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

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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Chlorothalonil -Fourth

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Antimicrobial Division (75010W)

THROUGH: Yiannakis M. Ioannou, Ph.D. J. M. Ioannou 8/6/97
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The Health Effects Division Carcinogenicity Peer Review Committee (HED/CPRC) met on June 11, 1997 to discuss and evaluate the weight-of-the-evidence on chlorothalonil in reference to its carcinogenic potential and to evaluate additional mechanistic data submitted by the registrant in support of the request for re-classification of the carcinogenicity of this chemical.

In accordance with the EPA Proposed Guidelines for Carcinogen Risk Assessment (April 23, 1996), chlorothalonil was characterized as "likely" to be a human carcinogen by all routes of exposure. This conclusion was based on evidence of increased incidence of renal adenoma, carcinoma, and adenoma/carcinoma combined in rats and mice following chronic administration of chlorothalonil at doses of 15 and 175 mg/kg/day, as well as increased incidence of forestomach carcinoma in CD-1 mice and papilloma and/or papilloma/carcinoma combined in Fischer 344 rats. The HED/CPRC concurred that the renal tumor type is rare, there is evidence that precursor lesions occur in the kidney at doses just below those producing tumors, and that a qualitatively (but not quantitatively) similar mechanism for renal tumor production is present in humans. For the forestomach tumors, the HED/CPRC concurred that the cell proliferation data supported a non-linear mechanism of action and that precursor lesions to forestomach tumors (including cell proliferation, hyperplasia, and hyperkeratosis) occur at doses and/or exposure times just below those producing tumors. Based on the discussion of the mode of action for production of renal and forestomach tumors by chlorothalonil, the HED/CPRC agreed that chlorothalonil met the risk assessment criteria for non-linearity, and that the Margin-of-Exposure (MOE) approach should be used for purposes of risk assessment.

SUMMARY

In the third meeting of the HED/CPRC on Chlorothalonil (January 3, 1996), data submitted by the registrant in support of the carcinogenicity re-classification request were considered by the committee. At that time, the additional data consisted of a carcinogenicity study in CD-1 male mice, a carcinogenicity study in Fischer 344 rats, one- and two-year toxicity studies in dogs, and mechanistic studies describing the biological basis for chlorothalonil-induced forestomach and kidney tumors. It was the registant's contention that the tumors observed in the forestomach and kidney from chlorothalonil administration were the result of non-genotoxic, threshold-based mechanisms. The HED/CPRC evaluated these data, and concluded from the January 3, 1996 meeting that the mechanistic studies submitted represented scientifically valid data, but that the data in support of the relationship between renal toxicity and carcinogenicity were not definitive enough to rule out other possible mechanisms of chlorothalonil-induced kidney tumors. Therefore, the B2 classification of chlorothalonil was retained with the recommendation of the use of linear low-dose extrapolation for quantitation of human risk.

In response to the conclusions of the third meeting of the HED/CPRC, the registrant submitted two studies that examined cell proliferation in the kidney from chlorothalonil administration. In the first study, 28 male Fischer 344 rats received technical chlorothalonil (97.9% a.i.) in the diet at 175 mg/kg/day for up to 91 days. Increased cell proliferation as well as histological lesions of degeneration of the proximal convoluted tubule and epithelial hyperplasia were observed at 175 mg/kg/day. In the second study, male SPF 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. PCNA immunostaining showed increased labeling of proximal convoluted tubule epithelial cells at 15 mg/kg/day on days 7, 14, and 21. BrDU labeling of rat forestomach showed increased labeling on day 28 at 15 mg/kg/day.

The fourth meeting of the HED/CPRC (June 11, 1997) was convened to discuss and evaluate the cell proliferation studies submitted with respect to the proposed mechanism of action for production of kidney tumors from chlorothalonil administration.

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 response occurs are 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.

The Peer Review Committee, in the meeting of January 3, 1996, considered additional mechanistic data submitted in support of a re-classification request by the registrant. The committee recognized that the data supporting the explanation for kidney tumor formation was scientifically valid. However, it was concluded that the evidence linking mitochondrial toxicity to carcinogenicity of chlorothalonil in the kidney was not definitive, and that other mechanisms could be operative.

A. Individuals in Attendance at the meeting:

1. Peer Review Committee (Signatures indicate concurrence with the peer review unless otherwise stated).

Karl Baetcke _____

Marion Copley _____

Kerry Dearfield _____

Yiannakis Ioannou _____

Hugh Pettigrew _____

Richard Hill _____

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of the committee report).

Timothy F. McMahon _____

Mark T. Butt[1] See 5a

3. Other Attendees:

Alan Levy _____

[1]signature indicates concurrence with pathology report.

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Timothy F. McMahon _____

Mark T. Butt[1] Mark T Butt

3. Other Attendees:

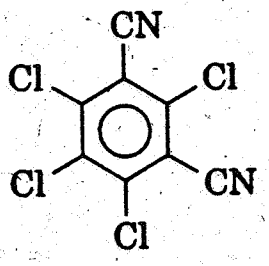
Alan Levy _____

[1]signature indicates concurrence with pathology report.

literature data, and previous Peer Review documents prepared and/or supplied by Dr. Timothy F. McMahon. The material reviewed is attached to the file copy of this report.

C. Background Information

Structure of chlorothalonil:



Chlorothalonil

Synonyms: 2,4,5,6-tetrachloroisophthalonitrile

Chlorothalonil is a fungicide registered for use on a wide variety of raw agricultural commodities (40 CFR §180.275). Chlorothalonil is also registered as a mildewcide in paints.

A summary of the animal carcinogenicity data for chlorothalonil follows:

Administration of Chlorothalonil in the diet to male and female Osborne-Mendel rats at 5,063 or 10,126 ppm (TWA) for 2 years resulted in an increase in the incidence of renal adenoma and carcinoma combined for both sexes at both dose levels. At the 10,126 ppm dose level, the increase was statistically significant for both sexes. A significant trend was also noted for incidence of renal adenomas and carcinomas combined in female rats.

Administration of chlorothalonil in the diet to male and female Fischer 344 rats for 129 weeks at dose levels of 0, 40, 80, and 175 mg/kg/day resulted in increased incidence of renal adenoma and carcinoma in male and female rats at the 175 mg/kg/day dose, which was statistically significant. In addition, a significant increase was noted at the 80 mg/kg/day dose level for renal adenoma and carcinoma in male and female rats. In the forestomach, a significant trend was identified for female rats in the incidence of gastric squamous mucosal papilloma and carcinoma combined.

In a second study in Fischer 344 rats, male and female rats received dietary chlorothalonil at doses of 0, 2, 4, 15, and 175 mg/kg/day for either 111 weeks (males) or 125 weeks (females). A statistically significant trend as well as pair-wise increase was noted for the incidence of kidney tubular adenomas, carcinomas, and adenomas/carcinomas combined, as well as stomach papillomas in both sexes at the 175 mg/kg/day dose level. At the 15 mg/kg/day dose level, a significant pair-wise difference was noted for male rats in the incidence of kidney tubular adenomas and/or carcinomas combined. A significant proportion of the rats observed with renal tumors at the high dose were also observed with tubular cell hyperplasia.

In a carcinogenicity study in B6C3F1 mice conducted by NCI, groups of male and female mice received chlorothalonil in the diet at 10,000 or 20,000 ppm for 91-92 weeks. There was no evidence of tumorigenicity in this study.

In a carcinogenicity study in CD-1 mice, male and female mice received chlorothalonil in the diet at dose levels of 0, 107, 214, and 428 mg/kg/day for 2 years. A significant trend was identified for the incidence of renal adenoma and carcinoma combined in male mice only. A significant increase in the incidence of squamous cell carcinoma of the stomach was also observed at the high dose in female mice.

In a second carcinogenicity study in CD-1 mice, male mice received chlorothalonil in the diet at doses of 1.42, 5.71, 25, and 107.1 mg/kg/day for two years. Tubular hyperplasia and karyomegaly were observed at the 25 and 107.1 mg/kg/day dose groups. Tubular hypertrophy was also observed at 107.1 mg/kg/day. Squamous hyperplasia and hyperkeratosis of the forestomach was observed at 5.71 mg/kg/day and above. There was no evidence of renal or forestomach neoplasms from dietary administration of chlorothalonil. The systemic NOEL was determined to be 5.71 mg/kg/day from this study based on renal tubular hyperplasia observed at 25 mg/kg/day.

Chlorothalonil is structurally related to a metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile, which is found as a major metabolite of chlorothalonil in meat and milk. The metabolite has been tested for carcinogenicity in both rats and mice and has been found to be negative. Published data on the stability of chlorothalonil dosing solutions suggests that this metabolite may actually be a degradation product and not a true metabolite. Hexachlorobenzene is also structurally related to chlorothalonil. The Agency possesses older carcinogenicity studies in mice and hamsters on hexachlorobenzene. These studies have not been evaluated for acceptability, but the data indicate an increased incidence of hepatomas when fed in the diet at 100 ppm to mice and 50 ppm to hamsters. Pentachlorophenol is also

structurally related to chlorothalonil and has been classified by the SAB/SAP as a B2 carcinogen based on increased incidence of liver tumors, pheochromocytomas, and hemangiosarcomas in mice.

Previous meetings of the Health Effects Division Carcinogenicity Peer Review Committee on Chlorothalonil Determined the Following (May 28, 1987 and January 3, 1996):

In the first Peer Review meeting, it was concluded that chlorothalonil be classified as a Group B2 (probable human carcinogen) based on an increased incidence of malignant and/or combined malignant and benign tumors in both sexes in two separate rat studies and in a mouse study. Based on these data (summarized above), the Q_1* of chlorothalonil was estimated as 1.1×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents [B. Fisher, 7/20/87].

In the second Peer Review Committee meeting, issues raised by the FIFRA Scientific Advisory Panel were discussed with respect to classification of the carcinogenicity of chlorothalonil. At this meeting, it was noted that the SAP panel agreed that the kidney tumors observed in CD-1 mice were biologically significant. The mutagenicity data for chlorothalonil was also reviewed by the Peer Review Committee with respect to the registrant's claim (and the SAP statement) that chlorothalonil is not genotoxic. The committee re-affirmed its classification of chlorothalonil as a Group B2 (probable human carcinogen).

In the third meeting of the Peer Review Committee, mechanistic data submitted by the registrant in support of a non-genotoxic mechanism of action for induction of renal tumors was discussed and evaluated by the committee. The summary of the committee's determination is shown below:

"The registrant has proposed that the classification of chlorothalonil be re-considered in light of presented data illustrating a threshold-based, non-genotoxic mechanism of action for induction of renal tumors in rats, and the lack of relevance of the rat model for evaluation of tumorigenic risk in humans. Based on the presented data, it would appear that a non-genotoxic mechanism for induction of renal tumors (and stomach tumors) by chlorothalonil is operative. However, there is a lack of information linking the toxicity of chlorothalonil to tumor development, particularly with regard to renal tumors. Disruption of mitochondrial function has not been definitively linked to eventual neoplasia in the kidney. The vacuolation observed appears to result from dilatation of the cisternae of the rough endoplasmic reticulum, which is not located in the mitochondrion. Thus, the full implication of the toxicity associated with formation of thiol metabolites from chlorothalonil has not been

established. 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. Differences in kidney toxicity between the rat and dog in response to administration of chlorothalonil appear to be based upon differences in conversion of the parent chemical to nephrotoxic metabolites within the kidney itself. Question regarding the relative absorption of chlorothalonil in humans, the delivered dose to the kidney, and relative susceptibility of the human are unanswered at this time. Based on this, the committee does not agree with the registrant's hypothesis regarding the relative suscepibility of humans to chlorothalonil induced renal toxicity at this time."

"The committee re-affirmed that the tumors observed from administration of chlorothalonil were related to administration of the chemical. In addition, the committee recognized that the data supporting the hypothesis that renal toxicity of chlorothalonil is associated with formation of toxic thiol metabolites in the kidney is scientifically valid. However, the committee concluded that the evidence sugesting that mitochondrial toxicity is linked to carcinogenicity of chlorothalonil in the kidney was not conclusive, and that other mechanisms could be operative. Based on these considerations, the committee voted to retain the B2 classification of chlorothalonil."

The Fourth Meeting of the Health Effects Division Carcinogenicity Peer Review Committee (June 11, 1997):

At this meeting, the weight-of-the-evidence for chlorothalonil was re-evaluated with reference to the carcinogenic potential of this chemical, based on new mechanistic data submitted by the registrant. These new data consisted of two cell proliferation studies, results of which are summarized 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. The apparent lack of cytotoxicity compared to the hypertrophic response in this study is not readily explained by the available data

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 at 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 ß-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 distrupted by the di- and tri-thiol analogs of chlorothalonil. Osmotic changes occur within the renal cotrical tubular cells as a result of toxic insult by the thiol metabolites of chlorothalonil, resulting in vacuolar degeneration follwed by cellular regeneration.

D. Summary of Carcinogenicity and Mechanistic Data

1. Human Data

There are limited data in humans with respect to sensitivity to nephrotoxicity of thiol metabolites of cysteine-S-conjugates. Literature data suggest that, based on *in vitro* measurements, activity of gamma-glutamyl transpeptidase in the rat kidney is approximately 10 times that of human. In addition, the amount of ß-lyase in human kidney is only about 10% of that found in rat kidney on a per gram basis. Therefore, it has been hypothesized that the human would be less sensitive to the nephrotoxicity and renal carcinogenicity of chlorothalonil than the rat. However, the tissue samples used for human study consisted of a mixture of cortical and medullary elements, and the source of the tissue also raises questions as to the influence of disease states and other variables on the actual susceptiblity of human kidney to thiol metabolites of chlorothalonil. In addition, there are unanswered questions regarding the relative absorption of chlorothalonil in humans as well as the delivered dose to the kidney and the actual metabolites of chlorothalonil in humans. Thus, any discussion of the relative susceptiblity of humans to the nephrotoxic and carcinogenic effects of chlorothalonil must at least address these areas.

2. Animal Data

Administration of Chlorothalonil in the diet to either male and female Osborne-Mendel rats (approximately 500 mg/kg/day) or Fischer 344 rats (175 mg/kg/day) resulted in a significant increase in the incidence of renal adenomas and carcinomas and adenomas/carcinomas combined. In Fischer rats, gastric squamous mucosal epithelial papilloma and carcinoma was observed at 175 mg/kg/day. In male CD-1 mice, a significant trend was observed for renal adenoma and carcinoma, while in female mice, increased incidence of squamous cell carcinoma was observed at 428 mg/kg/day. In a second study in CD-1 mice, tubular hyperplasia and karyomegaly were observed at 25 and 107.1 mg/kg/day, while tubular hypertrophy was also observed at 107.1 mg/kg/day. Squamous hyperplasia and hyperkeratosis of the forestomach was observed at 5.71 mg/kg/day and above.

In addition to the carcinogenic effects of chlorothalonil, significant non-neoplastic pathology of the kidney and stomach are observed after oral administration of chlorothalonil. These effects can be observed at doses

just preceding those doses which cause tumor development, and are considered precursor lesions to tumor development. The available data submitted to the Agency (and as also recognized by other regulatory agencies, notably Health and Welfare Canada) is summarized below:

Study Type	MRID #	Doses	Effects
2-yr mouse carcinogenicity	40122902	0, 10, 40, 175, 750 ppm	one year interim report. Renal hyperplasia at 750 ppm. Also, hyperplasia of gastric mucosa. Study graded supplementary.
2 yr rat carcinogenicity	00087376	unknown	kidney nephritis at 15000 ppm (750 mg/kg/day). No coregrade.
2 yr rat carcinogenicity	00087377	0.5% technical chlorothalonil	kidney hypertrophy. Study graded acceptable.
18 mo rat chronic toxicity	00087359	0.05% technical chlorothalonil	tubular hypertrophy reported. Study graded supplementary.
2 yr mouse carcinogenicity	00127858	0, 750, 1500, 3000 ppm	hyperplasia of stomach at 750 ppm. Tubular degeneration at 750 ppm. Study acceptable for cancer; supplementary for chronic.
2 yr mouse carcinogenicity	00030286	0, 10/15, 40, 175, 750 ppm	renal tubular hyperplasia at 175 ppm. Study graded acceptable.
2 yr rat carcinogenicity	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.
2 yr rat carcinogenicity	41250502	0, 2, 4, 15, 175 mg/kg/day	kidney tubular lesions; hyperplasia and hyperkeratosis of forestomach at 4.0 mg/kg/day. NOEL = 2.0 mg/kg/day. Study acceptable.

2 yr dog chronic	00114034	1.8, 3.5 mg/kg/day	kidney epithelium vacuolation at mg/kg/day. Ver by RfD committ Study acceptable.
90 day mouse study	00127857	15 and 50 ppm known; other doses possible	hyperplasia / hyperkeratosis of gastric mucosa at 5 ppm. NOEL = 15 ppm Study acceptable (doses need verification)
13 wk rat toxicity	00047936	0, 1.5, 3.0, 10, 40 mg/kg/day	increased intracytoplasmic inclusion bodies i proximal convolute tubules of males. NOEL = 1.5 mg/kg/day. Study acceptable.
13 wk cell proliferation - male rat	44223002	175 mg/kg/day	increased mean labeling index in kidneys; degeneration of proximal convolute tubules and epithelial hyperplasia. Study acceptable
cell proliferation - male rat	44240901	0, 1.5, 15, 175 mg/kg/day	increased labelinc of cells of proxima convoluted tubule a 15 mg/kg/day on day 7, 14, and 21 of treatment. increase labeling of stoma tissue at 15 mg/kg/day on day 28

The above data show a consistency in the type of non-neoplastic response of the kidney and stomach among species. The Peer Review Committee recognized that although there were a relative lack of data on the mechanism of toxicity of chlorothalonil in the mouse (as opposed to the rat), the morphologic appearance of the kidney lesions was similar to that observed in the rat, and there were no data indicating a mechanism to the contrary of what had been proposed thus far concerning the nephrotoxicity of chlorothalonil. The committee also recognized that rats and humans possess a similar mechanism for generation of nephrotoxic metabolites from chlorothalonil, but that it is likely that quantitative differences exist between the two species. Mutagenicity data for chlorothalonil show weak genotoxic activity of the chemical, but do not discount an overall non-linear response. Based on these data, the HED / CPRC agreed that a non-linear approach using the MOE would best characterize the human risk

for renal tumors produced by exposure to chlorothalonil.

E. Other Data

1. Mutagenicity and Cell Proliferation

Chlorothalonil has been tested in a variety of mutagenicity assays examining mutagenic/genotoxic potential. In the Salmonella assay, chlorothalonil was non-mutagenic at concentrations up to 10,000 µg/plate with and without metabolic activation (MRID # 40122908). In 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 (MRID # 00127854, 00147946, 00147947, and 00147948). However, in an *in vivo* cytogenetics assay (MRID # 00147948), 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. A positive response was observed in CHO cells at a dose of 0.3 µg/ml in the non-activated portion of an *in vitro* cytogenetics assay (MRID # 40559103). As agreed from the second HED/CPRC meeting (document dated July 20, 1988), it cannot be said that chlorothalonil is devoid of genotoxic activity; however, it should be noted that these 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 toxicity, proliferation, and genotoxicity come into play. These data are consistent with a non-linear mode of action for this chemical.

2. Structure-Activity Relationships

A metabolite of chlorothalonil, 4-hydroxy-2,5,6-trichloroisophthalonitrile (or DS-3701), was tested for carcinogenicity in both rats and mice (EPA Accession #'s 071527, 072270, 072276, and 071531) and was found to be negative in both species. Although some conversion of chlorothalonil to DS-3701 occurs in the gut (HED document # 003802), an abstract on the stability of chlorothalonil dosing solutions (Pharmacologist 22:3, 1980) suggested that DS-3701 was a degradation product of chlorothalonil in the dosing solution.

Hexachlorobenzene, also structurally related to chlorothalonil, has been shown to cause increased incidence of hepatomas in mice and hamsters when fed at doses of 100 ppm and 50 ppm respectively.

Pentachlorophenol, also structurally related to chlorothalonil, has been classified by the EPA as a B2 (probable human carcinogen), based on combined incidence of hemangiosarcomas, liver tumors, and pheochromocytomas observed in female mice.

F. Weight of the Evidence

The weight of the evidence for carcinogenicity of chlorothalonil is based on a) Long term studies demonstrating tumors of the kidney in rats and mice; b) species sensitivity of the response; and c) evidence for a non-linear mode of action for tumor induction in the kidney.

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 likely to be 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 response occurs are 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 risk assessment be applied to the chlorothalonil cancer data for the kidney and forestomach. A Margin of Exposure (MOE) approach is recommended for the kidney and forestomach tumors. Since this tumor type is 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 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).

G. Classification of Carcinogenic Potential

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, the HED/CPRC unanimously agreed that the weight of the evidence supported a classifcation of chlorothalonil as "likely" 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 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, should be used.