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DATA EVALUATION RECORD

Study Type: Oncogenicity Study (Non-guideline Study)

Test Animal: Fischer 344 Rats

EPA Identification No.s: MRID (Accession) No.: 412505-02
Record No.: Caswell No.: 215B
HED Project No.: 0-0030

Test Material: Technical Chlorothalonil, SDS-2787; T-117-12

Synonym: 2,4,5,6-Tetrachloroisophthalonitrile

Dosages: 0, 2.0, 4.0, 15.0, 175.0 mg/kg/day

Sponsors: Fermenta ASC Corp., 5966 Heisley Rd.,
P.O. Box 8000, Mentor, Ohio 544061-8000
and
SDS Biotech K.K., 12-7 Higashi Shimbashi 2-Chome,
Minato-ku, Tokyo 105, Japan

Study Number: 1102-84-0103-TX-007

Study Period: December 6, 1985 to March 6, 1988 (In Life Study)

Testing Facility: International Research and Development Corp.,
500 N. Main St., Mattawan, MI 49071
and
Experimental Pathology Laboratories, Inc.
P.O. Box 474, Herndon, Virginia 22070
and
Test Substance Analysis Laboratory Ricerca Inc.
P.O. Box 1000, Painesville, Ohio 44077

Title of Report: A Tumorigenicity Study of Technical Chlorothalonil in Rats

Author: Nelson H. Wilson, B.S. and James C. Killeen, Ph.D.

Report Issued: June 7, 1989

Background:

The present study is a follow up of another oncogenicity study in Fischer 344 rats fed with chlorothalonil in the diet at higher dose levels (40, 80, and 175 mg/kg/day). In that study,

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dose related tumors and hyperplasia/hyperkeratosis in the stomach, plus tubular tumors and a preneoplastic tubular hyperplasia in the kidneys were observed in all treated groups. The NOEL for these lesions could not be determined because it was below the lowest dose tested (40 mg/kg/day).

In the present study, chlorothalonil in the diet was fed to Fischer 344 rats at dose levels of 0, 2.0, 4.0, 15.0, and 175 mg/kg/day for a lifetime to determine the NOEL for the preneoplastic and neoplastic lesions in the kidneys as well as forestomach following chronic administration of chlorothalonil in rats.

The interim report covering the first year of the study was reviewed by the Toxicology Branch in June 8, 1988 (copy of DER is appended as Appendix K). The first year's findings included equal treatment related lesions in the kidneys of the low-mid (4.0 mg/kg/day), high-mid (15 mg/kg/day) and high dose (175 mg/kg/day) males, and in the high dose (175 mg/kg/day) female rats, as well as in the forestomach of the high-mid (15.0 mg/kg/day) and high dose (175 mg/kg/day) rats in both sexes. A renal tubular adenoma in one high dose male rat and dark urine in the high dose rats of both sexes were also observed.

Conclusions:

The present review covers the result of the entire oncogenicity study. In the 175 mg/kg/day group there was increased mortality, kidney weight and parathyroid enlargement, as well as decreased body weight and food consumption (g/kg/day) were noted in both sexes as compared to the control. An increased urine volume and decreased urine specific gravity were noted only in the high dose males. In the high-mid dose (15 mg/kg/day) group, a slightly increased kidney weight and an elevation of serum urea nitrogen level were observed. In the low-mid dose group (4.0 mg/kg/day), a slightly higher kidney weight was noted. No treatment related effect was observed in the low dose group (2.0 mg/kg/day).

The incidence and/or severity of epithelial hyperplasia in the proximal convoluted tubules of the kidney were increased in both sexes of the high-mid dose (15 mg/kg/day) and the high dose (175 mg/kg/day) groups, as well as in the males and possibly in the females of the low-mid dose group (4.0 mg/kg/day) as compared to the control. The incidence and/or severity of this hyperplasia increased with increasing dose. Increased renal tubular adenomas and carcinomas were observed in association with the above noted hyperplastic lesion in males of low-mid dose (4.0 mg/kg/day) and in both sexes of the high-mid dose (15 mg/kg/day) as well as in high dose (175 mg/kg/day) groups. No treatment related effect was observed in the low dose group (2.0 mg/kg/day).

An increased incidence and/or severity of hyperplasia, hyperkeratosis and ulcers or erosions of the squamous mucosa of the forestomach was observed in both sexes of the low-mid dose (4.0 mg/kg/day), high-mid dose (15 mg/kg/day) and the high dose (175 mg/kg/day) groups, as compared to the control. The incidence and/or severity of these lesions also increased with increasing dose. In association with these forestomach lesions, increased neoplastic lesions in some treated groups were also observed as compared to the controls, i.e. papillomas of the squamous mucosa of the forestomach in the low-mid dose (4.0 mg/kg/day) group as well as papillomas and carcinomas in the low-mid dose (4.0 mg/kg/day), high-mid dose (15 mg/kg/day) and high dose (175 mg/kg/day) groups. Lesions in the forestomach were considered to be the result of chronic irritation of the gastric mucosa by chlorothalonil. No treatment related effect was observed in the low dose group (2.0 mg/kg/day).

According to the result of this study, the overall NOEL was determined to be 2.0 mg/kg/day based on the urinalysis and clinical chemistry parameters, kidney weight, preneoplastic hyperplasia of the kidney, and chronic irritation of the forestomach data. The LOEL is determined to be 4.0 mg/kg/day, based on increased neoplastic lesions of the forestomach and the kidneys. It appears that the doses employed in this study were sufficient to produce a compound related effect and the MTD was reached. This study as presented is classified as core supplementary.

The present study confirms that chlorothalonil induces lesions similar to those identified in a previous oncogenicity study using higher dose levels of 40, 80, and 175 mg/kg/day.

In other oncogenicity studies, similar significant increases of renal and gastric lesions were also seen in Osborne-Mendel rats and CD-1 mice with chlorothalonil in the diet. It is noted that chlorothalonil was classified by the Toxicology Branch Peer Review Committee as a probable human carcinogen (B2) based on an increased incidence of malignant and/or combined malignant and benign tumors (both sexes) in two species (two rats strains and the CD-1 mouse).

The study accomplished its original purpose of determining the effects of chlorothalonil on kidneys and stomach at doses of up to 175.0 mg/kg/day. Although only the kidneys and stomachs were examined histopathologically, most other required tissues and organs were collected and preserved for further study if needed.

Core Classification: Core-Supplementary Data

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Study Title: A Tumorigenicity Study of Technical Chlorothalonil in Rats

Author: Nelson H. Wilson, B.S. and James C. Killeen, Ph.D.

Report Date: June 7, 1989 Study No.: 1002-84-0103-TX-007

Testing Facility: International Research and Development Corp., 500 N. Main St., Mattawan, MI 49071
and
Experimental Pathology Laboratories, Inc. P.O. Box 474, Herndon, Virginia 22070
and
Test Substance Analysis Laboratory Ricerca Inc. P.O. Box 1000, Painesville, Ohio 44077

Test Material: Technical Chlorothalonil, SDS-2787-1002; T-117-12; 2,4,5,6-Tetrachloroisophthalonitrile

Test Animal: Fischer 344 Rats

1. OBJECTIVE

The objective of this study was to establish the no-effect level for preneoplastic and oncogenic effects in the kidney and forestomach in Fischer 344 rats following chronic dietary administration of technical chlorothalonil.

2. MATERIALS AND METHODS

The in-life and necropsy phases of this study were conducted at the International Research and Development Corp., Mattawan, Michigan; the histopathologic phase was conducted at the Experimental Pathology Laboratories, Inc., Herndon, Virginia; and the test article and dietary analyses were conducted at the Test Substance Analysis Laboratory Ricerca Inc., Painesville, Ohio.

The following account described below is a summary of the study protocol including 16 protocol amendments (p. 46-162).

Test Material: Physical Description: A gray powder with a purity of approximately 98.3% and 4.3μ particle size
Lot No.: D-5840923 Compound ID #: SDS-2787-1002
Sources: Greens Bayou Production Plant, Fermenta ASC Corp., 2239 Haden Rd., Houston, Tx (compound used for diet preparation and denoted as blinded compound no. T-117-12, p.19); Ricerca Inc., Painesville, Ohio (compound used for standards for diet assay, p. 2140)
Storage: In closed container at room temperature in the dark

Test Animals: Species: Fischer 344 Rat
Source: Charles River Breeding Laboratory, Inc., Charles River Kingston, Route 209, P.O. Box 241, Stoneridge, N.Y. 12484
Total Number: 325 males and 325 females
Age: Approximately 6 weeks old at start of study
Body Weight: Males = 67-97 g; females = 59-83 g on day 0
Caging: In individual stainless steel cages
Acclimation period: At least 7 days
Feed: Purina Certified Rodent Chow #5002 & water were provided ad libitum. Feed was withheld one day prior to blood collection, and prior to scheduled necropsy

Environmental Parameters: Air temperature = 73°F ± 2.1°; Relative Humidity = 46% ± 10.9%; 12 hours dark/light cycle; fresh air exhanges = 8-12 per hour.

Study Design

This study was designed to establish the no-effect level for preneoplastic and oncogenic effects in the kidney and forestomach in Fischer 344 rats following chronic dietary administration of technical chlorothalonil.

Study Duration

The study was scheduled to terminate at the end of 30 months of compound administration. Ten animals/sex from each group were necropsied after 12 months of compound administration. Terminal necropsy of surviving high dose group males was conducted during month 23. Surviving animals in all other male groups were terminated during month 26. Surviving females in all groups were terminated during month 29.

Group Arrangement

Animals were assigned to the study using a computer-generated randomization as follows:

Test Group	Dosage (mg/kg/day)	Rats (Males/Females)
Control	0	65/65
Low Dose	2.0 (1.5@)	65/65
Low-Mid Dose	4.0 (3.5@)	65/65
High-Mid Dose	15.0	65/65
High Dose	175.0	65/65

@ Original design was to administer doses of 1.5 and 3.5 mg/kg/day, respectively, but because of dietary binding at low levels, diets were mixed with higher levels of chlorothalonil (see discussions on p. 9-10 of this DER).

Diet Preparation

The diets containing chlorothalonil were prepared weekly. Concentration of the test material was adjusted weekly for the first 14 weeks and every two weeks thereafter. In each week, portions of the diet were fed to the animals on the day of diet preparation and the remaining portions of the diet were frozen for use later in the week. The rats were fed twice per week with the portions of the diets that had been stored frozen being used to feed the animals for the last three days of the week.

Diet Analyses

Samples of the diets were collected and analyzed for chlorothalonil content on the day of diet preparation and four days after diet preparation. Homogeneity and stability studies were conducted prior to study initiation. Test material stability was analyzed prior to the initiation of the study, and at 6, 8, 12, 18 and 24 months as well as at study termination.

Clinical Observations

The rats were checked twice daily for mortality, moribundity and signs of toxicity. Detailed physical examinations were conducted weekly.

Body Weights

Individual body weights were taken weekly, from one week prior to study initiation through week 14 and every two weeks thereafter to termination.

Food Consumption

Individual food consumption was measured over a four day period once per week for the first 14 weeks and every two weeks thereafter to termination.

Individual Compound Intake

Individual compound intake was measured once per week for the first 14 weeks after initiation of test article administration, and every two weeks thereafter to termination.

Clinical Pathology Evaluation

Blood samples were collected from the orbital sinus of rats under anesthesia from each group at 12, 18, and 24 months, and at termination of the study. At each interval ten rats/sex were selected at random and fasted overnight prior to blood collection.

a. Hematology

Hematology tests were conducted at 12, 18, and 24 months, and at termination of the study. Parameters evaluated were hemoglobin, hematocrit, erythrocyte count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), leukocyte count (total and differential), and platelet count.

b. Clinical Chemistry

Clinical chemistry tests were conducted at 18 and 24 months and at termination of the study. No explanation was given why clinical chemistry tests were not conducted at 12 months. At each interval, blood serum from the same rats selected for hematologic study were used. Parameters examined were as follows: alkaline phosphatase, blood urea nitrogen (BUN), lactic dehydrogenase, alanine aminotransferase, aspartate aminotransferase, glucose, total protein, albumin, globulin, albumin/globulin (A/G) ratio, inorganic phosphate, calcium, sodium, potassium, chloride, creatinine (erroneously spelled as creatine on p. 114 of the study report), total bilirubin (direct bilirubin was also determined for those rats whose total bilirubin concentration was >1.0) and total cholesterol.

c. Urinalysis

Urine was collected overnight from ten randomly selected rats of each sex/group at 18 and 24 months and at termination of the study. No explanation was given why urinalysis tests were not conducted at 12 months of study. At each interval, the rats selected for hematologic and clinical chemistry evaluations were utilized for urinalysis. Parameters evaluated were the color, volume, appearance, specific gravity, occult blood, protein, pH, bilirubin, ketones, glucose, nitrites, urobilinogen and microscopic examination of sediment.

Ophthalmologic Examination

Ophthalmologic examination by a Veterinary Ophthalmologist was conducted on all surviving high dose males during month 23. All other survivors were subjected to ophthalmologic examination at 24 months (p. 17 & 20).

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Macroscopic Examinations

All rats which died or were sacrificed in extremis and all rats sacrificed at the scheduled intervals were subjected to macroscopic examination. At twelve months, an interim necropsy of 10 animals of each sex in each group was conducted. Terminal necropsy of surviving high dose male rats was conducted during month 23. Surviving males in all other groups were terminated during month 26 and surviving females in all groups were terminated during month 29. Rats sacrificed at scheduled necropsy were fasted overnight, anesthetized with ether, killed by exsanguination, and then necropsied. All moribund rats were also necropsied after they were killed by the same procedure described above.

Tissues harvested from all rats were fixed in Carson's modified Millonig's phosphate buffered formalin. These tissues are listed in the attached Appendix A. At each necropsy, the first organs to be removed were the kidneys, and prior to being weighed, the left kidney was cut in half longitudinally and the right kidney was cut in half transversely. The stomach was filled with fixative prior to being immersed in fixative. Two to four days after fixation, the stomach was opened along the greater curvature. The mucosal surface was examined for grossly observable lesions before the stomach was returned to the fixative. The lungs were inflated prior to fixation.

Organ Weights

The brain, liver and paired kidneys from all animals at the interim (12 months) and terminal necropsies were weighed.

Histopathological Evaluation

Only the kidneys, stomach, and renal and mesenteric lymph nodes were subjected to histopathological evaluation. These fixed tissues were trimmed, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined microscopically. All other tissues harvested were fixed and stored in fixative (see attached Appendix A, copied from p. 198 of the study report).

The first histopathological evaluation was conducted on tissues from all rats which died or were sacrificed in extremis and from all rats sacrificed at the one year interim necropsy. The second evaluation was conducted on all the remaining rats which died or were sacrificed in extremis after 12 months and at study termination.

Statistical analysis

Analysis of body weights, food consumption data (g/rat/day and g/kg/day), liver and kidney weights (absolute, relative to brain weight, and relative to body weight), brain weight (absolute and relative to body weight), and clinical observation incidence were conducted using analysis of variance (1-way classification), Bartlett's test (for equal or unequal variances) using Dunnett's multiple comparison tables to judge significance of difference.

Compliance Statements

- o A signed Statement of Confidentiality Claim was provided.
- o A signed Statement of compliance with EPA GLPs was provided.
- o A signed Quality Assurance Statement was provided.

RESULTS AND DISCUSSIONS

a. Analyses of Test Article and Diet

In the report submitted, two sources of the test compound were noted: Greens Bayou Production Plant, Fermenta ASC Corp., 2239 Haden Rd., Houston, Texas (p.19) and Ricerca Inc., Painesville, Ohio (p. 2140). Apparently, chlorothalonil that was used in the rat diet was different from that used as the standard for the diet assays. The former was identified as SDS-2787-1002 with a purity of 98.3% (p. 19) and the latter was identified as SDS-2787-0502 with a purity of 98.9% (p. 2140). Since no other identities were given, it can not be determined whether the test article used in this study came from the same batch.

Analysis of diet samples collected on the day of diet preparation indicated that the diets containing chlorothalonil were prepared at or near the intended concentrations, i.e. between 95% and 100% of nominal. Four days after the diets were prepared, concentrations of test material in the diets decreased to 79% and 86% for the low and low-mid dose groups, respectively. Decreased extractable chlorothalonil in the diet was due to binding of the test article to the basal diet. Diets for the low and low-mid dose groups were prepared at concentrations to deliver dose levels of 2.0 and 4.0 mg/kg/day on the day of preparation so that the rats would receive the intended dose (as per protocol) levels of at least 1.5 and 3.5 mg/kg/day, respectively.

The nominal and calculated mean test article concentrations derived from the mean compound consumption data corrected for potential binding in each dose level are as follows:

Dose Group	Mean Compound Consumption (mg/kg/day)@					
	Nominal		Analytical Availability			
			Completed[a]		Partial[b]	
	Males	Females	Males	Females	Males	Females
Low	2.1	2.1	2.0	2.0	1.8	1.8
Low-Mid	4.2	4.2	4.1	4.1	3.8	3.8
High-Mid	15.8	15.7	15.5	15.3	15.3	15.2
High	181	182	181	182	183	183

@ = derived from Table 2, p. 22 & 25 of the study report.
a = Assumes availability of chlorothalonil to the animals is unaffected by binding in the diet
b = Assumes binding of chlorothalonil in the diet has some effects on its availability to the animals

Since significant binding only occurred in the low and low-mid dose groups, the mean corrected compound consumptions for these groups were determined to be 1.8 and 3.8 mg/kg/day, respectively. For purposes of this study, however, these figures were rounded up to 2.0 mg/kg/day and 4.0 mg/kg/day and they were designated as the low and low-mid dose levels, respectively. The result of the diet assays indicated that the diets were prepared at or near their intended concentration throughout the study. Only on two occasions were the test article concentration levels low: for the low dose males (erroneously noted in the report as group I on p. 2150) at week 92 and for the low dose females (erroneously noted in the report as group I on p. 2150) at week 82; the mean assay values were only 59% and 58%, respectively, of the intended concentration. The basal diet contaminant analysis was not presented in the report.

f. Compound Intake

The calculated compound intake using the diet analysis data from day 0 of each study week yielded compound intake values comparable to those obtained by using nominal dietary concentrations. The calculated compound intake corrected for potential binding of chlorothalonil to the basal diet, yielded the following values, 1.8, 3.8, 15.3, and 183 mg/kg/day for the low, low-mid, high-mid, and the high dose groups, respectively. Thus the high dose group was given a dose of 183 mg/kg/day, or about 5% more than the intended 175 mg/kg/day dose (p. 338-348).

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b. Mortality

Fifty-three (not 54 as indicated in the report) control (23M/30F), forty-eight low dose (22M/26F), fifty-eight low-mid dose (22M/36F), sixty-nine high-mid dose (34M/35F), and eighty-nine high dose (44M/45F) rats died or were sacrificed in extremis prior to terminal necropsy. Starting from the weeks 79-91 interval a drastic increase in mortality was observed in the high dose males and after the weeks 92-104 interval for the females as compared to the controls (see attached Appendix B, copied from p. 216-219). Survival data of all except the high dose group was illustrated in a graph (see Appendix C, copied from p. 214-215 of report).

The study was scheduled for a maximum duration of 130 weeks, but since the remaining total number of high dose males was approaching 11 (20%), all surviving high dose males were terminated after 99 weeks of the study. Surviving males of all other groups were terminated after 111 weeks. All surviving females in all groups were terminated after 125 weeks of study.

c. Clinical Signs

The only two significant clinical signs observed were discolored urine and anogenital staining. These are summarized in a table on the next page.

As seen from that table, discolored urine (UD) was observed in both the high dose males and females throughout the study. Incidences of discolored urine in the high dose groups (both sexes) decreased after the weeks 53-55 interval. Discolored urine in the control and other treated groups (both sexes) was observed after the weeks 66-78 interval. The significance of these observations was unclear. It appears that the discolored urine observed in the high dose group (both sexes) is related to the administration of the compound.

As compared to the controls, a higher incidence of anogenital staining (AS) was observed in the latter half of the first year for the low-mid dose female (8/65), high-mid dose female (13/65), and the high dose female (17/65) groups as compared to the control (3/65). The significance of this observation in the treated female rat is unclear. However, since the incidence after the first year did not increase in the females and no increased incidence of anogenital staining was observed in the respective treated male groups throughout the study, it is suggested that anogenital staining was unrelated to treatment (see Table on the next page). The relationship between discolored urine and anogenital staining is unclear.

All other signs observed in the treated groups were comparable with the control or were not treatment related.

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Discolored Urine and Anogenital Staining Observations@

Dosage	UD AS	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Total Rats		65/65	65/65	65/65	65/65	65/65
Week 1-13	UD	0/0	0/0	0/0	0/0	65/57
	AS	0/1	0/0	0/2	0/1	1/0
Week 14-26	UD	0/0	0/1	0/0	0/0	65/52
	AS	0/2	0/0	0/2	0/5	1/3
Week 27-39	UD	0/0	0/0	0/0	0/0	65/60
	AS	0/3	0/3	0/8	0/13	1/17
Total Rats		65/65	65/65	65/65	64/65	65/65
Week 40-52	UD	0/0	0/0	0/0	0/0	65/64
	AS	#/1	#/3	#/7	#/12	#/16
Total Rats		55/55	54/54	54/55	54/53	55/55
Week 53-65	UD	0/0	0/0	0/0	0/0	55/53
	AS	0/3	1/0	0/3	0/7	0/2
Week 66-78	UD	0/0	0/0	0/0	0/0	38/47
	AS	0/1	1/1	1/4	1/4	0/0
Total Rats		54/54	54/52	51/52	52/52	51/52
Week 79-91	UD	1/0	1/1	1/0	1/1	8/39
	AS	3/4	1/2	3/5	2/8	0/1
Total Rats		49/46	51/50	46/51	50/47	25/50
Week 92-104	UD	9/2	5/0	2/5	4/2	8/35
	AS	6/4	3/4	2/6	5/9	0/3
Total Rats		38/42	39/44	41/43	37/37	$/40
Week 105-111	UD	5/1	0/3	6/3	8/1	-/18
	AS	1/4	4/2	7/8	3/6	-/3
Total Rats		$/38	$/39	$/34	$/32	$/32
Week 112-125	UD	-/8	-/1	-/6	-/5	-/13
	AS	-/8	-/5	-/10	-/7	-/6

@ = Derived from Table 2 on p. 220-291 of the study report;
$ = High dose males were sacrificed on week 99 and all other males were sacrificed on week 111;
# = Anogenital staining data were not recorded for the males in the Summary Table 2 on p.232-235 of the study report;
AS= Anogenital Staining; UD = Urine Discolored;

d. Body weight data

The mean body weights were calculated at predosing, weekly on weeks 1-14 for both sexes, and every two weeks on weeks 16-110 (to week 98 for high dose males) for the males and on weeks 16-124 for the females.

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Statistically significant differences in body weights were observed on a few occasions in the low and low-mid dose groups of both sexes and in high-mid dose group females as compared to the controls. These differences are of no biological significance. Significant reduction in mean body weight was noted in both the males (from week 2) and females (from week 6) from the high dose group and high-mid dose males (starting from week 6) as compared to the control (see attached Appendix D). The calculated percent mean body weight differences between the high dose group as compared to the controls during weeks 13 to 99 were between 9.2% and 25.4% for the males and between 4.9% and 16.6% for the females. These body weight reductions are considered dose related. The body weight data of all except the high dose group were illustrated in a graph (see attached Appendix D).

Body weight gain of animals was not calculated by the investigator nor was it discussed in the report. This reviewer calculated the body weight gain group means at various intervals and these values are presented in Appendix D1. As seen from this Appendix, body weight gain group mean reductions were observed primarily in the high dose groups at various intervals throughout the study. The overall body weight gain group mean values (BW at termination minus BW on week 0) for the high dose groups for both sexes were drastically reduced (32% for the males and 30% for the females). The fact that the food intake values were generally constant among the groups throughout the study (see discussions below), further suggests that the body weight gain reduction is related to treatment.

e. Food Consumption Data

Throughout the study there were scattered instances of statistically significant differences of mean absolute food intake (mg/animal/day) and mean relative food intake (mg/kg/day) in the low, low-mid, and high-mid dose group males and females as compared to the control rats. (p. 308-331).

Statistically significant reduction of mean absolute food consumption (mg/animal/day) was observed in high dose males. There was an increase or a decrease of mean absolute food consumption values for the high dose females starting from week one (Table 4 on p. 308-319 of study report). Throughout the study, on most occasions the mean relative food consumption (mg/kg/day) in the high dose rats (both sexes) was increased as compared to the control (Table 5 on p. 320-331 of study report). Since the difference between mean absolute and mean relative food consumption values among the low, low-mid, and high-mid dose groups was slight, it is not considered biologically significant.

The mean food intake efficiency data (body weight gain divided by food consumed multiplied by 100) did not show clear dose-related trends (see attached Appendix E).

The above results suggest that differences in food consumption data observed were not related to treatment.

f. Compound Intake

The average nominal compound intake over the course of the study (110 weeks for low, low-mid, and high-mid dose groups, 98 weeks for high dose male groups, and 124 weeks for all female dose groups) was as follows:

Dose Level	Average Compound Intake (mg/kg/day)	
	Male	Female
Control	0.0	0.0
Low	2.1	2.1
Low-mid	4.2	4.2
High-mid	15.8	15.7
High	181	182

Note: Derived from Table 2, p. 25 and Table 7, p. 338-318 of the study report.

g. Clinical Pathology

1. Hematology

The hematology measurements were conducted at 12, 18, 23, 24, 25, and 29 months. There were no observable compound related hematological changes in the treated groups as compared to the control at any interval tested. The few scattered instances of statistically significant differences between the treated groups as compared to the control were judged to be artifactual and were not considered to be dose related (p. 349-360).

2. Clinical Chemistry

Clinical chemistry parameters were conducted at 18 and 24 months and at termination of the study (p. 361-376). Selected clinical chemistry parameters are presented in a table on next page. As seen from that table, there were great variations among rats for these selected parameters and a pattern of compound related changes was not obvious. Elevated urea nitrogen and creatinine levels were observed in the high dose males as compared to the controls. Elevated serum phosphorus and cholesterol levels as well as depressed albumen levels were observed in both sexes of high dose animals. These values suggest a possible kidney function impairment in high dose males. In both sexes of the high dose group, at various intervals, alkaline phosphatase and alanine aminotransferase were generally lower as compared to the control. The toxicological significance of these decreased values is unknown since clinical significance is usually associated with elevated levels.

Selected Clinical Chemistry Parameters@

Dosage	Month	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Urea Nitrogen[a]	18	18.1/17.2	18.1/16.7	18.4/18.0	17.6/17.7	32.9#/16.7
	23	21.1/ -	- / -	- / -	- / -	51.3#/ -
	24	18.1/13.8	21.3/13.8	22.0/18.1	25.0*/15.3	$/19.3
	25	45.6/ -	26.5/ -	54.1/ -	31.9/ -	$ / -
	29	$ /19.9	$ /20.7	$ /24.8	$/26.1	$ /33.7
Creatinine	18	0.6/0.5	0.7/0.6	0.6/0.5	0.7/0.5	0.8*/0.5
	23	0.7/ -	- / -	- / -	- / -	1.8#/ -
	24	0.8/0.7	0.8/0.6	0.8/0.6	0.9/0.6	$ /0.7
	25	1.5/ -	1.0/ -	1.1/ -	1.3/ -	$ / -
	29	$ /0.8	$ /0.6	$ /0.8	$ /0.9	$ /0.8
Phosphorus[a]	18	8.0/5.8	7.9/6.5	8.4/7.0	7.5/6.7	9.5/7.8#
	23	5.3/ -	- / -	- / -	- / -	10.3#/ -
	24	6.8/6.4	6.9/6.5	7.3/7.4	7.3/6.3	$ /7.8
	25	7.6/ -	7.4/ -	7.2/-	7.7/ -	$ / -
	29	$ /5.7	$ /5.6	$ /6.2	$ /6.0	$ /8.9*
Cholesterol[a]	18	168/161	168/153	148/149	136/153	208/232*
	23	200/ -	$ / -	$ / -	$ / -	309*/ -
	24	169/227	192/168	257*/200	216/191	$ /278
	25	213/ -	251/ -	369/ -	284/ -	$ / -
	29	$ /256	$ /275	$ /200	$ /283	$ /382*
Albumin[b]	18	3.6/4.0	3.6/4.1	3.5/3.9	3.5/3.9	3.2#/4.0
	23	3.2/ -	- / -	- / -	- / -	2.7#/ -
	24	3.3/3.9	3.3/3.9	3.3/3.7	3.1/3.6	$ /3.5
	25	3.1/ -	3.0/ -	2.9/ -	2.9/ -	$ / -
	29	$ /3.6	$ /3.4	$ /3.5	$ /3.2	$ /3.0*
Alanine Aminotransferase[c]	18	56/18	66/46	49/61	45/37	28#/22#
	23	38/ -	- / -	- / -	- / -	19#/ -
	24	59/56	39/45	40/49	42/30*	$ /18#
	25	53/ -	48/ -	46/ -	34/ -	$ / -
	29	$ /48	$ /63	$ /40	$ /98[z]	$ /58
Alkaline Phosphatase[c]	18	57/41	52/43	70/46	52/36	39*/27#
	23	48/ -	- / -	- / -	- / -	43/ -
	24	49/53	47/41	38/36	49/36	$/65(22[y])
	25	46/ -	46/ -	43/ -	33/ -	$ / -
	29	$/45(34[z])	$/56(39[z])	$/53(39[z])	$/60(31[z])	$/171(29[z])

@ = Derived from Table 9, p. 361-376 and Appendix H, p. 1099-1148 of the study report; a = mg/dl; b = g/dl; c = IU/l; y = mean with two outliers excluded (calculated by this reviewer); z = means with one outlier excluded (calculated by this reviewer); M/F = Males/Females; $ = rats have been terminally sacrificed; - = parameter was not measured.

3. Urinalysis

As compared to the controls, urine volume was increased and urinary specific gravity was decreased in the high dose males at 18 and 23 months. Dark urine color was observed in the high dose rats of both sexes (65/65 males and 64/65 females) during the first year of study. These observations are judged to be related to treatment. All other parameters were comparable among the groups (see attached Appendix F, copied from p. 377-380 of the study report and discussions under clinical signs on p. 11 and 12 of this DER).

Ophthalmologic Examination

All surviving high dose male rats and an equal number of control males were subjected to ophthalmologic examination at 23 months. Ophthalmologic examination was conducted on all other surviving rats at 24 months. No compound related ophthalmologic abnormalities were observed.

Gross Macroscopic Findings

All rats which died or were sacrificed in extremis or at the scheduled necropsy were subjected to gross macroscopic examinations.

a. 0 to 12 months (see attached Appendix G)

Granular kidneys were only observed in the high dose male groups (7/10). Non-glandular stomach mucosal thickening was observed in the high dose males (10/10) and females (7/10), as well as in the high-mid dose male (2/10) and female (1/10) groups. These findings are considered to be related to treatment.

b. 12 Months to Termination (see Appendix G)

Although granular kidneys were observed in all groups after 12 months on study, increased incidence of granular kidneys was observed in both sexes of only the high dose group. Various lesions of the nonglandular stomach were also observed in all groups, but increased incidence of thickened mucosa, granular or irregular mucosa of the nonglandular stomach was only observed in both sexes of the high-mid and high dose groups. Increased incidence of ulcers and erosions of the nonglandular stomach was noted in females that died on study and in terminally sacrificed males and females of the high-mid and high dose groups as compared to the control. Increased incidence of erosions of the glandular stomach was only observed in the high dose males at terminal sacrifice. All the above increased incidents are judged to be related to treatment.

Parathyroid enlargement was observed at necropsy in both sexes of the high dose group rats which died during the study and at terminal sacrifice. Histopathological evaluation of another study revealed that parathyroid enlargement was the result of hyperplasia and was associated with renal toxicity.

Organ Weights

The brain, liver and paired kidneys from all animals killed at the interim (12 months) and terminal necropsies were weighed.

a. 12 Months Interim Sacrifice (p. 420-423)

The mean absolute and relative (to body and brain weights) kidney weights were significantly increased in both sexes of the high dose group as compared to the control. The kidney:body weight ratios in male of low-mid and high-mid dose groups, as well as the absolute kidney weights and kidney:brain ratios in females of the low-mid and high-mid dose groups were also significantly increased as compared to the control. The absolute and relative (to body and brain weights) in the male and the female relative (to body and brain weights) liver weights in the high dose group, were significantly increased as compared to the control (see attached Appendix H).

b. Terminal Sacrifice (p. 424-427)

The absolute kidney weight in the female high dose group was significantly increased, and the relative kidney weight (to body weight) in the high-mid dose male and in both sexes of the high dose groups was also significantly increased as compared to the control (see attached Appendix H).

Histopathological Evaluation

Only the kidneys, stomach, and the renal and mesenteric lymph nodes were subjected to histopathological evaluations.

The results of the histopathological data covering the first year of the study have been reviewed by the Toxicology Branch 6/8/88). Findings included epithelial hyperplasia, clear cell hyperplasia and karyomegaly in the kidneys of the low-mid, migh-mid and high dose male rats, and in the high dose female rats. Chlorothalonil also induced squamous epithelial hyperplasia and hyperkeratosis in the forestomach of both sexes of the high-mid and high dose groups. Tubular adenomas of the kidney were noted in only one high dose male rat (see DER dated 6/6/88 which is attached as Appendix K).

Discrepancies were noted in the Incidence Tables for the renal tubular adenomas and carcinomas as well as for the forestomach papillomas and carcinomas (Tables 3 and 6, p. 39 and 42 of the study report), and in the Summary Incidence and Neoplasm Summary Incidence Tables presented in the Histopathological Report (p. 1675-1718 of the study report). Data in these summary tables were checked against data presented in the Individual Animal Histopathological Incidence Table on p. 1719-1875 of the study report. Findings of these discrepancies are discussed in the Study Deficiencies section on p. 21 of this DER. Based on the Individual Animal Histopathological Data presented on p. 1719-1875 of the report new Incidence Neoplasm Summary Tables are constructed and are presented as the attached Tables A of Appendices I and J.

As seen from these appendices adverse histopathological findings are as follows:

a. Kidney

As seen from Table 5 in Appendix I, tumor incidence in the kidney for the low dose group was comparable to the controls. Increased incidence and severity of epithelial hyperplasia in the proximal convoluted tubules were noted in females and possibly in males from the low-mid dose group, and in both sexes from the high-mid and high dose groups as compared to the control (see Appendix I, Table 4). An increased incidence in tumors associated with hyperplasia was observed in both sexes of the high dose group (see Appendix I, Table 5). Similar dose related increased incidences of renal tubular adenomas and carcinomas were also observed in association with hyperplasia in both sexes of the high dose groups as compared to the controls. There were totals of 18 in the high dose males and 24 in the high dose females as compared to 1 male and no female in the controls were noted. Seven tubular adenomas were observed in the males with 11 in the females of the high dose group as compared to none in the controls (see Appendix I, Table A). These increased incidences are judged to be related to treatment.

b. Forestomach (see attached Appendix J)

As seen from Appendix J, tumor incidence in the forestomach for the low dose group was comparable to the controls. Throughout the study, increased incidence and/or severity of hyperplasia, hyperkeratosis and ulcers or erosions of the squamous mucosa of the stomach were observed in both sexes from the low-mid, high-mid, and the high dose groups as compared to the control (see attached Appendix J, Table 7). A slightly increased incidence of papillomas of the squamous mucosa was observed in the low-mid (3 males and 2 females), high-mid (2 males and 4 females), and the high (5 males and 7 females) dose groups as compared to the control (no male and one female).

One incidence of papilloma was observed in the female low dose group. The incidence and/or severity of the lesions increased with increasing dose. Squamous cell carcinoma was observed in one high-mid and three high female dose rats as compared to only one female control (see attached Appendix J, Table A). The pathologist noted that lesions in the stomach were considered to be the result of chronic local irritation of squamous mucosa by chlorothalonil. These findings are considered to be related to treatment.

c. Renal and Mesenteric Lymphnodes (p. 1675-1718)

Based on this study, no treatment related microscopic changes of the renal and mesenteric lymph nodes were noted.

CONCLUSIONS

Administration of technical chlorothalonil at 2.0, 4.0, 15.0, and 175 mg/kg/day in the diet of Fischer 344 rats did not produce compound related changes in the ophthalmologic and hematologic parameters evaluated in this study. Treatment related effects were observed in other parameters evaluated at dose levels of 4.0 mg/kg/day or greater.

In the high dose group (175 mg/kg/day), increased mortality and kidney weight, parathyroid enlargement, decreased body weight and food consumption relative to body weight (g/kg/day) were noted in both sexes as compared to the control. Increased urine volume and decreased urine specific gravity were only noted in the males.

In both sexes of the high-mid dose group (15 mg/kg/day), a slight increase in kidney weight and an elevation of serum urea nitrogen level were observed. In the low-mid dose group (4.0 mg/kg/day) a slightly higher kidney weight was observed. No treatment related effect was observed in the low dose group (2.0 mg/kg/day).

The incidence and/or severity of epithelial hyperplasia in the proximal convoluted tubules of the kidney was increased in both sexes of the high-mid dose (15 mg/kg/day) and the high dose (175 mg/kg/day) groups, as well as in the males and possibly in the females of the low-mid dose group (4.0 mg/kg/day) as compared to the control. The incidence and/or severity of this hyperplasia increased in a dose related fashion. Increased renal tubular adenomas and carcinomas were observed in association with the above noted hyperplastic lesion in males of low-mid dose (4.0 mg/kg/day) and in both sexes of the high-mid dose (15 mg/kg/day) and high dose (175 mg/kg/day) groups. No treatment related effect was observed in the low dose group

(2.0 mg/kg/day).

An increased incidence and/or severity of hyperplasia, hyperkeratosis and ulcers or erosions of the squamous mucosa of the forestomach was observed in both sexes of the low-mid dose (4.0 mg/kg/day), high-mid dose (15 mg/kg/day) and the high dose (175 mg/kg/day) groups as compared to the controls. The incidence and/or severity of these lesions also increased with increasing dose.

In association with these forestomach lesions, a slight increase in neoplastic lesions in some treated groups was also observed as compared to the controls, i.e. papillomas of the squamous mucosa in the low-mid dose (4.0 mg/kg/day) group and papillomas and carcinomas in the high-mid dose (15 mg/kg/day) and the high dose (175 mg/kg/day) groups. Lesions in the forestomach were considered to be the result of chronic irritation of the gastric mucosa by chlorothalonil. No treatment related effect was observed in the low dose group (2.0 mg/kg/day).

The NOEL was determined to be 2.0 mg/kg/day based on urinalysis, clinical chemistry, kidney weight data, preneoplastic hyperplasia of the kidney, and chronic irritation of the forestomach incidence data. The LOEL is determined to be 4.0 mg/kg/day. It appears that the doses employed in this study were sufficient to produce a compound related effect and the MTD was reached. This study accomplished its goal and it is classified as a core supplementary data.

Similar renal and gastric lesions were also seen in other oncogenic studies on animals treated with chlorothalonil: in Fisher 344 rats treated with higher doses of chlorothalonil, in CD-1 mice, and in Osborne-Mendel rats.

It is noted that chlorothalonil was classified by the Toxicology Branch Peer Review Committee as a probable human carcinogen (B2) based on increased incidence of malignant and/or combined malignant and benign tumors (both sexes) in two species (two rats strains and the CD-1 mouse).

CLASSIFICATION: Core-Supplementary Data

STUDY DEFICIENCIES:

- The table of contents of the report was not properly prepared; headings and subheadings of the appendices (individual study reports) were not clear and page numbers were not provided. Page numbers of the summary report were not appropriately renumbered.

- Two addresses were noted as the source of the test article for this study:
  + Greens Bayou Production Plant (a compound with a purity of 98.3% and with a 4.3μ particle size, ID# SDS-2787-1002; compound mixed in the rat diet);
  + Ricerca Inc. (a compound with a purity of 98.9% with an ID# SDS-2787-0503; used as standard solution analysis of chlorothalonil in the diet samples);
  + It was not clear whether the test article used in this study came from the same batch.

- Only the study director was filled out in the Study Assignment and Supervision form on p. 78.

- Body weight gain of animals was not calculated nor was it discussed in the report.

- There is no evidence that contaminants of the basal diet were presented in the report.

- Anogenital staining data for week 40-52 interval were not recorded for the males in the Summary Table 2 on p. 232-235 of the study report.

- Histopathological Data: one forestomach papilloma in the control and one tubular incidence in the high dose group male were not reported in the Neoplasm Summary Report on p. 1708 and 1710 of the study report. Adding errors were noted in Tables 3 and 6 (on p. 39 and 42 of the study report). In Table 3 the number of renal adenoma/carcinoma between the one year interim sacrifice and termination of study for the high dose should read 24 (males) and 35 (females), instead of 24 and 32, respectively. In Table 6 the total incidences of forestomach papilloma/carcinoma should read 2 instead of 1 for the control female and 10 instead of 9 for the high dose female group. In Table 5, Appendix I, the total tumor incidences for the high dose males should read 25 instead of 24 (was an adding error).

APPENDICES

APPENDIX A: List of Organs Harvested During Necropsy

APPENDIX B: Cumulative Mortality of all Rats (copied from p. 216-219 of the report)

APPENDIX C: Graph of Cumulative Mortality for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 214-215 of the study report)

APPENDIX D: Graph of Mean Body Weight and Summary of Body Weights for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 292-305 of the study report)

APPENDIX D1: Calculated Body Weight Gain Group Mean Values (Derived from Summary Mean Body Weight Values in Table 3, p. 294-305 of the study report)

APPENDIX E: Graph and Mean Food Intake Efficiency Values (copied from p. 332-337 of the study report).

APPENDIX F: Summary of Urinalysis Values (copied from p. 377-380 of the study report).

APPENDIX G: Summary Incidence of Macroscopic Observations of Selected Organs (extracted from Table 11, p. 381-419 of the study report).

APPENDIX H: Summary of Selected Organ Weights Values (extracted from Table 12, p. 420-427 of the report)

APPENDIX I: Summary of Selected Histopathological Findings in the Kidneys of Rats (Table A is derived from p. 1719-1875 and Tables 3-5 are copied 39-41 of the study report)

APPENDIX J: Summary of Selected Histopathological Findings in the Stomachs of Rats (Table A is derived from p. 1719-1875 and Table 7 is copied from p 42-44 of the study report)

APPENDIX K: DER of a One Year Interim Report of A Tumorigenicity Study of Technical Chlorothalonil in Rats (DER dated June 6, 1988).

APPENDIX D1
Calculated Body Weight Gain Group Mean Values
(Derived from Summary Mean Body Weight Values
in Table 3, p. 294-305 of the study report)

Body Weight Gain Group Means in Grams (Males/Females)@

Interval (Weeks)	Dosages				
	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High Dose M/F
0-2	45/23	44/23	42/24	43/25	36/22
2-5	71/32	74/33	70/33	69/34	64/30
5-10	58/22	56/21	55/21	53/22	52/19
10-16	39/17	36/17	38/17	35/17	33/13
16-20	19/8	16/7	14/7	16/8	14/7
20-26	24/11	24/11	25/13	25/10	19/9
26-38	29/12	25/13	27/14	24/14	24/11
38-52	16/15	16/18	20/16	19/16	13/13
52-66	21/19	19/21	19/13	13/19	7/15
66-82	7/25	6/25	5/27	7/20	-24/20
82-98	-20/15	-18/13	-13/14	-22/9	-35/-8
98-110	-27/1	-26/2	-36/-2	-32/-3	#/-5
110-124	#/-18	#/-9	#/-16	#/-11	#/-31
0-2	45/23	44/23	42/24	43/25	36/22
0-13	200/90	197/88	190/90	187/93	172/81
0-26	256/113	250/112	246/115	241/116	218/100
0-52	301/140	291/143	293/145	284/146	255/124
0-78	330/181	320/187	318/188	301/179	249/155
0-98	309/199	298/202	304/203	282/194	203/151
0-124	#/182	#/193	#/185	#/180	#/115

@ = Derived from Summary Mean Body Weight Values of Table 3, p. 294-305 of the report; # = sacrificed on week 99 of study

APPENDIX I
Summary of Selected Histopathological Findings in the Kidneys of Rats (Table A is derived from p. 1719-1875 and Tables 4-5 are copied from p. 40-41 of the study report)

Table A

Neoplasm Summary Incidence Table of the Kidney@

Dosage	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Tubular Adenoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	1/0
No. Examined 12 Month to Termination	55/55 1/0	54/54 1/0	54/55 1/0	54/53 3/0	55/55 17/24
Tubular Carcinoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined 12 Month to Termination	55/55 0/0	54/54 0/0	54/55 0/0	54/53 1/0	55/55 7/11
Grand Total	1/0	1/0	1/0	4/0	25/35

@ = Derived from Individual Animal Histopathological Data presented on p. 1719-1875 of the study report)

APPENDIX J
Summary of Selected Histopathological Findings in the Stomachs of Rats (Table A is derived from p. 1719-1875 and Table 7 is copied from p. 42-44 of the study report)

Table A

Neoplasm Summary Incidence Table of the Stomach@

Dosage	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Papilloma, Non-glandular					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined 12 Month to Termination	55/55 0/1	54/54 0/1	54/55 3/2	54/53 2/4	55/55 5/7
Squamous Cell Carcinoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined 12 Month to Termination	55/55 0/1	54/54 0/0	54/55 0/0	54/53 0/1	55/55 0/3
Grand Total	0/2	0/1	3/2	2/5	5/10

@ = Derived from Individual Animal Histopathological Data presented on p. 1719-1875 of the study report)

APPENDIX A
List of Organs Harvested During Necropsy

APPENDIX B
Cumulative Mortality of all Rats
(copied from p. 216-219 of the report)

APPENDIX C
Graph of Cumulative Mortality for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 214-215 of the study report)

APPENDIX D
Graph of Mean Body Weight and Summary of Body Weights for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 292-305 of the study report)

APPENDIX E
Graph and Mean Food Intake Efficiency Values (copied from p. 332-337 of the study report).

APPENDIX F
Summary of Urinalysis Values
(copied from p. 377-380 of the study report).

APPENDIX G
Summary Incidence of Macroscopic Observations of Selected Organs (extracted from Table 11, p. 381-419 of the study report).

APPENDIX H
Summary of Selected Organ Weights Values
(extracted from Table 12, p. 420-427 of the report)

APPENDIX I
Summary of Selected Histopathological Findings in the Kidneys of Rats (copied from Tables 3-5, p. 39-41 of the study report)

APPENDIX J
Summary of Selected Histopathological Findings in the Stomachs of Rats (copied from Tables 6-7, p. 42-44 of the study report)

APPENDIX K
DER of a One Year Interim Report of A Tumorigenicity Study of Technical Chlorothalonil in Rats
(DER dated June 6, 1988).

Notes only:
According to the results of this study, a statistically significant increased in the incidence of renal adenomas and carcinomas in both sexes, and increased incidence for combined papilloma/carcinoma of the forestomach in the female rats were observed in the high dose group (175 mg/kg/day).

APPENDIX D1

Calculated Body Weight Gain Group Mean Values
(Derived from Summary Mean Body Weight Values
in Table 3, p. 294-305 of the study report)

Body Weight Gain Group Means in Grams (Males/Females)@

Interval (Weeks)	Dosages				
	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High Dose M/F
0-2	45/23	44/23	42/24	43/25	36/22
2-5	71/32	74/33	70/33	69/34	64/30
5-10	58/22	56/21	55/21	53/22	52/19
10-16	39/17	36/17	38/17	35/17	33/13
16-20	19/8	16/7	14/7	16/8	14/7
20-26	24/11	24/11	25/13	25/10	19/9
26-38	29/12	25/13	27/14	24/14	24/11
38-52	16/15	16/18	20/16	19/16	13/13
52-66	21/19	19/21	19/13	13/19	7/15
66-82	7/25	6/25	5/27	7/20	-24/20
82-98	-20/15	-18/13	-13/14	-22/9	-35/-8
98-110	-27/1	-26/2	-36/-2	-32/-3	#/-5
110-124	#/-18	#/-9	#/-16	#/-11	#/-31
0-2	45/23	44/23	42/24	43/25	36/22
0-13	200/90	197/88	190/90	187/93	172/81
0-26	256/113	250/112	246/115	241/116	218/100
0-52	301/140	291/143	293/145	284/146	255/124
0-78	330/181	320/187	318/188	301/179	249/155

0-98	309/199	298/202	304/203	282/194	203/151
0-124	#/182	#/193	#/185	#/180	#/115

@ = Derived from Summary Mean Body Weight Values of Table 3, p. 294-305 of the report; # = sacrificed on week 99 of study

APPENDIX I
Summary of Selected Histopathological Findings in the Kidneys of Rats (Table A is derived from p. 1719-1875 and Tables 4-5 are copied from p. 40-41 of the study report)

Table A

Neoplasm Summary Incidence Table of the Kidney@

Dosage	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Tubular Adenoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	1/0
No. Examined 12 Month to Termination	55/55 1/0	54/54 1/0	54/55 1/0	54/53 3/0	55/55 17/24
Tubular Carcinoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined 12 Month to Termination	55/55 0/0	54/54 0/0	54/55 0/0	54/53 1/0	55/55 7/11
Grand Total	1/0	1/0	1/0	4/0	25/35

@ = Derived from Individual Animal Histopathological Data presented on p. 1719-1875 of the study report)

APPENDIX J
Summary of Selected Histopathological Findings in the Stomachs of Rats (Table A is derived from p. 1719-1875 and Table 7 is copied from p. 42-44 of the study report)

Table A

Neoplasm Summary Incidence Table of the Stomach@

Dosage	Control M/F	Low M/F	Low-mid M/F	High-mid M/F	High M/F
Papilloma, Non-glandular					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined	55/55	54/54	54/55	54/53	55/55
12 Month to Termination	0/1	0/1	3/2	2/4	5/7
Squamous Cell Carcinoma					
No. Examined	10/10	11/11	11/10	11/12	10/10
0-12 Months	0/0	0/0	0/0	0/0	0/0
No. Examined	55/55	54/54	54/55	54/53	55/55
12 Month to Termination	0/1	0/0	0/0	0/1	0/3
Grand Total	0/2	0/1	3/2	2/5	5/10

@ = Derived from Individual Animal Histopathological Data presented on p. 1719-1875 of the study report)

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Cumulative Mortality of all Rats
(copied from p. 216-219 of the report)

APPENDIX C
Graph of Cumulative Mortality for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 214-215 of the study report)

APPENDIX D
Graph of Mean Body Weight and Summary of Body Weights for the Control, Low, Low-mid, and High-mid Dose Groups (copied from p. 292-305 of the study report)

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Graph and Mean Food Intake Efficiency Values (copied from p. 332-337 of the study report).

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Summary of Urinalysis Values
(copied from p. 377-380 of the study report).

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Summary Incidence of Macroscopic Observations of Selected Organs (extracted from Table 11, p. 381-419 of the study report).

APPENDIX H
Summary of Selected Organ Weights Values
(extracted from Table 12, p. 420-427 of the report)

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Summary of Selected Histopathological Findings in the Kidneys of Rats (copied from Tables 3-5, p. 39-41 of the study report)

APPENDIX J
Summary of Selected Histopathological Findings in the Stomachs of Rats (copied from Tables 6-7, p. 42-44 of the study report)

APPENDIX K
DER of a One Year Interim Report of A Tumorigenicity Study of Technical Chlorothalonil in Rats
(DER dated June 6, 1988).

Notes only:

According to the results of this study, a statistically significant increased in incidence of renal adenomas and carcinomas in both sexes, and increased incidence for combined papilloma/carcinoma of the forestomach in the female rats were observed in the high dose group (175 mg/kg/day).

Chlorothalonil toxicology review

Page _____ is not included in this copy.

Pages 38 through 88 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

APPENDIX K
DER of a One Year Interim Report of A Tumorigenicity Study of Technical Chlorothalonil in Rats (DER dated June 6, 1988).

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

007631

[redacted]

JUN 16 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Lois Rossi, PM # 21
Fungicides/Herbicides Branch
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. 1/Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Dr. T. M. Farber, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

FROM: D. Ritter, Toxicologist
Rev. Sec. 1/Toxicology Branch
Hazard Evaluation Division (TS-769C)

Subject: EPA # 50534-7 - Chlorothalonil, submission of additional toxicity data.

Registrant: Fermenta Plant Protection, Mento, OH.

Caswell #: 215B

Fermenta is submitting additional toxicity data in support of an Amended Registration. The data include an interim report of a tumorigenicity study in rats. The DER is appended.

1. "A Tumorigenicity Study of Technical Chlorothalonil in Rats. One Year Interim Report." Study # 1102-84-0103-TX-004.

Chlorothalonil was fed in the diet of male and female rats (65 animals per dose per sex) at levels of 0, 2.0, 4.0, 15 or 175 mg/kg bw/day for one year. Ten animals per group were sacrificed and kidneys and stomachs were subjected to microscopic examination. Animals receiving 4.0 mg/kg bw/day or more demonstrated renal epithelial hyperplasia, clear cell hyperplasia and karyomegaly. One high dose male rat had a

renal tubular adenoma. Chlorothalonil also induced squamous epithelial hyperplaisa and hyperkeratosis of the forestomach in male and female rats receiving 15.0 and 175.0 mg/kg bw/day. The NOEL for renal effects is 2.0 mg/kg bw/day; for gastric effects the NOEL is 4.0 mg/kg bw/day.

2. Clarification of the In Vitro Chromosome Aberration Assay in Chinese Hamster Ovary Cells with Technical Chlorothalonil.Study # T4481.337. Toxicology Branch memo of 4/9/87, B. Dementi. Acc. # 405591-03. 1/4/88.

Dr. Chen's review of these data is appended. He found that they supported a classification of the study of "Acceptable".

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[redacted]

Reviewer: D. Ritter, Toxicologist_ [signature] 6-9-88 _Caswell #: 215B
Rev. Sec. # I/Toxicology Branch
Secondary Reviewer: R. Bruce Jaeger, Section Head [signature] 6/8/88
Rev. Sec. # I/Toxicology Branch

DATA EVALUATION RECORD

Study: Two Year Feeding Study in Rats: One year interim report.

MRID: 40559102.

Performing Laboratory: International Research & Development Corp.
Mattawan, MI.

Author(s): N. B. Wilson and J. C. Killeen.

Study ID Number: 1102-84-0103-TX-004.

Date of Study: 9/17/87.

Title: A Tumorigenicity Study of Technical Chlorothalonil in Rats.
A One Year Interim Report.

CORE Rating: Minimum Data. This is an interim report.

QA Statement: Satisfactory.

CONCULSIONS: Chlorothalonil induces microscopic alterations, consisting of epithelial hyperplasia, clear cell hyperplaisa and karyomegaly in the kidneys of male rats receiving dietary Chlorothalonil at 4, 15 and 175 mg/kg bw/day, and in female rats receiving 175 mg/kg bw/day. Chlorothalonil also induces squamous epithelial hyperplasia and hyperkeratosis in the forestomachs of male and female rats receiving 15 and 175 mg/kg bw/day. The overall NOEL based on hyperplastic changes in the renal cortex is 2.0 mg/kg bw/day.

Dark urine was reported in the high dose males and females. No explanation was offered for this finding.

One tubular adenoma was reported in a high dose male rat.

These findings are similar to those reported in earlier chronic studies using Chlorothalonil.

METHODS:

Purpose -

"This study was conducted to determine, if possible, the no-effect level for potentially preneoplastic and tumorigenic

[redacted]

effects in the kidney and forestomach in Fischer 344 rats following dietary adninistration of technical chlorothalonil."

Material Tested -

A standard batch of technical Chlorothalonil was used and was analyzed initially and at six, eight and twelve months. Blind No.: T-117-12.

Animals -

Fischer 344 rats were assigned to five groups of 65 male and 65 females each which were offered diets containing 0, 1.5, 3.0, 15 or 175 mg/kg bw/day of technical Chlorothalonil.

Diets -

The test material was mixed into standard laboratory diet at levels of 2.0 (1.5)*, 4.0 (3.0)*, 15 and 175 mg/kg bw/day. These preparations were assayed regularly throughout the study. The dietary preparations were made available to the animals ad libitum. Fresh diets were prepared every four days. Husbandry - Standard GLP. See Table I on compound ingestion.

Feed and water - Available ad libitum.

In-Life Measurements -

Animals were observed twice daily for mortality, morbundity and signs of toxicity.

Detailed physical examinations were done weekly.

Body weights were recorded from one week prior to initiation of diet, weekly through week 14, then biweekly thereafter.

Feed consumption was recorded similarly.

At 12 months blood samples were obtained from the orbital sinuses of 10 animals of each sex in each group and a hematological evaluation was done.

* Mixed at the higher level to account for possible dietary binding at low levels.

[redacted]

Blood parameters evaluated were:

Leukocytes
Red Cells
Hemoglobin
Hematocrit
MCV, MCH, MCHC
Platelets & Differentials

The same animals were then killed and subjected to a one year post-mortem examination.

Post Mortem Examinations -

All animals sacrificed in extremis were autopsied, as were all those that died during the one year period.

The brain, liver and kidneys were weighed. A full battery of tissues and organs were reserved for future histopathological examination. The kidneys, stomach and renal and mesenteric lymph nodes were prepared and examined histopathologically (W. M. Busey, DVM, PhD., Experimental Pathology Labboratories, Inc., of Herndon, VA).

RESULTS:

Morbidity and Signs of Toxicity -

One male in the 2, 4, and 15 mg/kg bw/day groups died on test. One 2 mg/kg bw/day female and two 15 mg/kg bw/day females died on test. These are not considered to be compound-related deaths.

The authors reported that dark yellow urine was noted in the majority of 175 mg/kg bw/day males and females, beginning at week five and persisting through week fifty-two. The females in the 4, 15 and 175 mg/kg bw/day groups exhibited yellow anogenital staining in the latter half of the interim period. No similar effects were reported for the males in these groups or for the 2 mg/kg bw/day animals of either sex.

Body Weights -

Statistically significant deviations from control values were reported in the three lower-dose groups at various times; however, these deviations occured at random and did not occur in a dose-related fashion. Those of the males and female in the highest dose groups were significantly reduced when compared to those of the controls. The differences became larger as the study progressed.

[redacted]

Diets and Compound Consumption -

The amount of extractable Test Material in the lower two doses decreased with time over each four day storage period. Freezing the diets prevented loss of biologically available Test Material.

Actual compound consumption was satisfactory for the study.

Feed consumption relative to body weight in the highest dose males became increasingly greater during the study when compared to that of the controls. The differences amounted to about ten percent. At the end of the interim period the females on the highest dose likewise were consuming 10 % more feed.

Blood Analyses -

No alterations in hematological values were reported at any level tested.

Organ Weights -

Kidney - Absolute kidneys weights were significantly increased in the 175 mg/kg bw/day males and females. Kidney weights relative to body weight and to brain weight also were significantly increased in these animals.

Liver - Absolute liver weights were significantly increased in the 175 mg/kg bw/day males and females. Liver weights relative to body weight and to brain weight also were significantly increased in these animals.

Gross Necropsy -

7/10 males in the high dose group exhibited granular kidneys. No other groups showed this effect. No other compound-related abnormalities were reported. See Table II.

Microscopic Examination -

A single tubular adenoma of the renal cortex was reported for one high dose male rat.

Interstitial fibrosis and regenerative epithelium occured in all groups and in both sexes. There was a trend toward greater severity as dose increased. See Table III.

[redacted]

Epithelial hyperplasia, clear cell hyperplasia and karyomegaly of the renal cortex were reported for all males receiving 4, 15 or 175 mg/kg bw/day, and for females receiving 175 mg/kg bw/day (Table IV). The severity of these lesions increased with increasing dose (Table V).

Male and female rats receiving 15 and 175 mg/kg bw/day showed squamous epithelial hyperplasia and hyperkeratosis of the gastric mucosa. No lesions were reported for groups receiving 2 or 4 mg/kg bw/day. This finding was associated with thickened mucosa (Table VI).

TABLE I

MEAN COMPOUND CONSUMPTION DURING THE FIRST YEAR OF THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Dose Group	Mean Compound Consumption, mg/kg/day					
	Nominal[a]		Analytical			
			Complete Availability[b]		Partial Availability[c]	
	Males	Females	Males	Females	Males	Females
Low	2.09	2.07	1.96	1.99	1.75	1.75
Low-Mid	4.18	4.15	4.03	3.98	3.65	3.63
High-Mid	15.7	15.7	15.2	15.1	15.2	15.2
High	181	181	180	180	183	181

[a] $\frac{\text{Food consumption (g/kg/day)}}{1000} \times \text{Nominal diet concentration (ppm)}$

[b] Assumes availability of chlorothalonil to the animals is unaffected by binding in the diet.

$$\frac{\text{Food consumption (g/kg/day)}}{1000} \times \text{Analytically determined diet concentration (ppm) on day of preparation (Day 0)}$$

[c] Assumes binding of chlorothalonil in the diet has some effect on its availability to the animals.

$$\frac{\text{Food consumption (g/kg/day)}}{1000} \times \frac{\text{Analytically determined Day 0 diet concentration (ppm)} + \text{Day 0 diet concentration adjusted for average binding (ppm)}}{2}$$

TABLE II

INCIDENCE[a] OF MACROSCOPIC AND MICROSCOPIC OBSERVATIONS
INDICATIVE OF CHRONIC PROGRESSIVE NEPHROPATHY AT ONE YEAR
IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Necropsy Observation	Histopathologic Observation	
		Granular Kidney	Regenerative Epithelium	Interstitial Fibrosis
Males/				
Control	0	0/10	9/10	6/10
Low	1.75	0/11	5/11	6/11
Low-Mid	3.65	0/11	9/11	7/11
High-Mid	15.2	0/11	10/11	6/11
High	183	7/10	10/10	10/10
Females/				
Control	0	0/10	5/10	1/10
Low	1.75	0/11	6/11	0/11
Low-Mid	3.63	0/10	6/10	1/10
High-Mid	15.2	0/12	8/12	1/12
High	181	0/10	9/10	2/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

[redacted]

TABLE III

SEVERITY OF INTERSTITIAL FIBROSIS AND REGENERATIVE EPITHELIUM IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Finding: Severity	Dose Group									
	Control		Low		Low-mid		High-mid		High	
	M	F	M	F	M	F	M	F	M	F
Interstitial Fibrosis:										
minimal	5	0	6	0	5	0	3	0	1	2
mild	1	1	0	0	1	1	3	0	3	0
moderate	0	0	0	0	1	0	0	0	6	0
marked	0	0	0	0	0	0	0	0	0	0
severe	0	0	0	0	0	0	0	0	0	0
Regenerative Epithelium:										
minimal	5	4	2	6	6	5	2	8	2	3
mild	3	1	3	0	2	0	6	0	2	6
moderate	1	0	0	0	1	1	2	0	5	0
marked	0	0	0	0	0	0	0	0	1	0
severe	0	0	0	0	0	0	0	0	0	0

TABLE IV

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Histopathologic Finding		
		Epithelial Hyperplasia	Clear Cell Hyperplasia	Karyomegaly
Males/				
Control	0	0/10	0/10	2/10
Low	1.75	0/11	0/11	5/11
Low-Mid	3.65	8/11	0/11	6/11
High-Mid	15.2	8/11	2/11	7/11
High	183	10/10	10/10	10/10
Females/				
Control	0	0/10	0/10	3/10
Low	1.75	0/11	0/11	3/11
Low-Mid	3.63	0/10	0/10	2/10
High-Mid	15.2	0/12	0/12	3/12
High	181	7/10	4/10	9/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

TABLE V

SEVERITY OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Finding: Severity	Dose Group									
	Control		Low		Low-mid		High-mid		High	
	M	F	M	F	M	F	M	F	M	F
Epithelial Hyperplasia:										
minimal	0	0	0	0	5	0	5	0	1	2
mild	0	0	0	0	3	0	3	0	1	2
moderate	0	0	0	0	0	0	0	0	3	3
marked	0	0	0	0	0	0	0	0	5	0
severe	0	0	0	0	0	0	0	0	0	0
Clear Cell Hyperplasia:										
minimal	0	0	0	0	0	0	2	0	1	2
mild	0	0	0	0	0	0	0	0	2	2
moderate	0	0	0	0	0	0	0	0	4	0
marked	0	0	0	0	0	0	0	0	3	0
severe	0	0	0	0	0	0	0	0	0	0
Karyomegaly:										
minimal	2	3	5	3	4	2	3	3	2	2
mild	0	0	0	0	1	0	3	0	5	6
moderate	0	0	0	0	1	0	1	0	3	1
marked	0	0	0	0	0	0	0	0	0	0
severe	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE VI

INCIDENCE[a] OF SELECTED MACROSCOPIC AND MICROSCOPIC FINDINGS IN THE FORESTOMACH AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Necropsy Finding	Histopathologic Finding	
		Thickened Mucosa	Squamous Hyperplasia	Hyperkeratosis
Males/				
Control	0	0/10	0/10	0/10
Lov	1.75	0/11	0/11	0/11
Lov-Mid	3.65	0/11	0/11	0/11
High-Mid	15.2	2/11	6/11	4/11
High	183	10/10	10/10	10/10
Females/				
Control	0	0/10	0/10	0/10
Lov	1.75	0/11	0/11	0/11
Lov-Mid	3.63	0/10	0/10	0/10
High-Mid	15.2	1/12	7/12	10/12
High	181	7/10	10/10	10/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

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PRIMARY REVIEWER: Jess Rowland, M.S, Toxicologist Jess Rowland 8/8/90
Section II, Tox.Branch II (H7509C)

SECONDARY REVIEWER: Alan Levy, Ph.D, Toxicologist Alan C. Levy
Section I, Tox.Branch II (H7509C) 8-8-90

DATA EVALUATION REPORT Stephen C. Dapson 8/8/90

STUDY TYPE: Teratogenicity Study in Rabbits GUIDELINE: 83-3(b)

TOX.CHEM.No.: 215B MRID No.: 412505-03 PROJECT NO.: 0-0030

TEST MATERIAL: Technical Chlorothalonil

STUDY NUMBERS: Ricerca Inc Reference No. 87-0060; Bio/dynamics Project No. 87-3196

SPONSOR: Farmenta Plant Protection Company, Mentor, Ohio.

TESTING LABORATORY: Bio/dynamics, East Millstone, New Jersey.

REPORT TITLE: A Teratology Study in Rabbits with Technical Chlorothalonil. (Doc.# 1544-87-0060-TX-002)

REPORT AUTHORS: N.H Wilson and J.C. Killeen

REPORT DATE: October 4, 1988

CONCLUSION: Groups of 20 mated female New Zealand White rabbits were given oral administrations of 0, 5, 10, or 20 mg/kg/day technical chlorothalonil suspended in 0.5% aqueous methyl cellulose during days 7 through 19 of gestation; dams were sacrificed on Day 30. No maternal, embryo, or fetotoxicity or teratogenicity was observed at the 5 or 10 mg/kg dose levels. At the high-dose (20 mg/kg), maternal toxicity was manifested by decreases in body weight gain and food consumption only during the period of dosing; no other treatment-related effects were observed at this level. No treatment-related effects were observed on fetal body weights or sex ratios. Chlorothalonil did not induce any external, visceral or skeletal malformations at any of the doses tested. Under the conditions of this study, the no-observed-effect-levels (NOELs) were 10 mg/kg for maternal toxicity and 20 mg/kg/day for developmental toxicity.

CORE CLASSIFICATION: Guideline; satisfies the requirements for a developmental toxicity study in rabbits.

QUALITY ASSURANCE: A quality assurance statement was provided.

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Study Title: A Teratology Study in Rabbits with Technical Chlorothalonil.

Testing Laboratory: Bio/dynamics, East Millstone, New Jersey.

Study (Project) No. 87-3196 (bio/dynamics) 87-0060 (Ricerca Inc)

Study Performance: December 5, 1987 thru January 22, 1988

Report Date: October 4, 1988

Authors: N.H Wilson and J.C Killeen

1. OBJECTIVE

The objective of this study was to assess the teratogenic potential of technical chlorothalonil in rabbits.

2. MATERIALS AND METHODS

a. Test Material

Trade Name: Technical chlorothalonil
Chemical Name: 2,4,5,6-tetrachloroisophthalonitrile
Ricerca Compound Number: T-117-12
Farmenta Identification Number: SDS-2787-1002
Farmenta Lot Number: D-5840923
Purity: 98.1%
Description: Light gray powder
Vehicle: 0.5% (w/v) methylcellulose in water

b. Test Animals

Species/Strain: Female New Zealand White [Hra:(NZW)SPF]
Age at receipt: 4 to 5 months
Body Weight: 3 to 4 kg
Source: Hazleton Research Products, Inc, Pennsylvania
Identification: Monel self-piercing ear tag.
Acclimation: 30 days, prior to mating.
Housing: Individually in stainless steel cages.
Food: Purina Certified High Fiber Diet No.5325 ad libitum.
Water: Tap water ad libitum
Environment: Temperature- 68 ± 5°C; Humidity- 50 ± 20%;
Air Exchange- 12/hr.; Light/Dark- 12 hr.cycle

Group Assignment: 20 mated females were randomly assigned to 1 control group and 3 treatment groups.

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c. Mating

Following acclimation, females were housed with sexually mature males (1:1) until mating had been observed (designated day 0 post coitum).

d. Preparation of Dosing Solutions

The test material/vehicle suspensions (w/v) were prepared fresh daily prior to dosing. The vehicle, 0.5% methylcellulose, was added to a weighed amount of the test material and the mixture was kept homogenous by constant stirring on a magnetic stirrer.

e. Analysis of the Dosing Solutions

Prior to initiation of the study, concentration, homogeneity, and stability analyses were determined for the 5 and 10 mg/kg dose levels (dosing suspensions at concentrations of 2.5 and 10.0 mg/ml,respectively). In addition, samples of dosing suspensions (all dose levels) used on days 1, 3, 7, 10, 14, 17, and 20 of dosing were also analyzed to confirm that the suspensions were prepared at their intended concentrations.

f. Administration of Test Article

The test article was administered daily orally via gavage at nominal dose levels of 5, 10, or 20 mg/kg from day 7 through 19 of gestation. The control group received the vehicle only. All groups received a dosing volume of 2 mL/kg body weight, with a daily adjustment of the individual volume to the actual body weight.

g. Observations

All animals were observed twice daily for morbidity, mortality, appearance, and clinical signs of toxicity. Dams were given a detailed physical examination on Days 0, 7, 10, 13, 16, 19, 24, and 30 of gestation. Body weights were measured on gestation days 0, 3, 7, 10, 13, 16, 19, 24 and 30. Food consumption was measured on Days 0, 3, 7, 10, 13, 16, 19, 24, and 29 post coitum. Dams aborting or delivering prematurely were sacrificed on the day such evidence was observed and given a gross postmortem evaluation.

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h. Termination

On day 30 post coitum, dams were sacrificed and post mortem examination included gross macroscopic examination of all internal organs with emphasis on uterus, uterine contents, position of fetuses in the uterus and the number of corpora lutea. The uteri (and its contents) of all females with live fetuses were weighed and the corrected body weights were calculated. Each fetus was weighed, sexed and examined for gross external abnormalities, and was prepared by fresh microdissection procedure for visceral examinations and stained with Alizarin Red S for skeletal examination.

3. RESULTS

a. Analysis of the Dosing Solutions

Homogeneity analysis indicated that the samples were accurately and homogeneously prepared; the mean values for the 2,5 and 10.0 mg/ml were 94 ± 3% and 103 ± 1%, respectively, of the nominal concentrations. Stability analyses of the two suspensions showed that the test material/methylcellulose suspensions were stable for at least a period of 24 hours at room temperature. Concentration analyses of the dosing suspensions collected during the dosing period (Days 1, 3, 7, 10, 14, 17, and 20) indicated that the suspensions were prepared accurately throughout the study. The means of the concentrations determined analytically for the 5, 10, and 20 mg/kg/day dosing suspensions were 96 ± 3%, 97 ± 5%, and 97 ± 3%, respectively, when expressed as a percentage of the expected concentrations.

b. Maternal Mortality

No mortality occurred during the study at the vehicle control, or at the low-dose (5 mg/kg) groups. The death of one female at the mid-dose (10 mg/kg) on Day 10 was considered to be non-treatment related; necropsy revealed fluid in the lungs and frothy fluid in the trachea, suggestive of an intubation error. The cause of death of one dam on Day 13 at the high-dose (20 mg/kg) was not established.

c. Maternal Body Weight Gain and Corrected Body Weight Gain.

Mean body weights for each recorded interval during gestation were comparable between the control and each treated group. Mean body weight gains were comparable for the control and each treated group during the pre-treatment period (Days 0-7 of gestation) and during the post-treatment period (Days 19-30). During the dosing period (Days 7-19 of gestation), a slight but statistically significant ($P < 0.05$) decrease in mean body weight gain was seen in dams at the 20 mg/kg/day when compared to control; dams at the 5 or 10 mg/kg/day did not show a similar effect (Table 1).

d. Maternal Food Consumption

No treatment-related effects were seen in the mean food consumption of dams between the vehicle control and the low- or mid-dose groups. At the high-dose, a slight, decrease in mean food consumption was observed during treatment days 7, 10, 13, 16, and 19; however, the difference was statistically significant ($p < 0.05$) only on Day 7 (Table 2).

e. Clinical Signs

No treatment-related clinical signs of toxicity were observed in dams at any dose groups.

f. Maternal Macroscopical Examination

No toxicologically significant macroscopical changes were observed in the dams that died or sacrificed at termination. The most frequent findings were discolored lungs (primarily red/tan/brown/ foci/areas) which were qualitatively similar in both the control and treated groups. The incidences in the control, low-dose, mid-dose, and high-dose groups were 60%, 60%, 70%, and 80%, respectively.

g. Reproduction Data

Pregnancy rates were comparable between the control and treated groups; 100% in the control, low- and mid-dose groups and 95% (19/20) in the high-dose group. There were no aborted pregnancies during the study. Premature delivery occurred in two control dams (10%), one low-dose dam (5%), two mid-dose dams (10%), and one high-dose dam (5%); the remaining 72 dams survived to scheduled sacrifice on Day 30. The mean number of corpora lutea and mean number of uterine implantations per dam were comparable between the control and treated groups. Although a slight increase in the mean pre-implantation loss index was seen at the mid-dose (0.153 ± 0.186) when compared to controls (0.077 ± 0.109), it was not considered to be treatment related since the difference was neither statistically significant nor was seen at the high-dose (0.081 ± 0.107) (Table 3).

h. Sex Ratios

The sex ratios of fetuses among the treated groups were comparable to that in the vehicle control group (Table 2).

i. Fetal Weight and Viability

Treatment altered neither fetal viability nor fetal weight.

No

j. External and Visceral Examinations

No treatment-induced external or visceral malformations were seen in fetuses from dams receiving 5, 10, or 20 mg/kg/day chlorothalonil. External malformations observed included a distended abdomen in one control fetus, the presence of a single external nare, and absence of upper incisors in one fetus from the low-dose group. No external malformations were seen in the 156 fetuses from the mid-dose or 158 fetuses from the high-dose Table 4).

The incidences of fetuses with visceral malformations for the control, low-, mid- and high-dose groups were 2/173 (1.2%), 1/170 (0.6%), 2/156 (1.3%), and 2/158 (1.3%). The type of malformations observed were similar to those commonly seen in rabbits of this strain (Table 5).

k. Skeletal Examination

The incidences of fetuses with skeletal malformation were: 9/173 (5.2%) in the vehicle control; 15/169 (8.9%) in the low-dose; 9/156 (5.8%) in the mid-dose; and 6/158 (3.8%) in the high-dose. The types of malformations included minor skeletal abnormalities (fused sternebrae, angulated arch of the hyoid) that are commonly seen in this strain of rabbits. No treatment-related differences in the level of skeletal ossification were seen between the vehicle control and the treated groups (Appendix 1).

4. DISCUSSION

Oral administration of chlorothalonil (0, 5, 10, or 20 mg/kg) to mated rabbits during the period of organogenesis (days 7 through 19) resulted in maternal toxicity characterized by slight decreases in mean body weight gain and mean food consumption at the high-dose (20 mg/kg) only during the dosing period; no embryotoxicity, fetotoxicity or teratogenicity was observed at this level. No maternal, embryo, fetotoxicity or teratogenicity was observed at the 5 or 10 mg/kg dose levels. Chlorothalonil did not induce external, visceral or skeletal malformations at any of the dose levels tested.

5. CONCLUSION

Based on these results, the NOELs and LOELs established are:

Maternal Toxicity NOEL: 10 mg/kg/day
LOEL: 20 mg/kg/day (HDT)
Developmental Toxicity NOEL: 20 mg/kg/day (HDT)

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3b) and is acceptable for regulatory purposes.

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Table 1. Maternal Body Weight Change In Rabbits Following Oral Administration of Chlorothalonil (grams).

Dose Level (mg/kg/day)		Gestation Days 0-7	7-10	10-13	13-16	16-19	19-30	7-19
0	Mean	106	6	24	86	21	94	136
	S.D	53	72	45	96	49	220	128
	N	20	20	20	20	20	18	20
5	Mean	107	8	3	78	22	38	111
	S.D	123	46	51	77	112	155	108
	N	20	20	20	20	20	18	20
10	Mean	89	-9	22	53	6	75	71
	S.D	59	80	66	78	84	185	183
	N	20	19	19	19	19	17	19
20	Mean	101	-26	8	21	-9	111	-8*
	S.D	81	73	59	106	104	226	206
	N	19	19	18	18	18	17	18

* Significantly ($p < 0.05$) different from controls (Dunnett's).

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Table 2. Maternal Food Consumption In Rabbits Following Oral Administration of Chlorothalonil (g/kg/day).

Dose Level (mg/kg/day)		Study Days 1	3	7	10	13	16	19	24	29
0	Mean	45	49	47	46	36	40	40	25	23
	S.D	12	6	6	8	11	11	13	15	14
	N	17	19	20	19	20	19	17	20	14
5	Mean	53	50	45	47	37	36	35	25	20
	S.D	6	7	9	7	10	16	12	9	15
	N	17	19	18	18	17	18	19	18	16
10	Mean	51	51	42	46	39	36	43	27	24
	S.D	9	7	14	7	7	18	15	14	15
	N	14	20	20	17	16	17	15	16	15
20	Mean	52	52	40*	42	29	26	28	28	23
	S.D	9	10	20	13	12	15	22	13	13
	N	16	18	15	18	17	16	13	16	15

* Significantly ($p < 0.01$) different from controls (Dunnett's).

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Table 3. Summary of Reproduction data in Rabbits Following Oral Administration of Chlorothalonil.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
No.of females mated	20	20	20	20
Female mortality	0	0	1	1
No. pregnant (%)	20 (100%)	20 (100%)	20 (100%)	19 (95%)
No.of pregnancies aborted	0	0	0	0
No.of premature births	2	1	2	1
No.of litters with viable fetuses	18	19	17	17
No.of litters with all resorption	0	0	0	0
No.of corpora lutea	198	192	191	184
Mean ± S.D.	11.0 ± 1.9	10.1 ± 1.9	11.2 ± 2.5	10.8 ± 2.0
No.of implantation sites	184	180	162	169
Mean ± S.D.	10.2 ± 2.4	9.5 ± 1.5	9.5 ± 2.9	9.9 ± 2.2
Preimplantation loss (M+S.D.)	0.077 ± 0.109	0.063 ± 0.091	0.153 ± 0.186	0.081 ± 0.107
No.of viable fetuses	172	170	156	158
Mean litter size ± S.D.	9.6 ± 2.2	8.9 ± 2.0	9.2 ± 2.7	9.3 ± 2.2
Mean no.of males ± S.D.	4.8 ± 1.9	3.9 ± 1.7	4.6 ± 2.1	4.5 ± 1.8
Mean no.of females ± S.D.	4.8 ± 2.1	5.1 ± 2.0	4.6 ± 2.5	4.8 ± 2.3
No.of dead fetuses	1	0	0	0
No.of resorption	11	10	6	11
Mean ± S.D.	0.6 ± 1.0	0.5 ± 1.9	0.4 ± 0.8	0.6 ± 0.6
Resorptions/implants (M±S.D.)	0.054 ± 0.001	0.049 ± 0.171	0.031 ± 0.063	0.067 ± 0.065
No.of litters with resorption (%)	7 (38.9)	2 (10.5)	4 (23.5)	10 (58.8%)
Mean fetal body weight (g)	45.68 ± 6.42	43.38 ± 5.25	43.32 ± 6.60	42.91 ± 9.37
Males	46.88 ± 6.72	43.19 ± 5.59	43.34 ± 6.94	43.89 ± 9.75
Females	44.93 ± 6.97	43.34 ± 5.28	41.99 ± 4.71	41.88 ± 8.84
Ratio of viable fetuses (Total m/f)	1.0	0.8	1.0	1.0

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Table 4. Summary of External Malformations in Fetuses of Dams Given Oral Administration of Chlorothalonil.

Types of Malformations	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Litters evaluated	18	19	17	17
Fetuses evaluated	173	170	156	158
Live	172	170	156	158
Dead	1	0	0	0
Distended abdomen				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Single external nare				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Absence of upper incisor				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Ascites of the abdominal cavity				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Total external malformations				
Fetal incidence	1	1	0	0
Litter incidence	1	1	0	0

Table 5. Summary of Visceral Malformations in Fetuses of Dams Given Oral Administration of Chlorothalonil.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Litters evaluated	18	19	17	17
Fetuses evaluated	173	170	156	158
Live	172	170	156	158
Dead	1	0	0	0
Lateral ventricles distended				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Persistent truncus arteriosus				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Interventricular septal defect				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Heart enlarged				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Renal pelvis(es) distended, Papilla(ae) absent				
Fetal incidence	0	0	1	2
Litter incidence	0	0	1	1
Ectopic kidneys				
Fetal incidence	0	0	1	0
Litter incidence	0	0	1	0
Total visceral malformations				
Fetal incidence	2	1	2	2
Litter incidence	2	1	2	1

APPENDIX 1.

M-2
APPENDIX M (cont.)
A TERATOLOGY STUDY IN RABBITS WITH T-117-12 87-3196

SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
NASAL(S) - MISSHAPEN					
FETAL INCIDENCE	N(Z)	1(0.6)	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	1(5.6)	1(5.3)	0	0
NASALS - FUSED					
FETAL INCIDENCE	N(Z)	1(0.6)	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	1(5.6)	1(5.3)	0	0
FRONTAL(S) - MISSHAPEN					
FETAL INCIDENCE	N(Z)	1(0.6)	0	0	0
LITTER INCIDENCE	N(Z)	1(5.6)	0	0	0
HYOID ARCH(ES) - ANGULATED					
FETAL INCIDENCE	N(Z)	5(2.9)	4(2.4)	3(1.9)	0
LITTER INCIDENCE	N(Z)	4(22.2)	4(21.1)	3(17.6)	0
UPPER INCISOR(S) - MISSING					
FETAL INCIDENCE	N(Z)	0	2(1.2)	0	0
LITTER INCIDENCE	N(Z)	0	2(10.5)	0	0
PALATINE SHELVES NOT FORMED ALONG MID-LINE					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
MALAR(S) - THICKENED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
ODONTOID - SMALL AND MISALIGNED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
FRONTALS - FUSED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	1(5.9)	0

M-3
APPENDIX M (cont.)
A TERATOLOGY STUDY IN RABBITS WITH T-117-12

87-3196

SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DA
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
PREMAXILLA - FUSED					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0
VOMER - ABSENT					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0
CERVICAL CENTRA - MISSHAPEN					
FETAL INCIDENCE	N(%)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(%)	0	1(5.3)	1(5.9)	0
THORACIC CENTRA - SMALL					
FETAL INCIDENCE	N(%)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(%)	0	1(5.3)	1(5.9)	0
THORACIC TRANSVERSE PROCESS(ES) - OVERSIZED					
FETAL INCIDENCE	N(%)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(%)	0	1(5.3)	1(5.9)	0
THORACIC TRANSVERSE PROCESS(ES) - SMALL					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0
THORACIC CENTRA - MISSHAPEN					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0
PRESENCE OF 8 LUMBAR VERTEBRAE					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	1(0.6)
LITTER INCIDENCE	N(%)	0	0	1(5.9)	1(5.9)
LUMBAR CENTRA - MISALIGNED					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	0
LITTER INCIDENCE	N(%)	0	0	1(5.9)	0

M-4

APPENDIX M (cont.)

A TERATOLOGY STUDY IN RABBITS WITH T-117-12

87-3196

SUMMARY OF FETAL SKELETAL MALFORMATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
SACRAL CENTRA - MISALIGNED					
FETAL INCIDENCE	N(Z)	0	0	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	0	1(5.9)	0
STERNEBRAE - FUSED					
FETAL INCIDENCE	N(Z)	4(2.3)	6(3.6)	1(0.6)	5(3.2)
LITTER INCIDENCE	N(Z)	4(22.2)	2(10.5)	1(5.9)	4(23.5)
STERNEBRAE - SCRAMBLED					
FETAL INCIDENCE	N(Z)	0	0	1(0.6)	1(0.6)
LITTER INCIDENCE	N(Z)	0	0	1(5.9)	1(5.9)
2ND STERNEBRA - IRREGULAR SHAPE					
FETAL INCIDENCE	N(Z)	0	0	0	1(0.6)
LITTER INCIDENCE	N(Z)	0	0	0	1(5.9)
RIBS - FUSED					
FETAL INCIDENCE	N(Z)	1(0.6)	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	1(5.6)	1(5.3)	1(5.9)	0
RIB(S) - BRANCHED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	1(5.9)	0
RIB(S) - CURVED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
	STAT SYMBOL				
TOTAL SKELETAL MALFORMATIONS					
FETAL INCIDENCE	N(Z)	9(5.2)	15(8.9)	9(5.8)	6(3.8)
LITTER INCIDENCE	N(Z) C-	8(44.4)	9(47.4)	9(52.9)	5(29.4)

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DA
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
HYOID ARCH(ES) - NOT OSSIFIED					
FETAL INCIDENCE	N(Z)	1(0.6)	0	0	0
LITTER INCIDENCE	N(Z)	1(5.6)	0	0	0
PRESENCE OF SUPERNUMERARY SUTURES					
FETAL INCIDENCE	N(Z)	3(1.7)	0	2(1.3)	1(0.6)
LITTER INCIDENCE	N(Z)	3(16.7)	0	2(11.8)	1(5.9)
HYOID BODY - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	10(5.8)	3(1.8)	7(4.5)	14(8.9)
LITTER INCIDENCE	N(Z)	6(33.3)	3(15.8)	6(35.3)	8(47.1)
HYOID BODY - NOT OSSIFIED					
FETAL INCIDENCE	N(Z)	1(0.6)	1(0.6)	1(0.6)	2(1.3)
LITTER INCIDENCE	N(Z)	1(5.6)	1(5.3)	1(5.9)	1(5.9)
INTERPARIETAL - SPLIT					
FETAL INCIDENCE	N(Z)	1(0.6)	0	0	0
LITTER INCIDENCE	N(Z)	1(5.6)	0	0	0
ANTERIOR FONTANEL - ENLARGED					
FETAL INCIDENCE	N(Z)	1(0.6)	0	0	7(4.4)
LITTER INCIDENCE	N(Z)	1(5.6)	0	0	2(11.8)
HYOID BODY - SPLIT					
FETAL INCIDENCE	N(Z)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	1(5.9)	0
FRONTAL(S) - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
PREMAXILLA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DAY
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
PARIETAL(S) - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	0	0	0	3(1.9)
LITTER INCIDENCE	N(Z)	0	0	0	3(17.6)
ODONTOID PROCESS - MISSHAPEN					
FETAL INCIDENCE	N(Z)	0	0	0	1(0.6)
LITTER INCIDENCE	N(Z)	0	0	0	1(5.9)
CERVICAL TRANSVERSE PROCESS(ES) - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	2(1.2)	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	2(11.1)	1(5.3)	1(5.9)	0
OSSIFICATION ADJACENT TO 7TH CERVICAL TRANSVERSE PROCESS					
FETAL INCIDENCE	N(Z)	6(3.5)	4(2.4)	6(3.8)	4(2.5)
LITTER INCIDENCE	N(Z)	2(11.1)	3(15.8)	5(29.4)	4(23.5)
CERVICAL CENTRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	1(0.6)	2(1.2)	3(1.9)	2(1.3)
LITTER INCIDENCE	N(Z)	1(5.6)	2(10.5)	2(11.8)	2(11.8)
CERVICAL CENTRA - NOT OSSIFIED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	1(0.6)
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	1(5.9)
DISCRETE OSSIFICATION VENTRAL TO 1ST CERVICAL CENTRA					
FETAL INCIDENCE	N(Z)	0	1(0.6)	0	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	0	0
THORACIC CENTRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(Z)	0	1(0.6)	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	1(5.3)	1(5.9)	0
THORACIC TRANSVERSE PROCESS - MISSING					
FETAL INCIDENCE	N(Z)	0	0	1(0.6)	0
LITTER INCIDENCE	N(Z)	0	0	1(5.9)	0

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DA
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
THORACIC TRANSVERSE PROCESS - MISSHAPEN					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	0
LITTER INCIDENCE	N(%)	0	0	1(5.9)	0
SACRAL TRANSVERSE PROCESS(ES) - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(%)	21(12.1)	22(13.0)	15(9.6)	25(15.8)
LITTER INCIDENCE	N(%)	12(66.7)	13(68.4)	10(58.8)	10(58.8)
6TH STERNEBRA - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	6(3.5)	5(3.0)	7(4.5)	14(8.9)
LITTER INCIDENCE	N(%)	4(22.2)	2(10.5)	3(17.6)	6(35.3)
6TH STERNEBRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(%)	5(2.9)	7(4.1)	9(5.8)	7(4.4)
LITTER INCIDENCE	N(%)	3(16.7)	4(21.1)	4(23.5)	5(29.4)
5TH STERNEBRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(%)	10(5.8)	7(4.1)	19(12.2)	14(8.9)
LITTER INCIDENCE	N(%)	6(33.3)	6(31.6)	9(52.9)	8(47.1)
5TH STERNEBRA - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	8(4.6)	9(5.3)	17(10.9)	3(1.9)
LITTER INCIDENCE	N(%)	5(27.8)	6(31.6)	5(29.4)	2(11.8)
SMALL OSSIFICATION ADJACENT TO 1ST STERNEBRA					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	0
LITTER INCIDENCE	N(%)	0	0	1(5.9)	0
6TH STERNEBRA - SPLIT					
FETAL INCIDENCE	N(%)	1(0.6)	2(1.2)	2(1.3)	6(3.8)
LITTER INCIDENCE	N(%)	1(5.6)	2(10.5)	2(11.8)	5(29.4)
1ST STERNEBRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DA
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
SMALL OSSIFICATION ANTERIOR TO 1ST STERNEBRA					
FETAL INCIDENCE	N(%)	1(0.6)	0	3(1.9)	0
LITTER INCIDENCE	N(%)	1(5.6)	0	2(11.8)	0
4TH STERNEBRA - SPLIT					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	0
LITTER INCIDENCE	N(%)	0	0	1(5.9)	0
5TH STERNEBRA - SPLIT					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	0
LITTER INCIDENCE	N(%)	0	0	1(5.9)	0
2ND STERNEBRA - INCOMPLETELY OSSIFIED					
FETAL INCIDENCE	N(%)	0	0	1(0.6)	2(1.3)
LITTER INCIDENCE	N(%)	0	0	1(5.9)	2(11.8)
RIB(S) - 13TH UNILATERAL					
FETAL INCIDENCE	N(%)	31(17.9)	18(10.7)	17(10.9)	17(10.8)
LITTER INCIDENCE	N(%)	15(83.3)	13(68.4)	9(52.9)	10(58.8)
RIB(S) - 13TH FLOATING					
FETAL INCIDENCE	N(%)	31(17.9)	11(6.5)	15(9.6)	10(6.3)
LITTER INCIDENCE	N(%)	14(77.8)	8(42.1)	8(47.1)	7(41.2)
RIB(S) - 13TH SHORT					
FETAL INCIDENCE	N(%)	46(26.6)	33(19.5)	41(26.3)	32(20.3)
LITTER INCIDENCE	N(%)	14(77.8)	14(73.7)	14(82.4)	15(88.2)
RIB(S) - BULBOUS					
FETAL INCIDENCE	N(%)	1(0.6)	0	0	0
LITTER INCIDENCE	N(%)	1(5.6)	0	0	0
RIB(S) - 13TH RUDIMENTARY					
FETAL INCIDENCE	N(%)	1(0.6)	2(1.2)	0	1(0.6)
LITTER INCIDENCE	N(%)	1(5.6)	2(10.5)	0	1(5.9)

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP		0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/DA
LITTERS EVALUATED	N	18	19	17	17
FETUSES EVALUATED	N	173	169	156	158
LIVE	N	172	169	156	158
DEAD	N	1	0	0	0
RIB(S) - 13TH SEGMENTED					
FETAL INCIDENCE	N(%)	1(0.6)	0	0	0
LITTER INCIDENCE	N(%)	1(5.6)	0	0	0
RUDIMENTARY STRUCTURE ADJACENT TO 1ST LUMBAR VERTEBRA					
FETAL INCIDENCE	N(%)	0	3(1.8)	0	1(0.6)
LITTER INCIDENCE	N(%)	0	2(10.5)	0	1(5.9)
RIB(S) - THICKENED					
FETAL INCIDENCE	N(%)	0	1(0.6)	0	0
LITTER INCIDENCE	N(%)	0	1(5.3)	0	0
METACARPAL(S) - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	9(5.2)	3(1.8)	6(3.8)	14(8.9)
LITTER INCIDENCE	N(%)	4(22.2)	2(10.5)	4(23.5)	5(29.4)
FORELIMB(S): MID-PHALANGE(S) - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	3(1.7)	4(2.4)	8(5.1)	8(5.1)
LITTER INCIDENCE	N(%)	2(11.1)	2(10.5)	2(11.8)	4(23.5)
FORELIMB(S): PROXIMAL PHALANGE(S) - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	0	0	0	2(1.3)
LITTER INCIDENCE	N(%)	0	0	0	2(11.8)
TARSAL(S) - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	0	1(0.6)	3(1.9)	3(1.9)
LITTER INCIDENCE	N(%)	0	1(5.3)	2(11.8)	1(5.9)
HINDLIMB(S): MID-PHALANGES - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	0	0	2(1.3)	2(1.3)
LITTER INCIDENCE	N(%)	0	0	1(5.9)	1(5.9)
HINDLIMB(S): PROXIMAL PHALANGE(S) - NOT OSSIFIED					
FETAL INCIDENCE	N(%)	0	0	0	1(0.6)
LITTER INCIDENCE	N(%)	0	0	0	1(5.9)

M-10
APPENDIX M (cont.)
A TERATOLOGY STUDY IN RABBITS WITH T-117-12 87-3196

SUMMARY OF FETAL SKELETAL VARIATIONS

DOSE GROUP			0 MG/KG/DAY	5 MG/KG/DAY	10 MG/KG/DAY	20 MG/KG/D
LITTERS EVALUATED	N		18	19	17	17
FETUSES EVALUATED	N		173	169	156	158
LIVE	N		172	169	156	158
DEAD	N		1	0	0	0
PUBIS(ES) - NOT OSSIFIED						
FETAL INCIDENCE	N(%)		0	0	0	2(1.3)
LITTER INCIDENCE	N(%)		0	0	0	1(5.9)
		STAT SYMBOL				
TOTAL SKELETAL VARIATIONS						
FETAL INCIDENCE	N(%)		111(64.2)	89(52.7)	103(66.0)	99(62.7)
LITTER INCIDENCE	N(%)	NS	17(94.4)	19(100.0)	17(100.0)	17(100.0)

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000201

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 8/22/90
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy 8/22/90
Section I, Toxicology Branch II (HFAS) (H7509C)
Stephen C. Dapson 8/29/90

DATA EVALUATION RECORD

STUDY TYPE: One-Generation Reproduction - Rat (Rangefinding Study)

MRID NUMBER: 412505-04

TEST MATERIAL: Chlorothalonil, Technical

SYNONYMS: 2,4,5,6-tetrachloroisophthalonitrile

STUDY NUMBER(S): 1722-87-0120-TX-001

SPONSOR: Fermenta ASC Corporation
5966 Heisley Road
P. O. Box 8000
Mentor, Ohio 44061-8000

TESTING FACILITY: Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
Painesville, Ohio 44077

TITLE OF REPORT: Reproduction dose-rangefinding study in rats with technical chlorothalonil

AUTHOR(S): N. H. Wilson, J. S. Chun and J. C. Killeen

DATE REPORT ISSUED: May 18, 1989

CONCLUSIONS: Based upon the results of this study, treatment levels of 500, 1500 and 3000 ppm in diet are appropriate for inclusion in a two generation reproduction study in rats.

Systemic NOEL = ~~1500~~ 750 ppm for female rats and ~~750~~ 375 ppm for male rats. EAD 9/5/91 ACK 9/5/91

Systemic LOEL = ~~750~~ 1500 ppm for female rats and ~~375~~ 750 ppm for males rats on the basis of body weight gain reduction EAD 9/5/91 ACK 9/5/91

Developmental NOEL = 1500 ppm

Developmental LOEL = 3000 ppm on the basis of reduced pup weight gain and reduced viability

CLASSIFICATION: Core - Supplementary
(This is not a guideline study.)

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I. PROTOCOL

A. Materials

1. Test Material: Technical grade chlorothalonil (stated purity of 98.1%); described as a light gray powder (Lot No. D-5840923).

2. Test species: 6-week old Charles River CD rats (Charles River Breeding Laboratory, Inc., Portage, MI.)

3. Diet preparation: Dietary mixtures containing the test material were prepared fresh weekly. Diet mixtures were stored at room temperature in the dark.

   Test diets, analyzed for homogeneity of mixtures and chemical stability in dietary mixtures for 14 days, were confirmed in test diets containing 200 and 3000 ppm. During the study, samples of each diet were collected and analyzed weekly for the first ten weeks and then during even weeks thereafter.

B. Procedures and Study Design

1. Mating: Each male was caged with one female from the same test group until a vaginal plug was observed or sperm cells were found in vaginal smears taken daily during the mating period. If sperm were not found, cohabitation was repeated each night until a positive smear was obtained or for 14 days, whichever came first.

   On day 18 of gestation, each pregnant female was individually placed into a cage with a solid bottom and Bed-O-Cobs bedding where they were kept through gestation and lactation.

2. Mating schedule: The F_0 parental animals were given test diets for ten weeks before they were mated.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups No.	Dose (ppm) *	Animals per group	
		Males	Females
1	0	15	15
2	200	15	15
3	375	15	15
4	750	15	15
5	1500	15	15
6	3000	15	15

* Diets were administered from the beginning of the study until the animals were sacrificed.

C. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Twice a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly until termination of the study for males and the 10 week growth period for females.
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0, 7, 14 and 21 post partum.
Food consumption	All	Weekly during premating period.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study.

The following indices were calculated:

$$\text{Male fertility index} = \frac{\text{No. females pregnant}}{\text{No. males mated}} \times 100$$

$$\text{Female fertility index} = \frac{\text{No. females pregnant}}{\text{Total no. females mated}} \times 100$$

3. Litter observations: At delivery, the date, number, weight and sex of live and stillborn pups and any external abnormalities were recorded. Daily observations for survival and overt toxic signs were conducted throughout lactation. The number and sex of live pups were recorded on days 0, 1, 4 (pre- and post-cull), 7, 14 and 21 of lactation. Total litter weights for live pups were measured on days 0, 4 (pre- and post-cull), 7 and 14. Individual body weights were measured on day 21.

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The following indices were calculated:

$$\text{Live Born Index} = \frac{\text{No. of pups born live}}{\text{Total no. of pups born}} \times 100$$

$$\text{Day 4 Viability Index} = \frac{\text{No. of pups alive at Day 4 (Precull)}}{\text{Total no. of pups born alive}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of pups alive on Day 21}}{\text{No. of pups alive on Day 4 (Postcull)}} \times 100$$

$$\text{Litter Viability Index} = \frac{\text{No. of litters with live pups on Day 21}}{\text{No. of litters with live pups on Day 0}} \times 100$$

4. Necropsy

a. Parental animals: All parental rats were sacrificed after completion of the lactation period. All animals were subjected to post mortem examinations.

b. Offspring: The surviving F1 offspring were sacrificed at 21 days of age. These animals were subjected to gross post mortem examinations. Stillborn pups or pups that died during the study were necropsied within six hours of discovery.

c. Necropsy observations: Gross necropsy included notation of the presence or absence of implantation scars. No tissues were saved for microscopic examination.

D. Statistical Analyses: Study data were divided into four general categories for statistical analysis.

1. Interval Scale Data - This category included adult body weights (male and female), food consumption, gestation length and litter weights. Bartlett's test was used to test for normality of the data and homogeneity of variance. If Bartlett's test indicated nonsignificance, data were subjected to a one-way analysis of variance followed by mean separation using Dunnett's test. Litter weights were also analyzed by analysis of covariance with litter size as the covariate. If Bartlett's test indicated significance, data were analyzed by nonparametric procedures described in part D,3 below.

For the parametric data, regression techniques, a test for trend in dose levels and a test for lack-of-fit were performed. Where significant lack-of-fit was indicated, the trend test was discounted.

All tests were two-sided and were conducted at the 5% level of significance.

2. Count Data - Day 0 data for litter size, number of live pups and number of stillborn pups were analyzed using these procedures. Per the study report, "These parameters were assumed to be Poisson distributed, and a square root transformation was used to achieve normality. Following the transformation, the analysis of these parameters was the same as that of the interval scale data-including performing Bartlett's test on the transformed data and the use of the nonparametric tests if the Bartlett's test shows significance.

"All tests were two-sided and were conducted at the 5% level of significance."

3. Percentage Data - These data included liveborn, stillborn, Day 4 pup viability and lactation indices and sex ratio on Day 0. "Statistical evaluation of the equality of the treatment means for these parameters (and, as needed, for parameters from the first group) was made by the Kruskal-Wallis test, a nonparametric one-way analysis of variance technique and by Dunn's summed rank test, a nonparametric technique for comparing the treatment groups to the control group. Also, Jonckheere's test was to test for a monotonic trend in the dose levels.

"Note that for these parameters, the index (or ratio) was computed separately for each litter. These litter indices, then, are the observations that were analyzed.

"All tests were two-sided tests and were conducted at the 5% level of significance."

4) Contingency Table Data - These data consisted of the mating and fertility indices. Per the study report, "Statistical analyses of these incidence data were performed using contingency tables. Each treatment group was compared to the control group using Fisher's Exact test for a 2x2 table. In addition, Armitage's test for a linear trend in the doses was performed.

"The Fisher's exact tests were done at the 1% level of significance (5% divided by the number of treatment groups) in order to maintain an overall significance level of approximately 5% for each parameter. Armitage's test was performed at the 5% significance level. All tests were two-sided."

II. REPORTED RESULTS

A. Analysis of test diets: Analyses of test diets for concentration, homogeneity, and stability were conducted prior to the initiation of the study. The following recoveries were reported: 96% ± 4% (200 ppm), 98% ± 5% (400 ppm), 98% ± 4% (700 ppm), 98% ± 3% (1500 ppm) and 99% ± 4% (3000 ppm). Outliers were reported for one of 28 samples analyzed from the 400 ppm diet (89%) and one of 34 samples analyzed from the 3000 ppm diet (112%).

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Homogeneity of the diets was evaluated by duplicate analyses of five samples each from the 200 and 3000 ppm diets. Concentrations were 202 ppm ± 1 ppm (101% ± 1%) and 3002 ppm ± 22 ppm (100% ± 1%), respectively.

Diet samples from the 200 and 3000 ppm diets collected after storage at room temperature for 4, 7 and 14 days. For the 200 ppm diet, concentrations were 210 ppm ± 1 ppm (104%), 181 ppm ± 1 ppm (90%) and 172 ppm ± 3 ppm (85%) for the 4, 7 and 14 day samples, respectively. For the 3000 ppm diets, comparable values were 2883 ppm ± 71 ppm (96%), 2850 ppm ± 9 ppm (95%) and 3060 ppm ± 78 ppm (102%).

Dietary concentration, homogeneity and stability with respect to the test material were acceptable.

B. Parental animals

1. Mortality and clinical signs: No deaths occurred during this study.

No treatment related clinical signs were reported.

2. Body weight and food consumption: During the premating period, body weights and body weight gains for males from the 1500 and 3000 ppm treatment groups were reduced relative to the control beginning with week 1 and persisting until mating. These observations were not paralleled by decreased food consumption. The only significant decrease noted in food consumption occurred during week 1 in the high dose group. This may have reflected an effect of the test material on palatability; food consumption increased in the subsequent weeks and no accompanying clinical signs were reported.

No treatment related effects in females on body weight, body weight gain or food consumption were reported during the premating period. Although statistical significance was indicated for food consumption in the 200 and 3000 ppm treatment groups, these increases are considered to be numerical anomalies rather than treatment effects.

Reported body weight and selected food consumption results for the premating period are summarized as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
F_0 Generation Males - Premating						
Mean body weight (g)						
0	208.0	210.7	207.8	208.9	206.1	208.0
10	529.9	510.9	498.9	501.8	471.4**	490.2
Mean weight gain (g)						
0 - 10	321.9	300.2	291.1	292.9	265.3	282.2
Mean food consumption (g/rat/day)						
1	24.5	25.3	24.0	24.0	22.7	20.4**
2	25.9	26.5	25.1	25.7	25.1	26.7
10	27.3	28.2	26.6	27.0	24.9	27.8
F_0 Generation Females - Premating						
Mean body weight (g)						
0	147.0	148.9	145.5	146.2	146.4	149.9
10	259.8	276.4	261.3	259.7	259.5	251.2
Mean weight gain (g)						
0 - 10	112.8	127.5	115.8	113.5	113.1	101.3
Mean food consumption (g/rat/day)						
1	15.9	17.4	15.8	16.0	15.7	16.4
2	16.7	17.7	16.1	16.5	16.3	17.4
10	16.1	18.3**	17.1	16.8	16.9	18.0**

**Statistically significantly different from control, $p<0.01$.
(Data excerpted from Study Tables 1 and 2, pages 37 and 41.)

No treatment related effects were reported on gestational or lactational maternal body weights. Lactational body weight gains exhibited a dose related increase in the 750, 1500 and 3000 ppm treatment groups relative to the control.

Selected group mean body weights for pregnant or nursing dams were summarized in the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Mean body weight (g)						
Gestation -						
Day 0	258.1	274.0	255.5	261.3	251.4	249.1
Day 20	387.1	389.8	384.2	384.3	372.5	372.2
Lactation -						
Day 0	300.7	311.9	297.0	297.9	293.5	287.9
Day 21	307.4	320.2	302.6	311.4	313.4	321.8
Mean body weight gain (g)						
Gestation -						
Days 0-20	129.0	125.8	128.7	123.0	121.1	123.1
Lactation -						
Day 0-21	6.7	8.3	5.6	13.5	19.9	33.9

(Data excerpted from Study Tables 9 and 10, pages 60 and 61.)

3. Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, males consumed 14, 25, 50, 101 and 207 mg/kg/day during the peak growth periods (weeks 1 to 10) for the 200, 375, 750, 1500 and 3000 ppm treatment groups, respectively. For females, the comparable intakes of the test material were 16, 29, 57, 115 and 245 mg/kg/day.

4. Reproductive performance: No treatment related effects on reproductive performance were reported.

Results for the parental animals are summarized from the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Males						
Mated (%)	100	93	93	93	100	100
Fertile (%)	87	100	71	93	93	87
Females						
Mated (%)	100	93	93	93	100	100
Fertile (%)	87	100	71	93	93	87
Median gestation interval (days)	22.7	22.1	22.3	22.0	22.5	22.2
Number of litters	13	14	10	13	14	13
Mean litter size						
Day 0	13.5	11.1	13.2	12.2	11.4	12.1
Day 21	8.0	7.8	8.0	8.0	7.6	7.8
Mean pup weight (g)						
Day 0	6.3	6.5	6.5	6.3	6.5	6.5
Day 21	53.2	53.4	54.5	50.3	52.6	46.8**

**Statistically significantly different from control, $p<0.01$.
(Excerpted from Study Tables 11-13, pages 62-64, and Study Tables 21 and 22, pages 72-75.)

5. Necropsy results

a. Organ weights: No organ weights were measured.

b. Pathology - Macroscopic examination: The only apparent treatment related gross pathology reported was the enlargement of the kidneys (bilateral) in 5/15 male rats from the 3000 ppm treatment group, with accompanying discoloration in 2/15. Pale, mottled and enlarged kidneys were reported in one high dose female. The authors indicated that this is a common lesion seen in response to treatment of rats with chlorothalonil and thus is probably treatment related in this study.

No other treatment related gross lesions were reported.

C. Offspring

1. Viability and clinical signs: No whole litter losses were reported in any treated groups. One control female delivered a litter containing a single stillborn pup. The percentages of pups born live surviving

at day 4 (pre-cull) were 100.0, 99.5, 98.8, 98.3, 100.0 and 95.7% for groups receiving 0, 200, 375, 750, 1500 and 3000 ppm of the test material in diet. The survival in the high dose group was significantly reduced ($p<0.01$) relative to the control. No pup loss occurred after the 4-day culling.

Sex ratio was not affected by treatment.

Changes in mean litter sizes were summarized in the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Day 0	13.5	11.1	13.2	12.2	11.4	12.1
Day 4						
(Pre-Cull)	13.5	11.0	13.0	11.9	11.4	11.6
(Post-Cull)	8.0	7.8	8.0	8.0	7.6	7.8
Day 7	8.0	7.8	8.0	8.0	7.6	7.8
Day 14	8.0	7.8	8.0	8.0	7.6	7.8
Day 21	8.0	7.8	8.0	8.0	7.6	7.8

(Excerpted from Study Table 21, pages 72-73.)

No apparent treatment related clinical observations were reported in F_1 pups.

2. Body weight: Pup weights for the 3000 ppm group were decreased relative to the control at the day 14 and 21 weighings. No other treatment related effects on pup body weights were reported.

Selected group mean body weights are summarized from the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Body weight (g)						
Day 0	6.3	6.5	6.5	6.3	6.5	6.5
Day 21	53.2	53.4	54.5	50.3	52.6	46.8**
Weight gain (g)	46.9	46.9	48.0	44.0	46.1	40.3

**Statistically significantly different from control, $p<0.01$.
(Excerpted from Study Table 22, pages 74-75.)

3. Necropsy results

a. Organ weights: Organ weights were not recorded.

b. Pathology - Macroscopic examination: No treatment related gross lesions were reported. Dilated renal pelvis was slightly more frequent in the 750 and 1500 ppm treatment group, but was similar to the control in the 3000 ppm group.

III. DISCUSSION

All treatment levels used in this study were well tolerated. No effects on reproductive parameters were reported. Males from the 1500 and 3000 ppm treatment groups exhibited slight body weight and body weight gain decrements relative to the control. Body weight gain was also slightly reduced in high dose females. Treatment related gross pathology was limited to kidney enlargement in the high dose males, an effect acknowledged by the registrant to be associated with chlorothalonil ingestion by rats.

Effects in offspring were decreased body weight gains in high dose pups and a slight reduction in viability prior to day 4 of lactation in the high dose pups.

On the basis of this study, the registrant selected 3000 ppm as a confirmed effect level, 500 ppm as a likely no effect level and 1500 ppm as an intermediate treatment level for a subsequent two generation reproduction study in rats with technical chlorothalonil. Tox Branch concurs with these selections.

CONCLUSIONS: Based upon the results of this study, treatment levels of 500, 1500 and 3000 ppm in diet are appropriate for inclusion in a two generation reproduction study in rats.

Systemic NOEL = 1500 ppm for female rats and 750 ppm for male rats
Systemic NOEL = 750 ppm for female rats and 375 ppm for males rats on the basis of body weight gain reduction
Developmental NOEL = 1500 ppm
Developmental LOEL = 3000 ppm on the basis of reduced pup weight gain and reduced viability

CLASSIFICATION: Core - Supplementary
(This is not a guideline study.)

CAS
000901

EPA No.: 68D80056
DYNAMAC No.: 324-B
TASK No.: 3-24B
September 4, 1991

DATA EVALUATION RECORD

CHLOROTHALONIL

Metabolism in Rats

STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Pilot study of the effect of the gamma-glutamyl transpeptidase inhibitor, AT-125, on the metabolism of ^{14}C-chlorothalonil. (Unpublished study No. 1376-86-0072-AM-002 by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; undated.) MRID No. 412505-06.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 9/4/91

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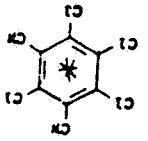
1. CHEMICAL: Terachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; 1,3-dicyano-2,4,5,6-tetrachlorobenzene; chlorothalonil.

2. TEST MATERIAL: [^{14}C]Chlorothalonil, uniformly labeled in the benzene ring, and unlabeled chlorothalonil were used. The radiolabeled test material had a specific activity of 134.8 mCi/mmol and a radiochemical purity of 99 percent. Analytical-grade unlabeled chlorothalonil was 98.9 percent pure. The structure and radiolabel position (*) of [^{14}C]chlorothalonil are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Pilot study of the effect of the gamma-glutamyl transpeptidase inhibitor, AT-125, on the metabolism of ^{14}C-chlorothalonil. (Unpublished study No. 1376-86-0072-AM-002 by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; undated.) MRID No. 412505-06.

5. REVIEWED BY:

Mary E. Cerny, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Robert J. Weir for
Date: 9/4/91

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 9/4/91

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6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: Nicolas P. Hajjar

Date: September 4, 1991

Alan C. Levy, Ph.D.
EPA Reviewer
Review Section IV
Toxicology Branch II
(H-7509C)

Signature: Alan C. Levy

Date: 9-5-91

Elizabeth A. Doyle, Ph.D.
EPA Reviewer
Review Section IV
Toxicology Branch II
(H-7509C)

Signature: E A. Doyle

Date: 9/5/91

7. CONCLUSIONS:

A. Groups of three male rats were given an intraperitoneal injection of either AT-125, an inhibitor of gamma-glutamyl transpeptidase (GGT), or phosphate-buffered saline (PBS) 1 hour before administration of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. (The first step in the metabolism of glutathione conjugates involves GGT.)

Gastrointestinal absorption of [^{14}C]chlorothalonil was low and roughly equivalent for both groups of animals, accounting for about 7.4 percent of the administered dose at 48 hours postdosing. Levels of radioactivity in the blood, liver, and kidneys also were low, representing no more than 0.2, 0.21, and 1 percent of the ^{14}C dose, respectively, at 24 hours after administration of the test material. Nearly all (76 to 83 percent) of the ^{14}C in the blood was associated with the plasma. Approximately 80 percent and more than 50 percent of the radiolabel in the plasma and kidneys, respectively, were bound to proteins.

Pretreatment with AT-125 had no effect on the amount of radioactivity (as ppm, total ^{14}C, and percent of ^{14}C dose) in the blood, plasma, red blood cells, and liver; the GGT inhibitor also did not alter the rate or total amount of ^{14}C eliminated in the urine. However, at 24 hours postdosing, the kidneys of pretreated rats contained approximately three times more radioactivity than the kidneys of nonpretreated animals (48 ppm, 105 g ^{14}C equivalents, and 0.88 percent of the ^{14}C dose for AT-125-pretreated rats versus 17 ppm, 39 g, and 0.33 percent for nonpretreated rats); in addition, 70 percent of the radiolabel in the kidneys of rats pretreated with AT-125 was extracted into buffered saline (pH 7), compared with 44 percent from nonpretreated animals. Extractability of radiolabel in the urine collected for up to 12 hours also differed: only 15 percent was extracted from acidified urine, whereas more than 75 percent was extractable from the urine of rats given only chlorothalonil. Extractability of radiolabel from urine collected between 12 and 24 hours was similar (≥60 percent) for both groups.

The nonextractable fractions of all urine samples contained two metabolites, tentatively identified as the di- and triglutathione (GSH) conjugates of chlorothalonil, which are probable precursors of the urinary thiol metabolites of chlorothalonil. A third, unidentified (but less polar, based on elution time) metabolite was isolated from all nonpretreated rats and from the 12- to 24-hour urine samples of AT-125-dosed animals. AT-125-pretreated rats may have also excreted a metabolite containing four

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glutathione moieties, and nonpretreated rats eliminated relatively large amounts of up to four additional (less polar) metabolites. For pretreated rats, the major metabolites were the diGSH and triGSH conjugates, which accounted for about 2 and 1.8 percent of the ^{14}C dose, respectively; between 11 and 45.5 percent of the urinary ^{14}C was the diGSH, and 18 to 41 percent of the urinary radiolabel was the triGSH conjugate. The major metabolite excreted in the urine of rats given only [^{14}C]chlorothalonil was not identified and represented approximately 2.71 percent of the ^{14}C dose and between 26 and 40 percent of the urinary radioactivity.

The data indicate that AT-125 affected the <u>in vivo</u> metabolism of [^{14}C]chlorothalonil by slowing the rate of conversion of initial metabolites to the polar products by increasing the amount of radioactivity in the kidneys at 24 hours after dosing. The study supports the prominent role of the glutathione pathway in the metabolism of chlorothalonil.

B. This study provides supplementary information on the metabolism of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg in male rats. This was a pilot study and it does not meet EPA Guidelines (85-1) for the following reasons: (1) the use of only males; (2) the administration of only one dose level of chlorothalonil, rather than single low, single high, and repeated low doses; (3) no collection and analysis of feces; and (4) incomplete tissue distribution data.

Items 8 through 10--see footnote 1.

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[1]Only the items appropriate to this DER have been included.

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11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) Chlorothalonil labeled uniformly with ^{14}C in the benzene ring had a specific activity of 134.8 mCi/mmol and a radiochemical purity of 99 percent. Analytical-grade unlabeled chlorothalonil was 98.9 percent pure. Methods used to determine specific activity and purity were not specified. Lot and/or batch numbers also were not reported.

2) Male CD Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Portage, MI), weighing between 221 and 246 g at the time of dosing, were used. Animals were quarantined for at least 7 days before dosing; rats were housed individually in stainless steel cages during the acclimation period.

3) The chlorothalonil dosing suspension was prepared by dissolving [^{14}C]chlorothalonil and unlabeled chlorothalonil in methylene chloride, evaporating the solvent, and then adding a "small" amount of 0.75 percent methylcellulose to the remaining solid material. The mixture was shaken, the material pulverized (mean particle size 3.4 microns), and the suspension diluted with 0.75 percent methylcellulose to a final concentration of 5.19 mg/mL (44.95 Ci/mL; 2,30 mCi/mmol); the radiochemical purity of the [^{14}C]chlorothalonil in the dosing suspension was 97.1 percent.

The AT-125 dosing solution was prepared by dissolving 10.0 mg AT-125 in 2 mL 0.9 percent saline/0.005 M phosphate buffer (PBS).

4) After an overnight fast, rats (three/group) received an intraperitoneal (ip) injection of either 10 mg buffered AT-125/kg or PBS. At 1 hour after ip dosing, animals in both groups were given a single oral (intubation) dose of 50 mg [^{14}C]chlorothalonil/kg. One additional rat received no treatment and served as a control. Immediately following administration of the radiolabeled test material, control and experimental rats were placed in individual metabolism cages containing water but no food. Urine was collected over dry ice at 6, 12, and 24 hours after oral dosing. Cages were rinsed with water at each collection time. All rats were killed at 24 hours after administration of [^{14}C]chlorothaonil; the liver and kidneys were removed, blood was collected, and carcasses were stored frozen.

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5) Aliquots of blood were combusted and then analyzed for [^{14}C]CO_2 by liquid scintillation counting (LSC). The remaining blood was centrifuged to separate plasma from red blood cells. Aliquots of plasma were assayed directly by LSC; aliquots of red blood cells were oxidized before counting. Plasma was analyzed further by adding 10 percent trichloroacetic acid (TCA); supernatants were counted directly, and precipitated material was assayed for ^{14}C content following digestion/solubilization with pepsin (pH 2).

Liver and kidney samples were combusted and analyzed for radioactivity by LSC. In addition, kidney tissue from one animal of each group was homogenized and extracted three times with buffered saline (pH 7), saline (pH 2), methanol, ethyl acetate, and hexane.

The ^{14}C content of each extract was determined via LSC. Aliquots of the pH 7-buffered saline extract were treated with a fourfold volume of 10 percent TCA, and TCA-soluble material was counted. The TCA precipitate was digested twice with pepsin (pH 2), and pepsin-solubilized material was assayed for radioactivity by LSC. Solids remaining after pepsin digestion were solubilized in KOH and counted.

Aliquots of urine were analyzed directly for ^{14}C content by LSC. The remaining urine was frozen and stored for metabolite identification. Prior to analysis for chlorothalonil metabolites, individual urine samples were thawed, acidified to pH 2, and extracted with ethyl acetate. Individual samples were then analyzed for metabolites with reverse-phase high-performance liquid chromatography (HPLC) and LSC. After this analysis, individual samples were pooled according to treatment group, collection time, and extractability, and reanalyzed by reverse-phase HPLC and LSC.

B. Protocol: The protocol for this study is presented in the Appendix.

12. REPORTED RESULTS:

A. Rats pretreated with AT-125 received an average (± S.D.) ip dose of 9.96 ± 0.04 mg AT-125/kg; both AT-125-pretreated and PBS-pretreated rats received an average oral dose of 50.38 ± 0.69 mg [^{14}C]chlorothalonil/kg (101.80 ± 3.74 μCi). The method(s) used to make these determinations were not described.

B. One AT-125-pretreated rat had loose stools after administration of chlorothalonil, and all three rats dosed with this enzyme inhibitor had red coloration in the abdominal cavity at necropsy. No other remarkable clinical observations were reported.

C. The concentrations and percent recoveries of radioactivity in the blood, plasma, and red blood cells were similar for both AT-125-pretreated and vehicle-only pretreated rats (Table 1). The average (± S.D.) amount of radiolabel in the blood was 13.0 ± 3.6 μg, which represented approximately 1 percent of the ^{14}C dose; the mean ^{14}C concentration in blood was 871 ± 224 ppm. Red blood cells contained much smaller amounts (about 400 ppb ^{14}C) of radioactivity, and it was determined that plasma radioactivity accounted for about 80 percent of the radiolabel in the blood. Of the ^{14}C recovered from the plasma of both groups of rats, only 8 percent was soluble following protein precipitation with TCA; the remaining radioactivity (about 93 percent of the plasma ^{14}C) was recovered following pepsin digestion of TCA precipitates.

The amount of radioactivity in the liver, measured as total (absolute) amount of ^{14}C, concentration (ppm), and percent of ^{14}C dose, was slightly increased after pretreatment with AT-125, when compared with livers of vehicle-only treated rats (Table 2). Average values for these parameters were 17.6 μg, 1.53 ppm, and 0.150 percent, respectively, for all AT-125-pretreated animals. The amount of radioactivity recovered from the kidneys of AT-125-pretreated rats was approximately 2.7 times greater than that in the kidneys of rats that received only chlorothalonil (Table 2). Kidneys of pretreated rats contained a total of about 105 μg equivalents, which represented 0.88 percent of the ^{14}C dose; mean concentrations of radioactivity averaged 48 ppm. Kidneys of rats pretreated with only the vehicle contained less than 40 μg equivalents, or about 0.33 percent of the ^{14}C administered. The average ^{14}C residue concentration in the kidneys of these animals was 17 ppm. Approximately 70 percent of the radiolabel in the kidney tissue of one AT-125-pretreated rat was extracted into pH 7-buffered saline, compared with 44 percent from the kidneys of an animal administered only [^{14}C]chlorothalonil. Individual extracts with other solvents (saline, pH 2; methanol; ethyl acetate; hexane) contained no more than 2 percent of the radiolabel in either pretreated or nonpretreated kidney samples and accounted for a total of less than 4 percent when combined (CBI p. 47). Approximately 42 to 44 and 33.5 to 38 percent of the saline-soluble radioactivity in the kidneys of AT-125-pretreated and untreated rats, respectively, was soluble in 10 percent TCA; nearly all (81.5 to 97 percent) of the ^{14}C in the protein that precipitated following TCA

TABLE 1. Mean (± S.D.) Percent Recovery, Distribution, and Concentration of Radioactivity in the Blood of Rats 24 Hours After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	^{14}C in blood: ppm[b]	Total µg equiv[c]	Percent of dose[d]	ppm ^{14}C (plasma)[e]	ppb ^{14}C (RBC)[f]	Percent in plasma[g]
AT-125-pretreated	899 ± 213[h]	13.4 ± 2.8	0.114 ± 0.028	1818 ± 552	352 ± 123	82.8 ± 8.4
Vehicle-pretreated	843 ± 318	12.5 ± 4.9	0.107 ± 0.041	1331 ± 303	403 ± 29	76.3 ± 4.0
All animals	871 ± 244	13.0 ± 3.6	0.111 ± 0.032	1574 ± 479	378 ± 85	79.6 ± 6.9

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Ng ^{14}C equivalents/mL blood.

[c]Equiv, equivalents.

[d]Percent of the ^{14}C dose recovered from the blood.

[e]Ng ^{14}C equivalents/mL plasma.

[f]Ng ^{14}C equivalents/g red blood cells (RBC).

[g]Percent of radiolabel in the blood found in the plasma.

[h]Each value represents three male rats, except "all animals" values, which represent six rats.

Source: CBI Table 2, CBI p. 43.

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TABLE 2. Mean (± S.D.) Percent Recovery and Concentration of Radioactivity in the Liver and Kidney of Rats 24 Hours After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	Liver ^{14}C			Kidney ^{14}C		
	ppm[b]	Total (mg)[c]	Percent of ^{14}C dose	ppm[b]	Total (μg)[c]	Percent of ^{14}C dose
AT-125-pretreated	1.80 ± 0.48[d]	19.27 ± 4.95	0.164 ± 0.047	47.75 ± 4.81	104.75 ± 19.59	0.884 ± 0.140
Vehicle-pretreated	1.26 ± 0.39	15.95 ± 4.82	0.136 ± 0.038	17.20 ± 3.83	38.75 ± 10.29	0.331 ± 0.080
All animals	1.53 ± 0.49	17.61 ± 4.73	0.150 ± 0.041	--[e]	--	--

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]μg ^{14}C equivalents/g tissue.

[c]Total ^{14}C equivalents (mg, liver; μg, kidney).

[d]Each value represents three male rats, except for "all animals" values, which represent six animals.

[e]Not calculated.

Source: CBI Tables 4 and 5, CBI pp. 45 and 46.

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treatment was solubilized by pepsin (CBI p. 48). Less than 5 percent of the saline (pH 7)-extracted radioactivity from the kidneys of AT-125-pretreated rats remained after pepsin digestion; approximately 7.5 to 10 percent was present after pepsin treatment of samples from nonpretreated rats.

D. Both pretreated and nonpretreated rats eliminated similar amounts of the ^{14}C dose at each collection interval (0 to 6 hours, 2 percent; 6 to 12 hours, 3 percent; 12 to 24 hours, 2.3 percent) and during the first 24 hours after administration of [^{14}C]chlorothalonil (7.4 percent) (Table 3). AT-125-pretreated rats eliminated slightly more radioactivity in the urine than nonpretreated rats during the first 6 hours after dosing (2.54 and 1.78 percent, respectively); the opposite was reported for the 6- to 12-hour postdosing collection period (2.54 and 3.39 percent, respectively).

Only 15 percent of the radiolabel in the acidified (pH 2) 0- to 6-hour and 6- to 12-hour urine samples of AT-125-pretreated rats was extracted into ethyl acetate; in contrast, more than 75 percent was extractable from the urine collected at 6 and 12 hours from rats given only [^{14}C]chlorothalonil (Table 4). Extractability of ^{14}C from urine collected between 12 and 24 hours after chlorothalonil administration was similar (60 versus 68 percent, respectively).

E. Three major peaks of radioactivity isolated (by HPLC/LSC) from individual urine samples accounted for 76.2 percent of the nonextractable urinary radiolabel and 66 percent of the total urinary ^{14}C excreted by AT-125-pretreated rats during the first 6 hours after administration of [^{14}C]chlorothalonil (CBI p. 51). Peaks eluting at 9 to 11, 5 to 7, and 2 to 4 minutes represented approximately 42, 15, and 9 percent of the urinary radioactivity, respectively (CBI p. 51). The 9- to 11-minute peak corresponded in elution time to a standard of the diglutathione (diGSH) conjugate of chlorothalonil; this peak also cochromatographed with the diGSH standard during a subsequent HPLC analysis. In addition, incubation with gamma-glutamyl transpeptidase (GGT) caused the amount of this metabolite (when isolated from a 6- to 12-hour urine sample) and the standard to decrease, and their elution times to change (per HPLC analysis). HPLC profiles of the radioactivity associated with the 5- to 7-minute peak--run before and after GGT treatment--also demonstrated a reduction in the amount of product in the presence of the enzyme. The 2- to 4-minute

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TABLE 3. Mean Percent Recovery of Radioactivity in the Urine of Rats After Oral Administration of [^{14}C]Chlorothalonil

Dosing Group[a]	Percent of ^{14}C administered			
	0–6 hr[b]	6–12 hr	12–24 hr	0–24 hr
AT-125-pre-treated	2.54 ± 0.41	2.54 ± 0.70	2.41 ± 0.49	7.49 ± 0.14
Vehicle-pretreated	1.78 ± 1.52	3.39 ± 2.26	3.39 ± 2.26	7.31 ± 0.97
All animals	2.16 ± 1.08[d]	2.97 ± 1.56[d]	2.97 ± 1.56[d]	7.40 ± 0.63

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Each value represents three male rats, except for "all animals" values, which represent six rats.

[d]Values calculated by the reviewers.

Source: Adapted from CBI Table 8, CBI p. 49.

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TABLE 4. Extractability of Urinary Radioactivity into Ethyl Acetate Following Oral Administration of [^{14}C]Chlorothalonil to Rats

Dosing Group	Percent of total ^{14}C extracted in samples collected at: 0–6 hr[b]	6–12 hr	12–24 hr
AT-125-pretreated	14.8 ± 7.8[c]	15.5 ± 4.5	59.8 ± 16.3
Vehicle-pretreated	78.9	75.3 ± 1.8	68.3 ± 5.3

[a]AT-125-pretreated rats received an ip dose of 10 mg AT-125/kg 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg. Vehicle-pretreated rats received an ip injection of phosphate-buffered saline 1 hour before a single oral dose of 50 mg [^{14}C]chlorothalonil/kg.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values represent the mean (± S.D.) of individual samples from three male rats, except for the 0- to 6-hour, vehicle-pretreated value, which represents only two rats.

Source: CBI Table 9, CBI p. 50.

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peak was not affected by GGT treatment (chromatogram not presented). Results of the HPLC analysis of the extractable fractions of individual urine samples were not presented.

According to the study authors, differences in elution times of radiolabeled peaks from individual urine samples (both nonextractable and extractable fractions) were observed; they attributed these differences to replacement of chromatography columns during the course of HPLC analysis. Thus, remaining urine samples were pooled and reanalyzed for chlorothalonil metabolites by HPLC and LSC.

For both groups of rats, pooled nonextractable fractions of urine collected at 6 and 12 hours contained metabolites that eluted between 3 and 7 minutes and 8 and 12 minutes (Tables 5 and 6). The later-eluting (7- to 11-minute) peak from AT-125-dosed rats was tentatively identified as the diGSH conjugate of chlorothalonil. The earlier-eluting (3- to 7-minute) peak of animals pretreated with AT-125 may have consisted of two metabolites: one that eluted at 2 to 4 minutes in the HPLC analysis of individual samples, and a second that eluted at 5 to 7 minutes (tentatively identified as the triGSH conjugate of chlorothalonil). An additional peak with an elution time between 12 and 15 minutes was present in all nonextractable fractions of rats administered only chlorothalonil and in the nonextractable fractions of the 12- to 24-hour urine samples collected from AT-125-pretreated animals. This compound was not identified.

HPLC profiles of extractable urinary material from both groups of rats indicated major peaks of radioactivity at 12 to 15 and 18 to 21 minutes at each urine collection interval (Tables 5 and 6). Minor peaks in samples from AT-125-pretreated rats eluted between 2 and 4 minutes and 9 and 11 minutes; a minor peak isolated from the 0- to 6-hour urine of pretreated animals and the 6- to 12-hour urine of nonpretreated rats eluted at 22 to 24 minutes.

More than 75 percent of the nonextractable radiolabel from 0- to 6-hour and 6- to 12-hour urine samples of AT-125-pretreated rats was contained in two peaks: the 9- to 12-minute peak (the diglutathione conjugate of chlorothalonil), which represented approximately 43 (6 hours) and 28 (12 hours) percent of the total urinary radioactivity and 1.8 percent of the administered ^{14}C dose; and the 3- to 7-minute peak (possibly a mixture of the triGSH conjugate and another metabolite), which accounted for about 20 (6 hours) and 41 (12 hours) percent of the

TABLE 5. Reverse-Phase HPLC Quantification of Radioactivity in the Urine of Rats Injected Intraperitoneally with AT-125 Before Oral Dosing with [^{14}C]Chlorothalonil

Collection interval[a]	Elution time	Percent of NE fraction[b]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	3-6 min	24.0[c]	20.2	0.53
	9-12 min	51.4	43.3	1.15
6-12 hr	3-7 min	50.5	41.0	0.88
	9-12 min	34.3	27.9	0.60
12-24 hr	3-5 min	40.9	17.9	0.43
	7-10 min	26.0	11.4	0.27
	13-15 min	15.7	6.9	0.17

Collection interval[a]	Elution time	Percent of EX fraction[d]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	2-4 min	11.6	2.1	0.05
	9-11 min	12.1	2.2	0.06
	12-15 min	15.7	2.9	0.07
	18-21 min	20.3	3.7	0.09
	24-26 min	13.9	2.5	0.06
6-12 hr	2-4 min	5.1	0.8	0.02
	11-15 min	38.5	5.7	0.16
	18-21 min	28.0	4.2	0.11
12-24 hr	12-15 min	34.7	22.3	0.54
	18-21 min	41.8	26.9	0.65

[a]Time after administration of [^{14}C]chlorothalonil.

[b]NE, nonextractable.

[c]Values are for samples pooled from three animals.

[d]EX, extractable.

Source: CBI Table 11, CBI p. 52.

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TABLE 6. Reverse-Phase HPLC Quantification of Radioactivity in the Urine of Rats Dosed with [^{14}C]Chlorothalonil

Collection interval[a]	Elution time	Percent of NE fraction[b]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	3-6 min	24.0[c]	5.2	0.14
	8-11 min	43.3	9.3	0.25
	13-16 min	21.9	4.7	0.13
6-12 hr	3-7 min	25.8	6.6	0.22
	8-11 min	41.7	10.6	0.36
	12-15 min	21.8	5.6	0.19
12-24 hr	3-6 min	25.1	8.1	0.17
	8-11 min	32.5	10.5	0.23
	13-15 min	27.7	9.0	0.19

Collection interval[a]	Elution time	Percent of EX fraction[d]	Percent of urinary ^{14}C	Percent of ^{14}C dose
0-6 hr	11-6 min	42.7	35.0	0.94
	17-19 min	14.3	11.7	0.32
	19-21 min	28.7	23.5	0.63
6-12 hr	12-15 min	26.9	20.1	0.68
	17-21 min	43.6	32.6	1.10
	22-24 min	9.4	7.1	0.24
12-24 hr	12-15 min	39.9	27.3	0.58
	18-21 min	40.0	27.4	0.59

[a]Time after administration of [^{14}C]chlorothaonil.

[b]NE, nonextractable.

[c]Values are for samples pooled from three animals.

[d]EX, extractable.

Source: CBI Table 12, CBI p. 53.

urinary ^{14}C and 1.4 [illegible] (see Table 5). The peaks [illegible] from the 12- to 24-[illegible] corresponded, respectively, to [illegible] conjugates described [illegible] 15-minute elution [illegible] urinary radioactivity [illegible] appeared to correspond [illegible] fractions of animals [illegible] Table 6). When combined, [illegible] from the extractable fractions collected from 125-pretreated rats through 6 hours [illegible] accounted for approximately 13 percent of the urinary radioactivity and only 0.5 percent of the ^{14}C dose; the three metabolites in the 6- to 12-hour extractable fraction accounted for about 11 percent of the urinary [illegible] and 2 percent of the ^{14}C dose. In contrast, the 12- to 24-hour extractable urine fraction contained [illegible] which represented more than 20 percent of the urinary radioactivity and more than [illegible] administered.

For nonpretreated rats, the [illegible] to 7, 8 to 11, and 12 to 16 [illegible] than 85 percent of the nonextractable [illegible] urine collected (see Table 6). [illegible] minute peak represented more than [illegible] radioactivity, and none accounted [illegible] of the administered dose at [illegible] metabolites were considered [illegible] further. Approximately 80 percent [illegible] consisted of three peaks, each [illegible] to 35 percent of the urinary [illegible] percent of the ^{14}C dose [illegible] study authors reported [illegible] analyzed further and [illegible] of these [illegible] available in another [illegible]

Evidence suggests that, in rats, [illegible] metabolism of glutathione conjugates, [illegible], conversion to less polar [illegible] During the first 12 [illegible] chlorothalonil, AT-125-[illegible] smaller amounts of [illegible] nonpretreated rats; in pretreated rats, both groups of rats contained roughly equivalent amounts of these polar products at 24 hours after dosing. In pretreated rats, the [illegible] about 2 percent of the [illegible] 12 minutes; these compounds were identified as the tri- and diglutathione conjugates of chlorothalonil, respectively. The [illegible] peaks eluting between [illegible] corresponded to approximately [illegible]

TABLE 7. Recovery and Distribution of Metabolites in the Urine of Rats Injected Intraperitoneally with AT-125 Before Oral Dosing with [^{14}C]Chlorothalonil[a]

Approximate Chromatographic Elution Time	Percent of ^{14}C in urine collected between: 0–6 hr[b]	6–12 hr	12–24 hr	Percentage of ^{14}C dose
2–4 min	2.1[c]	0.8	—[d]	0.07
3–7 min	20.2	41.0	17.9	1.84
7–12 min	45.5	27.9	11.4	2.08
11–15 min	2.9	5.7	29.2	0.94
18–21 min	3.7	4.2	26.9	0.85
24–26 min	2.5	—	—	0.06
Total	76.9	79.6	85.4	5.84

[a]Prepared by the study reviewers.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values are for samples pooled from three animals. Metabolites from both extractable and nonextractable fractions were combined.

[d]No peak detected.

Source: Adapted from CBI Table 11, CBI p. 52.

TABLE 8. Recovery and Distribution of Metabolites in the Urine of Rats Dosed Orally with [^{14}C]Chlorothalonil[a]

Approximate Chromatographic Elution Time	Percent of ^{14}C in urine collected between:			Percent of ^{14}C dose
	0–6 hr[b]	6–12 hr	12–24 hr	
3–7 min	5.2[c]	6.6	8.1	0.53
8–11 min	9.3	10.6	10.5	0.84
11–16 min	39.7	25.7	36.3	2.71
17–19 min	11.7	—[d]	—	0.32
17–21 min	—	32.6	—	1.10
18–20 min	—	—	27.4	0.59
19–21 min	23.5	—	—	0.63
22–24 min	—	7.1	—	0.24
Total	89.4	82.6	82.3	6.96

[a]Prepared by the study reviewers.

[b]Time after administration of [^{14}C]chlorothalonil.

[c]Values are for samples pooled from three animals. Metabolites from both extractable and nonextractable fractions are included.

[d]No peak detected.

Source: Adapted from CBI Table 12, CBI p. 53.

^{14}C dose (the latter value may consist of up to four metabolites, none of which were identified). Other peaks of radioactivity accounted for <1 percent of the dose. All peaks combined represented between 78 and 95 percent of the ^{14}C dose recovered from the urine of pretreated and nonpretreated rats, respectively.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the gamma-glutamyl transpeptidase (GGT) inhibitor, AT-125, affected the metabolism of chlorothalonil by altering the polarity of urinary metabolites for at least 12 hours and by increasing the amount of ^{14}C in the kidneys at 24 hours after oral dosing with [^{14}C]chlorothalonil.

AT-125 did not appear to affect concentrations of ^{14}C in the liver, blood, plasma, and red blood cells. For both AT-125-pretreated and nonpretreated rats, approximately 80 percent of the radioactivity in the blood was present in the plasma, and more than 80 percent of the plasma radioactivity was bound to proteins. Kidneys of AT-125-pretreated rats contained two to three times more radioactivity than kidneys of rats that received only chlorothalonil; approximately 70 percent of the radiolabel in the kidneys of rats pretreated with AT-125 was extracted into buffered (pH 7) saline, compared with 44 percent from nonpretreated animals. For both groups, more than 50 percent of the extracted radiolabel was bound to kidney proteins.

Pretreatment with AT-125 did not affect the rate of elimination of ^{14}C in the urine or the total amount of radioactivity excreted in the urine. However, the extractability of radiolabel in the urine varied considerably between the two groups. Only 15 percent of the radioactivity in the 0- to 6-hour and 6- to 12-hour urine samples (adjusted to pH 2) of pretreated rats was extractable with ethyl acetate, whereas more than 75 percent was extractable from acidified urine collected at 6 and 12 hours postdosing from rats given only chlorothalonil. Extractability of radiolabel from urine collected between 12 and 24 hours was similar (≥60 percent) for all groups of animals. The nonextractable fractions of all urine samples contained two metabolites, tentatively identified as diGSH and triGSH conjugates of chlorothalonil, probable precursors of the urinary thiol metabolites of chlorothalonil. A third unidentified (but less polar, based on a longer elution time) metabolite was isolated from all urine samples collected from nonpretreated rats and from the 12- to 24-hour urine samples of AT-125-dosed animals. Analysis of individual (rather than pooled) nonextractable radiolabel indicated

that AT-125-pretreated rats may have also excreted a metabolite containing four glutathione molecules or a metabolite not associated with glutathione conjugation.

The study authors concluded that AT-125 effectively inhibited the in vitro activity of GGT for a minimum of 12 hours. Evidence of this inhibition indicates the prominent role of the glutathione pathway in the metabolism of chlorothalonil and may suggest involvement of sulfur-containing metabolites in the nephrotoxicity of this fungicide.

B. A quality assurance statement and a statement of compliance with Good Laboratory Practices (GLPs), both signed and dated March 15, 1988, were included.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides supplementary information on the metabolism of orally administered [^{14}C]chlorothalonil and adequately describes the metabolism of a single oral dose of 50 mg [^{14}C]chlorothalonil/kg in male rats. This study also demonstrated that conjugation with glutathione is the major metabolic pathway for chlorothalonil in male rats. However, female rats were excluded (with no supporting rationale) from the study, and animals were given only one dose level of the test material, rather than a single low, single high, and repeated low doses as recommended by EPA (Guideline 85-1). Feces were not collected, and the only tissues analyzed for ^{14}C content were liver, blood, and kidneys; thus, absorption of [^{14}C]chlorothalonil was based on urinary elimination of radioactivity.

Gastrointestinal absorption of a single oral dose of [^{14}C]chlorothalonil was low, accounting for about 7.4 percent of the administered dose at 48 hours postdosing. Levels of radioactivity in blood, liver, and kidneys were low, representing no more than 0.2, 0.21, and 1 percent of the ^{14}C dose, respectively, at 24 hours after administration of the test material. As noted by the study authors, most radioactivity recovered from the plasma and kidneys was protein bound.

Pretreatment with AT-125 had no effect on the amount of radioactivity in the blood (including plasma and red blood cells) and liver; the GGT inhibitor also did not alter the rate or total amount of ^{14}C eliminated in the urine. However, AT-125 had a marked effect on kidney levels of radioactivity, which were about three times greater (as ppm, total residues, and percent of dose) in pretreated rats than in nonpretreated rats at 24 hours after [^{14}C]chlorothalonil administration. In addition, nearly twice as much of the radioactivity (i.e., 70

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versus 44 percent) in the kidneys of pretreated rats was soluble in pH 7-buffered saline when compared with animals given only [^{14}C]chlorothalonil; extraction of radiolabeled renal material into organic solvents and pH 2-buffered saline was minimal from both groups. Pretreatment with AT-125 produced only a slight increase in the amount of radioactivity in the liver. The differences in ^{14}C concentrations between the kidney and liver--both with and without pretreatment with AT-125--are consistent with the relatively low levels of GGT in the bile duct cells of the liver, as contrasted with high GGT levels in kidney parenchymal cells.

Differences in the extractability of ^{14}C from the urine and in the metabolites excreted by pretreated versus nonpretreated rats were also observed. Most (63.5 to 69 percent) of the radioactivity in the 0- to 12-hour urine samples collected from pretreated rats was not extractable into organic solvent (ethyl acetate) at pH 2, whereas only 11 to 13 percent was extractable from these samples. A shift in extractability (i.e., to 36 percent nonextractable and 49 percent extractable) was observed for samples collected from pretreated rats between 12 and 24 hours postdosing, indicating the elimination of a greater amount of less polar metabolites during this time. In contrast, extractability of ^{14}C from the urine of nonpretreated rats was relatively high, accounting for approximately 55 to 70 percent of the urinary ^{14}C at all time intervals. The increase in the extractability of ^{14}C from the urine of pretreated rats at 12 to 24 hours after dosing was accompanied by an increase in the amount of metabolites with longer elution times (i.e., less polar compounds) and a general drop in shorter-eluting (i.e., more polar) metabolites.

The data indicate a retardation in the rate of conversion to less polar material by pretreated rats, an effect most likely resulting from AT-125-induced inhibition of GGT. The data support the conclusion of the study authors that AT-125, an inhibitor of GGT (the enzyme involved in the early steps of metabolism of glutathione conjugates) affected the metabolism of chlorothalonil by increasing the polarity of urinary metabolites for at least 12 hours after oral administration of [^{14}C]chlorothalonil; the increase in polarity was attributed, in part, to the blocking of glutathione metabolism or cleavage from chlorothalonil. As the inhibitory effect of AT-125 diminished, metabolism of the glutathione conjugates of chlorothalonil proceeded more readily, and these less polar metabolites, possibly thiols, as suggested by the study authors, were excreted in the urine. The study supports the integral role of glutathione in the metabolism of chlorothalonil.

Items 15 and 16 -- see footnote 1.

APPENDIX

Materials and Methods
(CBI pp. 63-73)

Chlorothalonil toxicology review

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___ Description of product quality control procedures

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NATIONAL [illegible] (ID 12065)

EPA No.: 68D80056
DYNAMAC No.: 324-A
TASK No.: 3-24A
September 4, 1991

DATA EVALUATION RECORD

CHLOROTHALONIL

Metabolism in Rats

STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Study to determine the metabolic pathway for chlorothalonil following dermal application to rats. (Unpublished study No. 1625-87-0057-AM-001 performed by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; dated May 5, 1989.) MRID No. 412505-08.

APPROVED BY:

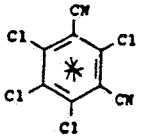
Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: Sept. 4, 1991

1. CHEMICAL: Terachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile; 1,3-dicyano-2,4,5,6-tetrachlorobenzene; chlorothalonil.

2. TEST MATERIAL: [^{14}C]Chlorothalonil, uniformly labeled in the benzene ring, and unlabeled chlorothalonil were used. The radiolabeled test material had a radiochemical purity of 97.6 percent (specific activity of original test material not reported). The unlabeled test material had a chemical purity of 95.4 percent. The structure and radiolabel (*) position of [^{14}C]chlorothalonil are shown below:



3. STUDY/ACTION TYPE: Metabolism in rats following dermal application.

4. STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen, J.C., Jr. Study to determine the metabolic pathway for chlorothalonil following dermal application to rats. (Unpublished study No. 1625-87-0057-AM-001 performed by Ricerca, Inc., Painesville, OH, for Fermenta ASC Corp., Mentor, OH; dated May 5, 1989.) MRID No. 412505-08.

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7. CONCLUSIONS:

A. Small amounts (mean 3.11 percent; range 2.52 to 4.00 percent) of a dermal dose of [^{14}C]chlorothalonil (equivalent to 5 mg/kg) were absorbed by male rats (five/group; four groups) during a 48-hour exposure period. About half of the ^{14}C absorbed (1.5 percent of the administered dose) was recovered from urine samples collected during the first 24 hours of exposure to the compound; the remaining 1.6 percent was recovered from 24- to 48-hour samples. The nature and amounts of urinary thiol metabolites of chlorothalonil varied quantitatively and qualitatively. The total amount of urinary thiol metabolites was low for all groups and varied considerably, accounting for approximately 0.04 to 2.7 percent of the total urinary radioactivity and 0.002 to 0.07 percent of the administered radioactivity. Detectable amounts of a tri-thiol metabolite of chlorothalonil were present in all groups of rats; in contrast, a di-thiol metabolite was present in the urine of two of the four groups, and the urine of only one group of rats contained mono-, di-, and tri-thiol metabolites. Differences in the detection of metabolites were most likely due to insufficient amounts of radiolabeled material in the urine.

B. This study provides supplementary information on the metabolism and elimination of dermally administered [^{14}C]chlorothalonil in male rats. Reproducibility of results was poor; specifically, the quantitative and qualitative recovery of urinary thiol metabolites varied considerably among similarly dosed animals. In addition, a rationale for using only males rats in this study was not provided.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1) [^{14}C]Chlorothalonil (lot No. not reported) was 97.6 percent pure, as determined by reverse-phase high-performance liquid chromatography (HPLC). The non-labeled, analytical grade test material (lot No. not specified) had a chemical purity of 95.4 percent, as determined by gas chromatography (GC). Impurities were not identified for either test material.

[1]Only the items appropriate to this DER have been included.

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2) Male CD Sprague-Dawley rats (Charles River Laboratories, Portage, MI), weighing between 211 and 267 g at the time of dosing, were used. Animals were quarantined for at least 7 days before application of the test material.

3) The dosing solution was prepared by diluting a stock solution of [^{14}C]chlorothalonil with nonlabeled chlorothalonil to the appropriate specific activity. The test material in solution was then divided equally among four vials, the solvent was evaporated, and the vials were frozen and stored until use.

The stock solution of radiolabeled chlorothalonil was prepared by dissolving 8.35 mg [^{14}C]chlorothalonil in 10 mL methylene chloride. A 0.01-mL aliquot of this solution was then diluted with methanol to a final volume of 10 mL and analyzed for radiochemical purity by HPLC and liquid scintillation counting (LSC). The purity and specific activity of the [^{14}C]chlorothalonil in the methanol-diluted stock solution were 98.7 percent and 120.5 mCi/mmol, respectively.

Unlabeled chlorothalonil (28.67 mg) was added to the stock solution of radiolabeled test material to give a final specific activity of 27.82 mCi/mmol (as determined by GC and LSC), and the solution was divided among four vials. The contents of the vials were evaporated to dryness under nitrogen and stored in the dark at -70°C until use.

Immediately prior to dosing, 6 mL of acetone was added to a vial (giving a chlorothalonil concentration of 1.58 mg/mL), and the appropriate dosing volume for each animal in a group was determined based on body weight and the specific activity and chlorothalonil concentration of the stored material.

4) Four groups (groups A, B, C, and D) of male rats (five/group) received a nominal dose of 5 mg [^{14}C]chlorothalonil/kg (target, 103 to 131 μCi/animal); the compound was administered in a single dermal application of approximately 1.5 mg test material/mL over an average skin area of 25 cm^2.

At 24 hours before dermal application of [^{14}C]chlorothalonil, the dorsal hair (approximately 40 cm^2) of each rat was clipped. Animals were then placed in individual metabolism cages, and predosing urine samples were collected. Immediately before dosing, animals were weighed, and dosing volumes were calculated. Rats were anesthetized with ether, and the

calculated dosing volume was applied with a syringe to a 25-cm^2 area of the clipped skin. The treated area was covered with a nonocclusive patch, and a neoprene rubber template/wire mesh screen device was glued to the back of the animal to prevent loss of the test material. Rats were exposed dermally to the [^{14}C]chlorothalonil solution for 48 hours; urine and feces were collected separately over dry ice at 24 and 48 hours postapplication.

5) Thiol metabolites in the urine were isolated by extraction of the urine with ethyl acetate and methylation of the extracts followed by column chromatography. The fractions corresponding to the thiol metabolites for groups A and B were analyzed by gas chromatography (GC)/mass spectroscopy (MS); those from groups C and D were analyzed by GC/selective ion monitoring (SIM).

The volume and pH of urine samples were determined, and aliquots were taken for LSC. Urine samples were filtered and pooled according to dosing group and collection period; aliquots were acidified to pH 2.0 and extracted three times with acidified ethyl acetate. Extractable organic fractions were reduced to dryness, methylated twice with diazomethane, and analyzed by reverse-phase HPLC/LSC. Aliquots of pooled 0- to 24-hour and 24- to 48-hour urine samples from all groups, and aliquots of the nonextractable aqueous phase and the methylated organic phase, were analyzed by reverse-phase HPLC/LSC.

For isolation and identification of the thiols for groups A and B, the methylated fractions from each group were pooled (0 to 48 hours) and placed on a flurasil column. The column was washed four times with hexane to remove interfering substances, eluted with ethyl ether:hexane (20:80 v/v) to elute thiols, and washed with methanol. The ether/hexane was evaporated, and florisil chromatography was repeated, followed by repeated cycles of HPLC and florisil chromatography of isolated peaks. The methanol fractions for group B were also concentrated in an attempt to recover additional radioactive material. Samples of interest were analyzed by GC/MS.

Individual derivatized samples (0 to 24 hours and 24 to 48 hours) from groups C and D were chromatographed by reverse-phase HPLC. The radioactive peaks that corresponded in elution time to methylated thiol standards of chlorothalonil thiol metabolites were collected, concentrated to dryness, and extracted with

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ethyl ether and ethyl acetate. These extracts were then concentrated by drying under nitrogen and reconstituted in either ethyl ether or ethyl acetate, depending on solubility. The reconstituted samples were analyzed by GC/SIM.

The limit of detection for samples analyzed by GC/MS was 50 ng; the sensitivity limit for samples analyzed by GC/SIM was 3 ng. The molecular ions monitored were the methylated mono-, di-, and tri-thiol metabolites of chlorothalonil.

No fecal samples were analyzed.

B. Protocol: The protocol and protocol amendments for this study are presented in the appendix.

12. REPORTED RESULTS:

A. Rats in all groups received an average (± standard deviation) of 4.6 ± 0.3 mg [^{14}C]chlorothalonil/kg (range 4.38 to 4.99 mg/kg). The average amount of radiolabel administered to each animal was 115.4 ± 7.0 µCi (range 106.5 to 123.25 µCi). A description of how the actual doses were determined was not provided.

B. Animals in all groups excreted approximately 3.11 percent (range 2.52 to 4.00 percent) of the ^{14}C dose in the urine during the 48 hours after compound application (Table 1). About half (1.5 percent of the ^{14}C dose) was recovered from samples collected during the first 24 hours; the remaining 1.6 percent was recovered from the 24- to 48-hour urine samples.

C. For groups A and B, total methylated thiols accounted for 2.74 and 0.26 percent of the total urinary radioactivity, respectively (Table 2); these values corresponded to 0.07 and 0.01 percent of the administered ^{14}C dose. Animals in group A excreted both di- and tri-thiols (1.58 and 1.16 percent of the urinary ^{14}C, respectively), but animals in group B excreted only the tri-thiol analog of chlorothalonil. Because of the large variability in the recovery of methylated thiols between groups A and B, the experiment was repeated (groups C and D) and the methodology for isolating thiol metabolites was modified.

In groups C and D, total thiols accounted for approximately 0.33 to 0.45 and 0.0376 to 0.0493 percent of the total urinary ^{14}C eliminated by rats, respectively (Table 2). The maximum amount of thiols recovered from the urine of these

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TABLE 1. Percent Recoveries of Radioactivity in the Urine of Rats Exposed Dermally to [^{14}C]Chlorothalonil for 48 Hours[a]

Group	Percent of ^{14}C administered:		
	0-24 hr[b]	24-48 hr	0-48 hr
A	1.22[c]	1.30	2.52
B	1.45	1.74	3.19
C	1.34	1.38	2.72
D	2.10	1.90	4.00
Mean (± S.D.)	1.53 ± 0.39	1.58 ± 0.29	3.11 ± 0.66

[a]Animals received an average dose of 4.6 mg [^{14}C]chlorothalonil/kg.

[b]Urine collection times.

[c]Each value is the mean of five animals.

Source: CBI p. 27.

TABLE 2. Distribution of Thiols in the Urine of Rats Exposed Dermally to 5 mg [^{14}C]Chlorothalonil/kg for 48 Hours

Group[a]	Time Period	Mono-SH[b]	Di-SH[b]	Tri-SH[b]	Total SH[c]
A	0-48 hr	ND[d]	1.5818	1.1600	2.7418
B	0-48 hr	ND	ND	0.2624	0.2624
C	0-24 hr	0.0055-0.0076	0.0092-0.0142	0.0422-0.0554	0.0569-0.0772
	24-48 hr	ND	0.0697-0.1074	0.2034-0.2622	0.2731-0.3696
	Total				0.3300-0.4468
D	0-24 hr	ND	ND	0.0203-0.0266	0.0203-0.0266
	24-48 hr	ND	ND	0.0173-0.227	0.0173-0.0227
	Total				0.0376-0.0493

[a]For groups A and B, quantitation was by GC/MS and for Groups C and D quantitation was by GC/SIM. Each group consisted of five male rats.

[b]Analyzed and quantitated as methylated thiols. Reported as a percent of the total radiolabel in urine.

[c]Total thiols are the sum of the individual thiols in each group.

[d]Not detected (3 ng sensitivity limit by GC/SIM; 50 ng limit of detection by GC/MS).

Source: CBI p. 29.

rats, as reported by the study authors, represented 0.007 percent of the radioactivity administered to animals in group C and 0.001 percent of that applied to rats in group D. These values were recalculated by the reviewers to be 0.012 and 0.002 percent of the administered ^{14}C dose, respectively. See Reviewers' Discussion of this DER for additional details.) The tri-thiol was identified in all urine samples from groups C and D. The di-thiol was recovered from the 0- to 24-hour and 24- to 48-hour urine samples of animals in group C; the group D urine samples did not contain this metabolite. The mono-thiol metabolite of chlorothalonil was present only in the 0- to 24-hour urine of group C rats.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Rats in each dose group eliminated approximately 1.5 percent of the ^{14}C dose in the urine during the first 24 hours after dosing and an additional 1.6 percent in the urine collected between 24 and 48 hours postapplication. The GC/MS and GC/SIM analyses of urine indicated that a maximum of only 0.07 percent of the administered dose was excreted as thiol metabolites. In contrast, urine from rats orally administered ^{14}C-chlorothalonil (5 mg/kg/day for 5 days) contained an estimated 1.6 percent of the administered dose and approximately 20 percent of the urinary rabiolabel as thiol metabolites (data presented in a footnote on CBI pp. 32 and 33; oral dosing data from SDS Biotech Corporation unpublished study No. 1173-84-0079-AM-003). This represents a 20-fold difference between the oral and dermal exposure groups with respect to the amounts of thiols excreted following the same dose (5 mg chlorothalonil/kg). The study authors noted that the glutathione pathway is involved in the metabolism of chlorothalonil to thiol metabolites and that the thiols generated may be responsible for the nephrotoxicity observed in animals exposed to this fungicide. They concluded that because excretion of these potentially nephrotoxic thiols was much less in the dermally exposed rats than in those exposed orally, dermal exposure may be associated with a lower risk of toxicity as compared with oral exposure (given the same dosage in mg/kg).

The GC/MS results for groups A and B also indicated a large discrepancy in the amounts of thiol metabolites recovered from the urine of rats in these two groups. For group A animals, total thiol metabolites accounted for 2.74 percent of the urinary radioactivity and 0.07 percent of the ^{14}C dose; in contrast, thiol metabolites of chlorothalonil accounted for only 0.26 percent of the urinary ^{14}C and 0.01 percent of the ^{14}C dose in animals of group B. In addition,

both the di- and tri-thiol metabolites were recovered from the urine of group A rats, accounting for approximately 1.58 and 1.16 percent of the urinary radioactivity, respectively, whereas only the tri-thiol metabolite was identified in the urine of rats in group B.

In an attempt to reconcile these differences, the study authors repeated the dermal exposure study using an additional 10 male rats (groups C and D). However, this repeated experiment did not yield markedly improved results (total thiols represented up to 0.45 and 0.049 percent of the urinary ^{14}C of group C and D rats, respectively), and the study authors provided no explanation for the poor reproducibility among the four groups. The total amounts of thiols excreted by rats from groups B and C were comparable (i.e., 0.26 and 0.45 percent of the urinary ^{14}C, respectively), but results from groups A and D were not in agreement with each other or with group B or C. It was suggested that the amount of thiols excreted in the urine of animals in group A (i.e., 2.74 percent of the total urinary ^{14}C) was probably high.

The study authors concluded that the urinary metabolite profiles of animals in the four exposure groups differed quantitatively but not qualitatively. They noted that the tri-thiol analog of chlorothalonil was the major urinary metabolite excreted by dermally exposed rats.

B. A quality assurance statement and a statement of compliance with Good Laboratory Practices, both signed and dated May 8, 1989, were included in the report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides only supplementary information on the metabolism and elimination of dermally administered [^{14}C]chlorothalonil in male rats. A major problem with this study involved the poor reproducibility and wide variability of the results, which made it difficult to determine how much of a 5-mg/kg dermal dose of [14C]chlorothalonil was eliminated, on an average, as thiol metabolites, and to evaluate the relative importance of thiol and glutathione metabolism of the fungicide following dermal exposure. In addition, values in the summary tables in the CBI could not be verified from the raw data. Another deficiency in this study, per EPA guidelines (85-1), was the use of only male rats; the study authors did not explain why female rats were excluded. The reviewers assumed that the urine and feces were collected until sacrifice (as stated in the protocol, CBI p. 54), rather than during the 48 hours after the 48-hour dermal exposure period, as is implied in the experimental section of the report (CBI p. 22).

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Urinary excretion data indicated that only a small amount (mean 3.11 percent; range 2.52 to 4.00 percent) of the dermal dose of [^{14}C]chlorothalonil was absorbed during the 48-hour exposure period. Information on the amount of ^{14}C excreted in the feces and remaining at the application site would have supported and complemented these data. Urinary elimination of ^{14}C appeared to be lower in dermally exposed rats than in orally dosed rats, indicating reduced absorption of the fungicide by the former route of exposure.

The total amount of thiol metabolites in the urine was low for all groups and varied considerably, accounting for approximately 0.04 to 2.7 percent of the total urinary radioactivity and 0.002 (as calculated by the reviewers) to 0.07 percent of the administered radioactivity. The maximum percent of the administered dose excreted as thiols appears to have been miscalculated by the study authors for groups C and D. They reported that 0.007 and 0.001 percent of the ^{14}C dose was excreted as thiols, respectively (CBI p. 30); however, when recalculated by the reviewers, these values corresponded to approximately 0.012 [0.004468 (the fraction (percent = 0.4468) of the urinary radiolabel excreted as thiol metabolites) x 2.72 percent (percent of administered dose excreted in the urine)] and 0.002 (0.000493 x 4.00) percent of the ^{14}C dose administered to rats in groups C and D, respectively.

The urinary excretion patterns of thiol metabolites also varied qualitatively: animals in groups B and C excreted detectable amounts of only the tri-thiol metabolite; both the di- and tri-thiol compounds were detected in group A samples; and all three methylated thiol compounds (mono-, di-, and tri-) were excreted by animals in group C. Differences in the detection of metabolites were most likely due to insufficient amounts of radiolabeled material, although samples from groups C and D were analyzed by GC/SIM at a detection level of 3 ng. Extraction, isolation, and cleanup procedures appeared to be appropriate, but repeated chromatographic analyses/extractions resulted in losses and reduced the amount of radioactivity available for metabolite identification. The tri-thiol metabolite was the principal urinary thiol metabolite, representing approximately 70 to 100 percent of the total thiols excreted by rats in groups B, C, and D, and 42 percent of that eliminated by group A rats. Glutathione and mercapturic acid conjugates of chlorothalonil and other metabolites were not identified.

Although direct comparisons between the oral dosing data presented by the study authors and the results of the dermal exposure experiments are not appropriate, the suggestion that rats administered oral doses of chlorothalonil eliminate much larger amounts of thiols in the urine (as percent of total

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urinary metabolites and percent of dose) than dermally exposed rats appears acceptable.

Items 15 and 16--see footnote 1.

APPENDIX

Protocol and Protocol Amendments
(CBI pp. 40-65)

RICERCA, INC.
DEPARTMENT OF TOXICOLOGY AND
ANIMAL METABOLISM

STUDY TO DETERMINE THE METABOLIC PATHWAY FOR CHLOROTHALONIL FOLLOWING DERMAL APPLICATION TO RATS

PROTOCOL
DOCUMENT NUMBER: 1625-87-0057-AM-000
SDS-2787

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Painesville, Ohio 44077

Unit Document Reference Number: 87-0057

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PROTOCOL

STUDY TO DETERMINE THE METABOLIC PATHWAY FOR CHLOROTHALONIL FOLLOWING DERMAL APPLICATION TO RATS

DOCUMENT NUMBER: 1625-87-0057-AM-000

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