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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

TXR No.: **0050341**

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: **11-20-01**

Subject: **KBR 3023:** Review of a 14-Day GLP Oral Toxicity Study in Rats.
DP Barcode: D269671, PC Code: 070705

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

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

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

The data base for KBR 3023 is almost entirely dermal. While these data are ideal for assessing dermal risk for a dermally applied product, they are inadequate for assessing oral risk resulting from hand-to-mouth transfer in children, so Bayer was asked to provide oral toxicity data.

Bayer Corporation performed a 14-day range-finding feeding study (MRID 45227601). OPPTS has no guideline for a subacute toxicity study, so this study was patterned after guideline OPPTS 870.3100 for a 90-day toxicity study in rodents, and has full clinical pathology and histopathology evaluations. Bayer performed it in compliance with the Good Laboratory Practices (GLPs). This subacute toxicity study is classified Acceptable. It cannot satisfy a guideline requirement because there is no guideline for a subacute oral toxicity study. The Data Evaluation Record (DER) is attached.

The high-dose of 20,000 ppm (1731 and 2826 mg/kg/day in males and females, respectively) was nearly twice the limit dose (1000 mg/kg/day). At this dose, absolute and (relative) liver weights were elevated 24% (32%) in males and 23% (33%) in females, minimal to slight liver hypertrophy (mostly centrilobular) was observed histopathologically, and there was no elevation in liver enzymes. This profile suggests an adaptive response to a xenobiotic in healthy livers. Minor decreases in body weight gain and food consumption, likely due to feed unpalatability, occurred during the first week only. Since adverse toxicity was not observed, there is no LOAEL, so the high-dose is a freestanding NOAEL.

KBR 3023

Subacute Oral Study (870.xxxx)

EPA Reviewer: John E. Whalan
Review Section: Registration Action Branch 2 (7509C)
EPA Secondary Reviewer: SanYvette Williams-Foy
Review Section: Registration Action Branch 2 (7509C)

John E. Whalan, Date 11-20-01

, Date 11/20/01

DATA EVALUATION RECORD

STUDY TYPE: Subacute Feeding Toxicity – Rats
[This is a GLP subacute range finding study patterned after OPPTS 870.3100]

DP BARCODE: D269671

SUBMISSION CODE: S5 86643

P.C. CODE: 070705

TOX. CHEM. NO.: 070705

TEST MATERIAL (PURITY): KBR 3023 Technical (97.1% a.i.)

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

CITATION(s): Wahle, B.S. (2000) Technical Grade KBR 3023: A Subacute Toxicity Testing Study in the Rat. Bayer Corporation, Agriculture Division, Toxicology, Stilwell, Kansas. Laboratory Study Number 109817, September 29, 2000. MRID 45227601. Unpublished

SPONSOR: Bayer Corporation, Agriculture Division, Kansas City, Missouri.

EXECUTIVE SUMMARY: In a subacute range-finding toxicity study (MRID 45227601), KBR 3023 (97.1% a.i.) was administered over 14 consecutive days to 5 Sprague-Dawley (Crl:CD®(SD)IGS BR) rats/sex/dose in the diet at dose levels of 1000, 1500, 2000, or 20,000 ppm (0, 90, 141, 183, or 1731 mg/kg/day in males; 0, 109, 163, 202, or 1826 mg/kg/day in females). Although this was a range-finding study, it was performed in compliance with the GLPs and patterned after guideline OPPTS 870.3100 (90-day oral toxicity in rodents).

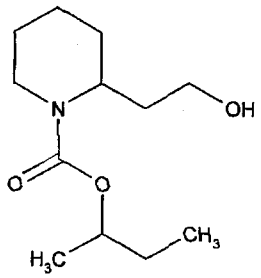
There were no deaths or clinical signs; no anomalous changes in body weight gain, food consumption, clinical pathology (including a thyroid panel), or organ weights; and no compound-related gross or histopathologic lesions at any dose level. At the 20,000 ppm dose, absolute and (relative) liver weights were elevated 24% (32%) in males and 23% (33%) in females, minimal to slight liver hypertrophy (mostly centrilobular) was observed histopathologically, and there was no elevation in liver enzymes. This profile suggests an adaptive response to a xenobiotic in healthy livers. Minor decreases in body weight gain and food consumption, likely due to feed unpalatability, occurred during the first week only. **The freestanding NOAEL is 20,000 ppm (1731 and 1826 mg/kg/day in males and females, respectively) which exceeds the limit dose of 1000 mg/kg/day. A LOAEL was not attained.** This subacute toxicity study is classified Acceptable. It cannot satisfy a guideline requirement because there is no guideline for a subacute oral toxicity study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided. Ophthalmologic examinations were not performed. Considering that this is a range-finding study, this deficiency is of no consequence. As a GLP study, this is a minor deficiency that has no impact on the classification of this study because the eyes were examined histopathologically. There was also no evaluation of clotting potential.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: KBR 3023 technical
   Description: Clear liquid
   Lot/Batch #: 030693
   Purity: 97.1% a.i.
   Stability of compound: See Section B3 (Diet preparation and analysis)
   CAS #: 119515-38-7
   Structure:



2. Vehicle: Diet

3. Test animals:
   Species: Rat
   Strain: Sprague-Dawley (Crl:CD®(SD)IGS BR)
   Age and weight at study initiation: Eight weeks old; mean weight of 244.0 and 174.6 g in males and females, respectively.
   Source: Charles River, Kingston, New York
   Housing: Individually housed
   Diet: PMI Certified Rodent Diet 5002 in meal form *ad libitum*
   Water: Tap water *ad libitum*
   Environmental conditions: Temperature: 18 to 26°C
   Humidity: 30 to 70%
   Air changes: At least 10/h
   Photoperiod: 12 hours light/dark
   Acclimation period: Two weeks

B. STUDY DESIGN:

1. In life dates - start: July 17, 2000 end: August 2, 2000

2. Animal assignment

   Groups of 5 males and 5 females were randomly assigned by body weight to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Diet Conc. (ppm)	Dose (mg/kg/day)	
		Male	Female
Control	0	0	0
Low	1000	90	109
Mid	1500	141	163
Mid	2000	183	202
High	20,000	1731*	1826*

*These doses exceed the limit dose of 1000 mg/kg/day

3. Diet preparation and analysis

Diet was prepared prior to study initiation by mixing appropriate amounts of test substance with PMI Certified Rodent Diet 5002, which was then stored under refrigeration. Homogeneity and stability of 500 and 20,000 ppm formulations were tested prior to study initiation. During the study, samples of all treated food were analyzed prior to study initiation for dose concentration.

Results - Homogeneity Analysis: ≤2%
Stability Analysis: Stable for a minimum of 7 days at room temperature, 14 days in freezer storage
Concentration Analysis: Within 5% of nominal. KBR 3023 was not detected in control feed.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage was acceptable.

4. Statistics - Continuous data that were examined statistically were evaluated initially for equality or homogeneity of variance using Bartlett's test. Group means were further analyzed by a one-way variance analysis (ANOVA) followed by Dunnett's test. In the event of unequal variances, and at the discretion of the study director, data were subject to non-parametric procedures consisting of a Kruskal-Wallis ANOVA followed by the Mann-Whitney-U test for between-group comparisons. Frequency data were initially examined for trends; data suggestive of a potential effect were then statistically evaluated using the chi-square, Fisher exact, or chi-square and Fisher exact tests. On a case by case basis, and at the discretion of the study director, data were subject to additional statistical procedures other than those mentioned above. For the Bartlett test, a probability (p) value ≤0.001 was considered significant; for all other statistical tests, differences with p values ≤ 0.05 were considered statistically significant. All statistical evaluations were performed using software obtained from either INSTEM Computer Systems or SAS Institute Inc.

C. METHODS:

1. Observations:

Animals were inspected once weekly for signs of toxicity and mortality.

2. Body weight

Animals were weighed weekly.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not calculated.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not performed.

5. Blood was collected from all fasted rats prior to termination for hematology and clinical chemistry analysis from all surviving animals. 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 corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Hemoglobin distribution width (HDW)
	(Thromboplastin time)	X	Red cell distribution width
	(Thromboplastin time)	X	Erythrocyte morphology
	(Clotting time)	X	Heinz bodies
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

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*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Direct bilirubin
	Cholinesterase (ChE)	X	Total serum protein (TP)*
X	Creatine phosphokinase	X	Triglycerides
X	Lactic acid dehydrogenase (LDH)		Serum protein electrophores
X	Serum alanine amino-transferase (also SGPT)*	X	Uric acid
X	Serum aspartate amino-transferase (also SGOT)*	X	Thyroxine (T4)
X	Gamma glutamyl transferase (GGT)	X	Triiodothyronine (T3)
	Glutamate dehydrogenase	X	Thyroid stimulating hormone (TSH)

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from all unfasted rats two days prior to blood collection. The CHECKED (X) parameters were examined.

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

* Not required for subchronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. These tissues were examined for all rats in the control and high dose groups as well as gross lesions from all animals. Additional tissues were examined as needed to establish a NOAEL. The (XX) organs, in addition, were weighed. The histopathologic grading ranged from 1 to 5 (minimal, mild, moderate, marked, severe), as well as "normal" and "present."

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)^{T}
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)^{T}
X	Jejunum*	XX	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+	X	Lacrimal gland^{T}
X	Rectum*	X	Urinary bladder*	X	Mammary gland^{T}
XX	Liver*$^{+}$	XX	Testes*$^{+}$		Parathyroids*$^{++}$
	Gall bladder*	XX	Epididymides	XX	Thyroids*$^{++}$
X	Pancreas*	X	Prostate	X	Harderian gland
		X	Seminal vesicle	X	Zymbal's gland
	RESPIRATORY	XX	Ovaries		
X	Trachea*	XX	Uterus*		OTHER
XX	Lung*	X	Cervix	X	Bone
	Nose	X	Vagina	X	Joint (femur/tibia)
X	Pharynx	X	Clitoral gland	X	Skull
X	Larynx	X	Preputial gland	X	Skeletal muscle
				X	Skin
				X	All gross lesions and masses*

* Required for subchronic studies based on OPPTS Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations :

1. Toxicity - No clinical signs were observed that could be attributed to the test article.

2. Mortality - There was no mortality.

B. Body weight and weight gain: Table 2 demonstrates that all groups gained body weight, but a slight decrease in body weight gain occurred during the first week only at the high-dose. Compared to controls, changes in absolute body weights and (body weight gains) were -7% (-43%) after the first week and -5% (+5%) after the second week in males; and -5% (-44%) after the first week and -7% (-19%) after the second week in females. These minor anomalies correspond to decreased food consumption during the first week (see Table 3), most likely due to unpalatability. Weight gain was comparable in all groups during the second week.

TABLE 2: BODY WEIGHT (study report pages 42 and 43)

Dose (mg/kg/day)	Males (grams)			Females (grams)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
0	244.0	296.9	337.7	174.6	200.9	221.9
1000	234.8	281.4	313.5	168.3	196.4	216.0
1500	241.3	295.6	334.3	173.3	205.2	225.1
2000	244.2	297.2	333.8	175.1	202.5	218.9
20,000	246.8	277.3	320.3	175.6	190.3	207.2

C. Food consumption and compound intake:

1. Food consumption - Table 3 shows that food consumption was only affected in the high-dose groups during the first week (-15% in males and -26% in females). These rats had food consumption comparable to the other groups during the second week.

TABLE 3: FOOD CONSUMPTION (study report pages 45 and 46)

Dose (mg/kg/day)	Males (g/rat/day)		Females (g/rat/day)	
	Week 1	Week 2	Week 1	Week 2
0	25.05	25.90	20.73	20.15
1000	23.77	24.31	19.59	21.48
1500	24.57	25.72	20.55	20.51
2000	24.67	25.75	18.76	20.19
20,000	21.32*	24.50	15.41*	18.68

* $p<5\%$

2. Compound consumption (time-weighted average) - See Table 1

D. Ophthalmoscopic examination - No ophthalmoscopic examinations were performed.

E. Blood work:

1. Hematology - No hematologic anomalies or abnormal erythrocyte morphology was observed.

2. Clinical Chemistry - Small dose-related decreases in levels of lactate dehydrogenase, creatine kinase, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were observed in both sexes at all doses. Although increases in these enzymes may be clinically significant, decreases are not. The thyroid parameters were unaffected. Slight increases in serum cholesterol were seen in high-dose males (+54%) and females (+38%). Small increases in cholesterol are typically seen during hepatic detoxification, and are not considered adverse.

F. Urinalysis — The urine of males and females in the high-dose was slightly acidic. No other urinalysis anomalies were found.

G. Sacrifice and Pathology:

1. Organ weight - Organ weight anomalies were observed only in the high-dose; all other groups resembled controls. There was an increase in absolute (and relative) liver weights of 24% (32%) in males and 23% (33%) in females. Kidney weights were increased 9% (17%) in males, but decreased 23% (17%) in females. Thyroid weights were increased 23% (28%) in males, but decreased 5% (3%) in females in the high-dose.

2. Gross pathology - There were very few gross lesions observed, and none could be attributed to the test article.

3. Microscopic pathology -

a) Non-neoplastic - All males and all females in the high-dose group had minimal to slight liver hypertrophy which was most evident in the centrilobular regions. In the high-dose, cystic thyroid follicles were observed in 5/5 females, compared to 2/5 controls, and in 2/5 males, compared to 2/5 controls; there was no increase in severity in either sex, so this lesion is not considered compound-related. There were no other lesions that could be attributed to the test article.

b) Neoplastic - No neoplastic lesions were observed.

III. DISCUSSION

A. The NOAEL is the high-dose of 20,000 ppm (1731 and 1826 mg/kg/day in males and females, respectively). At this dose, liver weights were elevated, but none of the liver enzymes were elevated. The only histopathologic finding was minimal to slight liver hypertrophy, primarily in the centilobular region. This profile suggests an adaptive response to a xenobiotic in healthy livers. While hepatotoxicity may be expressed in a longer duration study, there was no evidence of it in this study.

There were no deaths, clinical signs, clinically significant hematologic, chemical, or urinalysis anomalies, or gross or histopathologic lesions (other than centrilobular

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hypertrophy) at the high-dose. Minor decreases in body weight gain and food consumption were observed during the first week only, and were probably due to unpalatability. Even though the limit dose was exceeded, a LOAEL was not attained, so the NOAEL is freestanding. The next lower dose (183/202 mg/kg/day, M/F) resembled the controls.

A 9% (17%) increase and a 23% (17%) decrease in absolute and (relative) male and female kidney weights, respectively, was observed in the high dose. Although no clinical significance can be deduced from this finding, the kidney may be a target organ in a study of longer duration.

The thyroid weight anomalies in the high-dose were not clearly dose-related in the males and inconsequential in the females.

B. Study deficiencies: Ophthalmologic examinations were not performed. Considering that this is a range-finding study, this deficiency is of no consequence. As a GLP study, this is a minor deficiency that has no impact on the classification of this study because the eyes were examined histopathologically. There was also no measurement of clotting potential.