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Chronic toxicity and carcinogenicity testing in the Sprague–Dawley rat of a prospective insect repellant (KBR 3023) using the dermal route of exposure☆

Bradley S. Wahle, Ghona K. Sangha, Stephen G. Lake, Larry P. Sheets, Claire Croutch, Ware R. Christenson *

Agriculture Division, Toxicology, Bayer Corporation, 17745 South Metcalf, Stilwell, KS 66085-9104, USA

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Abstract

The chronic toxicology and carcinogenic potential of 1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine (KBR 3023), a prospective new insect repellent intended for human use, was studied in rats using the dermal route of application. Relying upon the toxicology profile that emerged in the subchronic rat bioassay that was conducted using dermally applied dosages of 0, 80, 200, 500 and 1000 mg KBR 3023/kg body wt/day, it was determined, in concert with the Environmental Protection Agency (EPA), that dermally applied dosages of 0, 50, 100 or 200 mg KBR 3023/kg body wt/day would be used in the conduction of all definitive forms of subchronic, chronic, and lifetime descriptive testing performed with the chemical. Using this testing approach, the specific results of this 2-year study are as follows. All in-life parameters, which included body weight, food consumption, clinical observations, survival, ophthalmology, clinical chemistry, hematology, and urinalysis, were unaffected by exposure to KBR 3023. Similarly, postmortem analyses, which included organ weights and gross pathology, were also unchanged following exposure to KBR 3023. Histopathology at the dose site/skin was characterized by a pattern of acanthosis and/or hyperkeratosis across all doses in 1- and 2-year rats. Beyond the dosing site, cystic degeneration of the liver was described in 2-year 200-mg KBR 3023/kg body wt/day males. No other compound-related non-dosing site lesion was identified at any dose tested. No evidence of a compound-induced neoplasia was suggested in this bioassay.

Keywords: KBR 3023; Insect repellant; Dermal toxicity testing; Risk assessment

Abbreviations: AAALAC, American Association for Accreditation of Laboratory Animal Care; CAS, Chemical Abstract Services; EPA, Environmental Protection Agency; NOEL, no-observed-effect-level; wt, weight.

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* Corresponding author. Tel.: +1-913-433-5225; fax: +1-913-433-5125.

E-mail address: russ.christenson.b@bayer.com (W.R. Christenson)

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1. Introduction

KBR 3023 is a piperidine derivative (Fig. 1), developed by the Bayer Corporation, that recently completed regulatory testing in support of a registration as an insect repellent intended for human use. The principal objectives of this 2-year dermal toxicity bioassay were: (1) to establish, under conditions of prolonged and repeated dermal exposure, a chronic toxicological profile for the KBR 3023-exposed rat, thus permitting determination of a maximum dose, uncomplicated by geriatric changes, which produces no observed adverse effects through at least 1 year (no-observed-effect-level (NOEL)) and (2) to characterize and possibly predict, based on the response in the rat, the oncogenic potential of KBR 3023 in the human.

The testing process was not typical for a pesticide agent in that (1) the entire data package was developed via the dermal route of administration and (2) identical dosage regimens were used for all the longer term bioassays. In the selection of this dosage regimen/approach to the testing of the agent, a significant and defining emphasis was placed upon the toxicological profile of the chemical that, paying particular attention to the response of the skin/dosing surface, emerged during the course of a 13-week dermal rat study that was performed over a dosage range of 0 (control), 80, 200, 500, or 1000 mg KBR 3023/kg body wt/day (Unpublished internal data, Bayer Corporation). The compound was applied neat to a shaved site ($\approx 10\%$ body surface) at a rate of 5 consecutive days/week. Upon termination of the study, microscopic analysis of the skin at the dosing site for all animals treated with the chemical showed signs of acanthosis, hyperkeratosis, and/or hypertrophy of the sebaceous glands around the hair follicle. Skin from untreated controls was unchanged. Despite the range of exposure (> 12-fold increase from 80 to the limit dose of 1000 mg KBR 3023/kg body wt/day), the severity grades for the lesions ranged only from minimal to slight for the treated skin examined at all dose sites. Such a pattern of microscopic changes is not unexpected as they have been observed, for example, when water is held in continuous contact with the skin of rats for 24–72 h (Jolly and Swan, 1980; Swan et al., 1986) or when medicinal grade petroleum is applied to animal or human skin on a daily basis for 10–15 days (Budhiraja et al., 1976). Therefore, for purposes of establishing the toxicological 'ground rules' with respect to the dosing surface under which to generate a complete testing package using the dermal route of exposure (i.e. metabolism studies as well as teratology, chronic dog, chronic and/or oncogenicity rat and mouse, and reproduction bioassays), the changes in the skin, in one sense unquestionably associated with exposure to the test substance, were on the other hand fundamentally non-dose responsive; best described as an adaptive, non-adverse, predictable/typical response to chronic exposure to an increasing volume (per dose) of a liquid material. In addition, reversibility of the skin changes at the dosing site was demonstrated following a 4-week period over which exposure was discontinued. From a regulatory perspective, these findings were not inconsistent with the Environmental Protection Agency's (EPA) experiences, and the mutually acceptable decision was made to assess the systemic chronic toxicity of KBR 3023 (in terms of a NOEL) as a separate and distinct consideration from the skin effects that emerged at the dosing site.

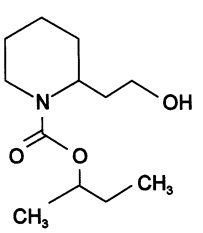

Fig. 1. The structure of KBR 3023 (1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)piperidine.

With the toxicological guidelines for the dosing site characterized, dose selection then proceeded based upon the non-dermal toxicological profile that emerged over the course of the 90-day exposure. Though body weight, food consumption, and mortality were unchanged at all doses tested, compound-related effects were noted in either one or both sexes of the 500 and 1000 mg KBR 3023/kg body wt/day dosages. Specifically, in-

creases in organ weight were measured in the liver and kidney. Microscopic lesions also were identified in these same tissues and were characterized by a diffuse hypertrophy in the liver and a hyaline degeneration of the kidney tubules. With the possible exception of declines in urinary pH and urobilinogen, no other compound-related and non-dosing site changes were indicated from the study data. Based on these non-skin-related changes, a 5-day/week dosing regimen of 0, 50, 100, or 200 mg KBR 3023/kg body wt/day in which to develop the entire toxicological package for KBR 3023 was proposed in concert with the EPA and mutually accepted. From this range of exposure, it was anticipated that a non-skin systemic NOEL, accommodating a factor of 100 as an appropriate margin of safety, would be established. Dispositional data obtained directly from the lifetime bioassays are not available. However, data obtained from a guideline-based single/repeated-dose dermal metabolism study with KBR 3023, indicated that absorption of the chemical ranged from 61–66% at a dosage of 20 mg/kg while an absorption range of 40–55% was observed at 200 mg/kg. Percent absorption was based on radioactivity measured in the plasma, excrete, tissues, and body, and refers to the sum of the parent compound and all biotransformation products (Unpublished internal data, Bayer Corporation). It is our expectation that this profile would be comparable for the studies reported herein.

Following the approach described above, the toxicological package for KBR 3023 was completed and submitted to the EPA for review. The following two coupled manuscripts, in the rat and the mouse, detail the effects of long-term dermal exposure to KBR 3023 as well as the technical problems associated with utilizing the dermal route of exposure for pesticide testing as opposed to oral exposure, which is used almost exclusively.

2. Materials and methods

2.1. Test substance

Technical grade KBR 3023 (Chemical Abstract Services (CAS) Registry No. 119515-38-7, minimum purity 97%, liquid at room temperature) was obtained from Bayer AG, Leverkusen, Germany and stored under freezer conditions ($\approx -$ 23°C). Aliquots from the test batch of KBR 3023 were obtained for dosing at 2-week intervals and maintained at room temperature during that time; room temperature stability was confirmed through 28 days (Unpublished internal data, Bayer Corporation).

2.2. Animals

This study was conducted under federal guidelines for the humane use and care of laboratory animals (National Institutes of Health, 1985) and was approved by the Institutional Animal Care and Use Committee of the toxicology department of the Agriculture Division of Bayer Corporation. Rats were housed in a temperature-, humidity-, light-controlled, and American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility (room temperature 18–26°C, relative humidity 40–70%, daily photoperiod of 12 h of fluorescent light [7:00–19:00 h] alternating with 12 h of darkness). Approximately 4-week old male and female Sprague–Dawley rats were obtained from Charles River Research Laboratories (Kingston, NY). All animals were examined upon receipt; those exhibiting deviations in general appearance and/or behavior were euthanized (CO_2 asphyxiation). Upon arrival and again after 1 month, a sample of animals was checked for serological evidence of common rodent infections, the results of which were unremarkable.

Animals were housed in either suspended stainless-steel wire-mesh cages over deotized cage board (Lab Supply, Fort Worth, TX) in the bedding tray or in polycarbonate shoebox-type enclosures containing Alpha-dri bedding (Shepards Specialty Papers, Kalamazoo, MI). Following 1 week of acclimation, animals were assigned to a control or one of three chemically treated groups using a weight stratification-based computer program (INSTEM Computer Systems, Stone,

Staffordshire, UK) and identified by tail tattoos. Food (Purina Mills Rodent Lab Chow® 5001-4 in 'etts' form, St Louis, MO) and municipal tap water (automatic watering) were provided for ad libitum consumption; food and feeders were replaced weekly. Cage racks were rotated weekly. Wire-mesh cages were changed every 3 weeks; shoebox-type enclosures were changed weekly.

2.3. Experimental design

All animals were ≈8 weeks old at initiation of exposure. During the study, rats were dermally administered undiluted KBR 3023 for ≈24 months at nominal dosages of 0 (concurrent untreated control), 50, 100, or 200 mg KBR 3023/kg body wt/day. Animals were randomly distributed into one of either four-dose groups within one of three major groupings arbitrarily designated as the 1-Year Sacrifice Group, 2-Year Sacrifice Group, or the Replacement Group. The 1-Year Sacrifice Group consisted of 20 males and 20 females in both the control and high-dose groups, and 10 males and 10 females in both the low and intermediate dose groups; The 2-Year Sacrifice Group consisted of 50 males and 50 females in all four dose groups; and The Replacement Group consisted of an additional five rats/dose/sex placed on study as part of the land 2-Year Sacrifice Groups that were maintained on study for approximately the first month of this 24-month study. The purpose of these animals was to serve as potential 'replacements' for any animals that unexpectedly died or developed non-compound-related problems, such as behavioral or physical abnormalities, at a very early stage in the study. Three animals each were replaced in the control, 50 mg/kg and 100 mg/kg dose groups (9 total): 4, maloccluded teeth; 1, found dead with teeth caught in feeder; 1, moribund; 1, missing upper incisors; 1, missing tooth/mouth lesion; and 1, excessive discharges and alopecia of the head.

On each of the 5 days of the normal work week, the undiluted test material was applied as uniformly as possible to a shaved area (≈10% of animal's total surface area) on the dorsal aspect of the trunk using an Easy-Step pipette (Continental Lab Products, San Diego, CA) and left uncovered.

As necessary, the exposure site was wiped with dry and/or water-dampened paper towels to remove any visible residue just prior to dosing. The skin was prepared by removing the hair on the dorsal and lateral area of the trunk, using Oster A-5 electric clippers (Oster Professional Products, McMinnville, TN) equipped with a Number 40 blade. Rats were clipped before the first dose was administered and once weekly or as necessary thereafter; care was taken to avoid abrading the skin. All animals were fitted with Elizabethan collars (EJAY International, Glendora, CA) for the duration of the study, beginning at least 1 week prior to the initiation of dosing. The collars were intended to reduce animal access to the dose site and thereby limit ingestion of the test substance (Fig. 2.). Dosage volumes were calculated, based on the mean weekly body weight determined for a given dose group; control animals were shaved and left untreated. The dose site area, representing ≈10% of the animal's total surface area (United States Environmental Protection Agency, 1984) was based on the following general relationship: $A_b = km^{2/3}$, where A_b is total surface area, k is the mass coefficient 8.79, and m is group mean body weight in grams (Spiers and Candas, 1984).

2.4. Antemortem observations

All animals were observed at least once daily (generally twice daily; AM and PM) for general appearance, behavior, signs of morbidity, and mortality. Each week, all animals were given a detailed physical examination that included palpation for the presence of tissue masses. Ophthalmoscopic examinations were performed pretest (only normal animals were assigned to the study) and prior to scheduled terminations. Individual body weights and food consumption were determined weekly for the duration of the study.

2.4.1. Clinical laboratory tests

Blood and urine were collected at ≈3, 6, 12, 18, and 24 months into the study from the first 20 surviving rats/sex/dose of the 2-Year Sacrifice Group. Blood was sampled via the orbital sinus following an overnight fast. Urine was collected

Fig. 2. Sprague–Dawley rat shaved and collared in preparation for long-term dermal exposure (shaved area on the dorsal aspect of the trunk represents ≈10% of the total surface area; Elizabethan collar from EJAY International, Glendora, CA).

during the night using a 'metabolism tray' attached to the bottom of the cage; to the extent possible, collected from the same animals (non-fasted) that were used the prior week for blood collection. Automated hematology parameters were measured with a TECHNICON H 1E (Miles Diagnostics Division, Tarrytown, NY) and included platelet count, leucocyte count, erythrocyte count, hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, leukocyte differential counts (atypical lymphocytes, band neutrophils, basophils, blasts, eosinophils, lymphocytes, metamyelocytes, monocytes, myelocytes, nucleated RBC, plasma cells, promyelocytes, and segmented neutrophils) and erythrocyte morphology (anisocytosis, basophilic stippling, hypersegmented neutrophils, hypochromasia, macrocytosis, microcytosis, poikilocytosis, polychromasia, spherocytosis, target cells, and toxic granulation). Manually measured parameters included reticulocyte and heinz body counts. Clinical chemistry parameters were assayed with a DACOS Analyzer (Courter Electronics, Inc., Hialeah, FL) and included sodium, potassium, chloride, urea nitrogen, glucose-fasting, creatinine kinetic, uric acid, triglyceride, cholesterol, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, total bilirubin, direct bilirubin, total protein, albumin, phosphorus, calcium, and globulin. Automated urinalysis parameters, including pH, urine protein, urine glucose, ketones, urine bilirubin, urine blood, urobilinogen, and nitrite were determined with an AMES CLINITEK 100 (Miles Diagnostic Division, Elkhart, IN) urine chemistry analyzer. Other parameters including urine appearance, urine clarity, urine color, specific gravity, and urine microscopic observation of solids were determined manually.

2.5. Postmortem examination

All animals were sacrificed by CO_2 asphyxiation or by exsanguination while under CO_2 anesthesia and subject to a postmortem examination. Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen, and testicles were weighed. The following standard tissue specimens were preserved in 10% buffered formalin: skin (protocol),

mammary gland, bone (femur, rib/cc jet, sternum), joint (fem/tib), muscle (protocol), skull, lungs, larynx, trachea, heart, aorta, spleen, bone marrow, lymph node (cervical), lymph node (mesenteric), thymus, liver, cecum, colon, esophagus, pancreas, rectum, salivary gland, small intestine (duodenum, ileum, jejunum), stomach, kidneys, ovaries, testicles, cervix, clitoral gland, epididymis, preputial gland, prostate, seminal vesicles, urinary bladder, uterus, vagina, adrenal glands, parathyroid, pituitary, thyroid, brain (cerebellum, cerebrum-midbrain, medulla/pons), optic nerve, sciatic nerve, spinal cord (cervical, lumbar, thoracic), exorbital lacrimal gland, eyes, harderian glands, and gross lesions.

2.6. Histopathology

Tissues to be examined were trimmed, embedded in paraffin, sectioned, mounted, stained with hematoxylin and eosin (H&E), and examined under a light microscope by a veterinary pathologist. Histopathological, ophthalmological lesions, and/or gross observations were assigned a semi-quantitative ranking of the severity grade/stage: normal; minimal, 1; mild or slight, 2; moderate, 3; marked, 4; or severe, 5 (Squire, 1989; Hardisty and Eustis, 1990). The 'grade number' was used to calculate an average grade for a particular observation within a dose group. Neoplastic lesions were generally not assigned a grade. A pathologist not associated with the Bayer Corporation reviewed the initial micropathologic evaluation of this study. Slides from a random selection of 10% of the animals/sex/dose group, as well as all tumors and potential target organs, were examined in the review.

2.7. Statistics

Continuous data that were examined statistically were evaluated initially for equality or homogeneity of variance using Bartlett's test (Snedecor and Cochran, 1967). Group means were further analyzed by a one-way variance analysis (ANOVA) (Snedecor and Cochran, 1967) followed by Dunnett's test (Dunnett, 1955, 1964). In the event of unequal variances, data were subject to nonparametric procedures consisting of a Kruskal–Wallis ANOVA (Hollander and Wolfe, 1973) 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 χ^2 and/or Fisher exact tests. On a case by case basis, 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, a probability (P) value < 0.05 was considered statistically significant. Statistical analyses were performed using software obtained from either INSTEM Computer Systems or SAS Institute Inc. (Cary, NC).

3. Results

3.1. Antemortem evaluations

3.1.1. Body weight and food consumption

Body weight gain (BWG) and food consumption remained unaffected in both sexes at all doses tested (Fig. 3).

3.1.2. Clinical observations and mortality

With the exception of the dosing surface, clinical and/or cageside observations attributable to exposure to KBR 3023 were not observed (Tables 1 and 2). Survival was unaffected by administration of KBR 3023 (Fig. 4).

3.1.3. Ophthalmology

No evidence of a KBR 3023-induced ophthalmic toxicity was observed in either sex at any dose tested (Table 3).

3.1.4. Clinical laboratory tests

Hematology, serum chemistry, and urinalysis data provided no suggestion of a compound-related effect in either sex at any dose tested.

3.2. Postmortem evaluations

3.2.1. Gross pathology/organ weights

Evaluation of the gross lesion incidence and

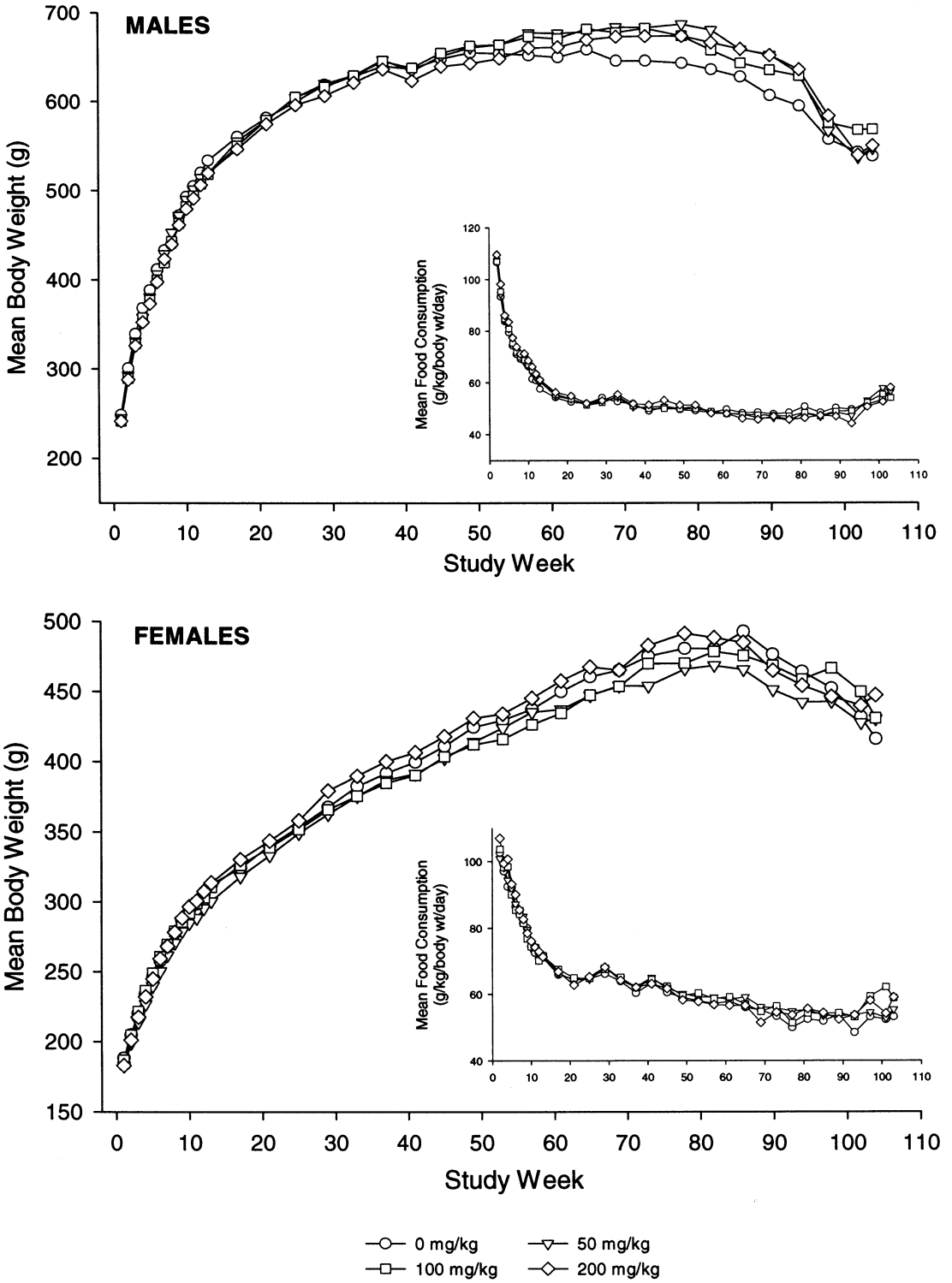

Fig. 3. Mean body weights by study week. Insets: Mean food consumption/kg body weight by study week.

Table 1
Summary of selected clinical observational findings[a]

		Chronic toxicity animals								Oncogenicity animals							
Sex		Males				Females				Males				Females			
Dose Level (mg/kg)		0	50	100	200	0	50	100	200	0	50	100	200	0	50	100	200
Animals examined		20	10	10	20	20	10	10	20	47	46	49	47	49	48	48	47
Hair	Alopecia	20	10	10	20	20	10	10	20	47	46	49	47	49	48	48	47
	Rough coat	8	5	3	7	1	0	1	1	4	10	3	6	4	3	6	10
Lesion	Ulceration									5	6	11	4	0	0	0	1
	Bleeding	0	1	0	1	0	1	0	0	0	5	2	2	0	2	1	0
	Sore	11	6	6	7	2	2	0	1	5	11	7	5	2	4	7	6
	Scab	19	10	10	20	17	8	8	16	46	46	49	47	41	41	38	36
	Abscess	3	3	0	1	1	1	0	1	9	6	10	5	11	7	6	7
	Redness	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	1
	Rash	10	5	5	12	9	2	3	5	26	22	22	20	36	35	29	24
	Raised zone	4	3	0	7	2	1	2	2	12	12	13	11	11	6	6	3
Dermal treatment	Edema	18	9	8	16	5	1	1	3	40	43	47	44	34	27	28	30

[a] Reflects data collected during the post-replacement period (after day 28) through respective terminal sacrifices.

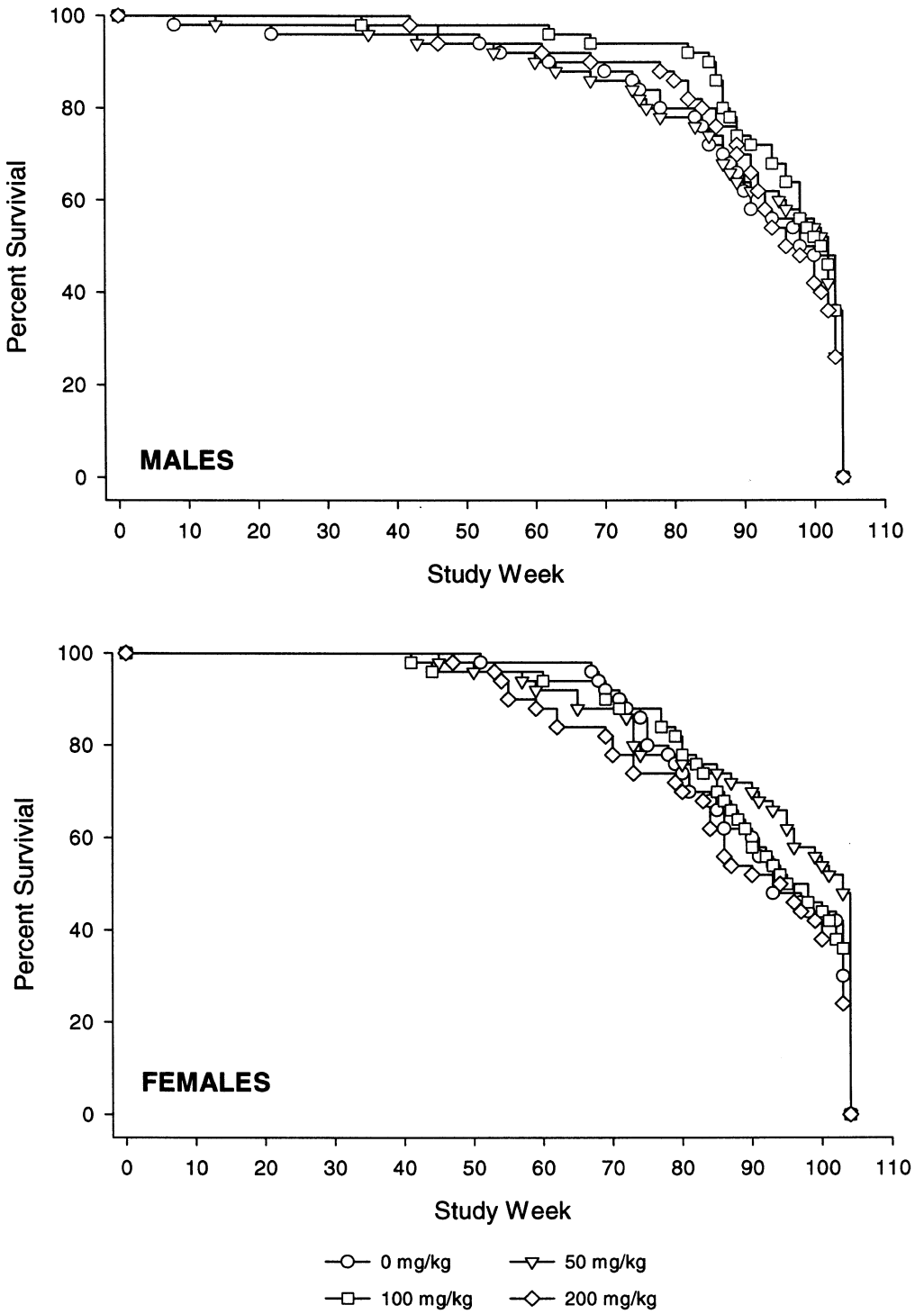

Fig. 4. Mean survival by study week.

Table 2
Location and number of clinically palpable masses

Dose (mg/kg)	Males				Females			
	0	50	100	200	0	50	100	200
Location								
Abdomen	1	1	5	3	18	17	20	12
Ano-genital	0	1	0	0	5	2	6	5
Dorsal head	0	1	0	1	1	0	1	1
Dorsal left neck	0	0	1	0				
Dorsal right forelimb	0	0	0	1				
Groin	0	1	1	0	22	16	15	13
Hindlimb					1	0	0	0
Left ear	0	0	0	I				
Left anterior dorsal					0	0	0	2
Left mid-dorsal					3	1	4	0
Left posterior dorsal	2	0	1	0	0	0	0	1
Left ventral neck	0	0	0	I				
Neck					2	0	0	0
Right anterior dorsal					2	0	0	0
Right mid-dorsal	1	2	0	0	3	0	7	0
Right posterior dorsal	3	0	2	0	0	0	1	1
Thorax	2	0	1	0	11	8	7	6
Ventral head					1	0	0	1

organ weight data provided no suggestion of a KBR 3023-induced change in either sex at any dose tested.

3.2.2. Micropathology

No evidence of chemically induced neoplasia was observed in this study (Table 4). Non-neoplastic lesions that were associated with exposure to KBR 3023 occurred in the treated skin and liver (Tables 5 and 6). As anticipated from the subchronic findings, an increased incidence of areas of acanthosis of the chemically treated skin was observed in 1-year 50, 100, and 200 mg/kg body wt/day males. The same lesion was not observed in 1-year females at any dose tested. Similarly, an increased incidence of areas of acanthosis and/or hyperkeratosis of the treated skin was observed in 2-year 50 and 100 mg/kg body weight/day females and in 2-year 200 mg/kg body wt/day males and females.

An increased incidence of areas of cystic degeneration of the liver (spongiosis hepatis) was noted in 2-year 200 mg/kg body wt/day males. These areas consisted of a multilocular cystic lesion containing a finely granular or flocculent eosinophilic material. Cystic degeneration of the liver occurs spontaneously in aging rats at a low incidence (Boorman et al., 1990); however, the biological significance of this age-related lesion is unknown. Moreover, alterations in liver function as measured by changes in weight and/or blood chemistries were not suggested from the study data.

3.2.3. Chronic toxicity profile and oncogenic assessment

Based on the lack of both adverse and nonskin compound-related effects through $1+$ years of continuous and repeated dermal exposure, a systemic chronic toxicity NOEL of 200 mg KBR 3023/kg body wt/day was established for the rat. Following ≈ 2 years of continuous and repeated dermal exposure to KBR 3023, the rat (like the mouse) showed no evidence of a chemically induced neoplastic response in any tissue examined.

Table 3
Summary of ophthalmoscopic findings

Sex	Chronic toxicity animals								Oncogenicity animals							
	Males				Females				Males				Females			
Dose Level (mg/kg)	0	50	100	200	0	50	100	200	0	50	100	200	0	50	100	200
Animals examined	20	10	10	18	20	10	9	20	24	23	23	19	22	26	19	19
Normal	18	9	9	15	19	9	9	19	5	3	5	5	5	7	5	9
Opacity, corneal	I	I	1	3	1	1	0	1	16	20	18	14	17	19	9	9
Degeneration, retinal	1	0	0	0					7	2	0	0	0	1	1	3
Exophthalmic													1	0	0	0
Microphthalmic									0	0	0	1	0	0	3	0
Synechia, anterior									0	0	0	1	0	0	1	0
Scar, corneal													2	0	0	0
Ulceration, corneal									3	1	0	1	1	1	2	1
Coloboma, iris									1	0	0	0				
Myosis									0	2	0	0	0	0	1	0
Vascularization, corneal									2	3	2	3	2	1	2	1
Descemetocele									1	1	0	1	1	1	0	1
Prolapse, iris													0	0	1	0
Phthisis bulbi									0	0	1	0	0	0	2	0
Cataract									3	2	4	2	1	2	6	2
Iritis									0	0	2	0	0	1	2	0

Table 4
Summary of selected neoplastic findings in oncogenicity animals[a]

	Total incidence							
	Males				Females			
Dose level (mg/kg)	0	50	100	200	0	50	100	200
Organ/lesion	[47][b]	[46]	[49]	[47]	[49]	[48]	[48]	[47]
Adrenal								
Pheochromocytoma	10	3	9	12	1	1	–	–
Pheochromocytoma, malignant	1	2	2	–	–	1	–	–
Adenoma, cortical	–	–	1	–	–	1	–	–
Brain								
Astrocytoma	3	–	2	–	1	1	–	–
Neoplasm, metastatic	1	–	–	–	–	–	1	–
Heart								
Sarcoma, histocytic	–	–	2	–	–	–	1	–
Kidney								
Adenoma	2	–	2	3	–	–	–	1
Carcinoma	–	1	–	–	1	–	–	–
Sarcoma, histocytic	2	–	1	1	–	–	1	1
Liver								
Carcinoma	1	2		1	2	–	–	–
Sarcoma, histocytic	–	–	2	1	–	–	3	1
Lungs								
Neoplasm, metastatic	2	1	–	–	2	–	1	–
Sarcoma, histocytic	1	–	2	–	–	–	2	1
Spleen								
Sarcoma, histocytic	–	–	–	1	–	–	3	1
Testes								
Interstitial Cell Tumor	2	4	2	2				
Ovaries					–	–	–	–

[a] Data are summarized for all animals (found dead, unscheduled sacrifice, and 1- and 2-year termination). Singular isolated findings (total incidence of 1; male and female combined) or findings noted in controls only are not included.
[b] [Number of tissues examined]; Female 50-ppm adrenal gland, $N = 47$.

4. Discussion

As anticipated from the subchronic findings, the toxicological response of the rat, following 2 years of continuous and repeated dermal exposure to KBR 3023, could be characterized as involving structural alterations primarily in the skin/dosing surface and to a far lesser degree the liver (males only). Histopathology at the skin/dosing surface was characterized by a pattern of acanthosis and/or hyperkeratosis at all doses and exposure periods. In males only, cystic degeneration of the liver was noted at 200 mg KBR 3023/kg body wt/day following 2 years. No other compound-related nondosing site lesion was identified at any dose tested. All other in-life and postmortem parameters were unchanged. Finally, no evidence of a compound-induced neoplasia was suggested in this bioassay.

Beyond the conclusions drawn from this inves-

Table 5
Summary of selected non-neoplastic findings in chronic toxicity animals[a]

	Total incidence (weighted grade)							
	Males				Females			
Dose Level (mg/kg)	0	50	100	200	0	50	100	200
Organ/lesion								
Liver	[20][b]	[I0]	[10]	[18]	[19]	[10]	[9]	[20]
Hyperplasia/fibrosis, biliary	1 (2.0)	–	–	1 (2.0)	–	–	–	–
Hyperplasia, Iymphoid	–	–	1 (2.0)	1 (1.0)	–	–	–	–
Necrosis	1 (2.0)	1 (2.0)	–	2 (2.0)	1 (2.0)	–	–	–
Parasite, cestode	–	–	–	–	–		1 (3.0)	1 (4.0)
Vacuolization	3 (2.0)	1(2.0)	1 (1.0)	3 (2.0)	1 (3.0)	–	–	2 (2.0)
Skin, treated	[20]	[10]	[10]	[18]	[19]	[10]	[9]	[20]
Acanthosis	4 (1.0)	9* (1.7)	6* (1.3)	13* (1.7)	1 (1.0)	1 (2.0)	–	4 (2.0)
Hyperkeratosis	17 (1.5)	8 (1.8)	8 (1.6)	12 (1.4)	–	–	2 (1.5)	1 (2.0)
Debris	–	4* (2.0)	–	–	1 (2.0)	–	1 (1.0)	3 (3.0)

[a] Data are summarized for all animals (found dead, unscheduled sacrifice, and 1- and 2-year termination). With the exception of singular isolated findings (total incidence of 1; male and female combined) and findings noted in controls only, all lesions are included for each tissue in which the compound-related finding was noted.

[b] [Number of tissues examined].

* Significantly different, $P \leq 0.05$.

tigation, several treatment-related effects were noted that appeared to be directly related to methodology and the inherent difficulties of conducting a lifetime bioassay via the dermal route.

4.1. Collar-related lesions

During the study, nonhealing open wounds developed around the necks of approximately 20% of the male animals of each dose group in the region adjacent to the rubber-like inner edge of the collar. The lesions were non-compound related (comparable incidence in all groups) and were essentially male-specific. A strategy to address this problem was developed. By removing the collars from five affected control animals, it was determined that a minimum of 2 weeks would be required for complete healing; to the point that wounds would not reopen shortly after replacement of the collars. It was also determined that a light daily coating of corn oil placed on the rubber portion of the collar was quite effective in preventing the full expression of the lesion, if initiated immediately following signs of onset. Following communication with the EPA, collars were removed and dosing was stopped for approximately 3 weeks (to maximize the healing process) for all animals with collar-related lesions. Collars were then replaced and exposure was resumed. In addition, corn oil was applied to the collars of all animals with the collar-related lesions for the remainder of the study. If the wound healed, application of corn oil ceased; if the lesion appeared to be returning, application of corn oil resumed. Following the steps described above, approximately half of the affected animals improved significantly and did not develop a reoccurrence of the neck lesions. However, the problem reoccurred in a little over half of the animals from which the collars were originally removed. It was anticipated that these lesions would never improve without removing the collars a second time. An alternate strategy was implemented. Those animals within each dose group originally scheduled to be sacrificed following 2 years (oncogenicity group) that continued to be affected (M: 8,9,5,10; F: 0,2,1,1)[1] were sac-

[1] Denotes incidence (50 animals/dose/sex) of the observation/lesion: control through high dose; an asterisk indicates statistical significance; $P < 0.05$.

rificed at 1 year in lieu of the corresponding animals originally scheduled to be sacrificed at that time (chronic toxicity group or satellite animals). The satellite animals, which were not sacrificed then continued on study, becoming part of the corresponding dose group of the animals scheduled to be sacrificed following 2 years. This allowed for the greatest number of animals without the collar-related lesions to start the second year of the study.

4.2. Foot lesions

In addition to the neck lesions, ≈40% of the male animals of each dose group developed lesions of the hind feet varying in severity from red swollen ulcerated areas to large (1 cm in diameter) elevated, ulcerated, bleeding areas of granulation tissue. Like the neck lesions, the lesions of the hind feet were non-compound related (comparable incidence in all groups) and were essentially

Table 6
Summary of selected non-neoplastic findings in oncogenicity animals[a]

	Total incidence (weighted grade)							
	Males				Females			
Dose Level (mg/kg)	0	50	100	200	0	50	100	200
Organ/Lesion								
Liver	[47][b]	[46]	[49]	[47]	[49]	[48]	[48]	[47]
Angiectasis	3 (2.3)	–	2 (3.0)	3 (2.7)	–	–	–	–
Hyperplasia/fibrosis, Biliary	16 (2.0)	7 (2.0)	17 (1.9)	12 (1.9)	12 (2.1)	6 (2.2)	7 (2.1)	2 (2.0)
Congestion	5 (2.2)	1 (2.0)	–	3 (2.0)	1 (2.0)	–	2 (2.0)	1(2.0)
Cyst	–	–	1 (2.0)	1 (2.0)	1 (4.0)	–	–	–
Degeneration,cystic	8 (2.3)	11 (2.0)	14 (2.1)	20* (2.3)	1 (2.0)	1 (3.0)	1 (2.0)	2 (2.0)
Fatty Change	1 (3.0)	–	1 (3.0)	–	–	–	–	–
Fibrosis	–	–	–	–	–	2 (2.0)	–	2 (2.5)
Focus/area of cellular alteration	3 (2.3)	1 (3.0)	3 (3.0)	–	1 (2.0)	–	2 (3.0)	–
Hematopoiesis, extramedullary	–	1 (1.0)	–	–	3 (1.7)	3 (1.0)	1 (2.0)	4 (1.8)
Hepatocytomegaly	–	–	1 (3.0)	1 (3.0)	–	–	–	–
Hyperplasia	–	–	–	–	–	∼	1 (2.0)	1 (3.0)
Inflammation, acute	–	–	3 (2.3)	1 (2.0)	–	–	–	–
Inflammation, chronic active	–	–	2 (2.5)	–	–	–	–	–
Necrosis	5 (2.6)	3 (2.0)	7 (2.9)	6 (2.5)	7 (2.0)	3 (2.3)	4 (2.8)	4 (2.8)
Pigmentation	–	–	1 (2.0)	1 (2.0)	–	1 (2.0)	–	1 (2.0)
Thrombosis	–	–	3 (2.0)	–	–	–	–	–
Vacuolization	4 (2.0)	5 (2.4)	5 (2.0)	1 (2.0)	17 (2.0)	11 (2.0)	4 (2.0)	14 (2.3)
Focus/area of cellular alteration, basophilic,tigroid	2 (2.0)	–	–	–	–	–	–	1 (2.0)
Focus/area of cellular alteration, vacuolated	1 (2.0)	4 (2.3)	2 (2.5)	4 (3.3)	1 (3.0)	2 (3.0)	–	1 (4.0)
Skin, treated	[47]	[46]	[49]	[47]	[49]	[48]	[47]	[46]
Acanthosis	–	5* (1.6)	4 (1.5)	16* (1.7)	1 (2.0)	4 (2.0)	6* (2.0)	12* (1.7)
Hyperkeratosis	22 (1.6)	24 (1.9)	32 (1.7)	37* (1.7)	3 (2.0)	10* (1.5)	10* (1.9)	22* (1.5)
Debris	–	1 (2.0)	1 (1.0)	3 (2.0)	–	–	1 (1.0)	1 (1.0)

[a] Data are summarized for all animals (found dead, unscheduled sacrifice, and 1- and 2-year termination). With the exception of singular isolated findings (total incidence of 1; male and female combined) and findings noted in controls only, all lesions are included for each tissue in which the compound-related finding was noted.

[b] [Number of tissues examined].

* Significantly different, $P \leq 0.05$.

male-specific. A strategy to address this problem was developed. The lesion appeared to be enhanced by the animal's weight on the wire-bottom cages. As such, all affected animals were moved to shoebox type polycarbonate cages and observed for approximately 2 weeks to see if a change in surface (wire-bottom to flat and supporting) would ameliorate the problem. This failed as the presence of bedding served to continue to irritate the wounds and in turn slow the healing process. Following examination by the staff veterinarian, Panalog ointment (SOLVAY Animal Health, Inc., Mendota Heights, MN) was applied daily during the regular workweek for up to 2 weeks resulting in significant improvement of the wounds. For the remainder of the study, all animals observed with the foot lesions were immediately transferred to shoebox type polycarbonate cages (all animals were eventually moved to shoebox cages as a preventative measure) and treated daily with Panalog ointment until the lesions had healed; if lesions healed, treatment was discontinued. As with the neck lesions, some of the hind foot lesions would not heal and were particularly severe. Several animals within each dose group of the 2-year sacrifice group (oncogenicity group) that continued to be affected with the more severe lesions, were sacrificed at 1 year as described above.

While a definitive cause of either lesion remains unclear, the analysis of both the character of the lesions and time of onset, supports the conclusion that both lesions were the result of and/or exacerbated by the species of rat and the type of caging employed for this study. The Sprague–Dawley is a very large and heavy strain of rat; attributes that undoubtedly played a role in both the collar and hind foot lesions. Larger collars were designed during the course of the study; however, it was very difficult to collar the large animals tight enough to ensure the animal would not remove the collar, but not so tight that the collar would irritate the animal's neck. As the animals grew, even the larger collars tended to fit around the neck more in the shape of a saucer than a cone (desired fit). In other words, on the smaller animals (i.e. females), the inside rubber edge of the collar tended to lay more parallel to the neck while on larger animals the inside edge tended to be more perpendicular and thus would dig into the neck even when secured properly (i.e. tight enough to ensure the animal would not remove the collar). The absolute size of the animals appeared also to play a role in the increased incidence of the hind foot lesions. The weight of the animals, while caged in wire-mesh cages, clearly had an effect on the formation and severity of the hind foot lesions as the animals would spend a good portion of time on their hind feet (e.g. when feeding, grooming, etc.). Most lesions first appeared as small red indentations on the sole of the foot, in the shape of the wire mesh. Over a period of weeks, the lesion would grow and eventually rupture. Had the animals not been placed on wire mesh, the resulting irritation and subsequent lesion may have been avoided.

In summary, it would appear that for dermal studies of this type, a smaller strain of rat, housed in polycarbonate, shoebox type caging may be the best choice.

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