



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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Lois Rossi, PM # 21
Fungicide-Insecticide Branch
Registration Division TS-769C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C *RBJ/4/7/88*

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

FROM: D. Ritter, Adjuvants Toxicologist
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C *DR 4-1-88*

Subject: Chlorothalonil; submission of supplemental data. *4/7/88*
Sponsor: Fermenta Plant Protection Co., Painesville, OH.
Caswell #: 215B
TOX Project #: 7-0704

Fermenta is submitting additional toxicity data in support of continued registration of products containing the fungicide, Chlorothalonil.

The company asserts that these data provide additional support for their contention that the carcinogenicity of Chlorothalonil is related to Glutathione-conjugates of Chlorothalonil, and that it is these metabolites that are inducing the neoplasms reported in the rat and mouse kidney and stomach. These data are reviewed below.

CHLOROTHALONIL

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D. Ritter

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1. A Tumorigenicity Study in Male Mice - a one year interim report. Document # 1099-84-0077-TX-003 (MRID 40122902).

Summary:

Charles River CD-1 male mice, sixty per group, are being offered diets containing 0, 10, 40, 175 or 750 ppm for two years. At week 18 the 10 ppm group was increased to 15 ppm. At one year blood samples were taken from ten animals per group for analysis of those parameters normally associated with an oncogenicity study in mice. The same mice were killed and the organ weights obtained. A complete gross and microscopic examination of the kidneys, renal lymph node, stomach and gastric lymph node was performed. The complete inventory of tissues and organs was taken and preserved for further histopathological analysis.

Results:

There was a dose-related increase in the kidney to body weight ratios and an increase in the severity of a hyperplastic lesion in the proximal tubules in the 750 ppm group. There was a slight increase in tubular hyperplasia at the 175 ppm level that was considered to be treatment related. It was considered to be a pre-neoplastic lesion. The NOEL for this effect at one year into the study is 40 ppm. Hyperplasia and hyperkeratosis of the squamous mucosa of the forestomach were reported for the 750 ppm group. The incidence of occurrence of these lesions is shown in Table I and II (attached).

No tumors were reported in the kidneys or in the forestomach at any level at one year. This study will be fully reviewed when it has been completed.

2. Report of the Status of a Tumorigenicity Study of Technical Chlorothalonil in Rats. Doc. # 1102-84-0103-TX-0011 (MRID 40122903).

Technical Chlorothalonil is being offered at dietary levels of 0, 2.0, 4.0, 15 and 175 mg/kg bw/day to groups of 65 male and 65 female Fischer 344 rats for two years. At one year, ten rats per sex per group were killed and necropsied. Mean body weights, food consumptions and survival were recorded. The histopathological examinations will be reported when the study is complete. There was a reduction in mean body weights in males and females receiving 175 mg/kg/day when compared to that of the corresponding controls; feed consumption was not affected by ingestion of Chlorothalonil at any test level. Rats of both sexes receiving 175 mg/kg/day demonstrated dark yellow urine (55/65 males; 38/65 females). A final report on this study will be issued when it is completed.

3. A 90 Day Study in Rats With the Monoglutathione Conjugate of Chlorothalonil. Doc. # 1108-85-0078-TX-006 (MRID 40122904). The DER by the Dynamac Corporation is attached.

Summary: 15 male Fischer 344 rats per group were dosed by gavage once daily with equimolar doses of 75 mg/kg/day Chlorothalonil, 150 mg/kg/day of Glutathione-Chlorothalonil conjugate or vehicle control (0.5 % methylcellulose in water) for 90 - 93 days. Routine clinical observations were made on blood and urine initially and at 7 and 13 weeks from fasted animals. 24 hour urine samples were collected after the first dose and from nonfasted animals on days 4 and 7, and after weeks 2, 4, 8 and 12. These samples were assayed for thiol metabolites. Stomach and kidneys and all gross lesions were fixed for histopathological examination. Left kidneys were prepared using Masson's Trichrome method.

Results:

Dark yellow urine was reported for 14/15 animals receiving Chlorothalonil. Neither the vehicle nor the Glutathione-Chlorothalonil groups showed this effect. Chlorothalonil and Glutathione-Chlorothalonil groups both had significantly reduced SGPT levels at 7 and 13 weeks. Both treatment groups had reduced liver to body weight ratios and significantly increased kidney ratios. Chlorothalonil-treated rats exhibited thickening of the gastric mucosa (13/15) and some ulceration (6/15). Controls and Glutathione-Chlorothalonil treated rats did not exhibit these lesions. The microscopic diagnosis was hyperplasia/hyperkeratosis of the forestomach (14/15) and gastritis (9/15) and ulcers and erosion (5/15).

Renal tissues from both treatment groups stained with H&E exhibited tubular epithelial hyperplasia and tubular hypertrophy. Those from the control group were normal. The lesions were also observed using the Masson trichrome stain.

The evidence did not support the author's claim that there was a common metabolic pathway for Chlorothalonil and its Glutathione-Chlorothalonil conjugate.

4. Histopathological Reevaluation of Stomach Tissue from a Mouse Tumorigenicity Study (Ref. 5TX-79-0102). Doc. #1107-85-0076-TX-006 (MRID 40122905).

In this study, the authors reexamined the relationship between gastric hyperplasia and hyperkeratosis and the tumors of the forestomach reported originally. They reported that in all tumor-containing forestomachs in which an evaluation was possible, squamous hyperplasia/hyperkeratosis was observed. Four animals with tumors had no "leftover" stomach tissue and

an evaluation of the presence or absence of hyperplastic/hyperkeratotic tissue was not possible. The authors reported that three additional mice bearing the gastric tumors likewise had these pre-existing lesions. The authors concluded that gastric hyperplasia/hyperkeratosis is a pre-neoplastic lesion in mice receiving dietary Chlorothalonil. They also concluded that "... no tumors would occur at dietary concentrations of chlorothalonil which do not produce hyperplasia and hyperkeratosis of the forestomach".

5. Pilot Study of the Gamma Glutaryl Transpeptidase Inhibitor, AT-125, on the Metabolism of Chlorothalonil. Interim Report. Doc. # 1376-86-0072-AM-001. (MRID 40122914). The DER by the Dynamac Corporation is attached.

Summary:

Pre-treatment of rats with AT-125 did not affect urinary excretion of radiolabeled 14-C Chlorothalonil, although there was a lower concentration of ethyl acetate-extractable metabolites. The interim study provides insufficient evidence for the authors' contention that conjugation with Glutathione is a major metabolic pathway for Chlorothalonil in rats.

6. In Vitro Studies on the Transfer of 14-C Chlorothalonil and/or its Metabolites from the Mucosal to the Serosal Surface of the Gastrointestinal Tract. Doc. # 1179-86-0020-AM-001. (MRID 40122913). The DER by the Dynamac Corporation is attached.

Summary:

About 7 % of a 14 C-Chlorothalonil dose placed inside a gut sac prepared from a male rat transferred to the outside (serosal) surface of the sac in 6 hours. HPLC analysis indicated that the products were metabolites of Chlorothalonil rather than Chlorothalonil itself. They were not identified, however.

7. Subcellular Fractionation of Kidneys from Male Rats Administered 14-C-Chlorothalonil. Doc. # 1178-86-0016-AM-001 (MRID 40122912). The DER by the Dynamc Corporation is attached.

Summary:

0.38 % of an orally administered dose of radio-labeled Chlorothalonil appeared in the kidneys of male rats prior to fractionation by ultracentrifugation. All fractions contained radioactivity. 81 % was found in the soluble portion with 0.2

% in the nuclear pellet; 7.0 % in the heavy mitochondrial pellet; 3.2 % in the light mitochondrial/lysosomal pellet; 2.0 % in the microsomal pellet and 6.3 % as cellular debris. The study contained numerous technical errors.

8. In Vitro Incubations of 14-C Chlorothalonil with stomach and Intestinal Mucosal Cells. Doc. # 1172-85-0081-AM-002. (MRID 40122911). DER attached.

Summary:

Cells lining the gastric squamous mucosa, the glandular mucosa and the small intestine metabolize Chlorothalonil to more polar metabolites though to be the mono- and di-gluthione conjugates of Chlorothalonil.

9. Mutagenicity Assays reviewed by Dr. John Chen are attached.

- a. Salmonella/Mammalian - Microsomal Assay using SDS-66471. Study # T-5079.1505 (MRID 40122907).

Rated Unacceptable due to lack of stability data.

- b. Salmonella/Mammalian - Microsomal Assay using SDS-66473. Study # T-5081.1505 (MRID 40122908).

Rated unacceptable due to lack of stability data.

- c. Salmonella/Mammalina - Microsomal Assay using SDS-66474. Study # 5080.1505 (MRID 40122909).

Rated Acceptable.