DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §82-1a, Subchronic Oral Toxicity Study in Rats

Work Assignment No. 1-01-35 F (MRID 45710205)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste. B
Durham, NC 27713

Primary Reviewer:
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr.
Date: 8/27/04

Secondary Reviewer:
Michael E. Viana, Ph.D.

Signature: Michael E Viana
Date: 8/27/04

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 8/27/04

Quality Assurance:
Steven Brecher, Ph.D.

Signature: David A. McEwan for Steven Brecher
Date: 8/27/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Lisa Austin
Registration Action Branch 1, Health Effects Division (7509C)
Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: Lisa Austin
Date 9/29/05
Signature: P.V. Shah
Date 12/15/05

Template version 11/01

DATA EVALUATION RECORD

TXR#: 0052493

STUDY TYPE: 90-Day Oral Toxicity [diet] - rats; OPPTS 870.3100 [§82-1a]; OECD 408.

PC CODE: 081901

DP BARCODE: 301496
SUBMISSION NO.: None

TEST MATERIAL (PURITY): Chlorothalonil (99.18% a.i.)

SYNONYMS: Tetrachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile

CITATION: Spencer-Briggs, D.J. (1994) Chlorothalonil: toxicity to rats by dietary administration for 13 weeks. Huntingdon Research Centre Ltd, Huntingdon, England. Laboratory Study No.: VCM 9/920338, June 20, 1994. MRID 45710205. Unpublished.

SPONSOR: Vischim S.r.l., 20031 Cesano Maderno, Milan, Italy

EXECUTIVE SUMMARY - In this subchronic oral toxicity study (MRID 45710205), 10 Crl:CD (SD) BR rats/sex/dose were exposed to Chlorothalonil (99.18% a.i.; Batch #: 71) in the diet at nominal concentrations of 0, 30, 60, 300, or 1500 ppm (equivalent to 0/0, 2.3/2.7, 4.7/5.5, 23.6/28.8, and 117/130 mg/kg/day in males/females) for up to 3 months.

No treatment-related adverse effects were observed on mortality, clinical signs, body weight, food or water consumption, food efficiency, ophthalmoscopic examination, hematology, clinical chemistry or organ weights.

Body weight gain was decreased ($p<0.05$) by 27-36% in the 1500 ppm group during the first week of treatment which was attributed to a palatability problem. The forestomach was roughened in all animals in the ≥300 ppm groups. The forestomach was also thickened in the 1500 ppm males (10 treated vs 0 controls) and females (4 treated vs 0 controls). The incidences of minimal to moderate epithelial hyperplasia and hyperkeratosis observed at the limiting ridge and the non-glandular region of the forestomach were increased in the ≥300 ppm groups (7-10/10 treated vs 0/10 controls). The incidence of minimal to moderate hyperplasia of the duodenum was increased in the 1500 ppm group (3/10 each treated vs 0/10 controls). The incidence of minimal epithelial hyperplasia and hyperkeratosis observed at the limiting region of the

forestomach was also increased in the 30 and 60 ppm males and the 60 ppm females (4-5/10 treated).

The LOAEL was 30 (equivalent to 2.3/2.7 mg/kg/day [M/F]) based on minimal epithelial hyperplasia and hyperkeratosis observed at the limiting ridge and/or nonglandular region of the forestomach in males and females. The NOAEL < 30 ppm (equivalent to < 2.3/2.7 mg/kg/day [M/F]).

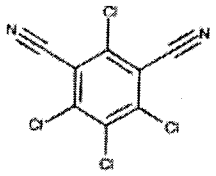
This study is classified as **acceptable/guideline** and does satisfy the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil

Description:	White powder
Batch #:	71
Purity (w/w):	99.18% a.i.
Stability of compound:	Stable in the diet for 3 days at room temperature or for 7 days refrigerated followed by 1 day at room temperature
CAS # of TGAI:	1897-45-6
Structure:	

2. Vehicle - diet

3. Test animals

Species:	Rat
Strain:	Crl:CD (SD) BR
Age and mean weight at Week 1:	Approximately 42 days; 178-185 g males; 153-160 g females
Source:	Charles River Breeding Laboratories, Margate, Kent, England
Housing:	In groups of 5/cage, sexes separated, in suspended cages with wire mesh floors
Diet:	Powdered Rat and Mouse No. 1 modified maintenance diet (Special Diet Services Limited, Essex, UK), *ad libitum* except overnight prior to blood collection
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	21±2°C
Humidity:	55±10%
Air changes:	Not reported
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	14 days

B. STUDY DESIGN

1. In-life dates - Start: 10/30/91 End: 2/3/92

2. Animal assignment - Animals were randomly assigned, stratified by weight, to the test groups presented in Table 1.

Table 1. Study design. [a]

Test Group	Nominal Dose (ppm)	Mean Chemical Intake in mg/kg bw/day for males/females	(# rats/sex)
Control	0	0/0	10
Low	30	2.3/2.7	10
Medium	60	4.7/5.5	10
Medium-High	300	23.6/28.8	10
High	1500	117/130	10

a Data were obtained from MRID 45710205 on pages 18 and 41.

3. **Dose-selection rationale** - A dose-selection rationale was not provided.

4. **Dose preparation, administration, and analysis** - Dietary formulations were prepared by first making a premix of the test compound and feed, and then diluting this premix with appropriate amounts of feed to achieve the desired concentrations. Test diets were prepared each week. Half of the prepared test diet was provided to the animals immediately. The remaining diet was stored in the refrigerator, until it was provided to the animals at midweek. Ten and 2500 ppm formulations were evaluated prior to treatment for homogeneity (top, middle, and bottom) and stability under two conditions (up to 10 days at room temperature, or 7 days refrigerated followed by 1 day at room temperature). Concentration analyses were reported for each dose preparation for Weeks 1 and 13.

Results: Homogeneity (range as %CV): 2.30-3.09

Stability (relative mean error in %, representing deviation from time 0):

Days (room temperature)	10 ppm	2500 ppm
3	-9.3	-5.6
4	-14.0	-3.2
10	-25.3	0.8
8 [a]	-2.8	2.4

a 7 days in the refrigerator followed by 1 day at room temperature

Concentration (range as relative mean error of nominal in %): -10.0-7.3

Under the conditions of the study, the analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. Statistics - Body weight gain, organ weight, food and water consumption, and clinical pathology data were subjected to the following statistical procedures (tested at $p<0.05$ and 0.01). In data sets where the relative frequency of the mode exceeded 75%, the proportion of animals with values different from the mode were analyzed with Fisher's test and Mantel's test. Otherwise, Bartlett's test was performed, and logarithmic transformation of data was performed when necessary to obtain homogeneous variances. One-way analysis of variance, followed by Student's t-test and Williams' test was performed when variance was homogeneous. Kruskal-Wallis analysis and non-parametric equivalents of the t-test and Williams' test were performed when variance was heterogenous. For organ weight data, the terminal body weight was used as a covariate when the within group relationship between organ weight and body weight was significant at the 10% level.

C. METHODS

1. Observations

1a. Cageside observations - Animals were observed twice daily during the study for mortality and signs of toxicity. Any signs of behavioral changes were recorded at least once daily (week days only).

1b. Clinical examinations - Detailed clinical observations, including palpations, were performed daily for the first 4 weeks and weekly thereafter.

1c. Neurological evaluations - Neurological evaluations were not performed.

2. Body weight - All animals were weighed prior to treatment, weekly during the study, and at termination. Body weight gain was also reported for Weeks 0-1, 1-13, and 0-13.

3. Food consumption and compound intake - Mean food consumption (g/animal/week) was reported in weekly intervals. Compound intake values (mg/kg/day) were calculated using the food consumption, body weight, and nominal dietary concentration data. Food efficiency (food consumption in g/body weight gain in g) was reported for the intervals Weeks 1-4, 5-8, 9-13, and 1-13. Water consumption (g/animal/day) was reported daily during Week 12.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals prior to initiation of treatment and on all animals in the control and 1500 ppm groups during Week 13.

5. Hematology and clinical chemistry - Hematology and clinical chemistry parameters were evaluated in all animals at termination. Blood was collected from the orbital sinus of animals fasted overnight and under light ether anesthesia. The CHECKED (X) parameters were examined for hematology and clinical chemistry analyses.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Blood cell morphology
	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total protein*
X	Alkaline phosphatase (ALK)*		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

7. Urinalysis - Urinalysis parameters were evaluated in 3-5 animals/sex/group on Day 1 and in Weeks 5, 9, and 13. The samples were collected from animals (not fasted) housed in metabolism cages over a period of 24 hours. The following CHECKED (X) parameters were examined.

	Appearance*		Glucose
X	Volume*		Ketone bodies
	Specific gravity*		Bilirubin
	pH*		Blood*
	Sediment (microscopic)		Nitrate
	Protein*		Urobilinogen

* Recommended (but optional) for 90-day oral rodent studies based on Guideline 870.3100

Urinary thiol, thioether, and creatinine concentrations were measured.

7. **Sacrifice and pathology** - All animals were necropsied. The following CHECKED (X) tissues were collected. The (XX) organs were weighed in animals sacrificed on schedule.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*[a]	XX	Brain*+[a]
X	Salivary glands*[a]	XX	Heart*+[a]	X	Peripheral nerve (sciatic)*[a]
X	Esophagus*[a]	X	Bone marrow*[a]	X	Spinal cord (3 levels)*
X	Stomach*[b]	X	Lymph nodes*[a]	XX	Pituitary*[a]
X	Duodenum*[a]	XX	Spleen*+[a]	X	Eyes (retina, optic nerve)*
X	Jejunum*[a]	X	Thymus*+[a]		GLANDULAR
X	Ileum*[a]			XX	Adrenal gland*+[a]
X	Cecum*[a]		UROGENITAL	X	Lacrimal gland
X	Colon*[a]	XX	Kidneys*+[b]	XX	Thyroid with parathyroid*[a]
X	Rectum*[a]	X	Urinary bladder*[a]		
XX	Liver*+[b]	XX	Testes with epididymides*+[a]		OTHER
	Gall bladder (not rat)*	X	Prostate*	X	Bone (femur and sternum)*[a]
	Bile duct (rat)	X	Seminal vesicles*	X	Skeletal muscle
X	Pancreas*[a]	XX	Ovaries*+[a]	X	Skin*
	RESPIRATORY	XX	Uterus (corpus and cervix)*+[a]	X	Femur joint
X	Trachea*[a]	X	Mammary gland*	X	Head
X	Lung*[b]	X	Vagina	X	All gross lesions and masses*[b]
X	Nose*				
X	Pharynx*				
X	Larynx*				

a All designated tissues from the control and 1500 ppm groups were examined microscopically.
b Designated tissues from all groups were examined microscopically.
* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.

Stomach, liver, lung, and kidney samples, and all gross lesion samples from all dose groups were processed and examined microscopically. Samples of salivary glands, esophagus, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, trachea, aorta, heart, bone marrow, lymph nodes, spleen, thymus, urinary bladder, testes with epididymides, ovaries, uterus (corpus and cervix), brain, peripheral nerve, pituitary, adrenal gland, thyroid with parathyroid, and bone from the control and 1500 ppm groups were also processed and examined microscopically.

8. Measurement of hepatic and renal non-protein thiol concentrations - Liver (1-2 g obtained from the same site of each animal) and kidney (one half of each kidney) were collected from 4-5 animals/sex/group, weighed, quick-frozen in liquid nitrogen, and then stored at -20°C. Tissues were thawed, homogenized in orthophosphate buffer (pH 7.4), diluted with sulphosalicylic acid solution, and centrifuged to remove protein. Non-protein thiol concentration was determined in duplicate analysis by a modification of the method of Ellman.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - No treatment-related clinical signs of toxicity were observed.

2. Mortality - No treatment-related mortality was observed. One control female died following blood sampling in Week 13.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weight gain was decreased ($p<0.05$) by 27-36% in the 1500 ppm group during the first week of treatment (Table 2). Body weight gain in the remainder of the study (Weeks 1-13) was similar to the control in all groups. Overall (Weeks 0-13) body weight gain remained decreased (not statistically significant [NS]) by 9% in the 1500 ppm males, due to reduced body weight gain during the initial week of treatment. Body weights were decreased by 6-10% throughout treatment in the 1500 ppm males, also due to reduced body weight gain during the initial week of treatment. No treatment-related effect was observed on body weight and body weight gain in the other groups.

Table 2. Mean body weights and body weight gains (g) at selected intervals in rats treated with Chlorothalonil for up to 3 months. [a]

Week(s)	Dose (ppm)				
	0	30	60	300	1500
Males					
0	181	185	178	180	178
4	372	381	364	364	335 (↓10)
7	442	451	428	433	406
12	508	532	499	508	477 (↓6)
13	507	522	494	512	474
BWG: 0-1	53±6.0	56±3.9	54±5.1	53±15.8	34±6.4** (↓36)
BWG: 1-13	273±45.4	282±42.1	262±33.1	279±77.7	262±43.8
BWG: 0-13	326±47.8	338±44.6	315±37.0	332±88.9	296±47.9 (↓9)
Females					
0	153	153	155	154	160
7	271	273	277	273	270
13	296	299	306	302	305
BWG: 0-1	26±5.3	26±5.2	29±3.1	27±6.2	19±7.4* (↓27)
BWG: 1-13	116±9.4	120±13.8	122±16.5	121±9.9	126±14.8
BWG: 0-13	143±7.6	146±16.9	151±18.0	148±14.0	145±13.4

a Data (n=10) were obtained from pages 29 and 38 of MRID 45710205. Standard deviations and statistical significance were reported only for body weight gains. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* Significantly different from controls; $p<0.05$

** Significantly different from controls; $p<0.01$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food and water consumption - No treatment related effect was observed on food and water consumption. A transient decrease (NS) of 16% was observed in the 1500 ppm males during Week 1.

2. Compound consumption- The mean achieved dosages are shown in Table 1.

2. Food efficiency - No treatment-related effect was observed on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were observed during ophthalmoscopic examination.

E. BLOOD ANALYSES

1. Hematology - No treatment-related effects were observed on hematology. Platelet count was decreased ($p<0.01$) by 16% in the 1500 ppm females; however, this effect was not considered adverse in the absence of corroborating evidence of toxicity. Other differences ($p<0.05$) were minor and/or unrelated to dose.

2. Clinical chemistry - No treatment-related effects were observed on clinical chemistry. Serum alanine aminotransferase was decreased ($p<0.05$) by 23-33% in the 1500 ppm males and the ≥300 ppm females; however, this slight effect was not considered adverse. Further experimentation suggested that this decrease was due to a decrease in pyridoxal-5'-phosphate, a necessary cofactor in the metabolism of Chlorothalonil and also required for the full expression of alanine aminotransferase. Other differences ($p<0.05$) were minor and/or unrelated to dose.

F. URINALYSIS: Concentrations of thiol, thioether, and creatinine were decreased ($p<0.05$) in the urine of the ≥300 ppm females (↓25-95%) at Week 13 (Table 3). The Sponsor stated that thioether was reported as nmoles thiol/μmole creatinine to provide a measure of the concentration of the urine itself; this measure was decreased on Day 1, but not at Week 13. This effect on urine concentration was not considered adverse. No treatment-related effects were observed in the other dose groups.

Table 3. Mean (±SD) thiol, thioether, and creatinine concentrations in urine of female rats treated with Chlorothalonil for 3 months.[a]

Time	Dose (ppm)				
	0	30	60	300	1500
Thiol (nmoles –Acetyl cysteine equivalents./mL urine)					
Pre-alkali hydrolysis					
Day 1	2.65±3.37	6.06±6.51	2.53±2.37	0	1.97±3.58
Week 13	4.91±2.63	6.81±5.14	1.75±3.84	0.78±1.28* (↓84)	0.23±0.51* (↓95)
Post-alkali hydrolysis					
Day 1	360±113	367±38	401±112	389±71	320±62
Week 13	530±216	407±107	452±84	270±180* (↓49)	393±187* (↓26)
Thioether (nmoles thiol/mL urine)					
Day 1	357±112	361±41	398±110	389±71	318±65
Week 13	525±215	400±109	451±87	269±180* (↓49)	393±187* (↓25)
Thioether (nmoles thiol/μ mole creatinine)					
Day 1	192±35	165±44	153±30	135±25* (↓30)	117±33** (↓39)
Week 13	102±26	133±14	118±38	125±42	128±39
Creatinine (μ moles/mL urine)					
Day 1	1.88±0.58	2.27±0.41	2.66±0.84	2.88±0.21	2.83±0.74
Week 13	5.43±2.21	3.08±1.08	4.25±1.90	2.30±1.61** (↓58)	2.99±1.14** (↓45)

a Data (n=3-5) were obtained from page 278-280 of MRID 45710205. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* Significantly different from controls; $p<0.05$

** Significantly different from controls; $p<0.01$

G. SACRIFICE AND PATHOLOGY

1. Organ weight - No treatment-related adverse effects were observed on organ weights. The following differences ($p<0.05$) were observed; however, clinical and pathological data did not corroborate an adverse effect: increased adjusted (for terminal body weight) liver and kidney weights in the 1500 ppm group (↑12-16%), and increased absolute pituitary weight in the 1500 ppm females (↑33%). Other organ weights were similar to controls.

2. Gross pathology - The forestomach was roughened in all animals (n=10) in the ≥300 ppm groups (vs 0 controls; Table 4). The forestomach was also thickened in the 1500 ppm males (10 treated vs 0 controls) and females (4 treated vs 0 controls). The incidences of other macroscopic lesions in the treated animals were similar to controls.

Table 4. Incidence (# affected/10) of selected macroscopic lesions in rats treated with Chlorothalonil for 3 months.[a]

Lesion	Dose (ppm)				
	0	30	60	300	1500
Males					
Forestomach					
Roughened	0	0	0	10	10
Thickened	0	0	0	0	10
Females					
Forestomach					
Roughened	0	0	0	10	10
Thickened	0	0	0	0	4

a Data were obtained from pages 47-50 of MRID 45710205.

3. **Microscopic pathology** - The incidences of minimal to moderate epithelial hyperplasia and hyperkeratosis observed at the limiting ridge and the non-glandular region of the forestomach were increased in the ≥300 ppm groups (7-10/10 treated vs 0/10 controls; Table 5). The incidence of minimal to moderate hyperplasia of the duodenum was increased in the 1500 ppm group (3/10 each treated vs 0/10 controls). The incidence of minimal epithelial hyperplasia and hyperkeratosis observed at the limiting region of the forestomach was also increased in the 30 and 60 ppm males and the 60 ppm females (4-5/10 treated). The incidences of other microscopic lesions in the treated groups were similar to controls.

Table 5. Selected non-neoplastic histological findings (# affected/10) in rats treated with Chlorothalonil for 3 months.[a]

Non-neoplastic lesion	Dose (ppm)				
	0	30	60	300	1500
Males					
Stomach					
Epithelial hyperplasia and hyperkeratosis at the limiting ridge (Total)	0	5	4	10	10
Minimal	0	5	4	5	8
Moderate	0	0	0	5	2
Epithelial hyperplasia and hyperkeratosis in the nonglandular region (Total)	0	0	1	10	10
Minimal	0	0	1	2	6
Moderate	0	0	0	8	4
Duodenum					
Epithelial hyperplasia (Total)	0	0	0	0	3
Minimal	0	0	0	0	2
Moderate	0	0	0	0	1
Females					
Stomach					
Epithelial hyperplasia and hyperkeratosis (minimal) at the limiting ridge (Total)	0	1	5	7	9
Epithelial hyperplasia and hyperkeratosis in the nonglandular region (Total)	0	1	2	9	10
Minimal	0	1	2	7	9
Moderate	0	0	0	2	1
Duodenum					
Epithelial hyperplasia (Minimal; Total)	0	0	0	0	3

a Data were obtained from pages 51-57 of MRID 45710205.

4. **Measurement of hepatic and renal non-protein thiol concentrations** - Non-protein thiol concentrations (mainly glutathione) were increased ($p<0.01$) in the liver of the 1500 ppm males (↑569%) and in the kidney of the 1500 ppm females (↑296%; Table 6). Non-protein thiol concentrations were also increased (NS) in the liver of the 1500 ppm females (↑142%) and in the kidney of the 300 ppm females (↑161%) and 1500 ppm males (↑185%).

Table 6. Mean (±SD) non-protein thiol concentrations (μmoles glutathione equivalents/g tissue) in livers and kidneys of rats treated with chlorothalonil for 3 months.[a]

Organ	Dose (ppm)				
	0	30	60	300	1500
Males					
Liver	0.597±0.435	0.270±0.131	0.228±0.176	0.399±0.299	3.396±1.162** (↑569)
Kidney	0.085±0.016	0.069±0.047	0.083±0.045	0.108±0.057	0.157±0.071 (↑185)
Females					
Liver	1.774±0.400	0.590±0.457	1.406±0.965	1.554±0.909	2.512±0.493 (↑142)
Kidney	0.077±0.047	0.111±0.071	0.071±0.065	0.124±0.097 (↑161)	0.228±0.066** (↑296)

a Data (n=5) were obtained from page 281 of MRID 45710205. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).
** Significantly different from controls; $p<0.01$

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS: The Sponsor stated that the NOAEL was 1500 ppm, the highest dose tested.

B. REVIEWER'S COMMENTS: No treatment-related adverse effects were observed on mortality, clinical signs, body weight, body weight gain, food or water consumption, food efficiency, ophthalmoscopic examination, hematology, clinical chemistry, organ weights, or gross and histological pathology.

A transient decrease (NS) of 16% was observed in food consumption in the 1500 ppm males during Week 1, indicating the possibility of a palatability issue. At termination, the forestomach was grossly roughened in all animals (n=10) in the ≥300 ppm groups (vs 0 controls). The forestomach was also thickened in the 1500 ppm males (10 treated vs 0 controls) and females (4 treated vs 0 controls). The incidences of minimal to moderate epithelial hyperplasia and hyperkeratosis observed at the limiting region and the non-glandular region of the forestomach were increased in the ≥300 ppm groups (7-10/10 treated vs 0/10 controls). The incidence of minimal to moderate hyperplasia of the duodenum was increased in the 1500 ppm group (3/10 each treated vs 0/10 controls). The incidence of minimal epithelial hyperplasia and hyperkeratosis observed at the limiting region of the forestomach was also increased in the 30 and 60 ppm males and the 60 ppm females (4-5/10 treated). The Sponsor stated that these changes reflected an adaption to gastric irritation. Gastric irritation may have played a role in the initial decrease in food consumption. A transient decrease ($p<0.05$) in body weight gain of 27-36% was observed in the 1500 ppm group during the first week of treatment. Body weight gain in the remainder of the study (Weeks 1-13) was similar to the control in all groups. Overall (Weeks 0-13) body weight gain in the 1500 ppm males did not fully recover to control levels, remaining decreased (NS) by 9%. Body weights were decreased by 6-10% throughout treatment in the 1500 ppm males, due to reduced body weight gain during the initial week of treatment. In

summary, effects observed on food consumption, body weight, and body weight gain were considered transient and not adverse.

The LOAEL was 30 (equivalent to 2.3/2.7 mg/kg/day [M/F]) based minimal epithelial hyperplasia and hyperkeratosis observed at the limiting ridge and/or nonglandular region of the forestomach in males and females. The NOAEL < 30 ppm (equivalent to < 2.3/2.7 mg/kg/day [M/F]).

This study is classified as **acceptable/guideline** and does satisfy the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were also noted, but do not effect the conclusions of this review:

- Mean corpuscular hemoglobin was not reported.
- Thymus weight was not reported.
- Although microscopic examination of the nose, pharynx, larynx, prostate, seminal vesicles, mammary gland, spinal cord, eyes, and skin is currently recommended, it was not required by the guidelines at the time this study was conducted.
- Standard deviations were not presented for body weight.
- A dose-selection rationale was not provided.