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OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Two-Generation Reproduction Study in Rats

Tox Chemical No.: 215B  
MRID No.: 417062-01C  
PC Code: 081901

DP Barcode: D179139  
Submission: S419158

FROM: Alan C. Levy, Ph.D., Toxicologist *Alan C. Levy 2/16/93*  
Review Section IV, Toxicology Branch II  
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TO: Walter Waldrop/Andrew W. Ertman, PM 71  
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THRU: Elizabeth A. Doyle, Ph.D., Section Head  
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*E. A. Doyle*  
*2/16/93*

and

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

*M. van Gemert 2/17/93*

REQUEST: Review a two-generation reproduction study in rats with Chlorothalonil

CONCLUSIONS:

Chlorothalonil, administered as a dietary admix at concentrations of 0, 500, 1,500 and 3,000 ppm to rats in a 2-generation (2 litters/generation) reproduction study, appeared to cause the following effects:



Parental body weight/body weight gain: lower than control primarily at 3,000 ppm

Parental kidneys and forestomachs: essentially at 500, 1,500 and 3,000 ppm (thickening/hypertrophy/hyperplasia of tissue)

Neonatal lower body weight on lactation day 21

Neonatal renal pelvis dilation: a suggestive increase in the incidence (only in the F1a generation)

Adult No Observed Effect Level (NOEL) = not attained (kidney and forestomach lesions at Lowest Dose Tested, 500 ppm = about 38 mg/kg as calculated by Reviewer from Report data)

Adult Lowest Observed Effect Level (LOEL) = 500 ppm (Lowest Dose Tested)

Reproduction NOEL = 1,500 ppm (about 115 mg/kg as calculated by Reviewer from Report data)

Reproduction LOEL = 3,000 ppm (about 234 mg/kg as calculated by Reviewer from Report data) - lower neonatal body weights on lactation day 21

**Classification: Core Minimum**

This study satisfies the Guideline requirements (§83-4) for a 2-generation reproduction study in rats.

Reviewed by: Alan C. Levy, Ph.D. *Alan C. Levy* 2/16/93  
Section IV, Tox. Branch II

Secondary reviewer: Elizabeth A. Doyle, Ph.D.  
Section IV, Tox. Branch II

*E. A. Doyle*  
2/16/93

#### DATA EVALUATION REPORT

**STUDY TYPE:** Two-Generation Reproduction and Fertility - Rats (§83-4)

**TEST MATERIAL:** Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile)

**SYNONYMS:** none

Tox. Chemical No.: 215B  
MRID No.: 417062-01C  
PC Code: 081901

DP Barcode: D179139  
Submission: S419158

**STUDY NUMBER:** 87-0121; 1722-87-0121-TX-003

**SPONSOR:** Fermenta ASC Corporation, Mentor, OH

**TESTING FACILITY:** Ricerca, Inc., Painesville, OH

**TITLE OF REPORT:** A Two Generation Reproduction Study in Rats with  
Technical Chlorothalonil

**AUTHORS:** F. Lucas and G. Benz

**REPORT ISSUED:** November 9, 1990

#### CONCLUSIONS:

Chlorothalonil, administered as a dietary admix at concentrations of 0, 500, 1,500 and 3,000 ppm to rats in a 2-generation (2 litters/generation) reproduction study, appeared to cause the following effects:

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primarily at 3,000 ppm

Parental kidneys and forestomachs: essentially at 500, 1,500  
and 3,000 ppm (thickening/hypertrophy/hyperplasia of  
tissue)

Neonatal lower body weight on lactation day 21

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Reproduction NOEL = 1,500 ppm (about 115 mg/kg as calculated by Reviewer from Report data)

Reproduction LOEL = 3,000 ppm (about 234 mg/kg as calculated by Reviewer from Report data) - lower neonatal body weights on lactation day 21

Classification: Core Minimum

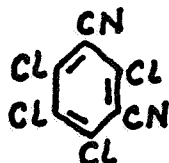
This study satisfies the Guideline requirements (§83-4) for a 2-generation reproduction study in rats.

# **I. MATERIALS AND METHODS**

## **A. Test Article Description**

Name: Chlorothalonil; 2,4,5,6-tetrachloroisophthalonitrile

Formula:



Lot Number: D-5840923

Purity: 98.1% (see section titled Purity)

Appearance: light gray powder

Storage: in the dark at room temperature

## **B. Dosing and Diet Preparation**

Chlorothalonil was administered as a dietary admix at concentrations of 0 (control), 500, 1,500 and 3,000 ppm. Fresh diets were made weekly. Test article and a small amount of basal diet were mixed using a mortar and pestle. This premix was then blended for 10 minutes with the rest of the basal diet in a Marion blender.

## **C. Dose Selection**

There was no indication in the Report as to how the concentrations of 0, 500, 1,500 and 3,000 ppm were chosen.

## **D. Purity, Concentration, Stability and Homogeneity of the Test Article**

### PURITY

A Report page indicated as Appendix F (Report page 1670 cross references this page) and stamped "Confidential Business Information", was entitled, "Analyses of Technical Chlorothalonil used in a Two Generation Reproduction Study in Rats with Technical Chlorothalonil, Study No. 87-0121."

### CONCENTRATION

Levels of chlorothalonil ( $\mu\text{g}$  test article/g of feed, or ppm) in this study were 0 (control), 500, 1,500 and 3,000. Duplicate assays were performed for each concentration weekly from weeks 1 through 13 and every other week from weeks 14

QUALITY CONTROL PROCEDURE INFORMATION IS NOT INCLUDED

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through 36. In addition, F1 diets were analyzed every other week from weeks 8 through 36 (duplicate assays). Individual assay results appeared on Report pages 1461-1465 with mean assay values presented on Report pages 1466-1468.

A summary of the mean (of 2 assays) values is presented in Table 1.

Table 1

A SUMMARY OF MEAN ASSAY RESULTS FOR CONCENTRATION OF CHLOROTHALONIL  
IN RAT FEED IN A TWO-GENERATION STUDY

ppm =	500	1,500	3,000
No. of duplicate assays .	38	40	38
Range ( $\mu\text{g/g}$ ) .....	462-535	1389-1606	2750-3224
Mean ( $\mu\text{g/g}$ ) .....	493	1,480	2,947
% of Nominal .....	98	99	99

Data extracted from Report pages 1466-1468.

STABILITY

Assays were conducted on 500 and 3,000 ppm 7 and 14 days after diet preparation. Results are presented in Table 2.

Table 2

A SUMMARY OF STABILITY ASSAY RESULTS IN A TWO-GENERATION RAT STUDY  
WITH CHLOROTHALONIL

ppm =	500	3,000
<u>7 Days</u>		
No. of assays .....	4	4
Assay values .....	517,514,504,504	3067,3058,2988,2930
Mean of assay values .	510	3011
% of nominal .....	102	100
<u>14 Days</u>		
No. of Assays .....	4	4
Assay values .....	463,460,454,450	2885,2881,2871,2868
Mean of assay values .	457	2877
% of nominal .....	91	96

Data extracted from Report pages 1459 and 1460.

# HOMOGENEITY

Concentrations of 500 and 3,000 ppm of chlorothalonil in feed were assayed after 10 and 20 minutes of mixing. Five duplicate (10 samples) assays per concentration were reported. See Table 3.

Table 3

A SUMMARY OF ASSAY RESULTS FOR HOMOGENEITY IN A TWO-GENERATION RAT STUDY WITH CHLOROTHALONIL

	ppm =	500	3,000
<u>10 Minute Mix</u>			
No. of duplicate assays .....		5	5
Range (ppm)† .....		481-512	2745-3060
Mean (ppm) .....		493	2945
% of nominal .....		99	98
<u>20 Minute Mix</u>			
No of duplicate assays .....		5	5
Range (ppm)† .....		494-515	2934-3179
Mean (ppm) .....		506	3049
% of nominal .....		101	102

† = values for 10 single assays

Data extracted from Report pages 1457 and 1458.

Values for purity, concentration, stability and homogeneity appear to be within acceptable limits.

## **E. Animals**

Male and female Sprague-Dawley (CD-VAF) rats were received from Charles River Laboratories, Portage, MI. The animals were 27 days old when received and were 40 days old at the start of chlorothalonil administration.

For the first 7 days after receipt, the rats were housed 2/cage (suspended stainless wire mesh). They were then housed individually (except during mating and lactation). A solid bottom, with corncob bedding, was placed in the cages of females from gestation day 18 through lactation. Room temperature and humidity were measured daily with target ranges of 70-76°F and 40-60%, respectively. There was a 12 hour light/dark cycle. Food and water were available ad libitum.



Randomization, after 7 days of acclimation and placement into groups, was based on body weights. Rats were subjected to a physical examination prior to randomization. There were 35 rats/sex/group.

#### F. Mating

There was a two-week mating period after 10 weeks for the F0 generation and after 14 weeks for the F1 generation. After weaning of the F1a or F2a litters, the F0 or F1 parents were rested 2 or 3 weeks prior to the second 2-week mating period (to produce F1b or F2b litters). Even if no litter was produced after the first mating, the adults were subjected to a second mating.

Brother-sister matings were avoided during the F1 generation matings. For the second mating period, different pairs of animals were mated.

Females were randomly assigned to males from the same test article concentration group (1:1). The female was placed in the male's cage each afternoon of the mating period and removed the next morning when it was examined for evidence of mating (copulation plug and/or sperm in a vaginal smear). The day evidence of mating was noted was considered to be gestation day 0. Cohabitation with the same male took place each night during the 2-week mating period until the plug/sperm were noted.

#### G. Gestation and Lactation

Females were returned to their individual cages after evidence of mating or after the 2-week mating period. Solid cage bottoms and corncob bedding were provided on gestation day 18 for females with evidence of mating and 4 days post-mating period for females with no evidence of mating.

Observations for signs of delivery were made twice each day. Lactation day 0 was the day on which all pups were delivered (if delivery was not complete by 3:00 P.M., the next day was considered to be lactation day 0).

Litters were culled to 8 pups (4/sex if possible) on lactation day 4. Culled pups were asphyxiated by CO<sub>2</sub> on day 4 and necropsied.

#### H. Selection of Offspring for F1 Generation

On lactation day 21, random selection was used to adjust the size of each F1b litter. For controls, 1/2 of the litters had 3 males and 2 females (when possible) chosen and 1/2

of the litters, 2 males and 3 females. For the 500, 1,500 and 3,000 ppm groups, adjustment was made to yield 2/sex if possible. There was an extra animal per sex in the control group litters in order to be sure there were sufficient numbers from which to select the F1 generation. [Report page 214, indicated the number of litters to be (ppm): 0 = 18, 500 = 23, 1,500 = 27 and 3,000 = 27; each litter with an average of 12.3-13.5 liveborn pups.]

F1 generation males and females (35/sex) were randomly selected from the F1b litters (after lactation day 21); at least one of each sex from each litter (if possible).

#### **I. Termination of Animals**

CO<sub>2</sub> asphyxiation was used to kill the animals and necropsies were performed on all rats.

Moribund - Killed at the descretion of the Study Director (or designee).

F0 and F1 Adults - All survivors killed after F1b or F2b litters weaned.

F1a, F1b, F2a and F2b Offspring - Lactation day 4, culled pups killed on that day. Except for F1b offspring to be used as F1 parents, all offspring were killed on lactation day 21.

#### **J. Parameters Evaluated**

##### **1. DURING LIFE**

The animals were observed once daily prior to treatment and twice/day (A.M. and P.M.) during treatment for signs of toxicity, moribundity and mortality.

##### **F0 and F1 Parental Animals**

Physical examinations as well as body weight and food consumption data were collected for the F0 rats from one week before test article administration and from day of selection of F1 animals.

Males had physical examinations weekly (entire study) and females, throughout the growth period as well as during the 2-week rest period.

F0 body weights were recorded weekly from one week prior to test article administration and F1 weights from day of selection. Except for

during mating, male weights were taken weekly. Females had weights recorded during the growth periods as well as during the 2-week rest. Females with positive mating signs had weights recorded on gestation days 0, 7, 14 and 20. Weights were also recorded on lactation days 0, 7, 14 and 21.

Food consumption was recorded weekly before mating and during the 2-week rest period but not during mating, gestation or lactation.

Test article consumption as mg/kg/day was calculated weekly before mating and during the 2-week rest period.

### Litters

The following were recorded: delivery date; number, weight and sex of live and stillborn pups; plus abnormalities. During lactation, pups were examined twice daily for survival and toxicity. The number and sex of live offspring were recorded on lactation days 0, 1, 4 (pre- and post-cull), 7, 14 and 21. Litter weights for live pups were recorded on lactation days 0, 4 (pre- and post-cull), 7 and 14; individual weights were recorded on lactation day 21.

## 2. POSTMORTEM EXAMINATION

All adults and pups were necropsied. The following tissues from 10/sex F0 and F1 randomly selected adults from each group were preserved in 10% neutral buffered formalin: adrenals, femur, sternum bone marrow, brain, esophagus, eyes, testes with epididymides as well as seminal vesicles, ovaries with uterus as well as vagina, heart with aorta, intestines (duodenum, jejunum, ileum, cecum, colon, rectum), kidneys, liver, lungs, pancreas, pituitary, prostate, salivary glands with cervical lymph nodes, skeletal muscle with peripheral nerve, skin with mammary tissue, spinal cord (cervical), spleen, stomach, thymus, thyroid/parathyroids, trachea, urinary bladder and gross lesions.

From all other F0 and F1 adults, the following tissues were preserved in 10% neutral buffered formalin: vagina, uterus, ovaries, kidneys, stomach, testes, epididymides, seminal vesicles, prostate and gross lesions.

Grossly abnormal pups were necropsied and preserved in 10% neutral buffered formalin.

Tissue microscopic evaluation was performed at Experimental Pathology Laboratories, Inc., Herndon, VA under the direction of Dr. William Busey. There was histologic preparation of the following tissues for all F0 and F1 adults: vagina, uterus, ovaries, kidneys, stomach, testes, epididymides, seminal vesicles, prostate and gross lesions. These tissues were microscopically evaluated from all control and 3,000 ppm adults. In addition, the stomach, kidneys and gross lesions of 500 and 1,500 ppm rats were examined. The prostate, epididymis and seminal vesicles from F0 males of the 500 and 1,500 ppm groups were evaluated microscopically. Only testes from the 500 and 1,500 ppm F1 males were examined. All other tissues from the F0 and F1 adults were kept in fixative.

### 3. REPRODUCTIVE INDICES

The following for each of the 4 groups were calculated from raw data (M = males, F = females):

Mating Index:  $\frac{\# \text{ M producing F with sign of mating}}{\# \text{ M mated}} \times 100$

Fertility Index:  $\frac{\# \text{ M producing pregnant F}}{\# \text{ M producing F with signs of mating}} \times 100$

Pup Indices: Calculated for individual litters and the mean for each group determined

Sex Ratio:  $\frac{\# \text{ live M}}{\# \text{ total live pups}} \times 100$

Liveborn:  $\frac{\# \text{ total liveborn}}{\text{total } \# \text{ pups born}} \times 100$

Stillborn:  $\frac{\# \text{ stillborn}}{\text{total } \# \text{ pups born}} \times 100$

Day 4 Viability:  $\frac{\# \text{ pups alive day 4 (pre-cull)}}{\# \text{ pups born alive}} \times 100$

Lactation:  $\frac{\# \text{ pups alive day 21}}{\# \text{ pups alive day 4 (post-cull)}} \times 100$

#### Litter Indices:

Liveborn:  $\frac{\# \text{ litters with live pups day 0}}{\# \text{ litters}} \times 100$

Stillborn:  $\frac{\# \text{ litters with stillborn pups}}{\# \text{ litters}} \times 100$

Day 4 Viability:  $\frac{\# \text{ litters with live pups day 4 (pre-cull)}}{\# \text{ litters with live pups day 0}} \times 100$

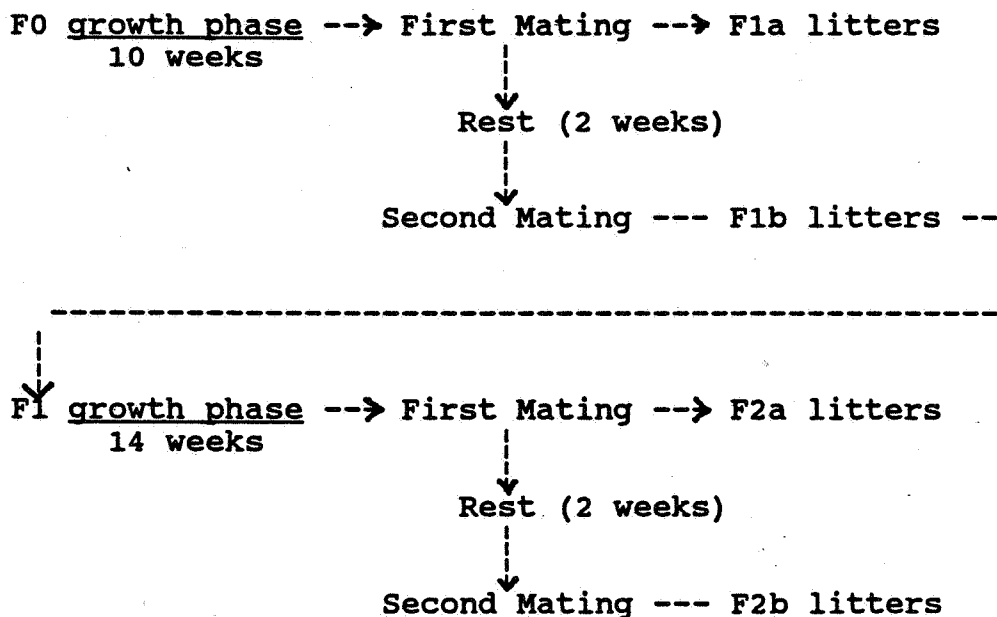
Lactation: # litters with live pups day 21/# of  
litters with live pups day 4  
(post-cull) x 100  
Viability: # litters with live pups day 21/# of  
litters with live pups day 0 x 100

#### 4. STATISTICAL EVALUATION

Detailed statistical methodology was presented on Report pages 46-50. "Statistical evaluations were conducted under the supervision of John M. Schollengerger, Ph.D., a professional statistician."

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#### SEQUENCE OF EVENTS (Report page 28)



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## II. RESULTS

### A. Mortality (Report pages 1513-1552 and 1604-1642)

FO GENERATION - none

F1 GENERATION -

500 ppm male 107419 - moribund week 21, gastritis  
1,500 ppm male 107493 - found dead week 35, chronic  
nephropathy  
1,500 ppm male 107507 - moribund week 21, chronic  
nephropathy

1,500 ppm female 107530 - found dead week 15, during delivery, gravid uterus  
 1,500 ppm female 107542 - found dead week 13, no suggestive cause of death  
 3,000 ppm male 107571 - found dead week 21, lymphoma of liver, spleen, thymus and kidney

### B. Clinical Signs

F0 GENERATION - Loose stools were reported in 7 males from the 3,000 ppm group with 6 animals having this finding only once. The Study Authors did not consider this a significant finding. This Reviewer agrees. [Not reported in F1 parental rats.]

F1 GENERATION - none

### C. Body Weights

Selected group mean male and female body weights and weight gains are presented in Tables 4, 5 and 6.

Table 4

SELECTED GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS FOR F0 AND F1 MALES IN A 2-GENERATION RAT STUDY WITH CHLOROTHALONIL

Body Weights (g)					Body Weight Gains (g)				
Week	0a	500	1500	3000	Week	0	500	1500	3000
F0 GENERATION									
0	159	158	162	159	0-1	56	54	51	38
1	215	212	212	197**	1-5	173	167	163	161
5	388	379	375	358**	5-10	105	99	98	99
10	493	478	473	457**	10-15	59	56	57	47
15	552	534	530	504**	15-20	53	44	42	37
20	605	578	572*	541**	20-25	27	23	22	20
25	632	601	594*	561**	25-30	18	21	20	16
30	650	622	614	577**	0-30	491	464	452	418
F1 GENERATION									
0	126	119	126	115**	0-1	62	53	60	55
1	188	172	186	170**	1-5	207	202	209	197
5	395	374*	395	367**	5-10	116	117	110	103
10	511	491	505	470**	10-14	65	57	57	52
14	576	548*	562	522**	14-20	52	54	51	51
20	628	602	613	573**	20-25	34	33	30	30
25	662	635	643	603**	25-30	15	3	7	5
30	677	638*	650	608**	30-34	25	20	14	19
34	702	658*	664	627**	0-34	576	539	538	512

FOOTNOTES ON NEXT PAGE

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NOTES: Values for weights or weight gains = mean for 35 rats  
No body weights taken during mating: weeks 15 and 16 or 26  
and 27 of F1 generation.  
Body weight gains calculated by this Reviewer.

a = ppm

Statistical Analyses: \* =  $p=0.05$ ; \*\* =  $p=0.01$

Statistically Significant Trend: 0.01 at all weighing intervals for  
body weights and weight gains

Data extracted from Report Tables 3, 5, 17 and 19; pages 98-103,  
107-112, 140-146 and 151-156.

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#### MALES (F0 AND F1) - TABLE 4

There was a statistically significant ( $p=0.01$ ) lower group mean body weight for the 3,000 ppm males compared with controls at all weighing intervals (weeks 1-30 for F0 and 1-34 for F1). In addition, there was a statistically significant trend toward lower weights at all of these intervals ( $p=0.01$ ). F0 body weight gains for the 30 week period were (ppm): 0 = 491, 500 = 464, 1,500 = 452 and 3,000 = 418 g. For F1 weight gains during the 34 week period, the values were (ppm): 0 = 576, 500 = 539, 1,500 = 538 and 3,000 = 512 g.

#### FEMALES (F1a AND F1b) - TABLE 5

Table 5 presents body weight and weight gain data for F0 females (prior to mating, during gestation F1a, during lactation F1a, during rest period between F1a and F1b, during gestation F1b and during lactation F1b). There were significant ( $p=0.05$  or  $0.01$ ) lower group mean weights in the 3,000 ppm group during the 10 weeks prior to mating (F1a). During gestation for F1a, the group mean body weight gains were essentially the same for all 4 groups (117-119 g). For the F1a lactation period (3 weeks), group mean gains were (ppm): 0 = -1, 500 = -3, 1,500 = 13 and 3,000 = 23 g, so that the group mean final lactation body weights for all 4 groups was essentially the same (307-317 g). The group mean gains during the 2 week rest period between F1a and F1b were 0-4 g.

For the F1b gestation period (Table 5), all 4 groups gained 121-125 g. During F1b lactation, the group mean body weight gains were (ppm): 0 = -16, 500 = -13, 1,500 = 3 and 3,000 = 17 g.

Table 5

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS FOR F0 FEMALES PRIOR TO MATING, DURING GESTATION AND DURING LACTATION IN A 2-GENERATION STUDY WITH CHLOROTHALONIL

Body Weights (g)					Body Weight Gains (g)				
Week	0a	500	1500	3000	Week	0	500	1500	3000
PRIOR TO MATING									
0	141	139	138	138	0-2	46	45	45	40
2	187	184	183	178**	2-4	32	32	32	31
4	219	216	215	209*	4-6	24	25	20	20
6	243	241	235	229**	6-8	16	15	16	15
8	259	256	251	244**	8-10	15	13	11	9
10	274	269	262	253**	0-10	133	130	124	115
DURING GESTATION (F1a)									
0	276	273	262*	256**	0-3	119	117	119	119
1	303	300	292	286*	.....				
2	332	332	318	315*					
3	395	390	381	375*					
DURING LACTATION (F1a)									
0	314	310	298*	294**	0-3	-1	-3	13	23
1	322	323	317	315	.....				
2	328	327	326	326					
3	313	307	311	317					
DURING REST PERIOD (BETWEEN F1a and F1b)									
20	312	308	304	291**	20-21	4	2	0	0
21	316	310	304	291**	.....				
DURING GESTATION (F1b)									
0	308	318	300	291	0-3	121	122	125	122
1	334	345	327	319	.....				
2	363	374	356	346					
3	429	440	425	413					
DURING LACTATION (F1b)									
0	343	350	338	326	0-3	-16	-13	3	17
1	345	357	351	339	.....				
2	349	355	351	338					
3	327	337	341	343					

a = ppm

NOTES: Values for weights or weight gains = mean for 35 rats except 34 at 1,500 ppm week 3 and at 0 ppm week 21 prior to mating; 18-31 during gestation or lactation

Body weight gains calculated by this Reviewer.

Statistical Analyses: \* = p=0.05; \*\* = p=0.01

Data extracted from Report Tables 4, 6, 29, 30, 44 and 45; pages 104-106, 113-115, 190, 191, 209 and 210.



Table 6

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS FOR F1 FEMALES PRIOR TO MATING, DURING GESTATION AND DURING LACTATION IN A 2-GENERATION STUDY WITH CHLOROTHALONIL

Body Weights (g)					Body Weight Gains (g)				
Week	0a	500	1500	3000	Week	0	500	1500	3000
PRIOR TO MATING									
0	112	105	108	102**	0-2	70	66	66	69
2	182	171**	174*	171**	2-4	47	35	37	39
4	229	206**	211**	210**	4-6	35	32	31	25
6	264	238**	242**	235**	6-8	18	17	17	14
8	282	255**	259**	249**	8-10	13	13	16	13
10	295	268**	275**	262**	10-12	13	12	6	10
12	308	280**	281**	272**	12-14	13	15	13	8
14	321	295**	294**	280**	0-14	209	190	186	178
DURING GESTATION (F2a)									
0	316	291*	295*	273**	0-3	116	118	127	121
1	341	319	328	304**	.....				
2	366	349	358	332**					
3	432	409	422	394**					
DURING LACTATION (F2a)									
0	342	326	334	312**	0-3	-10	-7	-3	12
1	350	339	351	328**	.....				
2	357	345	357	339					
3	332	319	331	324					
DURING REST PERIOD (BETWEEN F2a and F2b)									
24	344	323	324	308**	24-25	6	2	2	2
25	350	325*	326*	310**	.....				
DURING GESTATION (F2b)									
0	344	317**	316*	301**	0-3	116	124	122	117
1	368	347	345	328**	.....				
2	393	373	372	351**					
3	460	441	438	418**					
DURING LACTATION (F2b)									
0	370	353	356	334**	0-3	-16	-12	-4	8
1	374	368	372	352	.....				
2	380	371	377	363					
3	354	341	352	342					

a = ppm

NOTES: Values for weights or weight gains are means for the following numbers of rats: 33-35 prior to mating; 14-27 during gestation or lactation

Statistical analyses: \* = p=0.05; \*\* = p=0.01

Data extracted from Report Tables 18, 20, 59, 60, 74 and 75, pages 147-150, 157-160, 228, 229, 247 and 248.

FEMALES (F2a AND F2b) - TABLE 6

There were significant ( $p=0.05$  or  $0.01$ ) lower (from control values) group mean body weights for all 3 test article concentrations for the 14 weeks pre-mating for F2a. Body weight gains for this period were (ppm): 0 = 209, 500 = 190, 1,500 = 186 and 3,000 = 178. All 4 groups gained 116-121 g during F2a gestation, and during lactation, the 0, 500 and 1,500 ppm groups lost 3-10 g; whereas, the 3,000 ppm group gained 12 g. During the rest period, 2-6 g were gained (all 4 groups).

Although there were significantly ( $p=0.01$ ) lower body weights in the 3,000 ppm group during gestation (F2b) compared with controls, the weight gained during this 3 week period was 116-124 g for all 4 groups. The 3,000 ppm group gained 8 g during lactation compared with losses of 16, 12 and 4 g in the 0, 500 and 1,500 ppm groups.

**D. Food Consumption**

Food consumption was presented as absolute (g/rat/day) and relative (g/kg body weight/day).

F0 Males (Report Tables 7 and 9, pages 116-118 and 122-124)

Data were recorded for weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 21.

There were essentially similar amounts of food consumed/rat for all 4 groups (exceptions: week 1, 1,500 and 3,000 ppm; week 5, 3,000 ppm; and week 20, 500 ppm significantly,  $p=0.05$  or  $0.01$ , less than controls). As g/kg/day, there was significantly ( $0.01$ ) less eaten by the 3,000 ppm rats at week 1 while statistically ( $p=0.05$  or  $0.01$ ) more was eaten at weeks 2, 3, 4, 7, 8, 9, 10, 20 and 21. The greater amounts consumed (g/kg/day) appeared to be a reflection of body weights lower than controls. The 500 and 1,500 ppm males ate about the same amount of food as did controls.

F0 Females (Report Tables 8 and 10, pages 119-121 and 125-127.

Data were recorded for weeks 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 21.

Absolute (g/rat/day) food consumption was similar for all 4 groups with the following exceptions when it was less than control (statistically significant at  $p=0.05$  or  $0.01$ ): week 1, 500 and 3,000 ppm; week 3, 3,000 ppm; and week 6, 1,500 ppm. Relative (g/kg/day) amounts of food consumed were similar for all 4 groups with the following exceptions ( $p=0.05$  or  $0.01$ ): week 1, 500 and 3,000 ppm ( $<$  control); week 2, 3,000 ppm ( $>$  control); week 7, 3,000 ppm ( $>$  control); week 8, 3,000 ppm ( $>$  control); and week 21, 3,000 ppm ( $>$  control). The greater amounts of food eaten/kg body weight are likely to have been a reflection of body weights lower than controls.

F1 Males (Report Tables 21 and 23, pages 161-164 and 169-172)

Data were recorded for weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 24 and 25.

Presented as g/rat/day, similar amounts of food were consumed except for some isolated intervals when less was consumed than controls ( $p=0.05$  or  $0.01$ ): 500 ppm, 5/16 intervals; 1,500 ppm, 0/16 intervals; and 3,000 ppm, 2/16 intervals. Expressed as g/kg/day, the 3,000 ppm rats had significantly ( $p=0.05$  or  $0.01$ ) greater amounts of food consumed than did controls at all but one interval. There was also an increase ( $p=0.05$  or  $0.01$ ) in the 1,500 ppm animals at 6/16 measured periods. In the 3,000 ppm group at least, this appeared to be the result of body weights lower than those observed in controls.

F1 Females (Report Tables 22 and 24, pages 165-168 and 173-176)

Data were recorded for weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 24 and 25.

Only at weeks 6, 7, 24 and 25 was there a smaller ( $p=0.05$  or  $0.01$ ) amount of food consumed as g/rat/day in the 3,000 ppm group than in controls. The 500 ppm females ate less ( $p=0.05$  or  $0.01$ ) at weeks 3, 4, 5, 6, 7, 8, 9, 11, 24 and 25; whereas, at 1,500 ppm, lesser amounts ( $p=0.05$  or  $0.01$ ) were noted at weeks 3, 4, 6, 7, 9, 24 and 25. Relative consumption (g/kg/day) was greater ( $p=0.05$  or  $0.01$ ) in only the 3,000 ppm group at all intervals except weeks 3, 6, 24 and 25. These data are also an apparent result of lower body weights.

### E. Test Article Consumption

Selected group mean test article consumption data are presented in Table 7.

Table 7

SELECTED GROUP MEAN TEST ARTICLE CONSUMPTION (mg/kg/day) IN A  
2-GENERATION RAT STUDY WITH CHLOROTHALONIL

Week	ppm =	Males			Females		
		500	1500	3000	500	1500	3000
<u>F0 Parents</u>							
1 .....		53	157	292	50	154	278
5 .....		35	103	213	40	118	244
10a .....		28	86	178	34	106	216
21b .....		23	68	145	31	94	201
-----							
<u>F1 Parents</u>							
1 .....		57	178	370	60	181	382
5 .....		39	118	241	45	137	282
14c .....		26	79	164	33	98	210
25d .....		22	68	138	32	95	196

- a = last week before mating for F1a litters
- b = last week before mating for F1b litters
- c = last week before mating for F2a litters
- d = last week before mating for F2b litters

Data extracted from tables on Report pages 53 and 60. [Group mean weekly test article consumption was in Report Tables 11, 12, 25 and 26, pages 128-133 and 177-184.]

### F. Reproduction

Tables 8, 9, 10 and 11 present reproduction and neonatal data for the F0 parents, F1a offspring, F1b offspring, F1 parents, F2a offspring and F2b offspring.

Table 8

F0 GENERATION REPRODUCTION AND F1a NEONATAL DATA IN A 2-GENERATION  
RAT STUDY WITH CHLOROTHALONIL

Parameter	ppm =	0	500	1500	3000
Mating index (1) % (#/#)		74(26/35)	97(34/35)*	97(34/35)*	100(35/35)**
Fertility index (2) % (#/#) .....		96(25/26)	85(29/34)	91(31/34)	91(32/35)
Viability index (3) - %		99	96	99	98
Lactation index(4) - %		100	100	100	100
Liveborn index day 0 - %		98	99	99	98
Litter size, total pups (mean) .....		13.5	13.0	13.1	12.3
Live offspring (mean) ..					
Day 0 .....		13.1	12.9	12.9	12.1
Day 1 .....		13.0	12.7	12.9	11.9
Day 4 pre-cull .....		13.0	12.4	12.8	11.9
Day 4 post-cull .....		8.0	7.7	7.9	7.7
Day 7 .....		8.0	7.7	7.9	7.7
Day 14 .....		8.0	7.7	7.9	7.7
Day 21 .....		8.0	7.7	7.9	7.7
Mean weight of viable pups (g)					
Birth .....		6.2	6.2	6.3	6.3
Day 4 (pre- and post-cull) .....		9.6	9.8	9.5	9.8
Day 7 .....		15.4	15.7	15.5	15.5
Day 14 .....		31.0	31.1	30.8	30.2
Day 21 .....		49.6	49.2	47.9	46.1*

Statistical Analysis: \* = p=0.05; \*\* = p=0.01

(1) # males producing females with signs of mating/# males mated

(2) # males producing pregnant females/# males producing females  
with signs of mating

(3) # neonates viable day 4 (pre-cull)/# neonates viable at birth

(4) # pups alive day 21/# pups alive day 4 (post-cull)

Data extracted from Report Tables 31-42, pages 192-205.

Table 9

F0 GENERATION REPRODUCTION AND F1b NEONATAL DATA IN A 2-GENERATION RAT STUDY WITH CHLOROTHALONIL

Parameter	ppm =	0	500	1500	3000
Mating index (1) % (#/#)		77(27/35)	91(32/35)	91(32/35)	91(32/35)
Fertility index (2) % (#/#) .....		67(18/27)	72(23/32)	84(27/32)	84(27/32)
Viability index (3) - %		99	99	100	100
Lactation index (4) - %		100	100	100	96
Liveborn index day 0 - %		99	98	97	99
Litter size, total pups mean .....		12.4	12.7	13.9	13.0
Live offspring (mean)					
Day 0 .....		12.3	12.6	13.5	12.9
Day 1 .....		12.3	12.5	13.5	12.8
Day 4 pre-cull .....		12.2	12.5	13.5	12.8
Day 4 post-cull .....		7.8	7.7	8.0	7.7
Day 7 .....		7.8	7.7	8.0	8.0a
Day 14 .....		7.8	7.7	8.0	8.0a
Day 21 .....		7.8	7.7	8.0	8.0a
Mean weight of viable pups (g)					
Birth .....		6.6	6.3	6.2	6.3
Day 4 (pre- and post-cull) .....		10.9	10.5	9.4*	9.7
Day 7 .....		17.3	16.9	16.0*	15.9*
Day 14 .....		33.8	33.0	31.4*	29.5**
Day 21 .....		56.2	54.6	51.9**	48.3**

a = 26 litters instead of 27

Statistical Analyses: \* = p=0.05; \*\* = p=0.01

(1) # males producing females with signs of mating/# males mated

(2) # males producing pregnant females/# males producing females with signs of mating

(3) # neonates viable day 4 (pre-cull)/# neonates viable at birth

(4) # pups alive day 21/# pups alive day 4 (post-cull)

Data extracted from Report Tables 46-57, pages 211-224.

Table 10

F1 GENERATION REPRODUCTION AND F2a NEONATAL DATA IN A 2-GENERATION  
RAT STUDY WITH CHLOROTHALONIL

Parameter	ppm =	0	500	1500	3000
Mating index (1) % (#/#)		83(29/35)	83(29/35)	82(28/34)	77(27/35)
Fertility index (2) % (#/#) .....		79(23/29)	93(27/29)	86(24/28)	81(22/27)
Viability index (3) - %		99	95	98	97
Lactation index (4) - %		100	100	100	100
Liveborn index day 0 - %		95	96	94	99
Litter size, total pups mean .....		13.9	12.9	13.1	12.8
Live offspring (mean)					
Day 0 .....		13.2	12.4	12.9	12.7
Day 1 .....		13.0	11.8a	12.8	12.4
Day 4 pre-cull .....		13.0	12.2a	12.7	12.4
Day 4 post-cull .....		8.0	7.8	7.7	7.8
Day 7 .....		8.0	7.8	7.7	7.8
Day 14 .....		8.0	7.8	7.7	7.8
Day 21 .....		8.0	7.8	7.7	7.8
Mean weight of viable pups (g)					
Birth .....		6.3	6.1	6.3	6.3
Day 4 (pre- and post-cull) .....		10.3	10.0	10.3	10.0
Day 7 .....		16.2	15.7	16.4	15.8
Day 14 .....		32.5	31.0	33.2	31.1
Day 21 .....		53.3	50.2	52.5	48.7**

a = 27 litters on day 1 and 26 on day 4

Statistical Significance: \*\* = p=0.01

(1) # males producing females with signs of mating/# males mated

(2) # males producing pregnant females/# males producing females  
with signs of mating

(3) # neonates viable day 4 (pre-cull)/# neonates viable at birth

(4) # pups alive day 21/# pups alive day 4 (post-cull)

Data extracted from Report Tables 61-72, pages 230-243.

Table 11

F1 GENERATION REPRODUCTION AND F2b NEONATAL DATA IN A 2-GENERATION  
RAT STUDY WITH CHLOROTHALONIL

Parameter	ppm =	0	500	1500	3000
Mating index (1) %(#/#)		89(31/35)	85(29/34)a	67(22/33)	76(26/34)a
Fertility index (2) %(#/#) .....		45(14/31)	79(23/29)*	77(17/22)	62(16/26)
Viability index (3) - %		98	100	100	100
Lactation index (4) - %		100	100	100	100
Liveborn index day 0 - %		100	100	100	100
Litter size, total pups (mean) .....		13.0	13.1	12.3	13.2
Live offspring (mean)					
Day 0 .....		12.2	13.0	12.2	13.1
Day 1 .....		12.0	12.9	12.2	13.1
Day 4 pre-cull .....		12.0	12.9	12.2	13.1
Day 4 post-cull .....		7.7	7.9	7.6	7.8
Day 7 .....		7.7	7.9	7.6	7.8
Day 14 .....		7.7	7.9	7.6	7.8
Day 21 .....		7.7	7.9	7.6	7.8
Mean weight of viable pups (g)					
Birth .....		6.3	6.1	6.5	6.2
Day 4 (pre- and post-cull) .....		10.6	9.9	10.7	10.0
Day 7 .....		16.5	15.7	16.8	16.0
Day 14 .....		32.8	31.2	32.2	31.2
Day 21 .....		55.0	50.4*	52.1	48.6**

a = one male was mated to a second female after evidence of mating  
with a first female

Statistical Significance: \* = p=0.05; \*\* = p=0.01

(1) # males producing females with signs of mating/# males mated

(2) # males producing pregnant females/# males producing females  
with signs of mating

(3) # neonates viable day 4 (pre-cull)/# neonates viable at birth

(4) # pups alive day 21/# pups alive day 4 (post-cull)

Data extracted from Report Tables 76-87, pages 249-262.



## REPRODUCTION PARAMETERS -

NOTE: Mating Index (# males producing females with signs of mating/#males mated) and Fertility Index (# males producing pregnant females/# males producing females with signs of mating

F0 adults, controls: Of 35 pair mated/group, the number of females showing signs of mating (plug/sperm) were: 26, 34, 34 and 35 (0, 500, 1,500 and 3,000 ppm) for the F1a litters and 27, 32, 32 and 32 for the F1b litters. The fertility indices were similar for all 4 groups for the F1a litters (96, 85, 91 and 91% for 0, 500, 1,500 and 3,000 ppm groups). For the F1b litters, the fertility indices were 67, 72, 84 and 84%, respectively).

F1 adults, controls: The mating indices were similar for all 4 groups regarding the F2a litters (83, 83, 82 and 77% at 0, 500, 1,500 and 3,000 ppm). For the F2b litter, the mating indices were 89, 85, 67 and 76%, respectively. F2a litter fertility indices were 79, 93, 86 and 81%, respectively. However, for the F2b litters, fertility indices were 45 (14/31), 79 (23/29), 77 (17/22) and 62 (16/26) %.

Report page 69 states, "The pregnancy rates for all groups and especially the control group was lower than the pregnancy rate in the F1a littering. The difference may have been due to the age or size of the females at the time of the F1b littering."

There were no other reproduction parameters which were felt to show a difference between any of the 4 groups.

## NEONATAL PARAMETERS -

Live offspring (group mean) - The days 0, 1 and 4 (pre-cull) values for only the F1a pups, suggested (no statistical significance) a possible smaller litter size in the 3,000 ppm group compared with the control or other dose groups (500 and 1,500 ppm). For the 0, 500, 1,500 and 3,000 ppm groups, the values were: day 0 = 13.1, 12.9, 12.9 and 12.3; day 1 = 13.0, 12.7, 12.9 and 11.9; and day 4

(pre-cull) = 13.0, 12.4, 12.8 and 11.9.

Viable pup weights (group mean) -

F1a - lower ( $p=0.05$ ) weight only on day 21 at 3,000 ppm

F1b - lower ( $p=0.05$  or  $0.01$ ) weights on days 7, 14 and 21 at 1,500 and 3,000 ppm. [1,500 ppm, also on day 4 pre- and post-cull]

F2a - lower ( $p=0.01$ ) weight only on day 21 at 3,000 ppm

F2b - lower ( $p=0.05$  or  $0.01$ ) weight only on day 21 at 500 ( $0.05$ ) and 3,000 ( $0.01$ ) ppm

All other neonatal parameters appeared to be similar for all 4 groups.

**G. Parental Necropsy Findings**

**1. F0 ADULTS**

Gross - Table 12 presents gross necropsy findings which may have been the result of test article administration.

In males only, kidney findings of granular, slightly green and enlarged, were described in 4 or 5 3,000 ppm rats compared with none in controls. The forestomach of males and females appeared to be thickened and had mucosal foci at all 3 treatment levels compared with respective controls. Depressions/erosions/pits occurred in female forestomachs only at 500 and 3,000 ppm. There is a questionable increase in the number of males only with enlarged cervical lymph nodes at 1,500 and 3,000 ppm.

Table 12

POSSIBLE TEST ARTICLE RELATED GROSS NECROPSY FINDINGS OF F0 PARENTS  
IN A 2-GENERATION RAT REPRODUCTION STUDY WITH CHLOROTHALONIL

ppm =	Males				Females			
	0	500	1500	3000	0	500	1500	3000
<b>KIDNEY</b>								
Granular .....	0	1	0	4	0	0	0	0
Slightly green .....	0	0	1	5	0	0	0	0
Enlarged .....	0	0	1	5	0	0	0	0
Mottled .....	15	16	19	19	5	10	6	8
<b>STOMACH</b>								
Forestomach mucosa thickened .....	0	2	5	9	0	1	10	18
Forestomach mucosa foci .....	0	6	6	7	1	3	3	4
Forestomach depressions/erosions/pits .....	0	0	0	0	0	3	0	2
<b>CERVICAL LYMPH NODE</b>								
Enlarged .....	7	4	12	13	3	1	4	3

NOTE: 35 rats/sex/group

Data extracted from Report Tables 13 and 14, pages 135-137.

Microscopic - Table 13 presents microscopic findings which may have been the result of test article administration.

F0 parental microscopic observations indicated kidney changes in males and females at all 3 chlorothalonil concentrations. Forestomach hyperkeratosis and squamous epithelial hyperplasia were noted in all (35/sex) 1,500 and 3,000 ppm rats of both sexes compared with no more than 2/35 in a control group; in addition, there was a dose-related increase in severity.

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Table 13

POSSIBLE TEST ARTICLE RELATED MICROSCOPIC FINDINGS OF F0 PARENTS IN  
A 2-GENERATION RAT REPRODUCTION STUDY WITH CHLOROTHALONIL

ppm =	Males				Females			
	0	500	1500	3000	0	500	1500	3000
<b>KIDNEY</b>								
Epithelial hyperplasia	6	27	35	35	0	4	19	32
Tubular hypertrophy ....	0	9	24	33	0	2	14	26
Clear cell hyperplasia	0	8	27	25	0	0	0	1
Pigmentation .....	0	5	21	23	0	0	0	1
Karyomegaly .....	1	0	1	6	0	2	4	1
Adenoma, tubular carcinoma .....	0	0	0	2	0	0	0	0
Regenerative epithelium	0	0	0	0	5	4	7	6
<b>FORESTOMACH</b>								
Hyperkeratosis (total)	1	2	35	35	0	13	35	35
minimal .....	0	2	14	0	0	12	6	2
slight/mild .....	0	0	21	25	0	1	29	23
moderate/moderate severe .....	1	0	0	10	0	0	0	0
<b>Squamous epithelium</b>								
hyperplasia (total)	2	9	35	35	1	33	35	35
minimal .....	0	8	2	0	1	26	0	0
slight/mild .....	1	1	30	0	0	7	35	31
moderate .....	1	0	3	35	0	0	0	4

NOTE: 35 rats/sex/group

Data extracted from Report pages 85 and 87.

2. F1 ADULTS

Gross - Table 14 presents gross necropsy findings which may have been the result of test article administration.

In both males and females, kidney findings (granular, pale and enlarged) were noted primarily in the 1,500 and 3,000 ppm groups compared with 0 rats in controls with these observations. There were more animals with mottled kidneys in both sexes of 1,500 and 3,000 ppm groups than in respective controls. Forestomach mucosa with raised white areas/foci/nodule was described for all doses of both sexes compared with no controls.

Table 14

POSSIBLE TEST ARTICLE RELATED GROSS NECROPSY FINDINGS OF F1 PARENTS  
IN A 2-GENERATION RAT REPRODUCTION STUDY WITH CHLOROTHALONIL

ppm =	Males				Females			
	0	500	1500	3000	0	500	1500	3000
<b>KIDNEY</b>								
Granular .....	0	1	9	8	0	0	2	2
Green .....	0	0	0	2	0	0	0	0
Pale .....	0	1	5	3	0	0	1	1
Enlarged .....	0	0	5	9	0	0	3	4
Mottled .....	4	5	9	9	2	1	8	6
<b>STOMACH</b>								
Forestomach mucosa raised white areas/ foci/nodule .....	0	6	1	2	0	3	4	3
Non-glandular, diffuse dark red foci .....	0	2	0	0	0	0	0	0
Thickened .....	0	1	0	1	0	0	0	0
Glandular reddened areas/pitted areas ..	0	0	0	1	0	0	0	0
<b>CERVICAL LYMPH NODES</b>								
Enlarged .....	7	5	5	9	2	1	1	2

NOTE: Rats/group (0, 500, 1,500 and 3,000 ppm): males = 35, 34, 33  
and 34; females = 35, 35, 35 and 35  
Data extracted from Report Tables 27 and 28, pages 186-189.

Microscopic - Table 15 presents microscopic findings which may have been the result of test article administration.

F1 parental microscopic observations indicated kidney changes at all 3 concentrations in males and at 1,500 and 3,000 ppm in females. Forestomach hyperkeratosis and squamous epithelial hyperplasia were reported at all concentrations compared with none in control rats of either sex; in addition, there was a dose-related increase in severity.

Table 15

POSSIBLE TEST ARTICLE RELATED MICROSCOPIC FINDINGS OF F1 PARENTS IN  
A 2-GENERATION RAT REPRODUCTION STUDY WITH CHLOROTHALONIL

ppm =	Males				Females			
	0	500	1500	3000	0	500	1500	3000
<b>KIDNEY</b>								
Epithelial hyperplasia	1	22	34	35	0	1	15	28
Tubular hypertrophy ....	0	8	24	31	0	0	13	31
Clear cell hyperplasia	0	1	15	26	0	0	1	7
Pigmentation .....	0	0	7	18	0	0	1	2
Karyomegaly .....	0	0	4	13	0	0	4	11
Adenoma, tubular								
carcinoma .....	0	0	0	0	0	0	0	0
Regenerative epithelium	0	0	0	0	2	5	8	14
<b>FORESTOMACH</b>								
Hyperkeratosis (total)	0	11	34	35	0	25	25	35
minimal .....	0	9	7	1	0	22	20	2
slight/mild .....	0	2	27	26	0	3	5	31
moderate/moderate								
severe .....	0	0	0	8	0	0	0	2
Squamous epithelium								
Hyperplasia (total)	0	22	34	35	0	29	33	35
minimal .....	0	14	5	0	0	23	16	1
slight/mild .....	0	6	27	18	0	5	14	28
moderate .....	0	2	2	17	0	1	3	6

NOTE: 35 rats/sex/group

Data extracted from Report pages 85 and 87.

#### H. Neonatal Clinical Observations

No treatment related clinical signs were reported for neonates of the F1a, F1b, F2a or F2b generations.

#### I. Neonatal Necropsy Findings

1. F1a - One 3,000 ppm stillborn had an abnormal head (no eye lids, one apparent nostril and no visible external opening for the oral cavity).
2. F1b - No abnormalities reported.
3. F2a - In the control group, 2 males were joined from the thoracic region up to the head.

4. F2b - No abnormalities reported.

DILATION OF THE RENAL PELVIS

Table 16 presents renal pelvis dilation for F1a, F1b, F2a and F2b offspring.

Table 16

PUP AND LITTER INCIDENCE OF RENAL PELVIS DILATION IN OFFSPRING FROM  
A 2-GENERATION RAT STUDY WITH CHLOROTHALONIL

ppm =	# pups with finding				# litters with finding			
	0	500	1500	3000	0	500	1500	3000
F1a (number examined) ..	200	223	244	247	25	29	31	32
Unilateral (total) ..	6	12	8	10	5	10	6	9
slight .....	3	8	3	2	4	7	3	2
moderate .....	3	3	1	5	2	3	1	5
extreme .....	0	0	4	2	0	0	3	2
Bilateral (total) ...	2	4	4	6	2	2	4	4
slight .....	1	1	1	1	1	1	1	1
moderate .....	1	3	1	1	1	2	1	1
extreme .....	0	0	2	4	0	0	2	3
F1b (number examined) ..	70	106	146	138	18	23	27	26
Unilateral (total) ..	1	1	5	1	1	1	4	1
slight .....	1	0	4	0	1	0	3	0
moderate .....	0	1	1	1	0	1	1	1
F2a (number examined) ..	184	203	176	172	23	26	23	22
Unilateral (total) ..	16	17	10	11	10	9	8	7
slight .....	11	10	8	8	8	7	8	6
moderate .....	3	7	2	2	3	5	2	2
extreme .....	2	0	0	1	2	0	0	1
Bilateral (total) ...	6	4	2	3	4	4	2	3
slight .....	2	2	0	3	2	2	0	3
moderate .....	4	1	1	0	2	1	1	0
extreme .....	0	1	1	0	0	1	1	0
F2b (number examined) ..	108	182	130	132	14	23	17	17
Unilateral (total) ..	9	10	9	10	6	6	7	7
slight .....	3	5	4	1	3	4	4	1
moderate .....	5	3	2	7	4	1	2	5
extreme .....	1	2	3	2	1	2	3	2
Bilateral (total) ...	5	5	5	3	3	3	4	3
slight .....	1	1	0	2	1	1	0	2
moderate .....	2	2	2	1	1	2	2	1
extreme .....	2	2	3	0	1	2	2	0

Data extracted from Report Tables 43, 58, 73 and 88, pages 208, 227, 246 and 265.

The Reviewer has no comments regarding the Materials and Methods section.

A detailed description of the statistical analyses employed was included in the Report.

A Good Laboratory Practice Compliance Statement, a Quality Assurance Statement and a list of Quality Assurance inspections were included.

There were no historical control data included with the Report.

The Registrant stated that the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to the results of this study and that the study neither meets nor exceeds any of the applicable criteria. This Reviewer agrees.

### III. DISCUSSION

Analytical data for purity, concentration, stability and homogeneity appeared to have been within acceptable limits.

There was no parent mortality throughout the study which was considered attributable to test article administration. No F0 adults died. In the F1 generation, moribund sacrifices or deaths were noted as follows (ppm): 0 = 0, 500 = 1 male, 1,500 = 2 males and 2 females and 3,000 = 1 male.

Loose stools in 7 males from the F0 generation 3,000 ppm group with 6 rats having this finding only once were not considered to have been related to chlorothalonil administration.

In males of both the F0 and F1 generations, there were statistically significant lower body weights in the 3,000 ppm groups in addition to a trend for lower body weights. Body weight gains were reduced by (%) 5, 8 and 15 at 500, 1,500 and 3,000 ppm in F0 males and 6, 7 and 11, respectively in F1 males. The Report abstract on page 11 indicated that the No Observed Effect Level (NOEL) for body weights was 500 ppm in both generations. Group mean weights for the F0 rats at 500 and 1,500 ppm at week 30 were 622 and 614 g and in the F1 animals, 658 and 664 g, respectively. These values represent an approximate 4-6% decrease from control values. This Reviewer feels that if the 1,500 ppm values showed an effect, so did the 500 ppm values. However, 4-6% decrease is considered to be relatively small and therefore, the NOEL might have been 1,500 ppm.

Body weights for 3,000 ppm F0 females prior to mating were significantly lower than control values. During gestation for F1a and F1b, the weight gains were similar for all 4 groups. The NOEL for body weights in F0 females, prior to mating, appeared to be 1,500 ppm, not the 500 ppm stated in the Report abstract. For F1a and F1b lactation, the 3,000 ppm females gained weight compared with



controls which essentially maintained or lost weight, thus not indicating a negative effect by chlorothalonil.

F1 female body weights were statistically ( $p=0.01$ ) lower than controls prior to mating at all 3 concentrations with gains being reduced by 9, 11 and 15% at 500, 1,500 and 3,000 ppm. At least part of the severity of lower body weights in all chlorothalonil groups appeared to have been a reflection of the difference in the week 0 weights (112, 105, 108 and 102 g at 500, 1,500 and 3,000 ppm, respectively).

Absolute (g/rat/day) food consumption was generally similar for all groups in the F0 and F1 males and females. Relative (g/kg/day) reflected the decreases in body weight gain.

The fertility index (number of males producing pregnant females/number of males producing females with signs of mating) appeared to be lower for F0/F1b controls (67, 72, 84 and 84% for 0, 500, 1,500 and 3,000 ppm, respectively) and especially for F1/F2b controls (45, 79, 77 and 62%, respectively). Report page 69 indicated that the Authors felt the lower pregnancy rates may have been due to the age or size of the females in the F1b littering. There does not appear to be an explanation for the apparent lower pregnancy rates in the control groups.

The only neonatal parameter which seemed to be influenced by test article administration was a decrease in group mean viable pup weights, primarily on day 21 of lactation at 3,000 ppm for all litters (F1a, F1b, F2a and F2b).

F0 and F1 adult gross necropsy findings indicated kidney changes as evidenced by granular and/or green and/or pale and/or enlarged observations. These were described primarily for F0 3,000 ppm males as well as for 1,500 and 3,000 ppm F1 males and females. No controls had these findings. The forestomach of F0 males and females showed mucosal thickening and foci (all treated groups). In F1 parents, the primary forestomach finding was raised white areas/foci/nodule in both sexes at all concentrations (none in controls).

Tissue microscopic examination of F0 and F1 parents indicated the following kidney effects: epithelial hyperplasia, tubular hypertrophy, clear cell hyperplasia, pigmentation and karyomegaly. These appeared to involve more males than females and to be present essentially at all concentrations in males but primarily at 1,500 and 3,000 ppm in females. No more than one control rat showed any of the above findings except for 6 in F0 control male epithelial hyperplasia, compared with 27, 35 and 35 in the 500, 1,500 and 3,000 ppm groups. In females only, regenerative epithelium was described in 4-7 animals in all 4 F0 parents and at the following incidences in F1 parents (ppm): 0 = 2, 500 = 5, 1,500 = 8 and 3,000 = 14.

Forestomach microscopic findings in F0 and F1 males and females were hyperkeratosis and squamous epithelial hyperplasia. Severity was described as minimal, slight/mild and moderate/moderate severe.

No more than 3 F0 adult (both sexes) controls were reported to be affected as compared with essentially all F0 and F1 1,500 and 3,000 ppm rats of both sexes. In addition to a dose-response effect regarding the number of involved animals, there was a dose-response pertaining to severity of the findings. The 500 ppm animals also had these observations.

The only necropsy findings in neonates (F1a, F1b, F2a and F2b) suggestive of having been the result of chlorothalonil treatment, concerned renal pelvis dilation, and this appeared to have been only in the F1a generation. As seen in Table 16, there was the suggestion of an increase in the number of males and females with unilateral and bilateral involvement. Primarily, the descriptive severity "extreme" was not reported for the 0 or 500 ppm groups of either sex; but, for unilateral or bilateral, involved 2-4 rats (2-3 litters) from the 1,500 and 3,000 ppm groups.

#### IV. CONCLUSIONS

Chlorothalonil, administered as a dietary admix at concentrations of 0, 500, 1,500 and 3,000 ppm to rats in a 2-generation (2 litters/generation) reproduction study, appeared to cause the following effects:

Parental body weight/body weight gain: lower than control primarily at 3,000 ppm

Parental kidneys and forestomachs: essentially at 500, 1,500 and 3,000 ppm (thickening/hyperplasia/hypertrophy of tissue)

Neonatal lower body weight on lactation day 21.

Neonatal renal pelvis dilation: a suggestive increase in the incidence (only in the F1a generation)

Adult No Observed Effect Level (NOEL) = not attained (kidney and forestomach lesions at Lowest Dose Tested, 500 ppm = about 38 mg/kg as calculated by Reviewer from Report data).

Adult Lowest Observed Effect Level (LOEL) = 500 ppm (Lowest Dose Tested)

Reproduction NOEL = 1,500 ppm (about 115 mg/kg as calculated by Reviewer from Report data).

Reproduction LOEL = 3,000 ppm (about 234 mg/kg as calculated by Reviewer from Report data) - lower neonatal body weights on lactation day 21

**Classification: Core Minimum**

This study satisfies the Guideline requirements (§83-4) for a 2-generation reproduction study in rats.