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Carcinogenicity of Chlorothalonil:
Data in Support of a Non-Linear Mechanism for Carcinogenicity

by

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Summary

The Office of Pesticide Programs, U.S. Environmental Protection Agency (U.S. EPA), has recently characterized the fungicide Chlorothalonil as “likely” to be a human carcinogen² by all routes of exposure (Memorandum dated October 20, 1997 from Timothy F. McMahon to Walter Waldrop/Andrew Ertman). This decision is based on: 1) evidence of increased incidence of renal adenoma, carcinoma, and adenoma/carcinoma combined in Fischer 344 rats following chronic administration of chlorothalonil at doses of 15 and 175 mg/kg/day; 2) papilloma and/or papilloma/carcinoma of the forestomach combined in Fischer 344 rats at 175 mg/kg/day, and 3) increased incidence of forestomach carcinoma in CD-1 mice at 214 mg/kg/day. Based on the evidence characterizing the mode of action for production of renal and forestomach tumors, the Office of Pesticide Programs (OPP) concluded that Chlorothalonil met the cancer risk assessment guideline criteria for non-linearity of the dose response and that the Margin-of-Exposure approach be used for purposes of cancer risk assessment for chlorothalonil. Following is background material leading to this determination.

Background

Chlorothalonil [tetrachloroisophthalonitrile] is a non-systemic fungicide registered for use on a wide variety of food and feed crops. Chlorothalonil is also registered for use on the following crops grown for seed: field corn, grass, onions, and sugar beets. Antimicrobial uses for chlorothalonil include mold and mildew control in paint films and wood.

Carcinogenicity Studies - Rats

The carcinogenicity of chlorothalonil has been examined in a number of studies, as summarized from the following data submitted to and reviewed by the Agency:

1) NCI Rat Carcinogenicity Study

Reference: National Cancer Institute Study # NCI-CG-TR-41, 1978

In this study (MRID # 00030286), chlorothalonil (98.5% a.i.) was administered in the diet to groups of 50 male and 50 female Osborne-Mendel rats at doses of 0, 5,063 ppm or 10,126 ppm (approximately 250 and 500 mg/kg/day) for 2 years. Renal tubular epithelial adenomas and carcinomas were found in treated animals after 80 weeks of dietary exposure; no neoplasms were reported in concurrent controls. Statistical analysis of renal tumors in this study showed that at the high dose, a statistically significant increase in incidence of renal adenomas and carcinomas combined was observed for both male and female rats (4/49 high dose males vs. 0/10 control males; 5/50 high dose females vs. 0/10 controls, $p = 0.028$ for males, $p = 0.016$ for females). A significant trend was also reported for incidence of renal tumors combined in females ($p = 0.007$). Historical control incidence for combined renal adenomas/carcinomas was stated as 1.25% for male rats, and 0% for female rats. This study, while providing useful scientific data on the carcinogenicity of chlorothalonil, was graded supplementary by the Health Effects Division, Office of Pesticide Programs.

2) IRDC Rat Carcinogenicity Study

Reference: IRDC Tumorigenicity Study in Rats, Study # 099-5TX-80-234-008, MRID # 00146945 (1985).

In this study, chlorothalonil (98.1% a.i.) was administered in the diet to 60 male and 60 female Fischer 344 rats/dose at 0, 800, 1600, or 3500 ppm (0, 40, 80, or 175 mg/kg/day) for 129 weeks. A statistically significant increase in incidence of renal adenoma and carcinoma, separately and combined was observed in both male and female rats at the 175 mg/kg/day dose level, with a significant positive trend noted at control (incidence of 1/66, 5/61, 6/60, and 18/60 at 0, 40, 80, and 175 mg/kg/day for combined tumors in males, respectively; incidence of 0/60, 2/60, 7/61, and 19/59 at 0, 40, 80, and 175 mg/kg/day for females, respectively). In addition, a statistically significant increase in incidence of renal adenoma and carcinoma combined was observed at the 80 mg/kg/day dose for both male and female rats. Male rats also showed a significant increase in renal adenoma incidence at the 80 mg/kg/day dose. The only significant finding in this study with regard to forestomach tumors was the observation of a significant trend for the incidence of squamous mucosal papilloma and carcinoma combined in female rats (incidence of 0/60, 1/60, 3/61, and 3/59, $p < 0.05$ at control). There was no NOEL for non-neoplastic changes established in this study, based on the presence of glomerulonephritis, hyperplasia of the renal cortical tubules and pelvic/papillary epithelium, and tubular cysts at all dose levels.

3) IRDC and EPL Rat Carcinogenicity Study

Reference: A Tumorigenicity Study of Technical Chlorothalonil in Rats. Study conducted jointly by International Research and Development Corporation, Experimental Pathology Laboratories, Inc., and Test Substance Analysis Laboratory Ricerca, Inc. Submitted under MRID # 41250502 1989.

This study was performed in response to HED's review of the earlier (1985) rat chronic toxicity / carcinogenicity study (Accession # 258759), which determined that there was no NOEL with respect to stomach and kidney tumors at dose levels of 40, 80, and 175 mg/kg/day. In the present study, Charles River Fischer 344 rats (65/sex/group) received chlorothalonil (98.3% a.i.) In the diet at dose levels of 0, 2, 4, 15, and 175 mg/kg/day for 111 weeks (males) and 125 weeks (females). Carcinogenic potential was evidenced by statistically significant trends and pair-wise increases in the incidence of kidney tubular adenomas, carcinomas, and adenomas/carcinomas combined in male and female rats at the 175 mg/kg/day dose level. The incidence of forestomach papillomas and carcinomas was increased only at 175 mg/kg/day in males and at 15 and 175 mg/kg/day in females. At 15 mg/kg/day in male rats, there was a significant pair-wise difference in the incidence of kidney tubular adenomas and/or carcinomas combined. The NOEL for non-neoplastic changes was determined to be 2 mg/kg/day, and the LOEL was determined to be 4 mg/kg/day, based on increased kidney weights and hyperplasia of the proximal convoluted tubules in the kidney as well as forestomach hyperplasia and ulcers.

Carcinogenicity Studies in Mice

1) NCI Carcinogenicity Study

Reference: National Cancer Institute Study # NCI-CG-TR-41, 1978

In this study (MRID # 00030286), chlorothalonil was administered in the diet to groups of 50 male and 50 female B6C3F1 mice per dose at doses of 10,000ppm or 20,000ppm for 91-92 weeks. There was no evidence of tumorigenicity in treated mice.

2) SDS Biotech Study

Reference: Biodynamics Laboratory, East Millstone, NJ, Study # DTX-79-0102, MRID # 00127858 (1979).

Technical chlorothalonil (97.7% a.i.) was administered in the diet to groups of 60 male and 60 female CD-1 mice at 0, 750, 1500, or 3000 ppm (0, 107, 214, and 428 mg/kg/day) for 2 years. A statistically significant trend for the incidence of renal adenoma and carcinoma combined was observed in male mice (incidences of 0/57, 6/59, 4/59, and 4/56; $p < 0.01$ at control) but not in female mice. In male mice, a statistically significant increase in incidence of squamous cell carcinoma of the stomach was observed at the 214 mg/kg/day dose; for female mice, a significant increase in incidence of squamous cell carcinoma of the stomach at the 214 and 428 mg/kg/day dose levels was observed, with a significant trend at control. The incidence for squamous cell carcinoma in female mice was the same at the 214 and 428 mg/kg/day dose level (6/58 and 5/58, respectively). No NOEL for non-neoplastic effects could be determined in this study, based on the observations of bone marrow and splenic red pulp hyperplasia, increased kidney weight and surface irregularities, pelvic dilation, cysts and nodules, and stomach/esophageal hyperplasia and hyperkeratosis at all dose levels.

3) IRDC Carcinogenicity Study

Reference: A Tumorigenicity Study of Chlorothalonil in Male Mice - a Final Report. Study conducted by International Research and Development Corporation. Submitted under MRID # 40243701. (1987)

In this study, groups of Charles River CD-1 male mice (60/group) were administered technical chlorothalonil in the diet at doses of 0, 10, 40, 175, or 750 ppm (1.42, 5.71, 25, and 107.1 mg/kg/day) for 2 years. Chlorothalonil induced tubular hyperplasia and karyomegaly at 25 and 107.1 mg/kg/day. Increased incidence of tubular hypertrophy was also observed at 107.1 mg/kg/day. Squamous hyperplasia and hyperkeratosis of the forestomach were also increased at 5.71 mg/kg/day and above. There was no evidence of induction of renal or gastric neoplasms

from dietary administration of chlorothalonil in this study. This study was classified as core minimum data. Although only male mice were used, the study accomplished its purpose of defining the effects of chlorothalonil on kidneys and stomach at doses of 107.1 mg/kg/day and below. The NOEL was determined to be 5.71 mg/kg/day, and the LOEL 25 mg/kg/day, based on renal tubular hyperplasia observed at this dose level.

Non-Neoplastic Findings

In conjunction with the neoplastic effects of chlorothalonil observed from the above studies, non-neoplastic effects of chlorothalonil on the same target organs (kidney and stomach) have been reported at or just below those doses resulting in carcinogenicity. With regard to the IRDC Fischer 344 rat study and the SDS Biotech mouse study, renal tumors in these studies were accompanied by hyperplasia of the cortico-tubular epithelium and/or glomerulonephritis. In addition, hyperplasia / hyperkeratosis of the stomach was observed in the Fischer 344 rat study, but not in the CD-1 mouse study.

In the more recent 2-year rat study at the one year interim sacrifice, increased incidence of kidney epithelial hyperplasia was observed in male rats at 4.0 mg/kg/day and above. Increased incidence of clear cell hyperplasia was observed in male rats at 15 mg/kg/day, and increased incidence of karyomegaly of the forestomach was observed in female rats at 175 mg/kg/day. Female rats at the high dose were also observed with increased incidence of kidney epithelial cell hyperplasia and clear cell hyperplasia. At study termination, increased incidence of renal tubular epithelial cell hyperplasia and clear cell hyperplasia were observed in male and female rats at the 175 mg/kg/day. A significant portion of the rats observed with renal adenoma and/or carcinoma at this dose were also observed with tubular cell hyperplasia.

In its entirety, the available data submitted to the Office of Pesticide Programs on chlorothalonil show similar non-neoplastic effects in the kidney and stomach in several studies after repeated administration of the test chemical, as summarized below:

Study Type	MRID #	Doses	Effects
2 yr rat carcinogenicity (NCI)	00030286	0, 5063, 10126 ppm (0, 250, 500 mg/kg/day)	renal tubular epithelial adenomas and carcinomas (80 weeks) at 500 mg/kg/day
2 yr rat carcinogenicity (Hazleton)1109, 1108	00087376	unknown (1500, 15000 ppm) others?	kidney nephritis at 15000 ppm (750 mg/kg/day). Guideline

Study Type	MRID #	Doses	Effects
2 yr rat carcinogenicity (Hazleton)1101, -8, -9	00087377	0.5% (5000 ppm or 250 mg/kg/day) technical chlorothalonil	kidney hypertrophy. Study graded acceptable. (1-liners guideline)
18 mo rat chronic toxicity (Hazleton)	00087359	0.05% (500 ppm) technical chlorothalonil	growth depression, tubular hypertrophy reported. Study graded supplementary.
2 yr rat carcinogenicity (IRDC new 1989)	40559102	0, 2.0, 4.0, 15, 175 mg/kg/day	interim report. epithelial cell hyperplasia and clear cell hyperplasia and karyomegaly in kidneys at 4.0 mg/kg/day. NOEL = 2.0 mg/kg/day. Study acceptable.
	41250502		final report. Renal tubular adenomas/carcinomas in males at ≥ 15 mg/kg/day, females at 175 mg/kg/day. Forestomach papillomas/carcinomas in males at 175 mg/kg/day, females at ≥ 15 mg/kg/day. Kidney proximal tubular hyperplasia; forestomach hyperplasia and hyperkeratosis of at 4.0 mg/kg/day. NOEL = 2.0 mg/kg/day. Study acceptable.
30 month rat carcinogenicity IRDC - 1985)	acc258759 00146945	0, 800, 1600, 3500 ppm (0, 40, 80, 175 mg/kg/day)	renal adenomas and carcinomas, papillomas of stomach all doses. Renal hyperplasia of cortical tubules, tubular cysts all doses. Non-glandular stomach erosion and ulcers. (guideline when considered with MRID 41250502)
2 yr mouse carcinogenicity (NCI)	00030286	M - 0, 2688, 5375 ppm (0, 429, 851 mg/kg/day) F - 0, 3000, 6000 ppm (0, 429, 851 mg/kg/d)	no evidence of carcinogenicity
2-yr mouse carcinogenicity (IRDC new)	40122902	0, 10/15, 40, 175, 750 ppm	interim report. Renal hyperplasia at 750 ppm. Also, hyperplasia of gastric mucosa. Study graded supplementary.
	40243701		final report. renal tubular hyperplasia at 175 ppm. Study graded acceptable.
2 yr mouse carcinogenicity (Biodynamics)	00127858 071541acc	0, 750, 1500, 3000 ppm (0, 107, 214, 428 mg/kg/day)	renal neoplasms: all doses in males, sig only at 750 pp; renal tubular degeneration (all doses) Forestomach - hyperplasia and/or tumorigenesis in the squamous cell and epithelial layer of the esophagus and stomach, males (tumors > controls but non-sig all doses) and females (tumors sig ≥ 1500 ppm), hyperplasia of stomach at 750 ppm. . Study acceptable for cancer; supplementary for chronic.
2 yr dog chronic Hazelton	00114034	0, 60, 120 ppm (1.8, 3.5 mg/kg/day)	kidney epithelium vacuolation at 3.5 mg/kg/day (only at 12 months). Verified by RfD committee. Study acceptable.

Study Type	MRID #	Doses	Effects
1 yr dog chronic Pharmacology (LSR)	43653603	0, 15, 150, 500 mg/kg/day (capsule)	decreased body weight at 500 mg/kg/day NOEL = 150 mg/kg/day
90 day mouse study Concord woods (IRDC)	00138148 00258769 (00127857)	0, 7.5, 15, 50, 275, 750 ppm (0, 1.01, 2.14, 7.14, 39.3, 107)	hyperplasia / hyperkeratosis of gastric mucosa at 50 ppm. NOEL = 15 ppm. Study acceptable (doses need verification)
13 wk rat toxicity (Huntingdon)	00127852 00258768 (47936)	0, 1.5, 3.0, 10, 40 mg/kg/day	dilated renal tubules non-glandular stomach epithelial hyperplasia and hyperkeratosis at ≥ 10 mg/kg/day. After reread found: At ≥ 3 mg/kg/day increased intracytoplasmic inclusion bodies in proximal convoluted tubules of males. NOEL = 1.5 mg/kg/day. Study acceptable.
13 wk rat toxicity - male (Ricerca)	40243702	175 mg/kg/day	renal (epithelial regeneration, hyperplasia, vacuoles, loss of brush border, karyomegaly, tubular degeneration, hypertrophy) - starting 4 days. non glandular stomach (squamous epith. hyperplasia, keratosis, ulcer, erosion)
13 wk cell proliferation - male rat	44223002	175 mg/kg/day	increased mean labeling index in kidneys; degeneration of proximal convoluted tubules and epithelial hyperplasia. Study acceptable
cell proliferation - male rat	44240901	0, 1.5, 15, 175 mg/kg/day	increased labeling of cells of proximal convoluted tubule at 15 mg/kg/day on days 7, 14, and 21 of treatment. increased labeling of stomach tissue at 15 mg/kg/day on day 28.
Mechanistic study	43653604	175 mg/kg/day	small vacuoles appear early in the apical cells of the renal proximal tubular cell. They seem to coalesce and form large vacuoles, called osmotic nephrosis. This is suggestive of osmotic control within the proximal tubular cell.
Mechanistic study	43653608		di- and tri-thiol analogs of chlorothalonil impaired respiratory control in kidney cortical mitochondria.

Chlorothalonil Mutagenicity

The Health Effects Division (HED) of OPP discussed the mutagenicity data for chlorothalonil in light of the registrant's claim that chlorothalonil is not genotoxic. Chlorothalonil has been tested in a variety of mutagenicity assays. Among the *in vitro* data, chlorothalonil was non-mutagenic in the Salmonella assay at concentrations up to 10,000 $\mu\text{g}/\text{plate}$ with and without exogenous metabolic activation. On the other hand, several positive results were obtained in mammalian cultured cells; these include a positive mouse lymphoma assay (as reported by the NTP, Annual Report, 1986), a positive Chinese hamster ovary (CHO) aberrations and sister chromatid exchanges assays (Galloway et al., Environ. Mol. Mutagen. 10: 1-175, 1987), and another CHO aberrations assay submitted to the Agency (at 0.3 $\mu\text{g}/\text{ml}$ without activation). In a series of *in vivo* chromosome aberration tests, chlorothalonil did

not induce chromosomal abnormalities in bone marrow at doses up to 5000 mg/kg in mice, rats, and Chinese hamsters after one or two doses. However, in an additional *in vivo* cytogenetics assay, doses of 500, 2500, and 5000 mg/kg given once a day for 5 days to Chinese hamsters produced a weak clastogenic response at all dose levels, but no dose-response was noted. There was a series of micronucleus assays performed in mice, rats, and Chinese hamsters as well -- while these were considered acceptable by standards used in the late 1970's, they are unacceptable based on current guidelines. Other acceptable assays submitted to the Agency include a positive differential toxicity (DNA damage) assay with Salmonella without activation, a negative *B. subtilis* rec assay, and a negative cell transformation assay with F1706 P95 and H4536 P+2 cells.

As agreed upon at the HED Cancer Peer Review meetings, it cannot be said that chlorothalonil is devoid of genotoxic activity; however, it should be noted that the positive results are weak responses. The above data on mutagenicity show weak genotoxic activity of chlorothalonil at higher concentrations of the chemical, where probable chlorothalonil metabolites that cause cytotoxicity, proliferation, and genotoxicity come into play. It should be noted that while these are different types of toxic responses, they are not necessarily mutually exclusive for the development of tumors. At higher doses of chlorothalonil, mutagenic activity could bring added pressure to the existing mechanism of tumor induction, possibly contributing to the tumor response. Overall, with the interplay of the many responses to chlorothalonil exposure, these data appear consistent with a non-linear mode of action for this chemical.

Mode/Mechanism of Action

In previous meetings of the Health Effects Division's Carcinogenicity Assessment Review Committee (HED/CPRC)(May, 1987 and January, 1996), chlorothalonil was classified as a "Group B2" carcinogen, based on increased incidence of malignant and benign tumors of the kidney in male and female mice and rats. A q1 * of 1.1×10^{-2} was estimated from the data at that time. Subsequent to these two meetings, the registrant submitted data to the Office of Pesticide Programs in support of a re-classification request for carcinogenicity of chlorothalonil. The data submitted endorsed the hypothesis that chlorothalonil-induced tumors of the kidney and forestomach occur through non-genotoxic, threshold based mechanisms. In the stomach, it was contended that chlorothalonil-induced tumors arise through an inflammatory response of the squamous epithelium, resulting in sustained cell proliferation with restorative hyperplasia. In the kidney, it was proposed that certain thiol metabolites of

chlorothalonil exert a toxic effect upon kidney mitochondria, disrupting mitochondrial respiration with eventual cell death and compensatory proliferation that, if sustained, could eventually lead to neoplasia.

Stomach Tumors

The registrant submitted the following information in support of a mode/mechanism of action for chlorothalonil induced stomach tumors:

1) Chlorothalonil is a known skin and eye irritant, and contact with the forestomach of the rat would be expected to produce an inflammatory response. Study of the histopathological effects on the forestomach of rats during repeated oral administration of chlorothalonil showed multifocal ulceration and erosion of the mucosa that subsequently progresses to hyperplasia and hyperkeratosis. Such lesions have been observed in subchronic and chronic studies in rats and mice.

2) In evaluating the significance of chlorothalonil-induced rat forestomach tumors to possible human cancer risk, the following factors should be considered:

a) Chlorothalonil induces rodent forestomach tumors in a manner similar to other known forestomach carcinogens through a non-genotoxic mechanism involving irritation, cytotoxicity, cell necrosis, increased cell proliferation, and restorative hyperplasia.

b) The effect in the forestomach is threshold-based (i.e. non-linear).

c) The hyperplasia induced by repeated oral chlorothalonil administration is a reversible histopathological state. In a subchronic study in which groups of rats were treated with various doses of chlorothalonil for 13 weeks and allowed a 13 week recovery period, squamous cell epithelial cell hyperplasia and hyperkeratosis observed after 13 weeks of treatment at doses of 10 and 40 mg/kg/day were found to be reversible when treatment ceased.

d) There is no anatomical equivalent to the rodent forestomach in humans. The rodent forestomach is uniquely susceptible to the irritant effects of chlorothalonil due mainly to the time that chlorothalonil-containing food remains in the forestomach before it moves into the glandular stomach.

Kidney Tumors

In support of the hypothesis that a non-genotoxic mechanism is responsible for chlorothalonil-induced kidney tumors, the registrant submitted information based on published scientific literature. The proposed mode/mechanism is summarized below:

1) Following oral administration of chlorothalonil, conversion to the glutathione (GSH) conjugate occurs, most likely by GSH-transferase located within the gut mucosal cells. Subsequent absorption of the GSH-conjugate then occurs.

2) Following metabolism to and absorption of the GSH-conjugate, conversion to the cysteinyl glycine conjugate

occurs by the action of gamma-glutamyl transpeptidase; conversion of the cysteinyl glycine conjugate to the pre-mercapturic acid (also called the cysteine-S-conjugate) then occurs by cysteinyl glycine dipeptidase. The pre-mercapturic acid at this point can either be N-acetylated to the mercapturic acid, or can be converted to pyruvate, ammonia, and a variety of thiols by cysteine conjugate β -lyase. It is noted that the above biotransformations can occur at more than one site, i.e. in the bile or small intestine.

3) Both the GSH- and cysteine-S-conjugates appear to be absorbed by the intestinal epithelium and can re-enter the systemic circulation. Cysteine-S-conjugates may be taken up by the liver and pass into the bloodstream as intact conjugates or as mercapturic acids.

4) Cysteine-S-conjugates, GSH conjugates, or mercapturic acids reaching the kidney come into contact with proximal tubular epithelial cells. They can enter the kidney by filtration or by peritubular circulation. It is possible that entry occurs by both routes. Filtered GSH conjugates are transformed to the cysteine-S-conjugates by gamma-glutamyl transpeptidase. Mercapturic acids can also enter, be de-acetylated, and undergo bioactivation by β -lyase.

5) The “bioactivation” of pre-mercapturic acids to nephrotoxic thiols occurs through the action of cysteine conjugate β -lyase, an enzyme found in the cytosol and mitochondria of cells of the renal proximal tubule. That the action of β -lyase is a critical step comes from work demonstrating that aminooxyacetic acid, an inhibitor of β -lyase, blocks the *in vivo* toxicity of cysteine-S-conjugates.

6) While there is still speculation as to why the cellular damage by cysteine-S-conjugates is restricted to the S3 segment of the proximal tubule of the kidney, hypotheses include the presence of differences in cellular localization of one or more of the enzymes involved in forming the active toxicant, or differences in sensitivity of the cells along the nephron. The high activity of the gamma-glutamyl transpeptidase enzyme in the brush border membrane of the renal proximal tubule cell appears to play a role, probably through the accumulation of cysteine-S-conjugates in the tubular cell. Relative to other tissues, the kidney of both the rat and dog contain high levels of gamma-glutamyl transpeptidase (EPA MRID # 43653609).

7) The nephrotoxicity of cysteine-S- conjugates through activation to thiol metabolites is related to renal cortical mitochondrial dysfunction. Changes in the mitochondrial membrane (through inhibition of respiration and membrane-bound dehydrogenases) interfere with the availability of ATP and can affect membrane transport mechanisms, resulting in eventual cell death. Evaluation of mitochondrial respiration in the presence of mono-, di-, and tri-thiol analogs of chlorothalonil showed inhibition of mitochondrial respiratory control by the di- and tri-thiol analogs of chlorothalonil, possibly through inhibition of reducing equivalents from succinate to Coenzyme Q (MRID # 43653608).

8) Data are available which show an apparent species difference in the absorption and metabolism of chlorothalonil. In the rat, approximately 25% of an oral 50 mg/kg dose is absorbed, while in the dog, only about 8% of a 50 mg/kg dose is absorbed. Furthermore, approximately 1.6% of a 50 mg/kg dose in the rat is excreted in urine as dithiol- and trithiol analogs of chlorothalonil, while in the dog, very small percentages of dithiol and trithiol metabolites are found in urine (MRID # 43653611). The rate of absorption of chlorothalonil appears similar between rats and dogs (MRID # 43653612), but in the dog, there is less conversion to the di- and tri-methyl thio analogs of chlorothalonil (MRID # 43653612). This difference between rats and dogs is supportive of the higher NOEL for renal toxicity in the dog vs. rat, and may form the basis for the observed difference in renal toxicity of

chlorothalonil between rats and dogs.

9) With respect to human sensitivity (including age-related sensitivity) to the nephrotoxicity of thiol metabolites of cysteine-S-conjugates, data are limited. Literature data cited by the registrant including Lau et al., (MRID #'s 43653605 and 43653606) indicate that the activity of gamma-glutamyl transpeptidase (GGTP) in rat kidney is approximately 10 times greater than in human kidney. In addition, the data suggested that the amount of β -lyase in human kidney is only about 10% of that in rat kidney on a per gram basis. Based on these observations, the hypothesis is put forth that more cysteine-S-conjugates are generated in the rat than human. Thus, it should be assumed that, although there are quantitative difference among species in the activity of GGTP, qualitatively the potential for the formation of cysteine-S-conjugates exists in all species studied. Furthermore, it can be presumed that infants and children have the same ability to form this same metabolite.

OPP Position

Based on the above submitted data in support of the mechanism(s) for forestomach and kidney tumors induced by chlorothalonil, the Health Effects Division Carcinogenicity Assessment Review Committee in a third meeting (1996) concluded that "the tumors observed from administration of chlorothalonil were related to administration of the test chemical. In addition, the committee recognized that the data supporting the hypothesis that renal toxicity of chlorothalonil is associated with the formation of toxic thiol metabolites in the kidney are scientifically valid. However, the committee concluded that the evidence suggesting that mitochondrial toxicity is linked to carcinogenicity of chlorothalonil in the kidney was not conclusive, and that other mechanisms could be operative. With regard to interspecies comparison of susceptibility to the toxicity of chlorothalonil, the relevance or lack of relevance of the rat and dog model for evaluation of tumorigenic potential in humans has not been clearly established. Questions regarding relative absorption of chlorothalonil in humans, delivered dose to the kidney, and relative susceptibility have not been addressed. Based on these considerations, the committee voted to retain the [Group]B2 classification of chlorothalonil."

Cell Proliferation Studies

In response to the conclusions reached at the third carcinogenicity assessment review committee meeting, the registrant submitted two studies that examined cell proliferation in the kidney from chlorothalonil administration. Summaries of these 2 studies are presented below:

1) Citation: Mizens, M. (1996): A 90-Day Pilot Study for the Evaluation of Cell Proliferation in the Kidneys of Male Rats Following the Oral Administration of Technical Chlorothalonil. Study performed by Ricerca, Inc. MRID # 44223002. Unpublished.

Executive Summary: In a cell proliferation study, twenty-eight male Fischer 344 rats received technical chlorothalonil (97.9% a.i.) in the diet at 175 mg/kg/day for up to 91 days. Scheduled sacrifices occurred on days 7 (14 rats), 28 (7 rats), and 91 (7 rats) for the purpose of assessing the effect of chlorothalonil administration on cell proliferation in the kidney. Rats were implanted with Alzet minipumps containing bromodeoxyuridine 3.5 and 6.5 days prior to sacrifice (day 7), or 3.5 days prior to sacrifice (days 28 and 91). Mean labeling index was statistically increased in the kidneys of male rats treated with 175 mg/kg/day chlorothalonil at all scheduled sacrifice times. From day 7 to day 28, the fold increase in labeling index was relatively stable (approximately 10-fold over control), with a decrease to approximately 3.5-fold over control on day 91. Increased cell proliferation

correlated with histopathological lesions of degeneration of the proximal convoluted tubules and epithelial hyperplasia. The results of this study demonstrate a sustained cell proliferative response as a result of dietary administration of technical chlorothalonil at a dose of 175 mg/kg/day. This study is classified as acceptable (non-guideline). The study does not satisfy a particular guideline requirement, but demonstrates a cell proliferative effect of chlorothalonil on the kidney at a dose which also produces kidney tumors.

2) Citation: Hironaka, M. (1996): Analysis of Hyperplastic Changes in the Stomach and Kidney of Male Rats After 28-Day Induction by Chlorothalonil Technical. Study performed by the Center for Safety Assessment of Food, Agricultural Chemicals and Medical Drugs, Sumitomo. MRID # 44240901. Unpublished.

Executive Summary: In this study, 96 male SPF rats were divided into test groups of 6 animals per group. Rats received technical chlorothalonil (98.98% a.i.) in the diet at dose levels of 0, 1.5, 15, or 175 mg/kg/day for either 7, 14, 21, or 28 days (total of 24 rats per time point). Histological examination of kidney and stomach tissue was performed for each group after the appropriate exposure. In addition, kidneys were subjected to PCNA staining and stomach to BrdU staining, and the labeling index and labeling count of cell nuclei performed. Duodenum was used as a negative control for PCNA and BrdU staining. Increased absolute and relative weight of the kidneys was observed at 175 mg/kg/day at all time points, and in one animal at 15 mg/kg/day on Day 28. Increased incidence of vacuolization of the epithelium of the proximal convoluted tubules was observed at all time points at 175 mg/kg/day and on Days 7, 14, and 21 at 15 mg/kg/day. PCNA immunostaining of the proximal convoluted tubule epithelial cells showed increased labeling of cells at the 175 mg/kg/day dose level at all time points, and increased labeling at 15 mg/kg/day on Days 7, 14, and 21. BrdU labeling of the rat forestomach showed marked labeling at 175 mg/kg/day at all time points, and increased labeling on Day 28 at 15 mg/kg/day. The results of this study demonstrate a toxic response of the kidney and forestomach to repeated dietary administration of chlorothalonil at doses of 15 and 175 mg/kg/day. This study is classified as acceptable (non-guideline). This study does not satisfy a specific guideline requirement, but provides scientific data demonstrating a toxic response of the kidney and forestomach after repeated dietary administration of 15 and 175 mg/kg/day technical chlorothalonil.

The results of these two studies demonstrated: 1) a sustained cell proliferative response as a result of dietary administration of technical chlorothalonil at a dose of 175 mg/kg/day, and 2) a toxic response of the kidney and forestomach (including increased cell labeling) to repeated dietary administration of chlorothalonil at doses of 15 and 175 mg/kg/day. Based on the results of these two studies and the earlier submitted data, a more complete picture of chlorothalonil-induced toxicity and carcinogenicity is obtained. In brief, glutathione or cysteine-S-conjugates of chlorothalonil are absorbed from the gastrointestinal tract. Cysteine-S-conjugates, glutathione conjugates, or mercapturic acids reaching the kidney come into contact with proximal tubular cells, where eventual "activation" of pre-mercapturic acids occurs through the action of cysteine conjugate β -lyase, an enzyme found in the cytosol and mitochondria of the cells of the renal proximal tubules. Nephrotoxicity of cysteine-S-conjugates through activation to thiol metabolites is related to renal cortical mitochondrial dysfunction. Respiratory control has been shown to be disrupted by the di- and tri-thiol analogs of chlorothalonil. Osmotic changes occur within the renal cortical tubular cells as a result of toxic insult by the thiol metabolites of chlorothalonil, resulting in vacuolar degeneration followed by cellular regeneration.

The HED/CPRC recognized that, based on the mechanistic data submitted for the kidney tumor response and the review of these data, the mode of action for tumor induction of chlorothalonil is non-linear. The committee also recognized, however, that the non-neoplastic response observed in the kidney is considered a precursor to the neoplastic response, and that the dose(s) at which the non-neoplastic responses occur are very close to those at which a neoplastic response is observed. The tumor site itself is considered rare by the committee, adding to the weight of the evidence. In addition, the evidence for tumor production in mouse kidney is consistent with the mechanism proposed for tumor induction in the rat. Although the data in support of human sensitivity to the carcinogenic effects of chlorothalonil in the kidney were not conclusive, the committee agreed that qualitatively, a similar mechanism for tumor induction could occur in humans, but that quantitative differences were evident, based on the available data. At higher doses of chlorothalonil, mutagenic activity could bring added pressure to the existing mechanism of tumor induction, possibly contributing to the tumor response.

With regard to the forestomach tumors, the HED/CPRC recognized that precursor lesions to forestomach tumors (hyperplasia, hyperkeratosis, cell proliferation) were evident at doses close to those causing a tumorigenic response. Data submitted by the registrant showing cell proliferation and non-neoplastic pathology at doses near those producing a tumorigenic response were also recognized by the committee as supportive of a non-linear mode of action for chlorothalonil.

Based on the weight of the evidence presented to the HED/CPRC, the Committee agreed that a non-linear dose response assessment should be applied to the chlorothalonil cancer data for the kidney and forestomach. A Margin of Exposure (MOE) approach is recommended for both the kidney and forestomach tumors. Since ~~this~~ these tumor types are considered rare, for purposes of risk assessment, the MOE for the kidney and forestomach tumors should be determined using the 1.5 mg/kg/day dose from the 28-day SPF rat study as the "point of departure," as no tumor response or cell proliferation response was observed at this dose level. Tumor response in the kidney as well as cell proliferation were observed at the next highest dose level tested (15 mg/kg/day).

In considering the weight of the evidence for classification of the carcinogenicity of chlorothalonil, the Peer Review Committee utilized the *EPA Proposed Guidelines for Carcinogen Risk Assessment (April 23, 1996)*.

In accordance with these EPA proposed guidelines which are consistent with the existing 1986 cancer risk assessment guidelines, the HED/CPRC unanimously agreed that the weight of the evidence supported a classification of chlorothalonil as "likely to be a human carcinogen" by all routes of exposure. This conclusion was based on (1) the increased incidence of renal adenomas and carcinomas observed in both sexes of rats and mice; (2) the rarity of the tumor response in the kidney, and (3) the increased incidence of papillomas and/or carcinomas of the forestomach in rats and mice. While it was recognized that the mechanistic data supported a non-linear dose response for the mode of action for tumor production by chlorothalonil, the HED/CPRC also recognized that the kidney tumors were the result of administration of test chemical, were considered rare, and the submitted data supported the non-neoplastic pathology as directly related to eventual neoplasia.

The HED/CPRC agreed that a non-linear approach to risk assessment, using the Margin of Exposure **approach**, should be used.