DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §83-5; Combined Chronic Toxicity / Carcinogenicity Study in Rats

Work Assignment No. 2-01-35 A, formerly 1-01-35 A (MRID 45710212)

Prepared for
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DATA EVALUATION RECORD

TXR#: 0052493

STUDY TYPE: Combined chronic toxicity/carcinogenicity (diet)- rats; OPPTS 870.4300 [§83-5]; OECD 453.

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. (1996) Chlorothalonil: potential tumorigenic effects in prolonged dietary administration to rats. Huntingdon Life Sciences Ltd., *Huntingdon, England.* Laboratory Study No.: VCM 15/943286, January 17, 1996. MRID 45710212. Unpublished.

SPONSOR: Vischim S.r.l., Via Friuli, 55, 20031 Cesano Maderno, Milan, Italy

EXECUTIVE SUMMARY - In this combined chronic toxicity/carcinogenicity study (MRID 45710212), Chlorothalonil (99.28% a.i.; Batch No.: NF 28/01) was administered in the diet for 2 years to 50 Crl:CD (SD) BR rats/sex/dose at doses of 0, 15, 60, 240, and 1200 ppm (equivalent to 0/0, 0.7/0.9, 2.7/3.3, 10.6/13.9, and 54/70 mg/kg/day). Additionally, 20 rats/sex/dose were treated similarly and sacrificed at 12 months.

No treatment-related effect was observed on mortality, food and water consumption, food efficiency, ophthalmoscopic examinations, hematology, and non-protein thiol concentration in liver.

At 1200 ppm, an increased incidence of generalized yellow staining of the fur was observed in the interim and terminal groups (66-100% treated vs 0% controls). This sign was observed generally from Week 16 in males and Week 19 in females. Body weight gain was decreased ($p<0.05$) by 15-17% in the females during Weeks 0-1, 13-26, and 26-52; however, overall (Weeks 0-104) body weight gain was similar to controls, indicating recovery during the second year.

At 1200 ppm, nephrotoxicity was evident. Urinary protein concentration was increased ($p<0.05$) by 137-279% in the males at Weeks 52, 78, and 104. Adjusted (for terminal body weight) kidney weights were increased ($p<0.05$) in the males at Weeks 52 and 104 (incr 21-28%). Necropsy of the males, revealed uniform cortical scarring of the kidneys at the interim (15% treated vs 0% controls) and terminal (44% treated vs 14% controls) sacrifices. Kidney enlargement in the males (44% vs 20%) was also observed at the terminal necropsy. At the terminal sacrifice, there was an increased incidence of minimal to marked progressive glomerulonephrosis in the males (70% vs 52%) and the females (40% vs 26%).

At necropsy following the interim sacrifice, the forestomach in the ≥240 ppm groups was thickened, roughened, and white. Trace to moderate epithelial hyperplasia and hyperkeratosis in the non-glandular region and in the limiting ridge of the stomach was observed in these animals. At necropsy following the terminal sacrifice, the forestomach had depressions in the ≥60 ppm groups; and was thickened, roughened, and white in the ≥240 ppm groups; and limiting ridge thickened or prominent in the 1200 ppm males. There was an increased incidence in the following lesions in these animals: (i) epithelial hyperplasia and hyperkeratosis in the nonglandular region of the stomach in all treated animals; (ii) epithelial hyperplasia and hyperkeratosis in the limiting ridge of the stomach in ≥240 ppm males or the 1200 ppm females; (iii) stomach ulceration in the nonglandular region in the ≥60 ppm females; and (iv) submucosal fibrosis and inflammatory cells in the nonglandular region of the stomach in ≥60 ppm females.

The LOAEL is 15 ppm (0.9 mg/kg bw/day) in females based on an increased in the incidence and severity of epithelial hyperplasia, hyperkeratosis and ulceration of the nonglandular region of the stomach females, and 60 ppm (2.7 mg/kg bw/day) based on an increase in the incidence and severity of epithelial hyperplasia and hyperkeratosis of the nonglandular region of the stomach in males . The NOAEL is <15 ppm (0.9 mg/kg bw/day) in females and 15 ppm (0.7 mg/kg bw/day) in males.

In the 1200 ppm males, increased incidences were observed of squamous cell papilloma and carcinoma in the stomach (2-4% treated vs 0% controls $p\leq0.01$). The Sponsor stated that the stomach tumors resulted from prolonged gastric irritation. The reviewer agrees that chronic irritation may result in carcinogenesis. In the males in the 1200 ppm group, hepatocellular adenoma (8% treated vs 2% controls) was also increased in incidence. However, the increased incidence in all hepatocellular tumors was not significant. C-cell adenoma was increased in the thyroid of the ≥60 ppm males (2-4% treated vs 0% controls). C-cell carcinoma of the thyroid was only increased in males in the 240 ppm group. The magnitude of increase in each tumor incidence was low and may have been within biological variation; however, the carcinogenic potential of Chlorothalonil could not be adequately addressed due to the absence of historical control data.

Classification of the carcinogenic potential will be addressed by the Agency. Dosing was considered adequate based on decreased body weight in females and nephrotoxicity in both sexes.

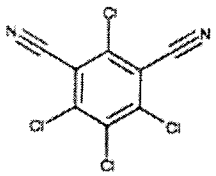
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4300; OECD 453) for a combined chronic toxicity/carcinogenicity study in rats.

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/Lot #:	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:	Rat
Strain:	Crl:CD (SD) BR
Age and mean weight at study initiation:	Approximately 7 weeks; 159-217 g males; 138-186 g females
Source:	Charles River Breeding Laboratories, Manston, Kent, England
Housing:	In groups of 5 (same sex) in suspended polypropylene cages with wire tops
Diet:	Ground Rat and Mouse No. 1 modified maintenance diet (Special Diet Services Limited, Essex, UK), *ad libitum* except during blood and urine sample collection
Water:	Tap water , *ad libitum* except during urine sample collection
Environmental conditions	
Temperature:	15-26°C
Humidity:	25-75%
Air changes:	Not reported
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	12-13 days

B. STUDY DESIGN

1. In life dates - Start: 11/30/92 End: 12/02/94

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

Table 1. Study design. [a]

Conc. in diet (ppm)	Dose to animal (mg/kg/day; M/F)	Terminal Sacrifice (24 months; rats/sex)	Interim Sacrifice (12 months; rats/sex)
0	0/0	50	20
15	0.7/0.9	50	20
60	2.7/3.3	50	20
240	10.6/13.9	50	20
1200	54/70	50	20

a Data were obtained from pages 17, 18, and 62 of MRID 45710212.

3. **Dose-selection rationale** - In a concurrently submitted subchronic oral toxicity study (MRID 45710205), 10 Crl:CD (SD) BR rats/sex/dose were exposed to Chlorothalonil in the diet at concentrations of 0, 30, 60, 300, or 1500 ppm nominally for up to 3 months. The LOAEL was not established; the NOAEL is 1500 ppm.

4. **Treatment preparation, analysis, and administration** - 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. Fifteen and 1500 ppm formulations were evaluated prior to treatment for homogeneity and stability (up to 4 days at room temperature, or 7 days refrigerated followed by 3 days at room temperature). Concentration analyses were reported for each dose preparation for Weeks 1, 13, 26, 39, 52, 65, 78, 91, and 104.

Results: Homogeneity (range as % CV): 2.49-7.71

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 (range as relative mean error in %, representing deviation from nominal): -10.0-6.7

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 was 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. Analysis included Peto's time-to-tumor methods, one-tailed test for trend against dose level, and one-tailed pairwise comparisons of each treated group against the combined control group. Where the trend test was statistically significant, the highest dose was excluded, and the trend test was repeated. This procedure was repeated until a statistically significant difference between the treated and control groups was no longer detected.

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 daily.

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, 1-4, 4-8, 8-13, 13-26, 26-52, 52-78, 78-104, and 0-104.

3. Food consumption and compound intake - The quantity of food consumed by each cage of rats was recorded twice each week, and group mean food consumption (g/animal/week) was reported weekly. Compound intake values (mg/kg/day) were calculated using the food consumption, body weight, and nominal dietary concentration data. Food efficiency (food consumption [g]/body weight gain [g]) was reported for 4-week intervals from Week 1-20, Weeks 21-26, and Weeks 1-26. Water consumption (g/animal/day) was reported daily during Weeks 12, 25, and 51 for the interim sacrifice groups.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals prior to initiation of treatment and on all surviving animals in the control and 1200 ppm groups during Weeks 52 and 104.

5. Hematology and clinical chemistry - Blood was collected from the orbital sinus of animals that were food-fasted overnight and under light ether anesthesia. Collections were made from 10 animals/dose/sex from the interim sacrifice groups on Weeks 13, 26, 52, and 54 and from the terminal sacrifice groups on Weeks 78 and 104. Samples collected at Week 54 were only analyzed for serum alanine aminotransferase and aspartate aminotransferase levels. Venous blood smears were prepared from all rats killed during the study, but were not examined. The CHECKED (X) hematology and clinical chemistry parameters were examined.

a. Hematology

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

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

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 (fasting)*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Sorbitol		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. Urinalysis - Urine was collected from 10 animals/dose/sex from the interim sacrifice groups on Weeks 13, 26, and 52 and from the terminal sacrifice groups on Weeks 78 and 104. The animals were fasted from food and water overnight for 16 hours during the collection. The following CHECKED (X) parameters were examined.

	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity / osmolality*	X	Bilirubin*
X	pH*		Blood/ red blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen
X	Total reducing substances (Clinitest)	X	–acetyl-β-D-glucosaminidase (NAG)
X	Heme pigments	X	Creatinine

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. **Sacrifice and pathology** - All animals, including those sacrificed on schedule or *in extremis* or found dead, were subjected to necropsy when possible. 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, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal/Harderian gland
X	Colon*	XX	Kidneys*+	XX	Thyroids*
X	Rectum*	X	Urinary bladder*	X	Parathyroids*
XX	Liver*+	XX	Testes with epididymides*+		OTHER
	Gall bladder* (not rat)	X	Prostate*	X	Bone (femur and sternum)
	Bile duct* (rat)	X	Seminal vesicle*	X	Skeletal muscle
X	Pancreas*	XX	Ovaries*+	X	Skin*
		X	Uterus*+	X	Knee joint
	RESPIRATORY	X	Mammary gland*	X	Head
X	Trachea*	X	Vagina	X	All gross lesions and masses*
X	Lung*++				
X	Nose*				
X	Pharynx*				
X	Larynx*				

a All designated tissues from the control and 1200 ppm groups were examined microscopically, except tongue, nose, pharynx, larynx, knee joint, head, and vagina. Additionally, all groups were examined in animals that were sacrificed *in extremis* or found dead. Also, tissues from the lung and liver from all survivors at Week 104 were examined microscopically, and tissues from the stomach, kidneys, and gross lesions were examined in all animals (all dose groups including those sacrificed on schedule or *in extremis*, or found dead).

* Recommended for combined chronic toxicity/carcinogenicity studies based on Guideline 870.4300

\+ Organ weight required in combined chronic toxicity/carcinogenicity studies

++ Organ weight required if inhalation route

8. **Measurement of non-protein thiol concentration in liver** - Liver samples were taken from 6 animals/dose/sex at study termination and assayed for non-protein thiol concentration by the

method of Boyland and Chasseaud. Tissues were homogenized in phosphate buffer, and the homogenate was diluted with sulphosalicylic acid solution. Samples were centrifuged, and non-protein thiol was quantified in the supernatant using DTNB by the method of Ellman.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - An increased incidence of generalized yellow staining of the fur was observed in the 1200 ppm group from the interim and terminal groups (66-100% treated vs 0% controls). This sign was observed generally from Week 16 in males and Week 19 in females.

Table 2a. Selected clinical observations (% affected) in rats treated with Clorothalonil for up to 1 year. [a]

Parameter	Dose (ppm)				
	0	15	60	240	1200
Males					
Generalized yellow staining of the fur	0	0	0	0	70
Females					
Generalized yellow staining of the fur	0	0	0	0	100

a Data (n=20) were tabulated by the reviewers from Appendix 9 of MRID 45710212.

Table 2b. Selected clinical observations (% affected) in rats treated with Clorothalonil for up to 2 years. [a]

Parameter	Dose (ppm)				
	0	15	60	240	1200
Males					
Generalized yellow staining of the fur	0	0	0	0	66
Females					
Generalized yellow staining of the fur	0	0	0	0	100

a Data (n=50) were tabulated by the reviewers from Appendix 9 of MRID 45710212.

2. Mortality - No treatment-related effect was observed. Mortality in treated groups was similar to controls. Survival exceeded guideline requirements of 50% at Week 78 and 25% at Week 104 in both sexes.

B. BODY WEIGHT AND BODY WEIGHT GAINS - Body weight was slightly decreased (statistical analysis not performed) in the 1200 ppm females throughout the study. Body weight gain was decreased ($p<0.05$) by 15-17% in the 1200 ppm females during Weeks 0-1, 13-26, and

26-52 (Table 3). A transient decrease ($p<0.01$) in body weight gain of 23% was observed in the 1200 ppm males during Weeks 0-1.

Table 3. Mean (±SD) body weights (g) at selected times in rats treated with Chlorothalonil for up to 104 weeks.[a]

Week(s)	Dose (ppm)				
	0	15	60	240	1200
Males					
0	194	194	194	193	193
13	552	546	548	543	531
52	802	788	781	793	776
104	772	826	759	845	760
BWG: 0-1	56±6.3	56±5.7	55±6.1	55±6.1	43±8.1** (↓23)
BWG: 13-26	115±32.4	109±28.8	107±28.9	116±30.1	112±34.6
BWG: 26-52	140±46.2	133±38.7	130±42.1	134±45.4	131±43.4
BWG: 0-104	579±118	634±108	570±118	655±156	568±113
Females					
0	165	162	161	161	161
13	324	316	315	314	309
26	375	366	363	361	352
52	483	464	477	466	441
104	616	548	545	527	548
BWG: 0-1	27±6.2	27±5.4	28±5.4	28±5.7	23±6.1** (↓15)
BWG: 13-26	51±19.5	50±23.4	48±22.5	46±16.6	43±14.2* (↓16)
BWG: 26-52	109±44.1	104±44.8	114±42.9	104±45.3	91±42.6* (↓17)
BWG: 0-104	453±124	388±79	385±129	367±107	387±87

a Data obtained from pages 50-53 of MRID 45710212. Percent difference from controls, calculated by reviewers, is included in parentheses.
* Significantly different from controls; $p<0.05$
** Significantly different from controls; $p<0.01$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption** - No treatment-related effect was observed on food consumption

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

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

4. **Water consumption** - No treatment-related effect was observed on water consumption.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related effects were observed during the ophthalmoscopic examinations.

E. BLOOD ANALYSES

1. **Hematology** - No treatment-related adverse effects were observed on hematology. Increases ($p<0.05$) in packed cell volume, hemoglobin, and erythrocytes, and decreases in mean corpuscular hemoglobin concentration were sometimes observed in the 1200 ppm males, but these differences were of no biological importance. Other minor differences ($p<0.05$) were also observed.

2. **Clinical chemistry** - Cholesterol was increased ($p<0.05$) by 38-60% in the 1200 ppm males at Weeks 52 and 78. Serum alanine aminotransferase was decreased ($p<0.05$) in the 1200 ppm males throughout the study (↓41-83%) and in the ≥240 ppm females at Weeks 13, 26, and 104 (↓26-60%). Further experimentation suggested that this decrease was due to a decrease in pyridoxal-5'-phosphate, a necessary cofactor in the metabolism of Chlorothalonil. Other differences ($p<0.05$) were minor, transient, and/or not considered adverse.

F. URINALYSIS - Urinary protein concentration was increased ($p<0.05$) by 137-279% in the 1200 ppm males at Weeks 52, 78, and 104. All other differences ($p<0.05$) were transient.

G. SACRIFICE AND PATHOLOGY

1. **Organ weights** - Adjusted (for terminal body weight) kidney weights were increased ($p<0.05$) in the ≥60 ppm males at Week 52 (↑9-28%) and the 1200 ppm males at Week 104 (↑21%). At Week 52, slight increases ($p<0.05$) in adjusted thyroid (↑19%), liver (↑14%), and kidney (↑13%) weights in the 1200 ppm females were observed, but were not observed at Week 104.

Table 4. Mean (±SD) absolute and adjusted (g) kidney weights in male rats treated with Chlorothalonil.[a]

Weight		Dose (ppm)				
		0	15	60	240	1200
Interim sacrifice, 52 weeks (n=18-28)						
Terminal body		755	782	795	758	736
Kidney	Absolute	4.40	4.67	4.92	5.15	5.57
	Adjusted[b]	4.43	4.62	4.83* (↑9)	5.17** (↑17)	5.66** (↑28)
Terminal sacrifice, 104 weeks (n=14-19)						
Terminal body		759	817	749	835	751
Kidney	Absolute	5.62	5.77	4.96	5.90	6.96
	Adjusted[b]	5.63	5.57	4.98	5.68	6.81** (↑21)

a Data were obtained from pages 90 and 92 of MRID 45710212. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Adjusted for terminal body weights
* Significantly different from controls; $p<0.05$
** Significantly different from controls; $p<0.01$

2. **Gross pathology** - At the interim sacrifice (Table 5a), the forestomach in the ≥240 ppm groups was thickened (90-100% treated vs 0% controls), roughened (60-95% vs 0%; excludes 240 ppm females), and white (15-95% vs 0%). In the ≥240 ppm males, uniform cortical scarring of the kidneys was observed (15% each treated vs 0% controls). Other gross observations or lesions were observed (% treated vs % controls) in the females at the interim sacrifice that were not observed at the terminal sacrifice including badly groomed fur at 1200 ppm (35% vs 0%), subcutaneous mass/es at ≥240 ppm (20% each vs 0%), and pale focus/i or area/s on the lung at 1200 ppm (30% vs 5%). The incidences of other gross lesions in the treated groups were similar to controls.

At the terminal sacrifice (Table 5b), the forestomach had depressions in the ≥60 ppm groups (42-68% treated vs 12-16% controls); and was thickened (36-70% vs 4-8%), roughened (18-60% vs 2-8%), and white (14-32% vs 2-6%) in the ≥240 ppm groups; and limiting ridge thickened or prominent in the 1200 ppm males (12% vs 0%). In the male kidney, uniform cortical scarring at ≥240 ppm (22-44% treated vs 14% controls) and enlargement at 1200 ppm (44% vs 20%) were observed. In the 1200 ppm males, the liver was enlarged (26% treated vs 10% controls) with pale subcapsular areas (34% vs 18%). An increased incidence of pale extra-orbital lachrymal glands were observed in the ≥60 ppm males (30-42% treated vs 20% controls), and ovarian masses were observed at 1200 ppm (12% vs 0%); however, microscopic pathology did not corroborate toxicity in these organs. The incidences of other gross lesions in the treated groups were similar to controls.

Table 5a. Incidence (%) of selected macroscopic lesions in rats treated with Chlorothalonil for up to 1 year.[a]

Lesion	Dose (ppm)				
	0	15	60	240	1200
Males					
Forestomach					
Thickened	0	0	0	90	100
Roughened	0	0	0	60	95
White	0	0	0	15	25
Kidneys, uniform cortical scarring	0	5	5	15	15
Females					
Forestomach					
Thickened	0	0	5	95	100
Roughened	0	0	0	5	75
White	0	0	0	35	95
Fur, badly groomed	0	0	0	0	35
Subcutaneous mass/es	0	5	0	20	20
Lung, pale focus/i or area/s	5	10	10	10	30

a Data (n=20) were obtained from pages 95-101 of MRID 45710212.

Table 5b. Incidence (%) of selected macroscopic lesions in rats treated with Chlorothalonil for up to 2 years. [a]

Lesion	Dose (ppm)				
	0	15	60	240	1200
Males					
Forestomach, Depression/s	16	16	42	60	62
Thickened	4	12	12	40	60
Roughened	2	4	4	20	52
White	2	6	6	14	22
Limiting ridge thickened or prominent	0	0	0	2	12
Kidneys, Uniform cortical scarring	14	10	14	22	44
Enlarged	20	26	12	22	44
Liver, Enlarged	10	14	4	16	26
Pale subcapsular area/s	18	20	16	22	34
Extra-orbital lachrymal glands, Pale	20	20	30	38	42
Females					
Forestomach, Depression/s	12	12	42	64	68
Thickened	8	0	14	36	70
Roughened	8	2	4	18	60
White	6	0	6	14	32
Ovaries, Mass/es	0	0	4	0	12

a Data (n=50) were obtained from pages 102-114 of MRID 45710212.

3. Microscopic pathology

a. Non-neoplastic - The incidence of selected Non-neoplastic lesions observed at the interim and terminal sacrifices are reported in Tables 6a and 6b, respectively. At the interim sacrifice, there was increased incidences in all treated groups in minimal to moderate centrilobular hepatocyte enlargement (30-94% treated vs 0% controls); and trace to moderate epithelial hyperplasia and hyperkeratosis in the non-glandular region (40-100% vs 0-6%) and in the limiting ridge (5-94% vs 0%; minimal or minimal to moderate) of the stomach. An increased incidence of ultimobranchial body in the thyroid was observed in the 1200 ppm males (28% treated vs 0% controls), but was not observed in the females nor at the terminal sacrifice. Other increases in lesion incidence were minor or were slight and not observed at terminal sacrifice.

At the terminal sacrifice, there was an increased incidence (% treated vs % controls) in the following lesions: (i) minimal to marked progressive glomerulonephrosis in the 1200 ppm males (70% vs 52%) and the ≥240 ppm females (36-40% vs 26%); (ii) minimal or minimal to moderate centrilobular hepatocyte enlargement in the 1200 ppm males (38% vs 18%) and ≥240 ppm

females (18-20% vs 4%); (iii) trace to marked or minimal to marked epithelial hyperplasia and hyperkeratosis in the nonglandular region of the stomach in all treated males and females (20-100% vs 12%); (iv) minimal or minimal to moderate epithelial hyperplasia and hyperkeratosis in the limiting ridge of the stomach in ≥240 ppm males (14-38% vs 4%) and the 1200 ppm females (22% vs 4%); (v) stomach ulceration in the nonglandular region in the ≥60 ppm females (20-40% treated vs 0% controls); and (vi) trace to moderate submucosal fibrosis and inflammatory cells in the nonglandular region of the stomach in ≥60 ppm females (18-28% vs 6%). Other differences in incidence and/or severity of lesions were minor or unrelated to dose.

Table 6a. Selected non-neoplastic histological findings (%) in rats treated with Clorothalonil for 1 year.[a]

Non-neoplastic lesion	Dose (ppm)				
	0	15	60	240	1200
Males					
Liver					
Centrilobular hepatocyte enlargement, total	0	37	56	89	94
Minimal	0	37	50	53	89
Moderate	0	0	6	37	6
Stomach					
Epithelial hyperplasia and hyperkeratosis - nonglandular region, total	6	42	78	100	100
Trace	0	0	0	0	0
Minimal	6	42	78	32	28
Moderate	0	0	0	68	72
Epithelial hyperplasia and hyperkeratosis - limiting ridge, total	0	5	6	16	94
Minimal	0	5	6	16	89
Moderate	0	0	0	0	6
Females					
Liver					
Centrilobular hepatocyte enlargement, total	0	30	35	63	56
Minimal	0	30	35	58	50
Moderate	0	0	0	5	6
Stomach					
Epithelial hyperplasia and hyperkeratosis - nonglandular region, total	0	40	50	100	100
Trace	0	0	5	0	0
Minimal	0	40	45	74	44
Moderate	0	0	0	26	56
Moderate epithelial hyperplasia and hyperkeratosis - limiting ridge, total	0	10	15	32	50

a Data (n=18-20) obtained from pages 117, 124, and 125 of MRID 45710212.

Table 6b. Selected non-neoplastic histological findings (%) in rats treated with Chlorothalonil for 2 years.[a]

Non-neoplastic lesion	Dose (ppm)				
	0	15	60	240	1200
Males					
Kidney					
Progressive glomerulonephrosis, total	52	60	44	60	70
Minimal	28	32	18	28	24
Moderate	14	18	16	24	14
Marked	10	10	10	8	32
Liver					
Minimal centrilobular hepatocyte enlargement, total	18	2	12	14	38
Stomach					
Epithelial hyperplasia and hyperkeratosis - nonglandular region, total	12	20	42	100	100
Minimal	6	10	20	26	34
Moderate	6	10	22	62	60
Marked	0	0	0	2	6
Minimal epithelial hyperplasia and hyperkeratosis - limiting ridge, total	4	0	8	14	38
Females					
Kidney					
Progressive glomerulonephrosis	26	28	30	36	40
Minimal	12	14	22	20	16
Moderate	10	10	2	10	14
Marked	4	4	6	6	10
Liver					
Centrilobular hepatocyte enlargement, total	4	10	10	18	20
Minimal	4	10	10	16	20
Moderate	0	0	0	2	0
Stomach					
Epithelial hyperplasia and hyperkeratosis - nonglandular region, total	12	26	52	92	98
Trace	0	4	0	2	0
Minimal	6	20	28	50	24
Moderate	6	2	22	36	68
Marked	0	0	2	4	6
Epithelial hyperplasia and hyperkeratosis - limiting ridge, total	4	4	6	8	22
Minimal	4	4	6	8	20
Moderate	0	0	0	0	2

(table continues next page)

Non-neoplastic lesion	Dose (ppm)				
	0	15	60	240	1200
Ulceration - nonglandular region, total	0	4	22	20	40
Submucosal fibrosis and inflammatory cells - nonglandular region, total	6	0	22	18	28
Trace	0	0	2	0	0
Minimal	2	0	16	10	26
Moderate	4	0	4	8	2

a Data (n=50) obtained from pages 142-194 of MRID 45710212.

b. Neoplastic - The incidence of hepatocellular adenoma was increased in the 1200 ppm males (8% treated vs 2% controls; Table 7). The increase in all hepatocellular tumors was not significant. Squamous cell papilloma and carcinoma in the stomach were also increased ($p \leq 0.01$) in the 1200 ppm males (2-4% treated vs 0% controls). C-cell adenoma was increased in the thyroid of the ≥60 ppm males (2-4% treated vs 0% controls). Historical control data were not reported. Neoplasia data are included in the Appendix.

Table 7. Selected neoplastic histological findings (%) in male rats treated with Clorothalonil in the diet for up to 2 years. [a]

Neoplastic lesion	Dose (ppm)				
	0	15	60	240	1200
Liver					
Hepatocellular adenoma	2	0	2	2	8
Hepatocellular carcinoma	4	2	4	4	4
Hepatocellular adenoma and/or carcinoma [b]	6	2	6	6	12
Stomach					
Squamous cell papilloma	0	0	0	0	2
Squamous cell carcinoma	0	0	0	0	4
Squamous cell papilloma and/or carcinoma [b]	0	0	0	0	6
Thyroid					
Adenoma, C-cell	0	0	2	2	4
Carcinoma, C-cell	2	0	0	4	0
C-cell adenoma and/or carcinoma [b]	2	0	2	6	4

a Data (n=50) were obtained from pages 131-132 of MRID 45710212.
b Data were tabulated by the reviewers from the individual data on pages 649-1691of MRID 45710212.

H. MEASUREMENT OF NON-PROTEIN THIOL CONCENTRATION IN LIVER: No treatment-related effect was observed on non-protein thiol concentration in liver.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The LOAEL was 1200 ppm based on histopathological changes in the stomach, liver, and kidney. There was no evidence of tumorigenic potential.

B. REVIEWER COMMENTS - No treatment-related effect was observed on mortality, food and water consumption, food efficiency, ophthalmoscopic examinations, hematology, and non-protein thiol concentration in liver.

An increased incidence of generalized yellow staining of the fur was observed in the 1200 ppm group from the interim and terminal groups (66-100% treated vs 0% controls). This sign was observed generally from Week 16 in males and Week 19 in females. Body weight gain was decreased ($p<0.05$) by 15-17% in the 1200 ppm females during Weeks 0-1, 13-26, and 26-52.

Nephrotoxicity was evident. Urinary protein concentration was increased ($p<0.05$) by 137-279% in the 1200 ppm males at Weeks 52, 78, and 104. Adjusted (for terminal body weight) kidney weights were increased ($p<0.05$) in the ≥60 ppm males at Week 52 (19-28%) and the 1200 ppm males at Week 104 (121%). Necropsy of the ≥240 ppm males revealed uniform cortical scarring of the kidneys at the interim (15% each treated vs 0% controls) and terminal (22-44% treated vs 14% controls) sacrifices. Kidney enlargement in the 1200 ppm males (44% vs 20%) was also observed at the terminal necropsy. At the terminal sacrifice, there was an increased incidence of minimal to marked progressive glomerulonephrosis in the 1200 ppm males (70% vs 52%) and the ≥240 ppm females (36-40% vs 26%). Although nephrotoxicity was evident at 240 ppm (cortical scarring in males and progressive glomerulonephrosis in females), the increased incidence was minor or slight and further histological evidence of toxicity was absent in either sex at this dose. Similarly, the minor increase in adjusted kidney weight in the 60 ppm males was not considered adverse in the absence of corroborating evidence of toxicity.

Effects were observed on the liver, but were not considered adverse. Cholesterol was increased ($p<0.05$) by 38-60% in the 1200 ppm males at Weeks 52 and 78; however, this was a transient effect. In the 1200 ppm males, the liver was enlarged (26% treated vs 10% controls) with pale subcapsular areas at the terminal sacrifice (34% vs 18%). Although pale subcapsular areas were observed grossly, microscopic pathology did not corroborate an adverse effect. There was increased incidences (% treated vs % controls) in all treated groups in minimal to moderate centrilobular hepatocyte enlargement at the interim sacrifice (30-94% vs 0%); and minimal or minimal to moderate centrilobular hepatocyte enlargement in the 1200 ppm males (38% vs 18%) and ≥240 ppm females (18-20% vs 4%) at the terminal sacrifice. Liver/hepatocyte enlargement was considered an adaptive response.

The stomach was adversely affected. The Sponsor stated that these changes reflect an adaption to gastric irritation. The reviewer believes that the increase in the incidence of epithelial hyperplasia and hyperkeratosis of the nonglandular region of the stomach was treatment related. At necropsy following the interim sacrifice, the forestomach in the ≥240 ppm groups was thickened (90-100% treated vs 0% controls), roughened (60-95% vs 0%; excludes 240 ppm females), and white (15-95% vs 0%). Trace to moderate epithelial hyperplasia and hyperkeratosis in the non-glandular region (40-100% vs 0-6%) and in the limiting ridge (5-94% vs 0%; minimal or minimal to moderate) of the stomach was observed in these animals. At necropsy following the terminal sacrifice, the forestomach had depressions in the ≥60 ppm groups (42-68% treated vs 12-16% controls); and was thickened (36-70% vs 4-8%), roughened (18-60% vs 2-8%), and white (14-32% vs 2-6%) in the ≥240 ppm groups; and limiting ridge thickened or prominent in the 1200 ppm males (12% vs 0%). There was an increased incidence (% treated vs % controls) in the following lesions in these animals: (i) trace to marked or minimal to marked epithelial hyperplasia and hyperkeratosis in the nonglandular region of the stomach in all treated males and females (20-100% vs 12%); (ii) minimal or minimal to moderate epithelial hyperplasia and hyperkeratosis in the limiting ridge of the stomach in ≥240 ppm males (14-38% vs 4%) or the 1200 ppm females (22% vs 4%); (iii) stomach ulceration in the nonglandular region in the ≥60 ppm females (20-40% treated vs 0% controls); and (iv) trace to moderate submucosal fibrosis and inflammatory cells in the nonglandular region of the stomach in ≥60 ppm females (18-28% vs 6%).

Other gross observations or lesions were observed (% treated vs % controls) in the females at the interim sacrifice that were not observed at the terminal sacrifice including badly groomed fur at 1200 ppm (35% vs 0%), subcutaneous mass/es at ≥240 ppm (20% each vs 0%), and pale focus/i or area/s on the lung at 1200 ppm (30% vs 5%). These transient increases were considered incidental.

The LOAEL is 15 ppm (0.9 mg/kg bw/day) in females based on an increased in the incidence and severity of epithelial hyperplasia, hyperkeratosis and ulceration of the nonglandular region of the stomach females, and 60 ppm (2.7 mg/kg bw/day) based on an increase in the incidence and severity of epithelial hyperplasia and hyperkeratosis of the nonglandular region of the stomach in males . The NOAEL is <15 ppm (0.9 mg/kg bw/day) in females and 15 ppm (0.7 mg/kg bw/day) in males.

In the 1200 ppm males, increased incidences were observed of squamous cell papilloma and carcinoma in the stomach (2-4% treated vs 0% controls $p \leq 0.01$). The Sponsor stated that the stomach tumors resulted from prolonged gastric irritation. The reviewer agrees that chronic irritation may result in carcinogenesis. In the males in the 1200 ppm group, hepatocellular adenoma (8% treated vs 2% controls) was also increased in incidence. However, the increased incidence in all hepatocellular tumors was not significant. C-cell adenoma was increased in the thyroid of the ≥60 ppm males (2-4% treated vs 0% controls). C-cell carcinoma of the thyroid was only increased in males in the 240 ppm group. The magnitude of increase in each tumor incidence was low and may have been within biological variation; however, the carcinogenic potential of Chlorothalonil could not be adequately addressed due to the absence of historical control data.

Classification of the carcinogenic potential will be addressed by the Agency. Dosing was considered adequate based on decreased body weight in males and nephrotoxicity in both sexes.

C. **STUDY DEFICIENCIES** - Historical control data for neoplasia were not provided. The following minor deficiencies were also noted, but do not effect the conclusions of this review:

- Mean corpuscular hemoglobin was not reported.
- Very few standard deviations were included with mean data.
- Some data (i.e. body weights were not statistically analyzed).
- Urine appearance was not reported, and urinary blood was not quantified.
- The bile ducts were not examined microscopically.
- The uterus was not weighed.

APPENDIX

Neoplastic findings

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	70		70		70		70		70	
Animals completed	Decedent 31	Terminal 19	Decedent 34	Terminal 16	Decedent 35	Terminal 15	Decedent 36	Terminal 14	Decedent 31	Terminal 19
Lymphoid/multicentric Tumours										
Examined	1	2	4	0	0	0	0	0	1	0
Histiocytic sarcoma (Malignant)	1	0	2	0	0	0	0	0	0	0
Myeloid leukaemia (Malignant)	0	1	0	0	0	0	0	0	0	0
Malignant lymphoma (Malignant)	0	0	2	0	0	0	0	0	0	0
Pleomorphic lymphoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Large granular lymphocyte lymphoma (Malignant)	0	1	0	0	0	0	0	0	0	0
Lungs										
Examined	31	19	34	16	35	15	36	14	31	19
Pulmonary adenoma (Benign)	1	0	0	0	0	0	0	1	0	0
Pulmonary adenocarcinoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Heart										
Examined	31	19	34	4	35	2	36	3	31	19
Endocardial Schwannoma (Benign)	1	0	0	0	0	0	0	0	0	0
Blood Vessels										
Examined	0	0	1	0	1	0	1	0	2	3
Osteosarcoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Lymph Nodes - Mesenteric										
Examined	31	19	33	0	34	1	36	0	31	19
Missing	0	0	1	0	1	0	0	0	0	0
Haemangiosarcoma (Malignant)	0	0	0	0	0	0	0	0	0	0
Haemangioma (Benign)	0	1	0	0	0	0	1	0	1	0
Spleen										
Examined	31	19	34	2	35	0	36	0	31	19
Haemangioma (Benign)	0	0	1	0	0	0	0	0	0	0
Liver										
Examined	31	19	34	16	35	15	36	14	31	19

(Neoplastic findings - continued)

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5		5
	70		70		70		70		70		
Animals completed	Decedent 31	Terminal 19	Decedent 34	Terminal 16	Decedent 35	Terminal 15	Decedent 36	Terminal 14	Decedent 31	Terminal 19	minal 9
Thyroids	(continued)										
Follicular adenoma (Benign)	1	0	0	0	0	0	1	1	1	0	1
Follicular carcinoma (Malignant)	0	0	1	1	0	0	0	0	0	0	2
C cell adenoma (Benign)	0	0	0	0	1	0	1	0	2	0	0
C cell carcinoma (Malignant)	0	1	0	0	0	0	2	0	0	0	
Adrenals											
Examined	31	19	34	14	35	12	36	12	31	19	9
Pheochromocytoma (Benign)	2	4	1	0	4	1	0	4	0	4	1
Cortical carcinoma (Malignant)	0	0	2	0	0	0	0	0	0	0	0
Malignant pheochromocytoma (Malignant)	1	3	0	2	2	0	2	0	1	1	3
Pituitary											1
Examined	31	19	33	8	35	5	36	7	31	19	9
Missing	0	0	1	0	0	0	0	0	0	0	3
Pituitary adenoma (Benign)	8	4	14	2	13	3	15	4	11	5	
Skeletal Muscle											
Examined	31	19	34	0	35	1	36	3	31	19	9
Rhabdomyosarcoma (Malignant)	0	0	0	0	0	0	0	0	1	0	1
Salivary Glands											
Examined	28	19	34	0	32	0	36	0	30	19	
Missing	3	0	0	0	3	0	0	0	1	0	0
Acinar epithelial adenoma (Benign)	0	0	0	0	0	0	1	0	0	0	0
Stomach											1
Examined	31	19	34	16	35	15	36	14	31	19	
Squamous cell papilloma - nonglandular region (Benign)	0	0	0	0	0	0	0	0	1	0	0
Squamous cell carcinoma - nonglandular region (Malignant)	0	0	0	0	0	0	0	0	0	2	0
Skin											
Examined	31	19	34	4	35	3	36	8	31	19	1

(Neoplastic findings - continued)

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	70		70		70		70		70	
Animals completed	Decedent 31	Terminal 19	Decedent 34	Terminal 16	Decedent 35	Terminal 15	Decedent 36	Terminal 14	Decedent 31	Terminal 19
Skin	(continued)									
Squamous cell papilloma (Benign)	1	0	0	0	0	2	0	0	0	1
Sebaceous adenoma (Benign)	1	0	0	0	0	0	0	0	0	0
Keratoacanthoma (Benign)	1	1	0	1	1	0	0	4	2	1
Basal cell carcinoma (Malignant)	0	0	0	2	1	0	1	0	2	0
Fibroma (Benign)	5	2	2	0	1	0	0	1	0	2
Fibrosarcoma (Malignant)	0	0	0	0	0	0	2	0	1	0
Dermal fibroma (Benign)	0	0	1	0	0	0	1	0	0	0
Ulcerated fibrosarcoma (Malignant)	0	0	0	0	1	0	0	0	0	0
Basal cell tumour (Benign)										
Subcutis										
Examined	12	4	6	4	9	3	11	2	4	6
Missing	0	0	0	0	0	0	0	1	0	0
Fibroma (Benign)	6	3	2	2	6	3	2	2	1	3
Fibrosarcoma (Malignant)	3	0	0	0	1	0	3	0	1	0
Lipoma (Benign)	1	1	3	2	1	0	5	0	1	1
Ulcerated fibrosarcoma (Malignant)	1	0	0	0	1	0	0	0	1	0
Malignant neurinoma (Malignant)	0	0	0	0	0	0	1	0	0	0
Ulcerated fibroma (Benign)										
Mammary Glands										
Examined	31	19	34	2	35	1	36	0	31	19
Mammary adenoma (Benign)	1	0	0	0	0	0	0	0	0	0
Mammary fibroadenoma (Benign)	1	0	1	0	1	1	0	0	0	0
Brain										
Examined	31	19	34	2	35	1	36	1	31	19
Astrocytoma (Malignant)	0	0	0	0	0	0	0	0	0	1
Meningioma (Benign)	0	0	0	0	0	0	1	0	0	0
Haemangioma (Benign)	0	0	1	0	0	0	0	0	0	0
Spinal Cord										
Examined	31	19	34	0	35	0	36	0	31	19

(Neoplastic findings - continued)

Males on study	Group 1 70		Group 2 70		Group 3 70		Group 4 70		Group 5 70	
Animals completed	Decedent 31	Terminal 19	Decedent 34	Terminal 16	Decedent 35	Terminal 15	Decedent 36	Terminal 14	Decedent 31	Terminal 19
Spinal Cord (continued)										
Astrocytoma (Malignant)	0	0	0	0	1	0	0	0	0	0
Harderian Glands										
Examined	0	0	2	0	0	0	0	0	0	0
Squamous cell carcinoma (Malignant)	0	0	1	0	0	0	0	0	0	0
Head										
Examined	0	0	0	0	1	0	2	0	0	0
Sebaceous squamous cell carcinoma of Zymbal's gland (Malignant)	0	0	0	0	0	0	1	0	0	0
Squamous cell carcinoma (Malignant)	0	0	0	0	0	0	1	0	0	0
Squamous sebaceous cell carcinoma - Zymbal's gland (Malignant)	0	0	0	0	1	0	0	0	0	0
Tail										
Examined	15	4	15	6	8	8	18	8	9	9
Squamous cell papilloma (Benign)	0	0	0	1	0	0	0	0	0	0
Abdominal Cavity										
Examined	1	0	0	0	0	0	0	0	1	0
Fibrosarcoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Lipoma (Benign)	1	0	0	0	0	0	0	0	0	0
Oral Cavity										
Examined	0	0	1	0	0	0	0	0	0	0
Ulcerated squamous cell carcinoma - left lip (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	
	70		70		70		70		70	
Animals completed	Decedent 33	Terminal 17	Decedent 30	Terminal 20	Decedent 37	Terminal 13	Decedent 35	Terminal 15	Decedent 34	Terminal 16
Head	(continued)									
Sebaceous squamous cell carcinoma of Zymbal's gland (Malignant)	0	0	0	0	1	0	0	0	0	0
Squamous sebaceous cell carcinoma - Zymbal's gland (Malignant)	0	0	0	0	1	0	0	0	0	0
Rhabdomyosarcoma (Malignant)	1	0	0	0	0	0	0	0	0	0
Tongue										
Examined	0	0	0	0	1	0	0	0	0	0
Squamous cell carcinoma (Malignant)	0	0	0	0	1	0	0	0	0	0
Diaphragm										
Examined	0	0	0	0	0	0	0	0	1	0
Anaplastic sarcoma (Malignant)	0	0	0	0	0	0	0	0	1	0
atypia (benign)	0	1	0	3	0	0	1	0	0	1
Mammary fibroadenoma (Benign)	18	12	13	17	18	8	22	11	18	11
Mammary fibroadenoma with epithelial atypia (Benign)	0	1	2	2	3	0	3	2	3	2
Mammary fibroma (Benign)	2	0	0	0	0	0	0	0	0	0
Mammary adenocarcinoma (Malignant)	8	2	6	7	7	2	2	4	5	4
Brain										
Examined	33	17	30	8	37	11	35	9	34	16
Astrocytoma (Malignant)	0	0	0	0	0	0	0	0	1	0
Meningioma (Malignant)	1	0	0	0	0	0	0	0	0	0
Head										
Examined	1	0	0	0	4	0	1	0	0	0

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Intergroup comparison of tumour incidence

Males on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	70		70		70		70		70	
Animals completed	Decedent 31	Terminal 19	Decedent 34	Terminal 16	Decedent 35	Terminal 15	Decedent 36	Terminal 14	Decedent 31	Terminal 19
Number of tumour bearing animals	28	15	27	13	28	9	29	10	20	16
Animals with malignant tumours	7	6	11	4	12	1	12	4	7	6
Benign tumours	25	14	19	12	22	9	25	9	17	14
Multiple tumours	13	11	11	6	14	4	22	8	13	10
Single tumours	15	4	16	7	14	5	7	2	7	6
Multiple malignant tumours	1	1	1	0	2	0	4	0	0	0
Multiple benign tumours	11	6	9	4	10	3	14	7	11	9
Metastatic tumours	0	1	0	0	0	0	2	0	0	0

(Intergroup comparison of tumour incidence - continued)

Females on study	Group 1		Group 2		Group 3		Group 4		Group 5	
	70		70		70		70		70	
Animals completed	Decedent 33	Terminal 17	Decedent 30	Terminal 20	Decedent 37	Terminal 13	Decedent 35	Terminal 15	Decedent 34	Terminal 16
Number of tumour bearing animals	32	14	29	20	37	13	34	15	34	14
Animals with malignant tumours	13	4	9	11	13	4	9	7	10	5
Benign tumours	31	14	25	20	35	13	32	15	31	14
Multiple tumours	24	10	11	17	23	10	20	14	20	10
Single tumours	8	4	18	3	14	3	14	1	14	4
Multiple malignant tumours	1	1	3	1	2	1	0	0	2	1
Multiple benign tumours	22	9	9	16	17	9	18	12	18	9
Metastatic tumours	1	0	0	1	0	0	2	0	3	1

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TABLE 12

Results of time-to-tumour analysis for hepatocellular tumours in males

All tumours (benign or malignant)

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	3	3.07	0.98		
15	70	1	3.00	0.33	0.77	
60	70	3	2.74	1.10	0.41	
240	70	3	2.98	1.01	0.43	
1200	70	5	3.20	1.56	0.35	0.11

Malignant tumours only

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	2	1.89	1.06		
15	70	1	1.79	0.56	0.64	
60	70	2	1.65	1.21	0.46	
240	70	2	1.74	1.15	0.43	
1200	70	2	1.92	1.04	0.54	0.41

▲ One-tailed pairwise comparisons against the control group (group 1).
One-tailed trend tests using groups with dose 0 up to the dose level for that row.

TABLE 15

Results of time-to-tumour analysis for renal tumours

All tumours (all tumours were benign), in males

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.48	0.00		
15	70	1	0.38	2.62	0.22	
60	70	0	0.48	0.00	0.50	
240	70	0	0.48	0.00	0.50	
1200	70	1	0.19	5.25	0.14	0.12

All tumours (benign or malignant), in females

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.42	0.00		
15	70	0	0.38	0.00	0.50	
60	70	1	0.40	2.53	0.22	
240	70	0	0.38	0.00	0.50	
1200	70	1	0.43	2.31	0.27	0.17

Malignant tumours only, in females

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.21	0.00		
15	70	0	0.25	0.00	0.50	
60	70	1	0.16	6.23	0.22	
240	70	0	0.19	0.00	0.50	
1200	70	0	0.20	0.00	0.50	0.46

▲ One-tailed pairwise comparisons against the control group (group 1).
One-tailed trend tests using groups with dose 0 up to the dose level for that row.

TABLE 16

Results of time-to-tumour analysis for pituitary tumours in females

All tumours (benign or malignant)

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	31	38.49	0.81		
15	70	35	37.85	0.92	0.31	
60	70	42	33.79	1.24	0.031	
240	70	40	34.18	1.17	0.055	
1200	70	34	37.69	0.90	0.39	0.68

Malignant tumours only

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.62	0.00		
15	70	0	0.62	0.00	0.50	
60	70	2	0.55	3.63	0.11	
240	70	1	0.53	1.89	0.23	
1200	70	0	0.68	0.00	0.50	0.61

▲ One-tailed pairwise comparisons against the control group (group 1).
\# One-tailed trend tests using groups with dose 0 up to the dose level for that row.

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TABLE 13

Results of time-to-tumour analysis forestomach squamous cell papilloma in the nonglandular region in males

All tumours (benign or malignant)

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.70	0.00		
15	70	0	0.56	0.00	0.50	
60	70	0	0.51	0.00	0.50	
240	70	0	0.60	0.00	0.50	0.50
1200	70	3	0.63	4.75	0.051	0.004

Malignant tumours only

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.46	0.00		
15	70	0	0.39	0.00	0.50	
60	70	0	0.36	0.00	0.50	
240	70	0	0.34	0.00	0.50	0.50
1200	70	2	0.46	4.37	0.12	0.025

▲ One-tailed pairwise comparisons against the control group (group 1).
\# One-tailed trend tests using groups with dose 0 up to the dose level for that row.

TABLE 14

Results of time-to-tumour analysis forestomach squamous cell papilloma in the nonglandular region in females

All tumours (benign or malignant)

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.62	0.00		
15	70	0	0.40	0.00	0.50	
60	70	0	0.71	0.00	0.50	
240	70	2	0.57	3.49	0.11	
1200	70	1	0.71	1.42	0.27	0.15

Malignant tumours only

Dose level in ppm	Initial group size	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison p-value▲	p-value for trend test#
		Observed (O)	Expected (E)			
0	70	0	0.21	0.00		
15	70	0	0.13	0.00	0.50	
60	70	0	0.24	0.00	0.50	
240	70	1	0.19	5.23	0.24	
1200	70	0	0.24	0.00	0.50	0.33

▲ One-tailed pairwise comparisons against the control group (group 1).
One-tailed trend tests using groups with dose 0 up to the dose level for that row.