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

006672

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".

006672

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

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.

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.

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.

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

006672

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

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

$\frac{\text{Food consumption (g/kg/day)}}{1000}$ x 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.

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

8

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

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

006672

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

006672

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

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
Low	1.75	0/11	0/11	0/11
Low-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
Low	1.75	0/11	0/11	0/11
Low-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

Review of Registrant's Response to the Previous TB Review Comments Concerning the In-Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary Cells with Technical Chlorothalonil, Study No. T448I.337 (Toxicology Branch Memo 4/9/87 B. Dementi) Accession No. 405591-03 January 4, 1988

Registrant's Response:

" The purpose of this report amendment is to include in the contract laboratory report in Appendix B a report amendment clarifying the selection of harvest times for the chromosomal aberration study from the preliminary toxicity test.

With metabolic activation at 3 ug/ml, the highest dose at which dividing cells were observed in the preliminary toxicity test, the percent of dividing cells in M_1, M_2 and M_3 was 20%, 79% and 1% respectively. In the solvent control the distribution was 5%, 94% and 1% respectively. The differences in percent of cells in first and second metaphase between the cells exposed to chlorothalonil and the solvent control were within experimental variation observed at the laboratory, and not due to cell cycle delay. Therefore, for the chromosomal aberration assay with metabolic activation the cells were harvested at the standard 10 hours. "

Reviewer's Comments:

The provided report amendment for clarifying the selection of harvest times for the study with S9 activation is considered to be justified.

Recommendation:

The test compound, chlorothalonil (T-117-12), was not considered to be a clastogenic agent in the S9 activated study at the concentrations tested (0.6 through 6 ug/ml). However, T-117-12 was considered positive in the nonactivated test system only. The study is upgraded to acceptable.

John H.S. Chen 6/7/88

Reviewed by John H.S. Chen
Review Section I
Toxicology Branch