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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Dermal Toxicity Study in Rats
OPPTS 870.3250 [§ 82-3]

DP BARCODE: D268402 SUBMISSION CODE: S584121
P.C. CODE: 0098901 EPA ID No.: 070465-00001

TEST MATERIAL: 1,2-Benzisothiazolin-3-one (87.1% a.i.)

SYNONYMS: XBINX 88®

CITATION: Cerven, R.(1998). 90 Day Repeated Dose Dermal Toxicity Study in Rats. MB Research Laboratories, Spinnerstown, PA., Study Project No. MB 99-7974.02. MRID 451846-01. Unpublished.

SPONSOR: Cincinnati Specialties, LLC, Cincinnati, OH 45217

EXECUTIVE SUMMARY:

In a subchronic dermal toxicity study [MRID 451846-01] the test article, 1,2-Benzisothiazolin-3-one (87.1% a.i.) [XBINX 88®], Lot No. 579 was applied dermally [10% of the body surface area] to the intact, shaved skin of 10 Wistar albino rats/sex/ group at doses [as received] of 0 (Sham Control), 100, 300 and 1000 mg/kg [limit dose], five days per week for a total of 90 days. The applied test article, moistened with distilled water was covered with a gauze patch. The patch and the truck was then wrapped with non-irritating tape. The patch and tape were allowed to remain in place for ≈6 hours after which it was removed and the site wiped with gauze moistened with distilled water.

The treated sites were scored for dermal irritation pretest and then once/week. All animals were observed once daily for signs of toxicity and twice daily for mortality. Detailed clinical

observations were made pretest and once per week during the study. Body weight were recorded pretest, weekly and at termination while food consumption was recorded weekly. Eyes were examined pretest and at termination. Hematology and clinical chemistry were evaluated only at study termination. A functional observational battery [FOB] was performed at termination only. On day 91, animals were anesthetized, exsanguinated and examined for gross and microscopic pathology. Organs were weighed and organ/body weight ratios calculated.

No changes occurred in mortality, body weight, body weight gain, food consumption, minimal functional observational battery parameters, ophthalmology or organ weights.

The irritant nature of the test compound produced local skin lesions in all test crops vs. the controls and were characterized by flaking and eschar macroscopically, and microscopically by hyperplasia and hyperkeratosis of the epidermis, sebaceous gland hyperplasia, focal superficial dermal inflammation, epidermal necrosis and dermal fibrosis.

Microscopic examination of the stomach of all treated groups vs. the control revealed hyperplasia and hyperkeratosis of the nonglandular mucosa, focal mucosal erosions, ulcers and or submucosal edema and inflammation. These changes are considered to have been produced by the local irritant nature of the test compound [as result of ingestion during grooming] and not the result of systemic toxicity.

No systemic LOAEL in males or females was established at the highest [limit dose] dose tested [1000 mg/kg/day]. No systemic NOAEL in either sex was established [>1000 mg/kg/day].

The non-systemic LOAEL in both sexes, based on stomach pathology, indicative of irritation, from the test compound was <100 mg/kg/day.

This study is classified as **Acceptable** (guideline) and satisfies the requirement for FIFRA Test Guideline § 82-3 for a Subchronic Dermal Toxicity Study in the rat.

COMPLIANCE: Signed and dated GLP and No Data Confidentiality statements were provided.

I. MATERIALS

A. Materials

Test Material:	1,2-Benzisothiazolin-3-one
Description:	Tan powder
Lot#:	579
Purity:	87.1% a.i. from analytical report
Stability:	Not specified
Solvent used:	Distilled Water
Other comments:	Test substance was stored at room temperature and humidity.

Note: XBINX 88® was used to describe the 87.1% a.i. commercial compound [from analytical report, p. A1 of MRID 451846-01]. Doses were based on a presumed a.i. concentration of 100% which was administered for the first 14 days (10 doses). Thereafter, the presumed a.i. concentration of 98.7% [actual 87.1% a.i.] was then administered for the duration of the study.

1. Control materials

Vehicle: Test material was moistened with 0.01 [low dose], 0.02 [mid dose] or 0.05 ml (control, high dose) of distilled water when applied to the rat skin.

B. Test Animals

Species:	Albino rat
Strain:	Wistar
Source:	Ace Animals, Boyertown, PA
Groups:	Four (4) groups of 10 animals each (sham control, 100, 300 and 1000 mg/kg dose groups)
Feed:	Purina Certified Rodent Chow #5002 *ad libitum*
Water:	Freely available
Weight:	220-277 gm (male), 162-196 gm (female)
Age:	Young adult
Acclimatization:	8 to 9 days
Housing:	Animals housed singlely in suspended stainless steel, wire bottomed cages
Environmental:	Temperature: 67-70° F, humidity: 33-39% Photoperiod: 12 hour light/dark cycle

II. STUDY DESIGN AND METHODS

In life start date: 1 Jan 2000, Completion: 6 April 2000

A. Animal assignment

Animals were assigned to one of four groups based on body weight using a computer randomization program. Ten rats/sex/ dose were utilized (Table 1).

TABLE 1. Dosing groups for subchronic dermal toxicity study with 1,2-Benzisothiazolin-3-one*

Experimental Group	Dose (mg/kg)	Number of animals/sex	Distilled Water Vehicle (ml)
Sham Control	0	10	0.05
High dose	1000	10	0.05
Mid dose	300	10	0.02
Low dose	100	10	0.01

*Data adapted from p.8, Table 1, MRID 451846-01.

B. Dose application

An area of skin equivalent to 10% of the body surface was clipped free of hair from the shoulders down to the wing of the hipbone and halfway down the flanks of each animal.

The test article was applied evenly to the prepared site and moistened with sufficient distilled water (0.01 to 0.05 mL) to ensure contact with the skin. The treated site of each rat was covered with a 4-ply gauze patch (Alco #052123) and further covered with Zonas® non-irritating tape around the trunk to retain the gauze dressing and to ensure that the animal could not ingest the test article. The animals were dosed once per day, at approximately the same time, five days per week, during a period of 90 consecutive days. After a 6 hours exposure, bindings were removed and the exposed skin was wiped with gauze moistened with distilled water.

The sham control group was handled in the same manner as the test group, but only distilled water (0.05 mL) was used to moisten the test site.

C. Observations

All animals were observed once daily for toxicity and pharmacologicl effects and twice daily for mortality.

1. Dermal Observations

The test sites were scored for dermal irritation prior to study initiation and once per week according to the scoring scale listed in Appendix 1. Only moderate ans/or severe irritation scores were reported.

D. Body weight

Animals were weighed at study initiation and once per week throughout the study and at termination.

E. Food consumption

Individual food consumption was calculated once per week throughout the study.

F. Ophthalmoscopic examination

Ophthalmological examinations were performed by a board-certified veterinary ophthalmologist prior to the study and within one week of termination.

G. Clinical Pathology

Blood was collected from all animals on day 91 after overnight fasting from the *dorsal aorta* for hematology and clinical analysis. The CHECKED (X) parameters were examined.

1. 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*		Reticulocyte count
	Blood clotting measurements*		
	Thromboplastin time		
	Clotting time		
x	Prothrombin time		

*Required for subchronic studies based on Subdivision F Guidelines.

2. Clinical chemistry

	ELECTROLYTES		OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
x	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose* [fasting]
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (SGPT)*		
x	Serum aspartate amino-transferase (SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
x	Sorbitol dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

3. Urinalysis: Not performed.

H. Functional Observational Battery

A functional Observational Battery (FOB) was performed and consisted of assessment of motor activity, grip strength and sensory reactivity to stimuli (visual, auditory and proprioceptive) during the last 2 weeks of the study. [Note: No motor activity portion or specialized staining of the nerves was included].

I. Sacrifice and pathology

Animals were sacrificed on study day 91 using ether and exsanguination.

1. Gross pathology

All animals were subjected to gross pathological examination which included examination of the external surfaces of the body, all orifices, the external and cut surfaces of the viscera, the cervical tissues and all organs and their contents.

2. Microscopic pathology

Organs and tissues were preserved in 10% neutral buffered formalin. All preserved tissues listed in the following Table

from all control and high dose animals, and in the low and mid dose levels the treated skin, untreated skin, kidneys, liver, mesenteric lymph node, spleen stomach, thymus, and other tissues with gross lesions were examined microscopically. The CHECKED (x) tissues were collected for histological examination. The (xx) organs, in addition, were trimmed and weighed [wet].

(a) Histopathology

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)^{T}
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.)
x	Jejunum*	x	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland^{T}
x	Rectum*	x	Urinary bladder*	x	Mammary gland^{T}
x	Liver*+	xx	Testes*+	x	Parathyroids*$^{++}$
	Gall bladder*	x	Epididymides	x	Thyroids*$^{++}$
x	Pancreas*	x	Prostate		
		x	Seminal vesicle		OTHER
	RESPIRATORY	x	Ovaries	x	Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
x	Lung*			x	Skin (treated & untreated)
	Nose			x	All gross lesions and masses*
	Pharynx				
	Larynx				

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

J. Statistics

Clinical chemistry, hematology, organ weight, body weights, body weight gains, organ body weight ratios and food consumption were tabulated and the means and standard deviations were calculated. An Analysis of Variance (ANOVA) was performed to identify statically different groups. Parametric data was analyzed using the Kruskai-Wallis analysis of variance with Dunn's post hoc test.

Interpretation of the results was made based on the presence or absence of pathology findings, abnormal physical signs and statistical evaluation of the various parameters checked.

II. RESULTS

A. OBSERVATIONS

No deaths occurred during the study. In addition, no treatment-related clinical signs were observed.

B. DERMAL OBSERVATIONS

The treated groups showed a similar incidence of reactions at the mid and high dose levels with a lower incidence at the low dose level during the first four weeks. Macroscopically, erythema/ edema, flaking skin and small areas of eschar were noted at all dose levels and times. Areas of necrosis were noted at week 4 in males and week 8 in females (Tables 2, 3).

Table 2. Dermal Observations in Ten Male Rats^

Dose [mg/kg]	Weeks on Test							
	1		4		8		13	
	R	E	R	E	R	E	R	E
0	0	0	0	0	0	0	0	0
1000	8	1	10 [2]	2	10 [8]	8	10 [7]	9
300	9	0	9	0	10 [2]	9	10 [2,1]	6
100	5	0	5	0	10 [1]	2	10	4

^ Adapted from Individual Dermal Observations, p. 94-101, MRID 451846-01.
R = # of rats showing erythema/small area of eschar/flaking skin/10 animals.
E = # of rats showing edema/10 animals.
[] = # of rats showing moderate eschar, necrosis.

Table 3. Dermal Observations in Ten Female Rats^								
Dose [mg/kg]	Weeks on Test							
	1		4		8		13	
	R	E	R	E	R	E	R	E
0	0	0	0	0	0	0	0	0
1000	9	1	9	1	10 [1,2]	7	10 [3,2]	6
300	7	0	6	0	9 [4]	4	9 [3]	4
100	3	0	3	0	10 [4]	5	9 [2]	3

^ Adapted from Individual Dermal Observations, p. 102-109, MRID 451846-01.
R = # of rats showing erythema/small area of eschar/flaking skin/10 animals.
E = # of rats showing edema/10 animals.
[] = # of rats showing moderate or severe eschar, # of rats with necrosis.

C. BODY WEIGHT AND WEIGHT GAIN

There were no statistically significant differences in body weight or body weight gain for any treatment group vs. the respective Control.

D. FOOD CONSUMPTION

No statistically significant differences in food consumption were observed in any of the male treatment groups.

E. OPHTHALMOSCOPIC EXAMINATION

No ocular lesions associated with the test substance were observed.

F. FUNCTIONAL OBSERVATIONAL BATTERY

Treated groups were comparable to the control.

G. BLOOD and CLINICAL CHEMISTRY ANALYSES

1. Hematology

The mean white blood cell count (7.4) of females at 1000 mg/kg was significantly [$p \leq 0.05$] greater than the control (5.8). Similar, though not significant, increases were noted in the 1000 and 300 mg/kg males and the 300 mg/kg females. Other hematology parameters were not remarkable in both sexes (Table 4).

Table 4. White Blood Cell Counts ($10^3/\mu l$) in Rats Treated with the Test Substance		
Dose (mg/kg)	Sex	
	Male	Female
0	7.8	5.8
1000	9.4	7.4*
300	9.6	7.2
100	7.4	5.7

^Adapted from Table 7, p. 137. MRID 451846-01.
*$p \leq 0.05$.

2. Clinical Chemistry

Males showed no statistically significant differences vs. the Controls.

The mean triglycerides value (30) in females at 300 mg/kg were significantly [$p \leq 0.01$] greater than the mean value (24) of the Control group. No dose response was seen (Table 5).

The mean albumin value (3.9) of females in the 1000 mg/kg group was significantly [$p \leq 0.01$] less than the mean albumin value (4.2) of the Control (Table 5). Although the value, in males, at 1000 mg/kg was not statistically significant, an increase was seen vs. the Control (Table 5).

The mean total protein value (5.7) of females at 1000 mg/kg was significantly [$p \leq 0.05$] less than the mean value (6.1) of the Control. A dose response was seen (Table 5).

Table 5. Clinical Chemistry Parameters in Females^			
Dose [mg/kg]	Triglycerides [mg/dl]	Albumin [gm/gl]	Total Protein [gm/dl]
0	24	4.2	6.1
1000	28	3.9**	5.7*
300	30**	4.2	5.9
100	25	4.3	6.0

^Adapted from Table 8, p. 140. MRID 451846-01.
*$p \leq 0.05$, **$p \leq 0.01$.

H. NECROPSY

1. Organ Weights and Organ/Body Weight Ratios

Organ weights between groups were not remarkable. In the males, a positive dose response was noted in the liver/body weight ratio with statistical significance [$p \leq 0.05$] being seen at 1000 mg/kg (2.76) vs. the mean control value of 2.50.

2. Macroscopic Pathology/Microscopic Pathology

(a) Skin Pathology

Macroscopic skin lesions, confirmed, microscopically were characterized microscopically by **hyperplasia and hyperkeratosis** of the epidermis [males: 0 (8)*, 1000 (9), 300 (10), 100 (10) mg/gm, females: 0 (2), 1000 (10), 300 (9), 100 (10), mg/gm], **sebaceous gland hyperplasia** [males: 0 (5), 1000 (9), 300 (10), 100 (10) mg/gm, females: 0 (0), 1000 (9), 300 (10), 100 (8) mg/gm], and **focal superficial dermal inflammation** [males: 0 (0), 1000 (8), 300 (10), 100 (4) mg/gm, females: 0 (0), 1000 (9), 300 (5), 100 (6) mg/gm]. Lower incidences of **epidermal necrosis** [males: 0 (0), 1000 (6), 300 (4), 100 (1) mg/gm, females: 0 (0), 1000 (5), 300 (4), 100 (4) mg/gm] and **dermal fibrosis** [males: 0 (0), 1000 (5), 300 (3), 100 (0) mg/gm, females: 0 (0), 1000 (2), 300 (3), 100 (3) mg/gm] were also seen in some compound exposed rats (Table 6).

In the control animals, repeated shaving of the skin for the sham application procedure produced minimal or mild hyperplasia and hyperkeratosis of the epidermis and minimal sebaceous gland hyperplasia. Pathology of the skin from the untreated sites also showed minimal or mild hyperplasia/hyperkeratosis of the epidermis and sebaceous gland hyperplasia. Minimal dermal inflammation was also seen at the high dose level vs. the control(Table 6a).

The lesions above were always more severe in the treated animals compared to either the control or untreated control animals.

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*() # of animals showing /lesions/10 animals total.

Table 6. Incidence of Skin Histopathology in Treated Animals^

	Male				Female			
mg/kg	0	1000	300	100	0	1000	300	100
No. of Animals/ Group	10	10	10	10	10	10	10	10
Fibrosis, dermal	0	5	3	0	0	2	3	3
Hyperplasia, sebaceous glands	5	9	10	10	0	9	10	8
Hyperplasia/ hyperkeratosis, epidermis	8	9	10	10	2	10	9	10
Inflammation, dermal	0	8	10	4	0	9	5	6
Necrosis, epidermis	0	6	4	1	0	5	4	4

^Adapted from Table 1, Appendix D, p. D13; MRID 451846-01.

Table 6a. Incidence of Skin Histopathology in Untreated Animals^

	Male				Female			
mg/kg	0	1000	300	100	0	1000	300	100
No. of Animals/ Group	10	10	10	10	10	10	10	10
Hyperplasia, sebaceous glands	2	5	3	2	0	0	0	0
Hyperplasia/ hyperkeratosis, epidermis	2	8	6	5	0	2	2	2
Inflammation, dermal	0	0	0	0	0	1	0	0

^Adapted from Table 1, Appendix D, p. D9; MRID 451846-01.

(b) Stomach Pathology

The most common pathology was thickening of the nonglandular mucosa due to **hyperplasia and hyperkeratosis**, oftentimes very prominent at the limiting ridge. In males the following incidence rate was noted at dose levels of 1000 (9), 300 (10) and 100 (4) mg/kg vs. 0 in the control group. In females the following incidence rate was noted at dose levels of 1000 (7), 300 (9), and 100 (2) mg/kg vs. 0 in the Control group (Table 7).

Other changes occurred at varying rates in the test compound treated groups included **erosions of the glandular mucosa** in males at 1000 (3) and 300 (2) mg/kg and females at 1000 (3) mg/kg.

Other changes in males included **submucosal edema and inflammation** in the nonglandular areas at 1000 (6), 300 (1) mg/kg/day and in the female at 1000 (1) mg/kg/day. **Submucosal edema and inflammation** of the glandular area occurred in the males at 1000 (3), 300 (4) and 100 (1) mg/kg and in females at 1000 (3) mg/kg. **Ulcers** in the non-glandular mucosa occurred at 1000 (2) mg/kg in males. For both males and females, the Control and the other dose levels showed a zero incidence for the lesions listed above (Table 7).

Table 7. Incidence of Stomach Histopathology^

	Male				Female			
mg/kg	0	1000	300	100	0	1000	300	100
No. of Animals/ Group	10	10	10	10	10	10	10	10
Dilation, mucosal glands	2	0	0	0	3	0	1	4
Edema/inflammation	0	3	4	1	0	3	0	0
Edema, inflammation, submucosa, non-glandular area	1	6	1	0	0	1	0	0
Erosion, glandular mucosa	0	3	2	0	0	3	0	0
Hyperplasia/ hyperkeratosis, nonglandular mucosa	0	9	10	4	0	7	9	2
Ulcers, nonglandular mucosa	0	2	0	0	0	0	0	0

^Adapted from Table 1, Appendix D, p. D10; MRID 451846-01.

Other microscopic lesions observed at all dose levels were similar to that found in the Control and suggested that no systemic toxicity was produced by the dermal application of the test substance.

III. DISCUSSION AND CONCLUSIONS

The treated skin abnormalities (macroscopic and microscopic) were caused by the local irritation effects of the test compound. The authors also believe that the stomach lesions probably were caused by ingestion of the test compound during grooming even though the treated area was cleaned after exposure for the stomach lesions were indicative of a local response of a highly irritating substance.

The statistically significant ($p \leq 0.05$), albeit minimal increases

in the white blood cell count in the females dosed at 1000 mg/kg vs. the controls were secondary to the local effect [inflammation] of the test compound. The males also showed an increasing trend, but statistical significance was never attained. These minimal changes were not appropriate for estimation of the LOAEL.

In the female, the significant ($p \leq 0.05$), although minimal differences noted in albumin and total protein at 1000 mg/kg and triglycerides at 300 mg/kg vs. the control appeared due to the test compound. Since liver and kidney pathology was not remarkable, these changes were considered to be of little toxicological importance. These minimal changes were not appropriate for estimation of the LOAEL.

Males at 1000 mg/kg showed a significant ($p \leq 0.05$) increase in the mean liver/body weight ratio vs. the control males. Since the liver pathology was not remarkable, and no change was seen in the mean liver weight, this change was considered to be of little toxicological importance.

The FOB was not performed at the appropriate time periods (pre, 4, 8 and 13th week). The motor activity portion, and the specialized nervous system pathology was also not included. Albeit, this minimal FOB information can be used as supplementary data.

Appendix 1

Dermal Scoring Code

Erythema:

No erythema..... 0
Very slight erythema (barely perceptible).. 1
Well-defined erythema 2
Moderate to severe erythema 3
Severe erythema (beef redness) to slight eschar formation (injuries in depth) 4

Edema:

No edema 0
Very slight edema (barely perceptible) 1
Slight edema (edges of area well-defined by definite raising) 2
Moderate edema (raised app. 1 mm) 3
Severe edema (raised more than 1 mm and extending beyond the area of exposure) 4