. By the final sampling interval (3-7 days post-treatment), 86-93% of the absorbed radioactivity had been excreted (12.0-24.1% dose), and radioactivity in the body and/or treatment site skin accounted for 1.5-3.0% of the dose. Urinary excretion (including cage wash) was the principle route of elimination of the absorbed radioactivity, accounting for 95-98% of the excreted radioactivity at the 8-hour sampling interval and for 75-88% of the excreted radioactivity at the final sampling interval.

- For the low dose group, an average of 1.84 mg/rat (0.118 mg/cm^2) of [^{14}C]KBR 3023 was applied to males and females. Following the 8-hour exposure period, 59.2-77.0% of the dose remained unabsorbed. Including the treatment site skin, males absorbed 16.4-20.4% of the dose and females absorbed 24.1-32.2% regardless of sampling interval. Immediately following the 8-hour exposure, the majority of the absorbed radioactivity was recovered in the body and treatment site skin (males, 14.5% dose; females, 18.7% dose), with males and females having excreted 5.4 and 13.5% of the dose, respectively, in the urine and feces. At the two subsequent sampling intervals, radioactivity in the body (including treatment site skin) declined steadily with a concomitant increase in excreted radioactivity. By the final sampling interval (3-7 days post-treatment), excreted radioactivity accounted for 18.2 and 24.1% of the dose for males and females, respectively, while 2.2-3.0% of the dose remained in the body and skin at the treatment site.

- For the middle dose group, an average of 9.25 and 9.59 mg/rat (0.596 and 0.617 mg/cm^2) of [^{14}C]KBR 3023 was applied to males and females, respectively. Following the 8-hour exposure period, 78.3-94.3% of the dose remained unabsorbed. Including the treatment site skin, males absorbed 15.7-19.1% of the dose and females absorbed 17.5-22.2% regardless of sampling interval. Immediately following the 8-hour exposure, the majority of the absorbed radioactivity was recovered in the body and treatment site skin (males, 14.4% dose; females, 15.6% dose), with males and females having excreted 3.0 and 5.8% of the dose, respectively. As with the low dose group, radioactivity in the body declined over time. By the final sampling interval, excreted radioactivity accounted for 17.6 and 15.0% of the dose for males and females, respectively, while 1.5-2.5% of the dose remained in the body and skin at the treatment site.

- For the high dose group, an average of 48.4 mg/rat (3.10 mg/cm^2) of [^{14}C]KBR 3023 was applied to males and females. Following the 8-hour exposure period, 65.7-81.2% of the dose remained unabsorbed. Including the treatment site skin, males absorbed 9.8-16.1% of the dose and females absorbed 17.4-22.6% regardless of sampling interval. Immediately following the 8-hour exposure, the majority of the absorbed radioactivity was recovered in the body and treatment site skin (males, 14.2% dose; females, 20.2% dose), with males and females having excreted 1.9 and 2.4% of the dose, respectively. As with the other dose groups, radioactivity in the body declined over time. By the final sampling interval, excreted radioactivity accounted for 12.0 and 20.6% of the dose for males and females, respectively, while 1.5-1.7% of the dose remained in the body and skin at the treatment site.

III. DISCUSSION

A. Investigator's Conclusion - The study author concluded that absorption of KBR 3023 following dermal dosing was moderate, accounting for 23.3, 18.9, and 17.0% of the dose from rats exposed for 8 hours at 0.133, 0.67, and 3.33 mg/cm^2, respectively. The absorption pattern was similar between males and females and between dose groups. Absorption of KBR 3023 did not appear to be concentration-dependent over the range of dosages used. For each dose group the primary route of excretion was through the urine, with the majority of urinary excretion being completed within 2 days. The data suggest that KBR 3023 did not bind to the site of dermal application. In addition, the *in vitro* study which mimicked the conditions in the dermal absorption study indicated that volatilization of KBR 3023 would be expected to account for ≤2% of the dosed material over the 8-hour exposure period.

Reviewer's Discussion - Sprague-Dawley rats (12/sex/group) were dosed dermally with neat [^{14}C]KBR 3023 at nominal doses of 2, 10, and 50 mg/rat (0.133, 0.67, or 3.33 mg/cm^2) and exposed for a duration of 8 hours. Actual dose levels averaged 1.8, 9.4, or 48.4 mg/rat (0.118, 0.606, or 3.10 mg/cm^2). Dosed radioactivity was quantitatively recovered from each dose group (85.6-111.7%).

After the 8-hour exposure, dermal absorption of KBR 3023 was moderate. Regardless of dose level and sampling interval, radioactivity absorbed and at the treatment site totaled 9.8-20.4% of the dose in males and 17.4-32.2% of the dose in females, indicating a difference in absorption between sexes. Dose level and sampling interval also had an effect on the percent dose absorbed and the percent dose remaining in or on the skin at treatment site. Immediately following the 8-hour exposure, the percent of the dose absorbed was 14.4, 9.8, and 6.9% for the low, mid, and high dose males, respectively, and 27.4, 12.6, and 8.9% for the low, mid, and high dose females, respectively. For skin at the treatment site, the situation was reversed with radioactivity remaining in or on the skin being highest for the high dose animals (males, 9.2% dose; females 13.7% dose) and lowest for the low dose animals (males, 5.5% dose; females 4.8% dose). At subsequent sampling intervals, the percent dose absorbed increased while radioactivity remaining at the treatment site declined. By the final sampling interval, dose level essentially had no effect on the percent of the dose absorbed or remaining on the skin.

Although there were differences between the sexes in the percent dose absorbed, the distribution and excretion of the absorbed radioactivity was similar between the sexes and dose groups. At each dose level for both sexes, the majority of the absorbed dose remained in the body (including blood) and treatment site skin (males, 14.2-14.5% dose; females, 15.6-20.2% dose) immediately following the 8-hour exposure period, with excreted radioactivity accounting for 1.9-13.5% of the dose. At subsequent sampling intervals, radioactivity in the body and in the treatment site skin declined steadily while the percentage of the dose excreted increased. By the final sampling interval (3-7 days post-treatment), 86-93% of the absorbed radioactivity had been excreted (12.0-24.1% dose), and radioactivity remaining in the body and/or treatment site skin accounted for 1.5-3.0% of the dose. Urinary excretion (including cage wash) was the principle route of elimination of the absorbed radioactivity, accounting for 75-98% of the excreted radioactivity.

IV. STUDY DEFICIENCIES

Study guidelines require at least three dosing durations in order to demonstrate variations in penetration over time. In this study, all test groups were dosed for the same 8-hour exposure period. This protocol mimics the way this insect repellent will be used. The maximum dosage used (3.33 mg/cm^2) in the study exceeded the maximum practical dose as specified by the guidelines and the study used both male and female rats. These are deviations from the guidelines, but not deficiencies.

The only deficiency noted was that detailed data were not provided on the stability and homogeneity of the [^{14}C]KBR 3023 dosing solutions. However, this deficiency does not adversely affect the acceptability of this study. This study is classified as **Acceptable (guideline).**

Table 1. Distribution of radioactivity following a single 8 hour exposure of rats to [^{14}C]KBR 3023.

Nominal dose (mg/cm^2)	Actual dose[a]		Sampling interval (hrs)[b]	Recovered radioactivity expressed as % of applied dose[c]									
	(mg/cm^2)	mg/rat		Protective Cover	Enclosure	Skin wash	Skin (test site)	Blood	Urine	Feces	Carcass	Cage wash	Total Recovered
Males													
0.133	0.122	1.909	8	9.8	1.4	55.6	5.5	0.2	4.7	0.2	8.8	0.5	86.6
	0.116	1.810	24	19.8	1.1	56.1	2.1	ND[d]	9.3	2.1	2.2	0.7	93.6
	0.115	1.803	168	30.6	1.4	40.4	1.9	ND	13.4	4.4	0.3	0.4	92.8
0.67	0.590	9.162	8	9.9	3.9	80.5	7.6	0.1	2.8	0.1	6.7	0.1	111.7
	0.593	9.204	24	20.5	3.6	68.5	3.0	ND	8.1	1.5	2.5	0.6	108.4
	0.604	9.370	168	13.0	2.9	69.9	1.0	ND	12.4	4.1	0.5	1.1	104.8
3.33	3.11	48.62	8	6.9	3.1	66.8	9.2	0.1	1.5	0.1	4.9	0.3	92.9
	3.09	48.26	24	7.4	4.8	69.0	4.0	ND	3.2	0.6	1.7	0.3	91.1
	3.11	48.62	168	13.8	2.6	59.0	0.8	ND	6.4	3.0	0.9	2.6	89.1
Females													
0.133	0.121	1.895	8	11.6	3.6	44.0	4.8	0.4	9.8	0.7	13.5	3.0	91.3
	0.118	1.839	24	18.3	1.9	45.1	2.1	ND	16.2	1.4	2.6	1.8	89.2
	0.115	1.791	168	15.5	3.0	52.6	2.2	ND	19.3	3.7	0.8	1.1	98.0
0.67	0.624	9.682	8	16.8	3.3	65.1	8.8	0.2	4.5	0.1	6.6	1.2	106.6
	0.619	9.610	24	15.6	3.5	59.2	3.4	ND	13.5	1.1	2.9	1.3	100.7
	0.610	9.464	168	8.8	1.2	69.0	1.0	ND	11.7	1.8	1.5	1.5	96.5
3.33	3.10	48.46	8	3.4	1.6	60.8	13.7	0.2	1.9	0.1	6.3	0.4	88.3
	3.07	47.94	24	6.8	3.2	59.6	6.2	ND	7.3	0.6	2.3	1.0	87.1
	3.09	48.36	168	3.2	3.0	59.5	1.0	ND	12.8	3.5	0.5	4.3	85.6

a Data were calculated by reviewer from data in Appendix III (pages 48-50) of study report.

b The 168 hour sampling interval represents animals that were sacrificed at the point when radioactivity in their urine declined to background levels, 3 to 7 days after the beginning of exposure.

c Data are the mean of 4 rats/sex/dose group and were obtained from Appendix III (pages 48-50) of the study report.

d ND = nondetectable.

Table 2. Summary of dose distribution values for [^{14}C]KBR 3023 following a single 8 hour dermal exposure of rats.[a]

Nominal dose (mg/cm²)	Actual dose		Sampling interval (hrs)[b]	Amount of dose							
				Not Absorbed[c]	Skin at test site		In Body[d]	Excreted[e]	Absorbed[f]		Total of skin and absorbed (%)
	mg/cm²	mg/rat		(% dose)	(% dose)	µg/cm²	(% dose)	(% dose)	(% dose)	µg/cm²	
Males											
0.133	0.122	1.909	8	66.8	5.5	6.71	9.0	5.4	14.4	17.57	19.9
	0.116	1.810	24	77.0	2.1	2.44	2.2	12.1	14.3	16.59	16.4
	0.115	1.803	168	72.4	1.9	2.19	0.3	18.2	18.5	21.28	20.4
0.67	0.590	9.162	8	94.3	7.6	44.84	6.8	3.0	9.8	57.82	17.4
	0.593	9.204	24	92.6	3.0	17.79	2.5	10.2	12.7	75.31	15.7
	0.604	9.370	168	85.8	1.0	6.04	0.5	17.6	18.1	109.3	19.1
3.33	3.11	48.62	8	76.8	9.2	286.1	5.0	1.9	6.9	214.6	16.1
	3.09	48.26	24	81.2	4.0	123.6	1.7	4.1	5.8	179.2	9.8
	3.11	48.62	168	75.4	0.8	24.88	0.9	12.0	12.9	401.2	13.7
Females											
0.133	0.121	1.895	8	59.2	4.8	5.81	13.9	13.5	27.4	33.15	32.2
	0.118	1.839	24	65.3	2.1	2.48	2.6	19.4	22.0	25.96	24.1
	0.115	1.791	168	71.1	2.2	2.53	0.8	24.1	24.9	28.64	27.1
0.67	0.623	9.682	8	85.2	8.8	54.91	6.8	5.8	12.6	78.62	21.4
	0.619	9.610	24	78.3	3.4	21.05	2.9	15.9	18.8	116.4	22.2
	0.610	9.464	168	79.0	1.0	6.10	1.5	15.0	16.5	100.7	17.5
3.33	3.10	48.46	8	65.8	13.7	424.7	6.5	2.4	8.9	275.9	22.6
	3.07	47.94	24	69.6	6.2	190.3	2.3	8.9	11.2	343.8	17.4
	3.09	48.36	168	65.7	1.0	30.90	0.5	20.6	21.1	652.0	22.1

a Data obtained from Table 1 of this report or calculated by reviewer from data in Table 1.
b The 168 hour sampling interval represents animals that were sacrificed at the point when radioactivity in their urine declined to background levels, 3 to 7 days after the beginning of exposure.
c Includes radioactivity in/on cover, enclosure, and skin wash.
d Includes radioactivity in carcass and blood.
e Includes radioactivity in urine, feces, and cage wash.
f Includes radioactivity in the body and excreted.