1-26-93

CAMPBELL FILE

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

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

009986

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

SUBJECT: 1,2-Benzisothiazolin-3-one - Review of 90-day feeding study in rats

EPA IDENTIFICATION NUMBERS: P.C. Code: 098901
DP Barcode: D182755
MRID No.: 419104-01

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 25 Jan 93
Toxicology Branch II, Section IV
Health Effects Division (H7509C)

TO: Linda Deluise
Product Manager (52)
Registration Division (H7505C)

THRU: Elizabeth Doyle, Ph.D. E.A. Doyle 1/25/93
Toxicology Branch II, Head Section IV
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 1/26/93
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: ICI Americas, Inc.

Chemical: PROXEL press paste
1,2-Benzisothiazolin-3-one

Action Requested: The registrant, ICI Americas Inc., has requested a review of the submitted 90-day feeding study.

Study: 'PROXEL' Press Paste: 90 Day Feeding Study in Rats

Study No: PRO798 dated 19 September 1990

Results: For 13 weeks, rats (Alpk:APfSD, Wistar derived) were given the test material incorporated in diet at 0, 200, 900, or 4000 ppm (equivalent mg/kg/day: 0, 15.3, 69.0, and 322 for males

and 0, 17.6, 78.3, and 356 for females, respectively). The LOEL is based on decreased body weights in both males and females and increased incidence of gross and non-neoplastic lesions in females.

	NOEL	LOEL
Male	200 ppm (LDT) (≈15.3 mg/kg/day)	900 ppm (MDT) (≈69 mg/kg/day)
Females	900 ppm (MDT) (≈78 mg/kg/day)	4000 ppm (HDT) (≈356 mg/kg/day)

Classification: core - guideline

This study satisfies guideline requirements (82-1) for a 90-day oral study in rats.

2

Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 25 Jan 93
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth A. Doyle, Ph.D. E. A. Doyle 4/29/93
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90 day oral - rat (82-1)

P.C. CODE: 098901

MRID NO.: 419104-01

TEST MATERIAL: PROXEL press paste

SYNONYMS: 1,2-Benzisothiazolin-3-one

STUDY NUMBER: PRO798

SPONSOR: ICI Americas, Inc
Wilmington, DE 19897

TESTING FACILITY: ICI Central Toxicology Laboratory
Alderley Park, Macclesfield
Cheshire, UK

TITLE OF REPORT: 'PROXEL' Press Paste: 90 Day Feeding Study in Rats

AUTHOR: A.J. Whiles

REPORT ISSUED: 19 September 1990

CONCLUSIONS: For 13 weeks, rats (Alpk:APfSD, Wistar derived) were given the test material incorporated in diet at 0, 200, 900, or 4000 ppm (equivalent mg/kg/day: 0, 15.3, 69.0, and 322 for males and 0, 17.6, 78.3, and 356 for females, respectively). The LOEL is based on decreased body weights in both males and females and increased incidence of non-neoplastic lesions (forestomach hyperplasia) in females.

	NOEL	LOEL
Male	200 ppm (LDT) (≈15.3 mg/kg/day)	900 ppm (MDT) (≈69 mg/kg/day)
Females	900 ppm (MDT) (≈78 mg/kg/day)	4000 ppm (HDT) (≈356 mg/kg/day)

Classification: core - guideline

This study satisfies guideline requirements (82-1) for a 90-day oral study in rats.

3

I. MATERIALS and METHODS

A. Test compound: Proxel press paste Description: yellow-brown powder Batch #: Y00180/025 Purity: 93.3% Contaminants: not given

B. Test animals: Species: Rat Strain: Alpk:APfSD (Wistar derived) Age: 6-7 weeks Weight (g): 152-230 (males), 133-170 (females) Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

C. Diet Preparation: A weighed amount of test compound, adjusted for purity, was mixed with basal diet (CT1 diet, Special Diets Services Limited, Stepfield, Witham, Essex, UK) to form a premix. The premix was mixed with appropriate amounts of basal diet to form the desired final concentrations of 200, 900 and 4000 ppm. Control animals received basal diet only.

The prepared diets were stored frozen until use. Fresh diet was presented to the animals every third day due to instability of test material in the 200 ppm diet. Test diets were prepared and analyzed for homogeneity, concentration and stability. Diets were mixed to homogeneity; samples from the top, middle and bottom had coefficients of variation within -9.1 and +6.5% of the target dose. The percent of target dose for the diets ranged from 91.3 to 115.5%. At room temperature, the test compound in the diet was stable for 2 days (200 ppm), 7 days (900 ppm) and 40 days (4000 ppm).

D. Study Design

1. Dose Selection: Dose selection was based on the results of a 28-day feeding study in rats.

2. Animal assignment: Animals were randomly assigned to study groups, as summarized in Table 1. Within each study group, animals were housed four per cage.

Table 1: Animal Assignment to Study Groups

Test Group		Dose in Diet (ppm)	Male	Female
Control	(CON)	0	12	12
Low	(LDT)	200	12	12
Mid	(MDT)	900	12	12
High	(HDT)	4000	12	12

E. Statistics: Analyses were carried out using the GLM procedure (SAS); least square means for each group were calculated using the LSMEAN option. Analysis of variance was used to evaluate initial body weights, weekly food consumption, total food consumption, food utilization, hematology and clinical chemistry data. Analysis of covariance was used to evaluate weekly body weight data and organ weights, using the initial and terminal body weights as the covariant, respectively. Pairwise comparisons between the controls and treated groups were carried out by comparing the least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Statistical significance of incidence data was not determined. The reviewer performed chi-square analysis to determine level of significance for data presented in Tables 5 and 6.

F. Quality assurance was documented by signed and dated GLP and quality assurance statements.

G. The sponsor applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of this study. This study neither meets nor exceeds any of the applicable criteria.

II. **RESULTS**

A. Observations: Animals were inspected daily for signs of toxicity, moribundity and mortality. Detailed examinations were preformed at least weekly.

1. Toxicity: Gross clinical observations performed during the study did not reveal any significant, treatment-related effects.

2. Mortality (survival): All animals survived until terminal sacrifice; no treatment-related effects were noted.

B. Body weight and Body weight Gain: Animals were weighed at the start of the study and weekly until terminal sacrifice.

1. Body weight: For high dose animals, the mean body weights were significantly decreased throughout most of the study (Appendix 1). The mean body weights of the males in the 900 ppm group were lower than control throughout the study with significant differences noted at Weeks 2, 3, 5, and 13. No differences in body weight were observed in the low dose animals and mid dose females.

2. Body weight Gain: Not determined

C. Food consumption, food conversion ratio, and compound intake: Food consumption was measured continuously throughout the study; data were summarized on a weekly basis.

1. Food consumption: No significant treatment-related changes in food intake were noted in any of the study groups.

2. Food conversion ratio: Food conversion ratios were calculated every four weeks. Significant decreases were observed in the high dose males during Weeks 1-4 and Weeks 1-13 (Table 2). No significant differences were noted in any of the female groups.

3. Compound intake results: The mean compound intake data are summarized in Table 3, below.

Table 2. Food Conversion Ratios for Male Animals (g growth/100 g food) (Data from Table 8B of the study)

Weeks	CON	LDT	MDT	HDT
1-4	20.08	20.90	19.38	17.75**
1-13	10.98	11.36	10.90	9.97**

** $p < 0.01$

Table 3: Compound Intake (mg/kg body weight/day) (From data presented in Table 5 of the study)

Study Group	Compound Intake	
	Male	Female
LDT	15.3	17.6
MDT	69.0	78.3
HDT	322	356

D. Ophthalmological examinations: Examinations were performed on control and high dose animals before the study was initiated and during Week 12. No treatment-related eye lesions were observed.

E. Hematology: At terminal sacrifice, blood was collected via cardiac puncture from all animals. Except for leukocyte differential count (control and 4000 ppm animals only), the following parameters were measured in all animals:

6

Hematocrit (HCT)	Mean corpuscular volume (MCV)
Hemoglobin (HGB)	Leukocyte differential count
Leukocyte count (WBC)	Mean corpuscular HGB (MCH)
Erythrocyte count (RBC)	Mean corpuscular HGB conc. (MCHC)
Platelet count	Kaolin-cephalin Time
Prothrombin time	

Statistically significant differences were noted in high dose males (decreased hemoglobin) and females (increased mean cell hemoglobin concentration and decreased hematocrit and kaolin-cephalin time). The differences were slight, however, and not of toxicological significance.

F. Clinical Chemistry: The following checked (X) clinical chemistry parameters were measured:

Electrolytes	Other
X Calcium	X Albumin
X Chloride	X Plasma creatinine
X Phosphorous	X Plasma urea
X Potassium	X Total cholesterol
X Sodium	Globulins
Enzymes	X Glucose
X Alkaline phosphatase (ALP)	X Total bilirubin
X Creatinine phosphokinase (CK)	X Total protein
X Alanine aminotransferase (ALT)	X Triglycerides
X Aspartate aminotransferase (AST)	
X γ-glutamyl-transferase	

Significant clinical chemistry findings are summarized in Table 4. Alkaline phosphatase in low and high dose males and cholesterol in mid and high dose females were significantly elevated. The magnitude of the responses, however, was not great enough to be of toxicological importance.

Table 4. Clinical Chemistry Data (Data from Table 11 of the study)

Parameter	Sex	CON	LDT	MDT	HDT
Alkaline Phosphatase (IU/ml)	Male	147	163*	157	185**
Cholesterol (mg/100 ml)	Female	70.3	69.8	77.9*	80.6**

* $p < 0.05$, ** $p < 0.01$

G. Sacrifice and Pathology: Detailed gross pathological examinations were performed on animals sacrificed in moribund condition, dying during the study, or surviving to

terminal sacrifices. All of the tissues listed below were fixed. Tissues (X) from animals in the control and 4000 ppm groups were examined histologically; additionally, the liver, kidney, lung and stomach of the 900 ppm group animals were also examined histologically. Tissues (XX) were weighed before histological examination.

Digestive system	Cardiovas./Hematol	Neurologic
X Salivary glands	X Heart	XX Brain
X Oral cavity	X Aorta	X Eyes
X Esophagus	X Bone marrow	X Periph. nerve
X Stomach	X Lymph nodes	X Spinal cord
X Duodenum	X Spleen	X Pituitary
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	X Harderian gland
X Cecum	XX Kidneys	XX Adrenals
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	X Epididymides	X Thyroids
X Pancreas	X Prostate	Other
Respiratory	X Seminal vesicle	X Bone
X Trachea	X Ovaries	X Skeletal muscle
X Lungs	X Uterus	X Skin
X Nasal cavity	X Cervix	X Gross lesions
		X Sternum

1. Organ Weights: In the high dose group, the absolute brain weights (males) and kidney weights (females) were slightly, but significantly, lower than controls. These differences, however, appear to be a reflection of the decreased mean body weight of these animals at terminal sacrifice. Although the absolute organ weights of the high dose animals were significantly lower than controls, no significant differences were noted in either the relative organ weight or organ weight adjusted for body weight.

2. Gross Pathology: Gross pathological lesions were limited to the stomachs of both males and females. The incidence of thickened limiting ridge was markedly increased in all high dose animals (Table 5).

Table 5: Gross lesions (Data summarized from Table 13 of study)

Lesion	Sex	CON	LDT	MDT	HDT
Stomach, thickened limiting ridge	Male	0/12	0/12	1/12	10/12*
	Female	0/12	0/12	1/12	10/12*

* $p \leq 0.05$

8

3. Microscopic Pathology

a) Nonneoplastic lesions: Microscopic examination of the stomach confirmed the gross pathological findings. Slight to moderate hyperplasia of the forestomach at the limiting ridge was observed in all high dose animals (Table 6). High dose females also showed slight mixed inflammatory cell infiltrate in the submucosa. Other lesions were noted, but were incidental in nature and not related to treatment.

b) Neoplastic lesions: None noted

Table 6: Nonneoplastic stomach lesions (Data summarized from Table 14 of study)

Lesion	Sex	CON	LDT	MDT	HDT
Hyperplasia forestomach limiting ridge	Male	0/12	0/12	0/12	11/12* (1,7,3)[a]
	Female	0/12	0/12	0/12	11/12* (0,8,3)
Mixed inflammatory cell infiltrate submucosa	Female	0/12	0/12	0/12	6/12* ((0,6,0)

* $p \leq 0.05$
[a] Numbers in parentheses represent the lesion grade (minimal, slight, moderate)

III. DISCUSSION: This study evaluated the subchronic toxicity of test compound, at dietary concentrations of 0, 200, 900, or 4000 ppm (equivalent mg/kg/day: 0, 15.29, 68.96, and 322 for males and 0, 17.63, 78.27, and 356.28 for females, respectively), when administered to rats for 13 weeks. All animals survived until scheduled sacrifice without any significant gross clinical signs.

During the study the body weights of all high dose animals were significantly decreased at 4000 ppm. Males in the 900 ppm group showed decreased body weights during the entire study with statistically significant decreases during Weeks 2, 3, and 13.

Throughout the study, food consumption was not significantly altered by treatment. The food conversion ratio, however, was significantly lower in the high dose males during Weeks 1-4 and 1-13.

Hematology and clinical chemistry parameters were within normal range. Although significant findings were noted, the magnitude

of the differences were not great enough to be of toxicological significance.

At terminal sacrifice, animals in the high dose group showed decreased absolute brain (males) and kidney (females) weights. No differences were noted in the relative organ weights of any of the animals. The observed differences do not appear to be treatment-related and are a reflection of the lower terminal body weights of the high dose animals.

Significant gross pathological and histopathological lesions were noted in males and females in the high dose group. On gross examination, there was an increased incidence of thickening of the limiting ridge of the stomach of all high dose animals. Histopathological examination revealed hyperplasia in the limiting ridge of the forestomach of high dose animals and increased incidence of mixed inflammatory cell infiltrate in the submucosa of high dose females.

	NOEL	LOEL
Male	200 ppm (LDT) (≈15.3 mg/kg/day)	900 ppm (MDT) (≈69 mg/kg/day)
Females	900 ppm (MDT) (≈78 mg/kg/day)	4000 ppm (HDT) (≈356 mg/kg/day)

The systemic LOEL is based on decreased body weights in both males and females, increased incidence of gross and non-neoplastic lesions in females.

Classification: core - guideline

This study satisfies guideline requirements (82-1) for a 90-day oral study in rats.

PROXEL PRESS PASTE : 90 DAY FEEDING STUDY IN RATS
TABLE 7A
INTERGROUP COMPARISON OF BODYWEIGHTS (g) - MALES

		Dietary Concentration of PROXEL press paste (ppm)			
		0(Control)	200	900	4000
Week 1	MEAN	192.2	184.3	185.5	187.9
	S.D.	19.1	17.8	15.3	18.8
	N	12	12	12	12
Week 2	MEAN	243.7	238.3	232.6*	225.3**
	S.D.	23.6	19.3	19.9	19.8
	N	12	12	12	12
Week 3	MEAN	288.3	281.8	271.8**	268.5**
	S.D.	24.7	23.5	21.1	21.7
	N	12	12	12	12
Week 4	MEAN	321.1	315.8	305.3	301.6**
	S.D.	25.0	27.8	27.9	23.2
	N	12	12	12	12
Week 5	MEAN	350.0	344.8	328.8*	322.5**
	S.D.	29.4	32.7	32.1	27.5
	N	12	12	12	12
Week 6	MEAN	375.9	370.6	353.9	346.4**
	S.D.	29.4	36.0	33.1	31.8
	N	12	12	12	12
Week 7	MEAN	397.7	391.6	375.0	366.4**
	S.D.	28.9	40.0	37.8	32.9
	N	12	12	12	12
Week 8	MEAN	407.3	406.1	388.2	381.4*
	S.D.	25.4	39.6	39.0	33.8
	N	12	12	12	12
Week 9	MEAN	420.8	421.8	400.9	391.1**
	S.D.	27.7	38.7	40.5	34.7
	N	12	12	12	12
Week 10	MEAN	436.6	438.6	414.2	407.4**
	S.D.	28.1	42.5	40.4	38.7
	N	12	12	12	12
Week 11	MEAN	449.6	450.6	423.2	419.1**
	S.D.	26.8	43.2	37.9	35.5
	N	12	12	12	12
Week 12	MEAN	462.0	458.1	433.7	428.7**
	S.D.	27.9	44.2	38.4	34.1
	N	12	12	12	12
Week 13	MEAN	467.6	463.8	437.1*	432.4**
	S.D.	29.0	43.3	39.2	33.9
	N	12	12	12	12
Week 13	MEAN	470.0	467.0	445.3	434.6**
	S.D.	27.7	42.0	37.0	32.5
	N	12	12	12	12

PROXEL PRESS PASTE : 90 DAY FEEDING STUDY IN RATS
TABLE 7 A
INTERGROUP COMPARISON OF BODYWEIGHTS (g) - FEMALES

		Dietary Concentration of PROXEL press paste (ppm) 0(Control)	200	900	4000
Week 1	MEAN	146.9	150.3	148.9	145.0
	S.D.	9.0	8.7	7.3	7.1
	N	12	12	12	12
Week 2	MEAN	169.7	172.8	174.3	164.1
	S.D.	13.2	11.5	11.7	7.2
	N	12	12	12	12
Week 3	MEAN	190.2	191.9	192.8	178.8*
	S.D.	17.4	13.4	14.4	8.9
	N	12	12	12	12
Week 4	MEAN	201.6	203.2	203.2	189.9*
	S.D.	17.7	15.3	16.7	11.0
	N	12	12	12	12
Week 5	MEAN	212.4	214.6	212.3	201.2**
	S.D.	16.6	12.4	15.3	11.4
	N	12	12	12	12
Week 6	MEAN	225.8	225.8	221.2*	209.0**
	S.D.	18.1	15.4	13.8	12.3
	N	12	12	12	12
Week 7	MEAN	231.2	229.4	228.5	216.7**
	S.D.	19.8	18.9	17.2	12.2
	N	12	12	12	12
Week 8	MEAN	232.8	237.2	231.7	220.3*
	S.D.	20.3	14.4	16.6	14.9
	N	12	12	12	12
Week 9	MEAN	239.3	245.6	234.9	223.8**
	S.D.	20.1	16.2	19.3	16.2
	N	12	12	12	12
Week 10	MEAN	247.2	253.9	243.4	232.7**
	S.D.	16.7	15.0	17.1	14.7
	N	12	12	12	12
Week 11	MEAN	249.3	253.8	246.2	230.9**
	S.D.	20.1	18.8	17.4	15.8
	N	12	12	12	12
Week 12	MEAN	250.0	254.6	248.8	234.1**
	S.D.	21.6	18.7	16.8	15.1
	N	12	12	12	12
Week 13	MEAN	254.0	261.3	258.1	242.4*
	S.D.	19.3	14.8	16.8	16.5
	N	12	12	12	12
Week 13	MEAN	258.0	261.4	257.2	241.3**
	S.D.	19.7	20.3	15.9	14.5
	N	12	12	12	12

12