UNDATED

DATA EVALUATION REPORT

PROXEL 098901

STUDY TYPE: SUBCHRONIC ORAL TOXICITY [CAPSULE] - DOG (82-1b)

Prepared for

Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2800 Crystal Drive
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-26

Primary Reviewer:
Eric B. Lewis, M.S. Signature: _____
Date: _____

Secondary Reviewers:
Claudia M. Troxel, Ph.D. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

EPA Reviewer: L. Morris, Ph.D. _____, Date _____
EPA Work Assignment Manager: P. Thompson, Ph.D._____, Date _____
Antimicrobials Division (7510W)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity (Capsule) - Dog
OPPTS 870.3150 [§82-1b]

DP BARCODE: N/A
P.C. CODE: N/A

SUBMISSION CODE: N/A
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): PROXEL (94.6% w/w)

SYNONYMS: 1,2 benzisothiazolin-3-one

CITATION: Allen, S.L. (1991)"Proxel" press paste: 90 day oral toxicity study in dogs. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Report number CTL/P/3399, 1991. MRID 42205701. Unpublished.

SPONSOR: ICI Specialities, Wilmington, DE 19897.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 42205701) Proxel press paste (94.6% w/w) was administered to 4 beagle dogs/sex/dose by capsule at dose levels of 0, 5, 20, or 50 mg/kg/day for 92 days.

No animals died during the study. The only treatment-related clinical observation was an increased incidence of emesis in both sexes of dogs, primarily at the higher dose levels. Treatment with Proxel resulted in no differences in body weight or total body weight gain compared to controls. Hematological alterations were sporadic and not considered to be dose-related. Clinical chemistry parameters that were altered significantly from controls ($p \leq 0.05$ or 0.01) at the high dose in both sexes for one or more weeks include plasma albumin (9-11% decrease), total protein (7-13% decrease), alanine transaminase (26-30% decrease), and triglycerides (30-49% increase). These results were accompanied by increased absolute liver weight in high-dose dogs of both sexes (17%) and mid-dose females (12%), and are consistent with an adverse effect on liver function. There were no treatment-related effects on gross or microscopic pathology, food consumption, or ophthamology. **The LOEL is 20 mg/kg/day for both male and female dogs, based on the incidence of emesis and clinical chemistry alterations at this dose. The NOEL is 5 mg/kg/day.**

This subchronic toxicity study is classified as **Acceptable (guideline)** and satisfies the guideline requirement for a subchronic oral study (82-1b) in dogs.

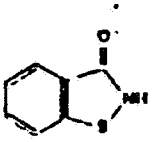
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Proxel

   Description: brown solid
   Lot/Batch #: Y00180/025
   Purity: 94.6% w/w
   Stability of compound: reported as stable at room temperature in a dark area
   CAS #: 2634-33-5
   Structure:



2. Vehicle and/or positive control: the test substance was suspended in corn oil and contained in gelatin capsules; negative controls received capsules containing only corn oil. There were no positive controls in this study.

3. Test animals

   Species: dog
   Strain: beagle
   Age and weight at study initiation: 23-25 weeks old; females, 8.1-13.1 kg; males, 9.2-13.3 kg.
   Source: ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, U.K.
   Housing: individually in 365 x 115 cm indoor pens with heated sleeping area.
   Diet: males were fed 400 g and females 350 g daily of an expanded dry diet (Laboratory Diet A from Special Diets Services, Ltd, Stepfield, Witham, Essex, U.K.).
   Water: potable, supplied *ad libitum*.
   Environmental conditions:
   Temperature: 19-22° C
   Humidity: not specified
   Air changes: approximately 10/hour
   Photoperiod: 12 hours light per 24 hours
   Acclimation period: 4-5 weeks

B. STUDY DESIGN

1. In life dates

Start: January, 1991; end: April, 1991

2. Animal assignment

Dogs were assigned randomly to the test groups in Table 1, such that there was an even distribution according to litter and body weight among the groups.

TABLE 1: Study design

Test group	Dose to animal (mg/kg/day)	Number of animals	
		Male	Female
Control	0	4	4
Low	5	4	4
Mid	20	4	4
High	50	4	4

Data taken from p.13, MRID 42205701.

3. Dose selection rationale

Dose levels were selected on the basis of results from a six-week oral dose ranging study undertaken at the same laboratory as the present study. No further details were provided by the study author.

4. Diet preparation and analysis

The test material was administered daily in gelatin capsules loaded for individual animals with dosages based on the most recent body weight and a purity of 94.6% w/w. It was not specified when the gelatin capsules were prepared.

Results -
Homogeneity analysis: Not applicable; test material was administered in gelatin capsules.

Stability analysis: Not applicable; test material was administered in gelatin capsules. The test material itself was noted as demonstrating stability under the conditions of storage.

Concentration analysis: Not applicable; test material (reported as 94.6% pure) was administered in gelatin capsules.

5. Statistics

Statistical analysis was done by analysis of either variance or covariance using the SAS GLM procedure (SAS Institute Inc. SAS User's Guide: Statistics, Version 5 Edition. Cary NC: SAS Institute, Inc., 1985). Differences from controls of the treated groups were represented by the differences in their least square means, and were tested statistically using a two-sided Student's t-test based on the error mean square in the analysis. Body weight changes were considered by analysis of covariance from day 1 weights, separately for males and females. Hematology and blood clinical chemistry data for both males and females were compared to pre-experimental values by analysis of covariance; a covariate adjustment was made based on the separate sex pre-experimental group means. Organ weights were compared by analysis of variance and covariance on final body weight, separately for males and females; data from paired organs were examined for differential effects on left and right components.

C. METHODS

1. Observations

Dogs were observed at least once daily, and usually on two other occasions each day, for gross clinical and behavioral abnormalities. A full clinical examination was performed in week 1 and prior to termination.

2. Body weight

Dogs were weighed before feeding on treatment day 1 and weekly throughout the pre-experimental and treatment periods.

3. Food consumption and compound intake

Food consumption for each dog was determined daily by measuring any food residues prior to giving the next meal.

4. Ophthalmoscopic examination

Eyes were examined by indirect ophthalmoscopy in week 1 and prior to termination.

5. Blood was collected from the jugular vein of all dogs before the morning feeding (i.e. after overnight fast) at weeks -1, 4, 8, and 13 for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
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
x	Blood clotting measurements*		
	(Thromboplastin time)		
x	(Kaolin-cephalin time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

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

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis

Urinalysis is not required for subchronic studies and was not conducted.

7. Sacrifice and pathology

All dogs survived to study termination, at which time they were sacrificed by sodium pentobarbitone anesthesia and exsanguination, and subjected to gross pathological examination. The CHECKED (X) tissues were collected from all animals and examined histologically. The (XX) organs, in addition, were weighed.

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

*Required for subchronic studies based on Subdivision F 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

A dose level of 100 mg/kg/day was initially given to dogs in the high dose group for up to 4 days, but was reduced to 50 mg/kg/day, as emesis was observed in all dogs shortly after dosing. The incidence of emesis was immediately reduced following dose adjustment, but a low incidence was seen throughout the study in the high-dose dogs. Emesis post-dosing was also noted to a lesser extent in the mid-dose dogs. The high-dose dogs also showed an increased incidence of fluid feces.

TABLE 2: Clinical observations in dogs given PROXEL						
Dose (mg/kg/day)	Males			Females		
	No. of affected animals	Study weeks affected	Total number of occurrences	No. of affected animals	Study weeks affected	Total number of occurrences
Fluid feces						
0	2/4	1,3,12	4	1/4	7	1
5	1/4	2,4	2	2/4	4-6, 9, 12	7
20	2/4	4-7	5	4/4	1-2,4,7,10-12	11
50	3/4	1-2,4-6,8,12-13	16	2/4	1-4,6-7,9,13	12
Incidence of emesis						
0	1/4	1	1	0	–	0
5	1/4	12	1	0	–	0
20	3/4	1-6,8-12	34	4/4	1-2,4-5,8,10	10
50	4/4	1-13	70	4/4	1-13	53

Data taken from pages 31,35,136-139,144-147; MRID 42205701

2. Mortality

All dogs survived to the scheduled termination date.

B. BODY WEIGHT AND WEIGHT GAIN

There were no statistically significant differences in body weight between control and treatment groups at the end of the treatment period. Slight (1-3%, $p \leq 0.05$) body weight increases compared to controls occurred in mid-dose females in some weeks. Body weight gain at the end of the study was slightly decreased (2%)compared to controls for high-dose females, and mid- and high-dose males (13% and 7%,respectively).

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

There was no effect on food consumption. All dogs consumed all the diet offered throughout the study.

8

2. Compound consumption

The compound was administered in gelatin capsules every morning before feeding, the amount given being determined by the most recent body weight measurement.

3. Food efficiency

Food efficiency was not calculated, as the treatment produced no differences in body weight between control and treatment groups.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects were observed.

E. BLOOD WORK

1. Hematology

There were few statistically significant differences in hematological parameters for the treated groups as compared to controls. For high-dose males, there was a slight (2%, $p \leq 0.05$) decrease in the mean cell volume at week 8. Mid- and high-dose females showed increased white blood cell count (21%, $p \leq 0.05$; 32%, $p \leq 0.01$; respectively)at week 8. Neutrophil count was also elevated (42%, $p \leq 0.01$) at week 8 in high-dose females.

2. Clinical chemistry

Statistically significant differences from controls ($p \leq 0.05$ or 0.01) for one or more weeks were seen in several clinical chemistry parameters (Table 3). The high-dose dogs of both sexes exhibited decreased plasma albumin (9-11%, all weeks), plasma total protein (7-12%, males, all weeks; females, weeks 8 and 13), and alanine transaminase (26-30%, weeks 8 and 13); and increased plasma triglycerides (30-49%, all weeks). Mid-dose dogs showed decreased albumin (9-10%, males, weeks 8 and 13), total protein (6-9%, males, all weeks; females, week 13),and alanine transaminase (17-26%, males, weeks 8 and 13; females week 13); and increased triglycerides (37%, females week 4). Other parameters altered at one or more weeks ($p \leq 0.05$ or 0.01) were incidental to treatment because they were small, transient, or lacked dose-response: plasma glucose (5-6% increase, females, all dose levels, week 13), plasma calcium (4 % increase, low-dose males, week 4; 5-7% decrease, females, all doses, weeks 8 and 13), plasma phosphorus (13% increase, high-dose males, week 4), plasma creatinine (8-10% increase, females, all doses, week 4), plasma creatine kinase (24-36% decrease, males, all doses, week 4), and total plasma bilirubin (28-31% decrease, low- and high-dose females, week 13).

TABLE 3: Clinical chemistry changes in dogs given Proxel[1]

Parameter	Week	Dose (mg/kg/day)			
		0	5	20	50
Males					
Albumin (g/100 mL)	-1	2.85	2.95	2.88	2.85
	4	2.92	2.86	2.73	2.64*
	8	2.99	2.91	2.70*	2.72*
	13	3.12	2.91	2.83*	2.77**
Total protein (g/100 ml)	-1	5.05	5.17	5.20	5.10
	4	5.21	5.29	4.89*	4.85**
	8	5.14	4.99	4.69**	4.76*
	13	5.50	5.31	4.98*	4.83**
Alanine transaminase (IU/L)	-1	19.3	16.8	16.5	17.8
	4	20.2	19.2	16.6	16.6
	8	24.7	21.4	18.3*	17.9*
	13	26.9	24.5	22.2*	19.9**
Triglycerides (mg/100 mL)	-1	32.3	31.5	31.8	35.8
	4	37.4	38.3	34.6	55.2**
	8	28.7	34.0	35.4	41.0**
	13	28.5	34.8	33.8	42.5**
Females					
Albumin (g/100 mL)	-1	3.10	3.05	3.00	3.13
	4	3.11	3.09	3.06	2.79**
	8	3.13	3.04	2.99	2.82**
	13	3.28	3.14	3.12	2.94**
Total protein (g/100 ml)	-1	5.45	5.25	5.42	5.45
	4	5.44	5.40	5.40	5.19
	8	5.21	5.16	5.07	4.81*
	13	5.73	5.52	5.32*	5.06**
Alanine transaminase (IU/L)	-1	19.5	20.0	14.3	17.8
	4	22.0	19.7	18.2	17.3
	8	23.8	22.1	18.8	16.6*
	13	27.0	25.9	21.3*	20.1**
Triglycerides (mg/100 mL)	-1	34.0	29.3	35.3	35.0
	4	34.6	36.1	47.3*	50.3**
	8	33.3	32.4	41.6	43.2*
	13	28.7	27.8	38.5	41.5*

Data taken from pages 52-60, MRID 42205701.
[1]The values presented for treatment weeks 4, 8, and 13 are "adjusted means" that were obtained by making a covariate adjustment based on the separate sex pre-experimental group means. [The pre-treatment values (week -1) were not adjusted.]
*Statistically different from control group mean at the 5% level
**Statistically different from control group mean at the 1% level

F. URINALYSIS

Urinalysis was not required and not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The absolute liver weights of males and females given 50 mg/kg/day were increased (17%, p≤ 0.01; 17%, p≤ 0.05, respectively) compared to controls (Table 4). Liver weights of females given 20 mg/kg/day were increased by 12% compared to controls. There was no evidence of any other treatment-related effects on organ weights, and no consistent evidence for a relationship between organ weight and final body weight. Since there was no evidence of any differential effects on the left and right components of paired organs, the data for combined weights only were statistically analyzed and tabulated by the study author.

TABLE 4: Liver weight changes in dogs given Proxel

	Dose (mg/kg/day)			
	0	5	20	50
Males				
Mean terminal body weight (kg)	14.07	14.05	13.63	14.02
Absolute liver weight (g)	473	477	494	554**
Organ/body weight ratio(%)[a]	3.36	3.40	3.62	3.95
Females				
Mean terminal body weight (kg)	12.07	12.32	12.47	11.95
Absolute liver weight (g)	379	382	423	445*
Organ/body weight ratio(%)[a]	3.14	3.10	3.39	3.72

Data taken from pages 36-37, 70-71; MRID 42205701.
[a]Calculated by reviewer
*Statistically different from control group mean at the 5% level
**Statistically different from control group mean at the 1% level

2. Gross pathology

There were no significant macroscopic alterations in either sex of dogs. Lesions were seen in one or two animals/sex in the brain (dilated ventricles), colon (thickened wall), duodenum and ileum (red area, thickened wall, firm), heart (red nodule), jejunum (fluid contents, firm, thickened wall), kidney (discolored area/spot, cyst),lung (firm, red spot, discolored area), prescapular lymph node (reduced), mesentery (black nodule), rectum (red spot/area, thickened wall), skin (hair loss), spleen (misshapen, black area), stomach (reddened spot, raised/ discolored area), thyroid gland (reduced, bilobed, discolored area), urinary bladder (red area), and ovary (enlarged, cyst). The low incidence of the macroscopic lesions indicated they were not toxicologically relevant. No treatment-related findings were detected.

3. Microscopic pathology

a. Non-neoplastic

Neither the incidence nor severity of any microscopic lesions was significantly elevated compared to controls. A small number of lesions were recorded, but were considered not to be treatment-related because they were sporadic or not dose-dependent.

b. Neoplastic

There was no evidence that treatment caused a neoplastic response in any group of animals.

III. DISCUSSION

A. DISCUSSION

Groups of four male and four female beagle dogs were given Proxel press paste in gelatin capsules at doses of 0, 5, 20, or 50 mg/kg/day for at least 90 days.

No animals died during this study. The most notable clinical signs in both sexes were emesis and fluid feces, primarily in the mid- and high-dose groups, throughout the treatment period (statistical analysis was not performed). The emesis appears treatment-related, based on the number of occurrences in mid- and high-dose dogs and the immediate reduction in incidence after the initial high-dose level was reduced from 100 to 50 mg/kg/day. The incidence of fluid feces is not clearly treatment-related; even though it increased at the higher dose levels, it was also present in five of the eight control dogs. The relationship between the treatment and the clinical signs is not known. The study author suggests the effects were the result of local irritation, but they were not accompanied by any pathological findings.

There were no significant differences in body weight between control and treated groups at the end of the study, and no changes in food consumption. Temporary elevation of white blood cell count(32%, $p \leq 0.01$) accompanied by neutrophilia was found at week 8 in high-dose females. There were other statistically significant hematological differences from control values, but these were sporadic and not considered to be dose-related.

Several clinical chemistry alterations occurred in both sexes at the mid and high doses, consistent with an effect on liver function. Changes included increased triglycerides (30-49%)and decreased plasma albumin (9-11%), alanine transaminase (26-30%), and total protein (6-9%). These changes suggest hepatic congestion, obviously mild since there were no histopathological correlates and no significant increases in relative liver weights. The study author attributed the reduction in plasma alanine transaminase to high values in some control animals and low pre-experimental values for animals in

treatment groups; the reviewer finds this explanation reasonable. Other statistically significant clinical chemistry changes were relatively minor or not dose-related, and considered to be of no toxicological significance. There were no pathological findings in the liver considered to be toxicologically significant. No treatment-related effects were revealed in either sex by ophthamology or gross or microscopic pathology.

Based on the clinical observation of incidence of emesis and the changes in clinical chemistry, the LOEL is 20 mg/kg/day for both sexes of dogs under the conditions of this study. The NOEL for both sexes is 5 mg/kg/day based on a lack of toxic effects.

B. STUDY DEFICIENCIES

There were no deficiencies that would impact the interpretation or classification of this study.