DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §83-2b; Carcinogenicity Study in Mice

Work Assignment No. 2-01-35 B; formerly 1-01-35 B (MRID 45710211)

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

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

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

Signature: Ronnie J. Bever Jr.
Date: 11/30/04

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

Signature: Michael E Viana
Date: 12/1/04

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

Signature: Mary L Menetrez
Date: 12/6/04

Quality Assurance:
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 11/30/04

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EPA Reviewer: William B. Grecar, M.P.H., D.A.B.T. **Signature:** William Grecar
Registration Action Branch 1, Health Effects Division (7509C) **Date** 9/22/2005
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/21/05

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DATA EVALUATION RECORD

TXR#: 0052493

STUDY TYPE: Carcinogenicity study in mice [feeding] OPPTS 870.4200 [§83-2b]; OECD 451.

PC CODE: 081901

DP BARCODE: 301496
SUBMISSION NO.: None

TEST MATERIAL (PURITY): Chlorothalonil (99.28% a.i.)

SYNONYMS: 2,4,5,6-tetrachloro-1,3-benzo-dicarbonitrile

CITATION: Spencer-Briggs, D.J. (1995) Chlorothalonil: potential tumorigenic effects in prolonged dietary administration to mice. Huntingdon Life Sciences Ltd, Huntingdon, England. Laboratory Study No.: VCM 16/943065, December 20, 1995. MRID 45710211. Unpublished.

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

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 45710211), Chlorothalonil (99.28% a.i.; Batch # NF 28/01) was administered in the diet to Crl:CD(SD)BR mice (50/sex/dose) at doses of 0, 15, 60, 240, or 960 ppm (0/0, 1.9/2.5, 7.8/9.9, 30.4/40.6, and 130/157 mg/kg/day in males/females) for up to 18 months.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, food utilization, or hematology.

Minimal to moderate epithelial hyperplasia of the nonglandular region of the stomach in al treated male groups (23-42 vs 10) and epithelial hyperplasia (limiting ridge) of the stomach in all treated male groups (13-32 vs 3) was observed.

Minimal to moderate epithelial hyperplasia and of the nonglandular region of the stomach was observed in ≥60 ppm females (27-42 vs 9). Folding of the epithelium of the nonglandular region of the stomach was observed in the ≥60 ppm males (16-18 vs 3). Hyperkeratosis of the nonglandular region of the stomach was observed in the ≥240 ppm males (39-45 vs 23) and ≥240 ppm females (45-47 vs 26). At ≥60 ppm in the kidney, increased ($p \leq 0.05$) incidences (# affected/50 in treated vs controls) of the following histological lesions were observed in males:

(i) minimal to moderate basophilic cortical tubules vs minimal in the controls (23-32 vs 12); (ii) cystic atrophic glomerulus/i with hypertrophic parietal epithelium (13-22 vs 4); and (iii) foci of mineralization in the cortex (17-19 vs 9; not statistically significant at 240 ppm). The increase in this spectrum of lesions indicates a treatment-related effect that is considered adverse because it suggests an acceleration of the natural, age-related degeneration of the kidney. Urinalysis or PAS staining (evaluating the integrity of the basement membrane) could have provided further information regarding the impact of these lesions on renal function.

At 960 ppm, eosinophilic droplets in keratin was observed in the stomach (7 vs 0) of males; and an increased incidence of enlarged kidneys were observed in males, and in uniform cortical scarring of the kidneys in both sexes. Additionally, the adjusted kidney weight was increased ($p \leq 0.01$) in both sexes. In the 960 ppm males, increased incidences of sinus histiocytosis and erythrophagocytosis were observed ($p \leq 0.05$) in the mesenteric lymph node. Focal epithelial hyperplasia of the nonglandular region of the stomach (7 vs 0) and papillomatous hyperplasia (nonglandular region and limiting ridge) was observed in the 960 ppm females (5 vs 0, each region).

The LOAEL is 15 ppm (equivalent to 1.9 mg/kg bw/day) in males, based on epithelial hyperplasia of the nonglandular region of the stomach and epithelial hyperplasia (limiting ridge) of the stomach. The NOAEL is < 15 ppm (equivalent to 1.9 mg/kg bw/day) in males. The LOAEL is 60 ppm (equivalent to 9.9 mg/kg bw/day) in females based on epithelial hyperplasia and of the nonglandular region of the stomach. The NOAEL is 15 ppm (equivalent to 2.5 mg/kg bw/day) in females.

In the 960 ppm females, increased incidences were observed of pulmonary adenocarcinoma (4/50 treated vs 1/50 controls) and multiple pulmonary adenocarcinoma (1 vs 0). Statistical analysis of the incidences of these lung neoplasia were not reported, nor were historical control data provided for any neoplastic lesion. Increased incidences of benign squamous cell papilloma (nonglandular type) in the stomach was observed in the 960 ppm males (4/50 treated vs 1/50 controls) and females (5/50 treated vs 0/50 controls). These increases were positive in the trend test at $p \leq 0.01$ in both sexes, and pairwise comparison to controls was significant ($p \leq 0.05$) in the 960 ppm females.

Classification of the carcinogenic potential will be addressed by the Agency. Dosing was considered adequate based on gastro- and nephrotoxicity.

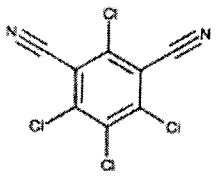
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil

Description:	White powder
Batch #:	NF 28/01
Purity (w/w):	99.28% a.i.
Stability of compound:	Stable in the diet for 4 days at room temperature or for 7 days refrigerated followed by 3 days at room temperature
CAS #:	1897-45-6
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Mouse
Strain:	Crl:CD(SD)BR
Age and mean weight at Week 1:	Approximately 7 weeks; 24-35 g males; 18-27 g females
Source:	Charles River Breeding Laboratories (Portage, MI)
Housing:	In same sex pairs in suspended cages with polypropylene bottoms and stainless steel wire tops
Diet:	Powdered SDS Rat and Mouse No. 1 modified maintenance diet, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	16-30°C
Humidity:	23-74%
Air changes:	Not reported
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	15 days

B. STUDY DESIGN

1. In life dates: Start: 11/27/92 End: 6/23/94

2. Animal assignment/dose levels: The animals were randomly assigned, stratified by weight, to the test groups shown in Table 1.

Table 1. Study design [a]

Nominal dose (ppm)	Actual Intake (mg/kg/day)	(#/sex)
0	0/0	50
15	1.9/2.5	50
60	7.8/9.9	50
240	30.4/40.6	50
960	130/157	50

a Data obtained from pages 16, 17, and 50 of MRID 45710211.

3. **Dose-selection rationale:** The Sponsor stated that the dose selection was based on the results of a 13-week toxicity study (HRC Report No. VCM 1/920342); however, data were not provided.

4. **Dose preparation 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. It was stated that in a prior study (HRC report VCM 1/920342), homogeneity and stability of 10 and 2500 ppm formulations were evaluated (results not presented in this study). As the diet was the same as that used in a combined chronic toxicity/carcinogenicity study in rats (MRID 45710212), stability data reported in that study are presented here. Homogeneity data were not presented. Samples of each dose formulation for Weeks 1, 13, 15 (60 ppm only), 26, 39, 52, 65, and 78 were analyzed to determine concentration.

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

Days (room temperature)	15 ppm	1500 ppm
3 [a]	-3.3	12.9
4	-6.7	4.8

a 7 days in the refrigerator followed by 3 days at room temperature

Concentration (% of nominal in all dose formulations): 91.0-104.5%, except 242% in the 60 ppm formulation on Week 13

The analytical data indicated that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Body weight gains, organ weight, food consumption, and clinical pathology data were subjected to the following statistical procedures (tested at $p \leq 0.05$ 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 heterogeneous. 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. Mortality was analyzed using log rank methods. The Sponsor stated that tumor incidence rates were analyzed according to IARC recommendations. The incidences of stomach squamous papilloma were analyzed by logrank methods. The incidence of tumors was evaluated using a test for heterogeneity, a one-tailed test for trend against dose level, a test for non-linearity in any dose-related trend, and one-tailed pairwise comparisons for each treated group against the control group.

C. METHODS

1. Observations: Animals were observed twice daily for morbidity and mortality, and once daily for signs of toxicity. A detailed palpation was performed daily for the first 4 weeks and weekly thereafter.

2. Body weight and body weight gain: All animals were weighed prior to treatment, weekly during the study, and at termination. Overall (Weeks 0-80) body weight gain was also reported.

3. Food consumption and utilization, and compound intake: Mean absolute food consumption (g/animal/day) was recorded twice weekly and reported weekly. Food utilization (food consumption/body weight gain) was reported for Weeks 1-4, 5-8, 9-12, 13-16, 17-20, 21-26, and 1-26. Compound intake values (mg/kg/day) were calculated using the food consumption, body weight, and nominal dietary concentration data.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed and are not required by the Guidelines (OPPTS 870.4200b/OECD 451).

5. Hematology and clinical chemistry: Venous blood smears were prepared from all mice killed during the study, where possible, and from 10 mice/sex/dose during Weeks 52 and 80/81. A leukocyte differential count was performed on the blood smears from mice killed during the study and from the control and 960 ppm groups at Weeks 52 and 80/81.

a. Hematology

	Hematocrit (HCT)	X	Leukocyte differential count*
	Hemoglobin (HGB)		Mean corpuscular hemoglobin (MCH)
	Leukocyte count (WBC)		Mean corpuscular hemoglobin concentration (MCHC)
	Erythrocyte count (RBC)		Mean corpuscular volume (MCV)
	Platelet (thrombocyte) count		Reticulocyte count
	Blood clotting measurements	X	Cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (Control and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451.

b. Clinical chemistry: Clinical chemistry evaluations were not performed and are not required by the Guidelines (OPPTS 870.4200/OECD 451).

6. Urinalysis: Urinalysis was not performed and is not required by the Guidelines (OPPTS 870.4200/OECD 451).

7. Sacrifice and pathology: All animals that died or were sacrificed *in extremis* and those sacrificed on schedule were subjected to gross pathological examination when possible (n=49-50), and the following CHECKED (X) tissues were collected for histological examination. Additionally, the (XX) organs were weighed from all survivors, as well as animals that died or were sacrificed *in extremis* at the discretion of the pathologist.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	X	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (optic nerves)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*		UROGENITAL	X	Adrenal glands*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Parathyroids*
X	Rectum*	XX	Testes*+ with epididymides	X	Thyroid*
XX	Liver*+	X	Prostate*	X	Harderian gland
X	Gall bladder* (not rat)	X	Seminal vesicle*		OTHER
	Bile duct* (rat)	X	Ovaries*+	X	Bone (femur and sternum)
X	Pancreas*	X	Uterus*+	X	Skeletal muscle
	RESPIRATORY	X	Mammary gland*	X	Skin*
X	Trachea*	X	Vagina	X	Joint (femurotibial)
X	Lung*++			X	Head
X	Nasal cavity*			X	All gross lesions and masses*
X	Pharynx*				
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200
\+ Organ weight required in carcinogenicity studies
++ Organ weight required if inhalation route

The Harderian gland, nasal cavity, lacrimal gland, head, larynx, pharynx, tongue, and vagina were not examined microscopically. All the other collected tissues were examined microscopically in all animals that were sacrificed *in extremis* or found dead; and in all animals in the 960 ppm and control groups. Additionally, samples of the lungs, livers, kidneys, and gross lesions were examined from all dose groups.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity: No treatment-related effect was observed on clinical signs.

2. Mortality: No treatment-related effect was observed on mortality. The guideline survival requirements of 50% at Week 65 and 25% at Week 78 were met.

B. BODY WEIGHT: No differences ($p \leq 0.05$) from controls were observed in body weights in any treated group throughout treatment (Table 2). Overall (Weeks 0-80) body weight gain was decreased (not statistically significant) by 10-11% in the 960 ppm group; however, the group

variation was large, and this effect was considered incidental due to the lack of an appreciable effect on body weights throughout the study.

Table 2. Mean body weights and body weight gains (g) at selected intervals in mice treated with Chlorothalonil in the diet for up to 18 months.[a]

Weeks	Dose (ppm)				
	0	15	60	240	960
Males (n=29-50)					
0	30	29	29	29	30
13	43	42	41	43	41
80	50	51	49	49	48
Weeks 0-80	19.9±6.2	21.7±5.9	20.2±5.6	20.0±5.4	17.9±7.1 (↓10)
Females (n=36-50)					
0	22	22	22	22	22
13	30	30	30	30	30
80	40	42	41	39	37
Weeks 0-80	17.5±7.4	19.3±8.2	18.7±7.9	16.8±7.0	15.6±4.4 (↓11)

a Data were obtained from pages 41-43 of MRID 45710211. Percent difference from controls, calculated by the reviewers, is included in parentheses.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: No treatment-related effects were observed on food consumption. Overall (Weeks 1-80) group mean food consumption was decreased ($p \leq 0.05$) by 8% in the 960 ppm females. This effect was considered minimal. There were no other differences ($p \leq 0.05$) observed.

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

3. Food utilization: No treatment-related effect was observed on food utilization.

D. BLOOD ANALYSES: No treatment-related effect was observed on hematology.

E. SACRIFICE AND PATHOLOGY

1. Organ weights: Terminal body weight-adjusted kidney weight was increased ($p \leq 0.01$) in the 960 ppm males (↑26%) and ≥240 ppm females (↑10-19%; Table 3). Other organ weights in the treated groups were similar to controls.

Table 3. Mean absolute and adjusted (for terminal body weight) kidney weights (g) in mice treated with Chlorothalonil in the diet for up to 18 months.[a]

Parameter	Dose (ppm)				
	0	15	60	240	960
Males (n=29-40)					
Terminal body weight	48.9	49.3	48.6	48.2	46.5
Kidney, Absolute weight	0.796	0.820	0.857	0.855	0.987
Adjusted weight	0.791	0.814	0.855	0.856	0.998** (126)
Females (n=36-43)					
Terminal body weight	38.5	40.7	40.0	38.6	36.7
Kidney, Absolute weight	0.478	0.494	0.522	0.540	0.573
Adjusted weight	0.48	0.48	0.50	0.53** (110)	0.57** (119)

a Data were obtained from pages 53-54 of MRID 45710211. Percent difference from controls, calculated by the reviewers, is included in parentheses.

** Significantly different from controls; p≤0.01

2. **Gross pathology:** At 960 ppm, increased incidences (# affected/50 in treated vs controls) were observed of enlarged kidney in males (5 vs 0) and uniform cortical scarring of the kidney in males (12 vs 8) and females (8 vs 3; Table 4). Increased incidences of the following lesions were observed in the forestomach in both sexes: (i) excrescence/s in the 960 ppm males (6 vs 1) and females (12 vs 0); (ii) thickened in the 960 ppm males (10 vs 6) and ≥240 ppm females (8-15 vs 3); (iii) white in the 960 ppm males (11 vs 6) and females (12 vs 2); (iv) roughened in the ≥240 ppm males (11-38 vs 4) and females (16-38 vs 6); and (v) limiting ridge prominent in the 960 ppm males (14 vs 2) and ≥240 ppm females (5-14 vs 0). Increased incidences of the stomach corpus mucosa of the 960 ppm males being pale (7 vs 2) and thickened in area/s (10 vs 4) were observed. The incidences of other gross lesions in the treated groups were similar to controls.

Table 4. Incidence (# affected/50) of selected gross lesions in mice treated with Chlorothalonil in the diet for up to 18 months. [a]

Gross lesion	Dose (ppm)				
	0	15	60 [b]	240	960
Males					
Kidney					
Enlarged	0	1	1	2	5
Uniform cortical scarring	8	7	5	8	12
Forestomach					
Excrescence/s	1	0	0	2	6
Thickened	6	2	7	5	10
White	6	6	7	3	11
Roughened	4	7	7	11	38
Limiting ridge prominent	2	3	2	2	14
Stomach Corpus Mucosa					
Pale	2	3	2	2	7
Thickened area/s	4	6	5	7	10
Females					
Kidney					
Uniform cortical scarring	3	4	5	6	8
Forestomach					
Excrescence/s	0	1	2	2	12
Thickened	3	3	4	8	15
White	2	3	6	4	12
Roughened	6	7	3	16	38
Limiting ridge prominent	0	0	1	5	14

a Data were obtained from pages 55-64 of MRID 45710211.
b n=49 in females

3. Microscopic pathology

a. Non-neoplastic: Treatment-related non-neoplastic lesions (# affected/49-50) are detailed in Tables 5a and 5b. Increased ($p \leq 0.05$) incidences in the following lesions (# affected/50 in treated vs controls) were observed in the male kidney: (i) minimal to moderate basophilic cortical tubules at ≥60 ppm vs minimal in the controls (23-32 vs 12); (ii) cystic atrophic glomerulus/i with hypertrophic parietal epithelium at ≥60 ppm (13-22 vs 4); (iii) minimal dilated cortical tubules at 960 ppm (8 vs 2); and (iv) minimal foci of mineralization in the cortex at ≥60 ppm (17-19 vs 9; NS at 240 ppm). In the esophagus, eosinophilic droplets of keratin were observed in the ≥240 ppm males (30-40 vs 0) and females (25-36 vs 0), and minimal to moderate hyperplasia was observed in the 960 ppm males (16 vs 0). In the stomach, increased incidences (# affected/49-50 in treated vs controls) of the following lesions were observed:

(i) hyperkeratosis (nonglandular epithelium) in the ≥240 ppm males (39-45 vs 23) and ≥240 ppm females (45-47 vs 26); (ii) eosinophilic droplets in keratin in the 960 ppm males (7 vs 0); (iii) minimal to moderate epithelial hyperplasia (nonglandular region) in all treated males (23-42 vs 10) and ≥60 ppm females (27-42 vs 9); (iv) minimal to moderate (except 2 marked conditions in the 960 ppm males) epithelial hyperplasia (limiting ridge) in all treated males (13-32 vs 3) and the 960 ppm females (19 vs 6); (v) folding of the epithelium (nonglandular) in the ≥60 ppm males (16-18 vs 3) and ≥240 ppm females (23-29 vs 5); (vi) moderate to marked focal epithelial hyperplasia (nonglandular region) in the 960 ppm females (7 vs 0); and (vii) papillomatous hyperplasia (nonglandular region and limiting ridge) in the 960 ppm females (5 vs 0, each region).

In the 960 ppm males, increased incidences (# affected/50 in treated vs control; $p \leq 0.05$) were observed in sinus histiocytosis (25 vs 12) and erythrophagocytosis (13 vs 5) in the mesenteric lymph node, but were considered equivocally treatment-related. It was stated that these abnormalities could be related to the toxicity observed in the GI system. An increased incidence of areas of hypertrophic cells in the zona fasciculata of the adrenals was observed in the ≥60 ppm males (17-19 vs 8; $p \leq 0.05$ at 60 and 960 ppm); however, there was no other corroborating evidence of toxicity to the adrenals. Therefore, this effect was not considered adverse. The incidence of all other microscopic lesions were considered unrelated to treatment.

Table 5a. Incidence (# affected/50) of selected non-neoplastic microscopic lesions in male mice treated with Chlorothalonil in the diet for up to 18 months. [a]

Microscopic lesion	Dose (ppm)				
	0	15	60	240	960
Kidneys					
Basophilic cortical tubules (Total)	12	20	23*	24*	32**
Minimal	12	20	22*	22*	30**
Moderate	0	0	1	2	2
Cystic atrophic glomerulos/i with hypertrophic parietal epithelium [b]	4	1	13*	22**	22**
Dilated cortical tubules (Minimal)	2	3	2	1	8*
Foci of mineralization in the cortex (Minimal)	9	7	19*	17	19*
Esophagus					
Eosinophilic droplets in keratin [b]	0	0	0	30**	40**
Epithelial hyperplasia (Total)	0	2	0	3	16**
Minimal	0	1	0	3	14
Moderate	0	1	0	0	2
Stomach					
Hyperkeratosis - nonglandular epithelium [b]	23	22	27	39**	45**
Eosinophilic droplets in keratin [b]	0	0	0	2	7**
Epithelial hyperplasia - nonglandular region (Total)	10	23**	31**	37**	42**
Minimal	8	18*	14	18*	15
Moderate	2	5	17**	19**	27**
Epithelial hyperplasia - limiting ridge (Total)	3	13**	13**	23**	32**
Minimal	1	10**	11**	19**	21**
Moderate	2	3	2	4	9*
Marked	0	0	0	0	2
Folding of the epithelium - nonglandular [b]	3	6	18**	16**	16**

a Data were obtained from pages 30-33 and 72-95 of MRID 45710211.
b Severity data were not provided.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

Table 5b. Incidence (# affected/50) of selected non-neoplastic microscopic lesions in female mice treated with Chlorothalonil in the diet for up to 18 months.[a]

Microscopic lesion	Dose (ppm)				
	0	15[b]	60[b]	240	960
Esophagus, Eosinophilic droplets in keratin[b,c]	0	0	0	25**	36**
Stomach					
Hyperkeratosis - nonglandular epithelium[c]	26	20	27	47**	45**
Epithelial hyperplasia - nonglandular region (Total)	9	14	27**	42**	41**
Minimal	8	12	22**	12	15
Moderate	1	2	5	30**	26**
Epithelial hyperplasia - limiting ridge (Total)	6	5	3	7	19**
Minimal	6	3	3	6	13
Moderate	0	2	0	1	6*
Folding of the epithelium - nonglandular[c]	5	7	8	23**	29**
Focal epithelial hyperplasia - nonglandular region (Total)	0	2	1	3	7**
Minimal	0	1	0	0	0
Moderate	0	1	0	2	1
Marked	0	0	1	1	6*
Papillomatous hyperplasia - nonglandular region[c]	0	2	1	2	5*
Papillomatous hyperplasia - limiting ridge[c]	0	0	0	1	5*

a Data were obtained from pages 31-32 and 96-117 of MRID 45710211.
b n=49
c Severity data were not provided

b. Neoplastic: Neoplasia data are included in the Appendix. Increased incidences of benign squamous cell papilloma (nonglandular type) in the stomach was observed in the 960 ppm males (4/50 treated vs 1/50 controls) and females (5/50 treated vs 0/50 controls; Table 6). These increases were positive in the trend test at $p \leq 0.01$ in both sexes, and pairwise comparison to controls was significant ($p \leq 0.05$) in the 960 ppm females. In the 960 ppm females, increased incidences were observed of pulmonary adenocarcinoma (4/50 treated vs 1/50 controls) and multiple pulmonary adenocarcinoma (1 vs 0). Although the Sponsor did not specifically address the pulmonary adenocarcinoma, it was stated that, other than squamous cell papilloma in the stomach, the observed tumor profile was that expected for this strain of mouse in this laboratory. Statistical analysis of the incidences of these lung neoplasia were not reported, nor were historical control data provided for any neoplastic lesion. Consequently, Chlorothalonil was considered to have carcinogenic potential. The incidences of other neoplastic lesions in the treated groups were similar to controls.

Table 6. Incidence (# affected/50) of selected neoplasia in mice treated with Chlorothalonil in the diet for up to 18 months.[a]

Neoplastic lesion	Dose (ppm)				
	0	15[b]	60[b]	240	960
Males					
Stomach, Squamous cell papilloma-nonglandular type (Benign)	1[c]	0	0	2	4
Malignant tumors	0	0	0	0	0
Benign and/or malignant tumors	1	0	0	2	4
Females					
Lungs					
Pulmonary adenoma (Benign)	6	3	3	2	1
Pulmonary adenocarcinoma (Malignant)	1	2	2	2	4
Pulmonary adenocarcinoma-multiple (Malignant)	0	0	1	0	1
Benign and/or malignant adenomas[d]	7	2	6	4	6
Stomach, Squamous cell papilloma-nonglandular type (Benign)	0[c]	0	1	0	5*
Malignant tumors	0	0	0	0	0
Benign and/or malignant tumors	0	0	1	0	5

a Data were obtained from pages 66, 68, and 69 of MRID 45710211
b n=49 in females, except lungs at 15 ppm
c Statistically significant positive trend ($p \leq 0.01$)
d Data were tabulated by the reviewers from the individual data on pages 1016-1658 of MRID 45710211.
* Statistically significant pairwise comparison to control ($p \leq 0.05$)

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The LOAEL was 60 ppm based on nephrotoxicity. The NOAEL was 15 ppm. There was no evidence of tumorigenic potential.

B. REVIEWER COMMENTS: No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, food utilization, or hematology.

Minimal to moderate epithelial hyperplasia of the nonglandular region of the stomach in al treated male groups (23-42 vs 10) and epithelial hyperplasia (limiting ridge) of the stomach in all treated male groups (13-32 vs 3) was observed.

Minimal to moderate epithelial hyperplasia and of the nonglandular region of the stomach was observed in ≥60 ppm females (27-42 vs 9). Folding of the epithelium of the nonglandular region of the stomach was observed in the ≥60 ppm males (16-18 vs 3). Hyperkeratosis of the nonglandular region of the stomach was observed in the ≥240 ppm males (39-45 vs 23) and ≥240 ppm females (45-47 vs 26). At ≥60 ppm in the kidney, increased ($p \leq 0.05$) incidences (# affected/50 in treated vs controls) of the following histological lesions were observed in males:

(i) minimal to moderate basophilic cortical tubules vs minimal in the controls (23-32 vs 12); (ii) cystic atrophic glomerulus/i with hypertrophic parietal epithelium (13-22 vs 4); and (iii) foci of mineralization in the cortex (17-19 vs 9; not statistically significant at 240 ppm). The increase in this spectrum of lesions indicates a treatment-related effect that is considered adverse because it suggests an acceleration of the natural, age-related degeneration of the kidney. Urinalysis or PAS staining (evaluating the integrity of the basement membrane) could have provided further information regarding the impact of these lesions on renal function.

At 960 ppm, eosinophilic droplets in keratin was observed in the stomach (7 vs 0) of males; and an increased incidence of enlarged kidneys were observed in males, and in uniform cortical scarring of the kidneys in both sexes. Additionally, the adjusted kidney weight was increased ($p \leq 0.01$) in both sexes. In the 960 ppm males, increased incidences of sinus histiocytosis and erythrophagocytosis were observed ($p \leq 0.05$) in the mesenteric lymph node. Focal epithelial hyperplasia of the nonglandular region of the stomach (7 vs 0) and papillomatous hyperplasia (nonglandular region and limiting ridge) was observed in the 960 ppm females (5 vs 0, each region).

The LOAEL is 15 ppm (equivalent to 1.9 mg/kg bw/day) in males, based on epithelial hyperplasia of the nonglandular region of the stomach and epithelial hyperplasia (limiting ridge) of the stomach. The NOAEL is < 15 ppm (equivalent to 1.9 mg/kg bw/day) in males. The LOAEL is 60 ppm (equivalent to 9.9 mg/kg bw/day) in females based on epithelial hyperplasia and of the nonglandular region of the stomach. The NOAEL is 15 ppm (equivalent to 2.5 mg/kg bw/day) in females.

In the 960 ppm females, increased incidences were observed of pulmonary adenocarcinoma (4/50 treated vs 1/50 controls) and multiple pulmonary adenocarcinoma (1 vs 0). Statistical analysis of the incidences of these lung neoplasia were not reported, nor were historical control data provided for any neoplastic lesion. Increased incidences of benign squamous cell papilloma (nonglandular type) in the stomach was observed in the 960 ppm males (4/50 treated vs 1/50 controls) and females (5/50 treated vs 0/50 controls). These increases were positive in the trend test at $p \leq 0.01$ in both sexes, and pairwise comparison to controls was significant ($p \leq 0.05$) in the 960 ppm females. It was stated that the stomach tumors may have resulted from chronic gastric mucosa irritation.

Classification of the carcinogenic potential will be addressed by the Agency. Dosing was considered adequate based on nephrotoxicity.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

C. **STUDY DEFICIENCIES:** Historical control data for neoplasia were not provided. Additionally, the following minor deficiencies were noted:

- Data for the dose-selection rationale were not provided.
- Very few standard deviations were included with mean data.

- Some data (i.e. body weights) were not statistically analyzed.
- Homogeneity data on the formulated diets were not submitted.
- The nasal cavity, pharynx, and larynx were not examined microscopically.
- Tabulated summary data for clinical signs of toxicity were not provided.
- The heart, spleen, ovaries, uterus, and adrenal glands were not weighed.

APPENDIX

Neoplastic findings

	Group 1		Group 2		Group 3		Group 4		Group 5	
Males on study	50		50		50		50		50	
Animals completed	Decedent 21	Terminal 29	Decedent 17	Terminal 33	Decedent 12	Terminal 38	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Lymphoid/multicentric Tumours										
Examined	0	2	1	1	2	2	1	0	0	3
Malignant lymphoma (Malignant)	0	2	0	1	1	0	1	0	0	1
Pleomorphic lymphoma (Malignant)	0	0	1	0	0	2	0	0	0	2
Histiocytic sarcoma (Malignant)	0	0	0	0	1	0	0	0	0	0
Lungs										
Examined	21	29	17	33	12	38	12	38	10	40
Pulmonary adenoma (Benign)	4	11	2	6	0	7	2	6	1	10
Pulmonary adenomata - multiple (Benign)	0	1	0	0	0	0	0	0	0	0
Pulmonary adenocarcinoma (Malignant)	1	2	1	2	1	4	1	0	0	4
Pulmonary adenocarcinomata - multiple (Malignant)	2	0	0	0	0	0	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Heart										
Examined	21	29	17	2	12	3	12	2	10	40
Haemangiosarcoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Lymph Nodes - Mesenteric										
Examined	21	29	17	33	12	38	12	38	10	40
Haemangioma (Benign)	0	1	0	0	0	0	0	0	0	0
Spleen										
Examined	21	29	17	2	11	2	12	2	10	40
Haemangiosarcoma (Malignant)	0	0	2	0	0	0	0	0	0	0
Liver										
Examined	21	29	17	33	12	38	12	38	10	40
Hepatocellular adenoma (Benign)	3	6	1	10	0	3	3	5	0	7
Hepatocellular adenomata (Benign)	1	1	0	0	0	2	0	2	0	1

(Neoplastic findings - continued)

	Group 1		Group 2		Group 3		Group 4		Group 5	
Males on study	50		50		50		50		50	
Animals completed	Decedent 21	Terminal 29	Decedent 17	Terminal 33	Decedent 12	Terminal 38	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Liver	(continued)									
Hepatocellular carcinoma (Malignant)	1	1	0	2	1	2	1	1	1	0
Hepatocellular carcinomata (Malignant)	0	0	1	1	0	0	0	1	0	1
Haemangiosarcoma (Malignant)	2	0	0	0	0	1	0	0	0	0
Kidneys										
Examined	21	29	17	33	12	38	12	38	10	40
Renal adenoma (Benign)	0	0	0	1	0	1	0	1	0	0
Epididymides										
Examined	21	29	17	3	12	1	12	1	10	40
Interstitial cell tumour (Benign)	0	0	0	0	0	0	0	0	0	1
Testes										
Examined	21	29	17	6	12	3	12	5	10	40
Haemangioma (Benign)	0	0	0	0	0	0	0	1	0	0
Interstitial cell tumour (Benign)	0	1	0	2	0	2	0	2	0	0
Adrenals										
Examined	21	29	17	33	12	38	12	38	10	40
Cortical adenoma (Benign)	0	1	0	0	0	0	0	0	0	1
Cortical adenoma (fusiform cell type) (Benign)	0	0	0	1	0	0	0	0	0	0
Skeletal Muscle										
Examined	21	29	17	0	12	0	12	0	10	40
Haemangiosarcoma (Malignant)	1	0	0	0	0	0	0	0	0	0
Stomach										
Examined	21	29	17	33	12	38	12	38	10	40
Squamous cell papilloma - nonglandular region (Benign)	1	0	0	0	0	0	0	2	0	4
Skin										
Examined	21	29	17	3	12	3	12	1	10	40

(Neoplastic findings - continued)

	Group 1		Group 2		Group 3		Group 4		Group 5	
Males on study	50		50		50		50		50	
Animals completed	Decedent 21	Terminal 29	Decedent 17	Terminal 33	Decedent 12	Terminal 38	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Skin	(continued)									
Basal cell tumour (Benign)	0	1	0	0	0	0	0	0	0	0
Harderian Glands										
Examined	0	0	0	0	0	0	0	1	0	1
Adenocarcinoma (Malignant)	0	0	0	0	0	0	0	1	0	0
Cystadenoma (Benign)	0	0	0	0	0	0	0	0	0	1
Femur/Joint										
Examined	21	29	17	0	12	0	12	0	10	40
Haemangioma (Benign)	0	0	1	0	0	0	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Adipose Tissue										
Examined	4	0	1	0	1	1	2	3	0	0
Malignant schwannoma (Malignant)	0	0	1	0	0	0	0	0	0	0

(Neoplastic findings - continued)

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent 7	Terminal 43	Decedent 11	Terminal 39	Decedent 14	Terminal 35	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Lymphoid/multicentric Tumours										
Examined	1	8	1	5	6	6	2	2	1	8
Malignant lymphoma (Malignant)	0	5	1	4	2	3	0	0	0	6
Pleomorphic lymphoma (Malignant)	0	2	0	0	2	2	1	1	1	1
Histiocytic sarcoma (Malignant)	1	1	0	1	2	1	1	1	0	1
Lungs										
Examined	7	43	11	39	14	35	12	38	10	40
Pulmonary adenoma (Benign)	1	5	1	2	0	3	0	2	0	1
Pulmonary adenocarcinoma (Malignant)	0	1	2	0	0	2	1	1	1	3
Pulmonary adenocarcinomata - multiple (Malignant)	0	0	0	0	1	0	0	0	0	1
Spleen										
Examined	7	43	11	3	14	5	12	5	10	40
Haemangioma (Benign)	0	0	0	0	0	0	0	0	1	0
Haemangiosarcoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Liver										
Examined	7	43	11	39	14	35	12	38	10	40
Hepatocellular adenoma (Benign)	0	2	0	1	0	0	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	0	1	0	0	0	0	0	0
Ovaries										
Examined	7	43	11	20	14	18	12	16	10	40
Cystadenoma (Benign)	0	0	0	1	0	0	0	1	0	0
Luteoma (Benign)	0	1	0	1	0	0	0	0	0	0
Granulosa cell tumour (Malignant)	0	0	0	0	0	1	0	0	0	0
Haemangioma (Benign)	0	1	0	0	0	0	0	1	0	0
Uterus										
Examined	7	43	11	32	14	30	12	35	10	40

(Neoplastic findings - continued)

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent 7	Terminal 43	Decedent 11	Terminal 39	Decedent 14	Terminal 35	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Uterus	(continued)									
Endometrial adenocarcinoma (Malignant)	0	0	0	0	1	0	0	1	0	0
Endometrial stromal sarcoma (Malignant)	0	1	0	0	1	0	1	0	0	2
Haemangioma (Benign)	0	0	0	0	0	1	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Leiomyoma (Benign)	0	1	0	2	0	0	0	1	0	0
Leiomyosarcoma (Malignant)	0	1	1	0	0	0	0	1	0	1
Cervix										
Examined	7	43	11	0	14	0	12	0	10	40
Fibrosarcoma (Malignant)	0	0	0	0	0	0	0	0	0	1
Stomach										
Examined	7	43	11	38	14	35	12	38	10	40
Squamous cell papilloma - nonglandular region (Benign)	0	0	0	0	0	1	0	0	0	5
Mammary Glands										
Examined	7	43	11	2	14	0	12	0	10	40
Mammary adenocarcinoma (Malignant)	0	0	1	2	0	0	0	0	0	0
Adenoacanthoma (Malignant)	0	0	0	0	0	0	0	0	0	1
Bone										
Examined	0	1	0	0	0	0	0	0	0	0
Osteoma (Benign)	0	1	0	0	0	0	0	0	0	0

Intergroup comparison of tumour incidence

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent 21	Terminal 29	Decedent 17	Terminal 33	Decedent 12	Terminal 38	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Number of tumour bearing animals	11	18	6	17	3	19	5	17	2	25
Animals with malignant tumours	7	5	4	5	3	9	3	3	1	8
Benign tumours	8	17	4	16	0	13	4	17	1	20
Multiple tumours	4	7	3	7	1	5	2	5	0	6
Single tumours	7	11	3	10	2	14	3	12	2	19
Multiple malignant tumours	0	0	2	1	1	0	0	0	0	0
Multiple benign tumours	1	6	0	4	0	2	1	2	0	5
Metastatic tumours	0	0	2	0	0	0	0	0	0	0

(Intergroup comparison of tumor incidence - continued)

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	50		50		50		50		50	
Animals completed	Decedent 7	Terminal 43	Decedent 11	Terminal 39	Decedent 14	Terminal 35	Decedent 12	Terminal 38	Decedent 10	Terminal 40
Number of tumour bearing animals	2	18	4	11	8	11	4	9	4	18
Animals with malignant tumours	1	11	4	6	8	9	4	5	4	14
Benign tumours	1	10	1	7	0	4	0	5	1	6
Multiple tumours	0	4	2	3	1	2	0	1	1	5
Single tumours	2	14	2	8	7	9	4	8	3	13
Multiple malignant tumours	0	0	1	2	1	0	0	0	0	3
Multiple benign tumours	0	1	0	0	0	1	0	0	0	0
Metastatic tumours	0	0	2	0	2	1	0	0	1	0

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Results of logrank analysis

Male mice

Dose level (ppm)	Number of animals with Tumours		Relative rate (O/E)	Pairwise comparison P-value*
	Observed (O)	Expected (E)		
0	1	1.23	0.81	
15	0	1.36	0.00	0.50
60	0	1.28	0.00	0.50
240	2	1.61	1.24	0.74
960	4	1.51	2.64	0.27

Treatment effect	χ^2	df	p-value
Heterogeneity	7.036	4	0.13
Trend*	6.045	1	0.007
Non-linearity	0.952	3	0.81

Female mice

Dose level (ppm)	Number of animals with Tumours		Relative rate (O/E)	Pairwise comparison P-value*
	Observed (O)	Expected (E)		
0	0	1.30	0.00	
15	0	1.24	0.00	1.00
60	1	1.06	0.94	0.46
240	0	1.18	0.00	1.00
960	5	1.21	4.12	0.028

Treatment effect	χ^2	df	p-value
Heterogeneity	15.973	4	0.003
Trend*	14.174	1	< 0.001
Non-linearity	1.736	3	0.63

\+ One-tailed test for trend against the dose levels.
* Comparisons are against Group 1 and are one-tailed
Initial group size = 50