MEMORANDUM
FEB 28 1996

SUBJECT: Carcinogenicity Peer Review of Chlorothalonil (3rd)

TO: Walter Waldrop / Andrew W. Ertman
   Product Manager #71
   Special Review and Reregistration Division (7508W)

FROM: Timothy F. McMahon, Ph.D.
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       Toxicology Branch II, Health Effects Division (7509C)

       and

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       Toxicologist, Review Section I
       Toxicology Branch II, Health Effects Division (7509C)

THRU: Yiannakis M. Ioannou, Ph.D.
       Section Head, Review Section I
       Toxicology Branch II, Health Effects Division (7509C)

       and

       Stephanie R. Irene, Ph.D.
       Acting Chief, Toxicology Branch II
       Health Effects Division (7509C)

Summary

The Health Effects Division Carcinogenicity Peer Review Committee (HED/CPRC) met on January 3, 1996 to discuss and re-evaluate the weight of the evidence on Chlorothalonil with particular reference to its carcinogenic potential, based on mechanistic and other data provided by the registrant. These data were not requested by the Agency, but were submitted by the registrant in support of a request for re-classification of the carcinogenicity of Chlorothalonil. The classification of Chlorothalonil prior to this meeting was a Group B2 - probable human carcinogen, with a recommendation that a low dose extrapolation model be applied to the animal data for quantitation of human risk ($Q_1^*$).
New data submitted by the registrant 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 basis for Chlorothalonil-induced forestomach and kidney tumors. The mechanistic data were submitted to the Agency in support of the hypothesis that the tumors observed in the forestomach and kidney from Chlorothalonil administration occur through non-genotoxic, threshold based mechanisms. In the stomach, it is proposed 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 is proposed that certain thiol metabolites of Chlorothalonil exert a toxic effect upon the kidney mitochondria, disrupting mitochondrial respiration with eventual cell death and compensatory proliferation that if sustained, could eventually lead to neoplasia.

The HED/CPRC evaluated the additional carcinogenicity data submitted for Chlorothalonil, as well as the mechanistic data in support of the registrant's hypotheses regarding the mechanism of induction of forestomach and kidney tumors. It was the Committee's determination that the mechanistic studies submitted in support of the re-classification request 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 mechanism(s) of renal tumor induction. Therefore, the HED/CPRC voted to retain the Group B2 classification for Chlorothalonil and the recommendation for the use of the linear low dose extrapolation for quantitation of human risk.

**Synopsis of Data Evaluated by the HED/CPRC**

Administration of Chlorothalonil in the diet to Osborne-Mendel rats for two years resulted in a significant increase in the incidence of renal adenomas and carcinomas combined for both sexes, and a significant trend for the incidence of combined renal tumors in female rats.

Administration of Chlorothalonil in the diet to Fischer 344 rats for 129 weeks resulted in a significant increase in the incidence of renal adenoma and carcinoma in male and female rats. Significant trends were observed in female rats for the incidence of gastric squamous mucosal papilloma and carcinoma combined.

In a second study conducted in Fischer 344 rats, administration of Chlorothalonil in the diet for 111 weeks (males) or 125 weeks (females) resulted in significant trends and pair-wise comparisons for increased incidence of kidney tubular adenomas, carcinomas, and adenomas and/or carcinomas combined, as well as stomach papillomas in male and female rats. A significant portion 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 the National Cancer Institute, dietary administration of Chlorothalonil resulted in no evidence of tumorigenicity.

In a carcinogenicity study in CD-1 mice, dietary administration of Chlorothalonil resulted in a significant trend 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 a second study in CD-1 mice, male mice receiving dietary Chlorothalonil showed increased incidences of tubular hyperplasia and karyomegaly. Squamous hyperplasia and hyperkeratosis of the forestomach were also observed at the mid dose and above. There was no evidence of induction of renal or gastric neoplasms from dietary administration of Chlorothalonil in this study.

Mechanistic studies submitted addressed the question of whether Chlorothalonil induces forestomach and renal tumors through a non-genotoxic mechanism. In the stomach, it is hypothesized 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 is proposed that certain thiol metabolites of Chlorothalonil exert a toxic effect upon the kidney mitochondria, disrupting mitochondrial respiration with eventual cell death and compensatory proliferation that if sustained, could eventually lead to neoplasia.

The HED/CPRC considered all of the above data in the re-classification request for Chlorothalonil. While the data were recognized as scientifically sound, it was also concluded that there were uncertainties with regard to the relationship between the toxicity of Chlorothalonil and the induction of tumors, particularly with regard to induction of renal tumors. Based on this and the above tumor data, the Group B2 classification of Chlorothalonil was retained.
A. Individuals in Attendance at the Peer Review Meeting:

1. Peer Review Committee: (signature indicates concurrence with the peer review unless otherwise stated).

Yiannakis M. Ioannou
William T. Burnam
Stephanie R. Irene
Karl P. Baetcke
Marion Copley

2. Reviewers: (Non-committee members responsible for data presentation; signature indicates technical accuracy of panel report).

Yiannakis M. Ioannou¹
Timothy F. McMahon¹
Alan Levy¹

¹Also a member of the CPSC for this chemical; signature indicates concurrence with the Peer Review unless otherwise stated.
B. Material Reviewed

The material available for review consists of previous peer review documents (dated September 4, 1987 and July 20, 1988), one liner, additional toxicology data (rat chronic toxicity / carcinogenicity study, dog chronic toxicity studies), and non-guideline mechanistic and histopathologic studies investigating the mechanism of Chlorothalonil-induced stomach and renal tumors. The additional data submitted were reviewed by Drs. Timothy McMahon and Alan Levy, Health Effects Division. Statistical analysis of the data from the rat chronic toxicity/carcinogenicity study was performed by Lori Brunsman, Science Analysis Branch.

C. BACKGROUND

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), is a fungicide registered for use on a wide variety of raw agricultural commodities (40 CFR §180.275). In addition, Chlorothalonil is also registered as a mildewcide in paints. The carcinogenicity of Chlorothalonil has been considered previously in two separate meetings of the Health Effects Division Carcinogenicity Peer Review Committee (May 28, 1987 and May 9, 1988). In both of these meetings, Chlorothalonil was classified as a Group B2 carcinogen (probable human carcinogen), based upon the observations of increased incidence of renal tubular adenomas and carcinomas in rats and mice as well as increased incidence of forestomach tumors in rats and mice. The registrant (ISK Biotech Corporation) has submitted additional toxicology and metabolism data in support of a request for the re-classification of Chlorothalonil's carcinogenic potential. The purpose of the present document is to: 1) recapitulate previous data on carcinogenicity of Chlorothalonil; 2) present the additional data in support of the re-classification request; and 3) present the Agency's interpretation of these additional data as related to the re-classification request.
Structure of Chlorothalonil

\[
\begin{array}{c}
\text{CN} \\
\text{Cl} \\
\text{Cl} \\
\text{Cl} \\
\text{Cl} \\
\text{CN} \\
\end{array}
\]

Chlorothalonil

D. Evaluation of Carcinogenicity Data

i. First Peer Review

The Toxicology Branch Peer Review Committee met on May 28, 1987 to discuss and evaluate the weight of the evidence on Chlorothalonil with particular reference to its carcinogenic potential.

a. Individuals in Attendance

1. Peer Review Committee: Theodore M. Farber, Ph.D.; William M. Burnam; Reto Engler, Ph.D.; Robert Beliles, Ph.D.; Richard Levy, M.S.; Judith Hauswirth, Ph.D.; Esther Rinde, Ph.D.

2. Reviewers: David Ritter, Ph.D. (toxicology data review); H. Lacayo and B. Fisher (statistical analyses).

b. Material Reviewed

The material available for review consisted of DER's, one-liners, and other data summaries prepared by David Ritter. Tables and statistical data analyses for the mouse and rat studies were provided by H. Lacayo [Memo, 5/17/85] and B. Fisher [Memo, 7/20/87].
1. NCI Rat Oncogenicity Study

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

Chlorothalonil (98.5% pure) was administered in the diet to groups of 50 male and 50 female Osborne-Mendel rats at 5,063 or 10,126 ppm (TWA) for 2 years. Renal tubular epithelial adenomas and carcinomas were found in treated animals after 80 weeks dietary exposure; no neoplasms were reported for concurrent controls. This study was rated "supplemental" by the Toxicology Branch, based on the usual deficiencies in NCI protocols. The tumor incidences are presented in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Incidence of Renal Neoplasms (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control 5,063 ppm</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>M 0/10</td>
</tr>
<tr>
<td></td>
<td>F 0/10</td>
</tr>
<tr>
<td>Adenoma</td>
<td>M 0/10</td>
</tr>
<tr>
<td></td>
<td>F 0/10</td>
</tr>
<tr>
<td>Combined</td>
<td>M 0/10 (0)</td>
</tr>
<tr>
<td></td>
<td>F 0/10 (0)</td>
</tr>
</tbody>
</table>

The statistical analysis of the renal tumors in this study showed that at the high dose, there was a statistically significant increase in the incidence of renal adenomas and carcinomas combined for both male and female rats (males, \( p = 0.028 \); females, \( p = 0.016 \)). In addition, a significant trend was observed for combined incidence of renal tumors in females (\( p = 0.007 \)). According to the first Peer Review document, historical control incidence for renal neoplasms combined was 3/240 [1.25%] for male rats, and 0/235 [0%] for female rats.

2. IRDC Rat Oncogenicity Study

Reference: IRDC Tumorigenicity Study in Rats, Study # 099-5TX-80-234-008, Accession # 258759, 5/28/85.

Chlorothalonil (98.1% pure) was fed in the diet to Fischer 344 rats, 60 per sex per group, at 0, 800, 1600 or 3500 ppm (0.40, 80 and 175 mg/kg/day, respectively) for 129 weeks. Renal tumors of epithelial origin (adenoma and carcinoma) were found in treated rats, but not in concurrent female controls. Incidences of these lesions are given in Table 2. Incidences of papillomas and carcinomas of the squamous epithelium of the forestomach are given in Table 3; concurrent female controls had no gastric neoplasms.
Table 2
Chlorothalonil - IRDC Fischer 344 Rat Study
Incidence (%) of Renal Tumors

<table>
<thead>
<tr>
<th></th>
<th>Dose (mg/kg/day)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>40</td>
<td>80</td>
<td>175</td>
</tr>
<tr>
<td>Renal Tumor Incidence¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1/66 (2) *</td>
<td>3/61 (5)</td>
<td>1/60 (2)</td>
<td>6/60 (10) *</td>
</tr>
<tr>
<td>Adenoma²</td>
<td>0/66 (0) **</td>
<td>2/61 (3)</td>
<td>5/60 (8) *</td>
<td>12/60 (20) **</td>
</tr>
<tr>
<td>Combined</td>
<td>1/66 (2) **</td>
<td>5/61 (8)</td>
<td>6/60 (10) *</td>
<td>18/60 (30) **</td>
</tr>
<tr>
<td>B. Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0/60 (0) **</td>
<td>1/60 (2)</td>
<td>3/61 (5)</td>
<td>12/59 (20) **</td>
</tr>
<tr>
<td>Adenoma²</td>
<td>0/60 (0) **</td>
<td>1/60 (2)</td>
<td>4/61 (7)</td>
<td>7/59 (12) **</td>
</tr>
<tr>
<td>Combined</td>
<td>0/60 (0) **</td>
<td>2/60 (3)</td>
<td>7/61 (11) **</td>
<td>19/59 (32) **</td>
</tr>
</tbody>
</table>

¹Number of tumor bearing animals / number of animals examined
²does not include animals with carcinoma
* p < 0.05; ** p < 0.01; significance of trend denoted at control.

As the above data show, a significant increase in incidence of renal adenoma and carcinoma was observed in both male and female rats at the high dose of Chlorothalonil, with a significant trend noted at control. In addition, a significant increase in incidence of renal adenoma and carcinoma combined was noted at the mid dose for both male and female rats. Male rats also showed a significant increase in renal adenoma incidence at the mid dose.
Table 3
Chlorothalonil - IRDC Fischer 344 Rat Study
Incidence (%) of Forestomach Tumors (Gastric Squamous Mucosa)

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>40</th>
<th>80</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma (%)</td>
<td>1/66</td>
<td>0/60</td>
<td>0/60</td>
<td>1/60</td>
</tr>
<tr>
<td>(2)</td>
<td>(0)</td>
<td>(0)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td><strong>B. Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma (%)</td>
<td>0/60</td>
<td>0/60</td>
<td>1/61</td>
<td>1/59</td>
</tr>
<tr>
<td>(0)</td>
<td>(0)</td>
<td>(2)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>Papilloma&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0/60</td>
<td>1/60</td>
<td>2/61</td>
<td>2/59</td>
</tr>
<tr>
<td>(0)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Combined %</td>
<td>0/60*</td>
<td>1/60</td>
<td>3/61</td>
<td>3/59</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.
<sup>1</sup>Number of tumor bearing animals / number of animals examined
<sup>2</sup>Does not include animals with carcinoma

As the above data on forestomach tumors indicate, the only significant observation was that of a significant trend in female rats for the incidence of gastric squamous mucosal papilloma and carcinoma combined.
3. NCI Mouse Oncogenicity Study

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

Chlorothalonil was administered in the diet to groups of 50 male and 50 female B6C3F1 mice at 10,000 or 20,000 ppm (nominal dosage) for 91-92 weeks. No significant increase in tumor incidence was found in treated mice.

4. SDS Biotech Mouse Study

Reference: Biodynamics Laboratory, East Millstone, NJ, Study # DTX-79-0102, Accession # 071541, 1979.

Chlorothalonil technical (97.7%) was fed 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, respectively) for 2 years. Renal tubular adenomas and carcinomas and gastric mucosal squamous and glandular carcinomas were increased in males, but not in females; no tumors were reported for concurrent controls of either sex. Tumor incidences and accompanying statistical analysis are given in Table 4.

<table>
<thead>
<tr>
<th>Renal Tumor Incidence</th>
<th>0</th>
<th>107</th>
<th>214</th>
<th>428</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>0/57 (0)</td>
<td>3/59 (5)</td>
<td>4/59 (7)</td>
<td>2/56 (4)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0/57 (0)</td>
<td>3/59 (5)</td>
<td>0/59 (0)</td>
<td>2/56 (4)</td>
</tr>
<tr>
<td>Combined</td>
<td>0/57 (0)**</td>
<td>6/59 (10)</td>
<td>4/59 (7)</td>
<td>4/56 (7)</td>
</tr>
</tbody>
</table>
Table 4, continued

<table>
<thead>
<tr>
<th>Stomach Tumor Incidence</th>
<th>Dose (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

A. Males

<p>| | | | | |</p>
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<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Squamous Cell</td>
<td>0/55</td>
<td>2/59 (3)</td>
<td>5/59 (9)*</td>
<td>1/51 (2)</td>
</tr>
<tr>
<td>Glandular</td>
<td>0/55</td>
<td>1/59 (2)</td>
<td>2/59 (3)</td>
<td>0/51 (0)</td>
</tr>
</tbody>
</table>

B. Females

<p>| | | | | |</p>
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<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous Cell</td>
<td>0/57*</td>
<td>2/60 (3)</td>
<td>6/58 (10)*</td>
<td>5/58 (9)*</td>
</tr>
<tr>
<td>Glandular</td>
<td>0/57</td>
<td>1/60 (2)</td>
<td>1/58 (2)</td>
<td>2/56 (4)</td>
</tr>
</tbody>
</table>

1 number of tumor bearing animals / number of animals examined
2 does not include animals with carcinoma
* p < 0.05; ** p < 0.01; significance of trend denoted at control.

The above data show that for renal tumors, a significant trend was observed for the incidence of combined renal adenoma and carcinoma in male mice only. With regard to stomach tumors, the data indicated that for male mice, a significant increase in the incidence of squamous cell carcinoma was observed at the mid dose; for female mice, a significant increase in incidence of squamous cell carcinoma at the mid and high dose was observed, as was a significant trend at control. It is noted that the incidence for squamous cell carcinoma in female mice was the same at the mid and high dose.

With regard to the IRDC Fischer 344 rat study and the SDS Biotech CD-1 Mouse study, renal tumors observed 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. These observations indicate that tumor formation is observed at doses which also cause significant non-neoplastic effects.
c. Classification of Carcinogenic Potential from the 5/28/87 Meeting:

The Peer Review Committee concluded that Chlorothalonil should be classified as Group B2 (Probable Human Carcinogen) based on increased incidences of malignant and/or combined malignant and benign tumors (both sexes) in 2 rat studies and in the mouse study, as follows:

- In Fischer 344 rats, statistically significant increases in the incidence of renal adenomas and carcinomas in both sexes, and a dose-related increase in papillomas of the forestomach in female rats;

- In an NCI study with Osborne Mendel rats, a statistically significant increase in combined renal adenoma/carcinoma in both sexes, which the Committee considered as part of the weight of evidence, despite deficiencies in protocol;

- In CD-1 mice, a statistically significant increase in the incidence of carcinoma of the forestomach in both sexes, with a positive dose-related trend in females. In addition, there was a positive dose-related trend for combined renal adenoma/carcinoma in male mice, which the Committee considered significant because of their rareness, and because renal tumors of the same type and location were seen in the adequate rat study.

Based on the female F344 rat renal tumors (carcinomas and adenomas) the potency (\(Q_1^*\)) of Chlorothalonil was estimated as \(1.1\times10^{-2} \text{ (mg/kg/day)}^{-1}\) in human equivalents [B. Fisher, 7/20/87].
d. Toxicology Issues Reviewed in the 5/28/87 Meeting:

In the IRDC Fischer 344 rat study, the adequacy of dosing for carcinogenic potential appeared to have been exceeded for males at the highest dose tested (3500 ppm), based on reduced survival after 27 months compared to concurrent controls. For female rats, the 3500 ppm dose was felt to be an adequate dose for assessment of carcinogenic potential.

In the SDS Biotech CD-1 mouse study, the adequacy of dosing for carcinogenic potential appeared to have been exceeded at 1500 ppm (high dose), based on decreased survival in male mice.

It was mentioned in the peer review document that nephrotoxicity leading to possible tumor formation through formation of thiol metabolites in the kidney could be a mechanism of Chlorothalonil induced renal tumorigenesis.

ii. Second Peer Review

The Peer Review Committee met on May 9, 1988 to examine the issues raised by the Science Advisory Panel (SAP) with respect to the classification of carcinogenicity for Chlorothalonil.

a. Individuals in Attendance:

1. Peer Review Committee: Theodore Farber, Ph.D.; William Burnam; Robert Beliles, Ph.D.; Lynnard Slaughter, Ph.D.; Judith Hauswirth, Ph.D.; Richard Levy, M.S.; Kerry Dearfield, Ph.D.; Esther Rinde, Ph.D.

2. Reviewers: David Ritter, Ph.D.; Bruce Jaeger.

b. Material Reviewed

The SAP Panel response (10/1/87); Peer Review Memo (9/4/87) Toxicology Chapter of the Registration Standard (2/24/88); Reviewer's summaries of additional data (Memo, D. Ritter to L. Rossi, 4/7/88 and attached DERs); Reviewer's memo and DER for interim report of a 2-year feeding study in F344 rats (Memo to L. Rossi, 6/7/88 and DER, 6/9/88)
The carcinogenicity studies presented in the first Peer Review Document were presented to the Science Advisory Panel. The SAP did not comment specifically on the Agency evaluation and classification of Chlorothalonil, although they did agree that the renal tumors in the CD-1 male mouse were biologically significant at concentrations below the maximum tolerated dose. The Panel expressed concern regarding additional data which had not been reviewed at the time of the Peer Review.

Additional data were reviewed and presented in the second Peer Review Document. These data included interim reports (after 1 year) for the following 2 studies:

1. A 2-year dietary feeding study (0, 2, 4, 15 or 175 mg/kg/day Chlorothalonil) in Fischer 344 rats, which the Registrant is conducting to determine the no-effect level for "potentially preneoplastic and tumorigenic effects in the kidney and forestomach". The interim findings included hyperplasia and karyomegaly of the renal cortex in males at 4, 15 and 175 mg/kg/d, and in females at 175 mg/kg/d; and squamous epithelial hyperplasia and hyperkeratosis of the gastric mucosa in both sexes at 15 and 175 mg/kg/d.

2. A 2-year dietary feeding study (0, 10, 40, 175 or 750 ppm; 0, 1.42, 5.71, 25, and 107.1 mg/kg/day Chlorothalonil) in Charles River CD-1 male mice also reports a slight increase in renal tubular hyperplasia at 175 ppm, and hyperplasia and hyperkeratosis of the squamous mucosa of the forestomach at 750 ppm.

The registrant stated that with respect to the forestomach lesions, these “result from the locally irritating effects of Chlorothalonil itself” [SAP Transcript 9/23/87, pg. 73]. However, it was pointed out by Dr. Slaughter that hyperplasia and/or hyperkeratosis could be caused by factors other than local irritation, such as decreased Vitamin A intake. Dr. Hauswirth also offered that she is aware of some chemical carcinogens which are known to deplete hepatic storage of Vitamin A.

The Committee also discussed the mutagenicity data for Chlorothalonil, in light of the Registrant's claim (and the Panel's statement) that Chlorothalonil is not genotoxic. The in vitro data included a positive mouse lymphoma assay [as reported by NTP, Annual Report, 1986]; a positive CHO aberrations and positive CHO Sister Chromatid Exchange assays [Galloway,S. et al.: Environ. Molecular Mutagen 10:1-175, 1987]; and a positive CHO Aberration assay (data submitted to the Agency - in Peer review file). These results indicate that Chlorothalonil has at least weak clastogenic activity. Most in vivo studies for clastogenic activity appear negative after 1 or 2 doses; however, after 5 consecutive doses (over 5 days), there was a weak clastogenic response [Ibid]. (It was also pointed out that the
protocol for the three submitted micronucleus assays, which were acceptable by standards used in the late 1970's, are unacceptable based on current guidelines).

The Committee agreed that based on the above data, it cannot be said that Chlorothalonil is devoid of genotoxic activity; however, it should be noted that these are all weak responses.

c. Classification of Carcinogenic Potential from the 5/9/88 Meeting:

The review of the supplemental data did not provide a basis for either increasing or decreasing the initial classification (B2) for Chlorothalonil which was based on: malignant and/or combined malignant and benign tumors (both sexes) in 2 strains of the rat and in the CD-1 mouse.

The Peer Review Committee concluded, on consideration of all of the available data for Chlorothalonil, that the evidence satisfies the criteria contained in the EPA Guidelines [FR51:33992-34003, 1986] for sufficient evidence, and reaffirmed its classification of Chlorothalonil as a Group B2 (Probable Human Carcinogen).

E. Additional Toxicology Data

i. Carcinogenicity in Mice


In a mouse carcinogenicity study, groups of Charles River CD-1 male mice (60 per group) were administered diets containing technical Chlorothalonil at doses of 0, 10, 40, 175, or 750 ppm (1.42, 5.71, 25, and 107.1 mg/kg/day nominal) for two years. Chlorothalonil induced tubular hyperplasia and karyomegaly in the 175 and 750 ppm dose groups. Animals in the 750 ppm dose group also showed an increased incidence of tubular hypertrophy. Squamous hyperplasia and hyperkeratosis of the forestomach were also increased at 40 ppm and above. There was no evidence of induction of renal or gastric neoplasms from dietary administration of Chlorothalonil in this study. The systemic LEL in this study was determined to be 175 ppm, based upon renal tubular hyperplasia. The systemic NOEL was determined to be 40 ppm.

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 750 ppm and below.
ii. Carcinogenicity in Rats


This study was performed in response to review of a previous 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 dietary levels of 0, 2, 4, 15, and 175 mg/kg/day Chlorothalonil (98.3% a.i.) 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 and/or carcinomas combined as well as stomach papillomas in male and female rats at the 175 mg/kg/day dose level. In male rats, additional evidence of tumorigenicity was observed in the form of a significant trend and pair-wise comparison in the incidence of kidney tubular carcinomas at 175 mg/kg/day. 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. At 4 mg/kg/day, stomach squamous cell papillomas were observed in increased incidence, and this difference was statistically significant.

In female rats, a significant trend and pair-wise comparison was observed for the incidence of stomach squamous cell papillomas and papillomas and/or carcinomas combined at 175 mg/kg/day vs control. There was also a significant trend for stomach squamous cell carcinoma in female rats.
Table 3. Chlorothalonil - Fischer 344 Rat Study

**Male Kidney Tubular Tumor Rates** and Peto's Prevalence Test Results (p values)

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>15</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma (%)</td>
<td>1/54</td>
<td>1/54</td>
<td>1/54</td>
<td>3/53</td>
<td>17a/54</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>0.102</td>
<td>0.000**</td>
</tr>
<tr>
<td>Carcinoma (%)</td>
<td>0/54</td>
<td>0/54</td>
<td>0/50</td>
<td>1/52</td>
<td>7b/48</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>0.109</td>
<td>0.011*</td>
</tr>
<tr>
<td>Combined (%)</td>
<td>1/54</td>
<td>1/54</td>
<td>1/54</td>
<td>4/53</td>
<td>23c/54</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>0.045*</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

aFirst adenoma observed at week 71, dose 175 mg/kg/day.
bFirst carcinoma observed at week 83, dose 175 mg/kg/day.
cOne animal in the 175 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One animal in the 175 mg/kg/day dose group of the interim sacrifice group had an adenoma.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level. If *, then p < 0.05. If **, then p < 0.01.
Table 4. Chlorothalonil - Fischer 344 Rat Study

**Male** Stomach Squamous Cell Tumor Rates\(^{+}\) and Peto's Prevalence Test Results (p values)

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>15</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma (%)</td>
<td>0/54</td>
<td>0/54</td>
<td>3/49</td>
<td>2/52</td>
<td>5(^{a}/46)</td>
</tr>
<tr>
<td>P</td>
<td>0.000(^{**})</td>
<td>-</td>
<td>0.042(^{*})</td>
<td>0.076</td>
<td>0.001(^{**})</td>
</tr>
</tbody>
</table>

\(^{+}\)Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

\(^{a}\)First papilloma observed at week 85, dose 175 mg/kg/day.

Note: Interim sacrifice animals are not included in this analysis.
   No animals in the interim sacrifice group had papillomas.
   Significance of trend denoted at control.
   Significance of pair-wise comparison with control denoted at dose level.
   If \(^{*}\), then p < 0.05. If \(^{**}\), then p < 0.01.
Table 5. Chlorothalonil - Fischer 344 Rat Study

Female Kidney Tubular Tumor Rates\(^*\) and
Peto's Prevalence Test Results (p values)

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>15</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma (%)</td>
<td>0/54</td>
<td>0/53</td>
<td>0/51</td>
<td>0/52</td>
<td>24(^a)/53</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000**</td>
</tr>
<tr>
<td>Carcinoma (%)</td>
<td>0/39</td>
<td>0/39</td>
<td>0/33</td>
<td>0/35</td>
<td>11(^b)/35</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000**</td>
</tr>
<tr>
<td>Combined (%)</td>
<td>0/54</td>
<td>0/53</td>
<td>0/51</td>
<td>0/52</td>
<td>32(^c)/53</td>
</tr>
<tr>
<td>p =</td>
<td>0.000**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

\(^*\)Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

\(^a\)First adenoma observed at week 79, dose 175 mg/kg/day.

\(^b\)First carcinoma observed at week 111, dose 175 mg/kg/day.

\(^c\)Three animals in the 175 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. No animals in the interim sacrifice group had adenomas or carcinomas.

Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then \( p < 0.05 \). If **, then \( p < 0.01 \).
Table 6. Chlorothalonil - Fischer 344 Rat Study

Female Stomach Squamous Cell Tumor Rates* and Peto's Prevalence Test Results (p values)

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>15</th>
<th>175</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papilloma (%)</td>
<td>1/44</td>
<td>1/45</td>
<td>2/47</td>
<td>4/41</td>
<td>7^a/46</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(10)</td>
<td>(15)</td>
</tr>
<tr>
<td>p =</td>
<td>0.011*</td>
<td>0.404</td>
<td>0.168</td>
<td>0.095</td>
<td>0.020*</td>
</tr>
<tr>
<td>Carcinoma (%)</td>
<td>1/54</td>
<td>0/53</td>
<td>0/52</td>
<td>1/52</td>
<td>3^b/54</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(0)</td>
<td>(0)</td>
<td>(2)</td>
<td>(6)</td>
</tr>
<tr>
<td>p =</td>
<td>0.007**</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.094</td>
</tr>
<tr>
<td>Combined (%)</td>
<td>1^c/54</td>
<td>1/53</td>
<td>2/52</td>
<td>5/52</td>
<td>9^c/54</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
<td>(4)</td>
<td>(10)</td>
<td>(17)</td>
</tr>
<tr>
<td>p =</td>
<td>0.001**</td>
<td>0.404</td>
<td>0.168</td>
<td>0.060</td>
<td>0.003**</td>
</tr>
</tbody>
</table>

*Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^aFirst papilloma observed at week 99, dose 175 mg/kg/day.

^bFirst carcinoma observed at week 75, dose 175 mg/kg/day.

^cOne animal in each of the 0 and 175 mg/kg/day dose groups had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. No animals in the interim sacrifice group had papillomas or carcinomas.

Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.
Examination of non-neoplastic effects in this study yielded the following observations:

1) With increasing dose of Chlorothalonil, a significant increasing trend for mortality was observed in both male and female rats. At the 175 mg/kg/day dose level, survival in both sexes was significantly reduced when the study period as a whole was considered. The majority of deaths at the 175 mg/kg/day dose occurred between weeks 79 and the termination of the study in both sexes. The percent mortality for male rats (80%) and female rats (82%) appears to have slightly exceeded the guideline limit at this dose.

2) At the one year interim sacrifice, increased incidence of 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 above, and increased incidence of karyomegaly was observed at 175 mg/kg/day. In female rats at the interim sacrifice, increased incidence of these kidney lesions was observed only at the 175 mg/kg/day dose level.

3) At study termination, increased incidences of renal tubular epithelial cell hyperplasia and clear cell hyperplasia were observed in male and female rats at 175 mg/kg/day. A significant portion of the rats observed with renal adenoma and/or carcinoma at this dose was also observed with tubular cell hyperplasia (HED document # 008901, review by David S. Liem, Ph.D, dated 9/5/91).

4) At the 4.0 mg/kg/day dose level and above, hyperplasia and hyperkeratosis of the forestomach were observed in increased incidence in both male and female rats at the terminal exam. At the interim sacrifice, these lesions of the forestomach were observed at the 15 mg/kg/day dose level and above. The observation that these lesions were observed in increased incidence at lower dose levels as the length of treatment increased supports the hypothesis of chronic irritation of the forestomach by Chlorothalonil.

5) Other non-neoplastic effects observed in this study included:

   a. At 175 mg/kg/day: significant alterations in clinical chemistry; decreased body weight and body weight gain; significantly increased kidney and liver weight.

   b. At 15.0 mg/kg/day: significantly increased kidney weight in female rats; increased urea nitrogen in male rats.

   c. At 4.0 mg/kg/day: increased kidney weight in male rats.

Based on these observations, the systemic NOEL was determined to be 2.0 mg/kg/day for male and female rats, and the systemic LEL was determined to be 4.0 mg/kg/day. The 175 mg/kg/day dose was determined to be adequate for assessment of carcinogenic potential (HED document # 008901, memorandum from Alan C. Levy, Ph.D. to Cynthia Giles-Parker), although it is noted that survival at this dose was slightly below the guideline limit.
iii. Chronic Toxicity in Dogs

One Year Toxicity Study


In a chronic toxicity study (MRID No. 43653603), Chlorothalonil (98.3% purity) was administered orally by gelatin capsules to Marshall beagle dogs (5/sex/group) at doses of 0 (empty capsules), 15, 150 and 500 mg/kg/day for 52 weeks.

There were decreases in body weight gains in males and females at 500 mg/kg/day. Alanine aminotransferase levels were reduced about 90% in all dosed animals compared with controls as well as with pretreatment levels. There were no effects on the following parameters: mortality, clinical signs, food consumption, ophthalmology, hematology, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

The Systemic Toxicity NOEL = 150 mg/kg/day
The Systemic Toxicity LOEL = 500 mg/kg/day, based on decreased body weight gains in both sexes

This study is Acceptable and satisfies the data requirement (§83-1) for a chronic toxicity study in dogs.
Two Year Toxicity Study


In a 2-year chronic toxicity study, Chlorothalonil was administered by dietary admix to beagle dogs (8/sex/group with 4/sex/group scheduled for a 52-week interim sacrifice) at doses of 0 (control), 60 (1.8 mg/kg/day) and 120 (3.5 mg/kg/day) ppm. [MRID No. 00114034]

There was an increase in kidney vacuolated epithelium in 120 ppm males only, and only at the 12-month sacrifice.

The NOEL was 60 ppm (1.8 mg/kg/day)
The LOEL was 120 ppm (3.5 mg/kg/day) based on kidney vacuolated epithelium.

Core classification is Minimum. This study satisfies the data requirement (§83-1) for a chronic toxicity study in dogs.

This study was conducted during the period of 1/25/68 and 1/27/70.
iv. Mechanistic Studies

The chronic oral administration of Chlorothalonil to rats and mice has been shown to result in development of forestomach and kidney tumors. The registrant has submitted data to the Agency in support of the hypothesis that the tumors observed in the forestomach and kidney from Chlorothalonil administration occur through non-genotoxic, threshold based mechanisms. In the stomach, it is 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 is proposed that certain thiol metabolites of Chlorothalonil exert a toxic effect upon the kidney mitochondria, disrupting mitochondrial respiration with eventual cell death and compensatory proliferation that if sustained, could eventually lead to neoplasia.

Chlorothalonil-Induced Forestomach Tumors

The registrant presented the following with regard to Chlorothalonil-induced stomach tumors:

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 Chlorothalonil administration 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.

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

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

2) The effect in the forestomach is threshold-based.

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

4) 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 stored in the forestomach before it moves into the glandular stomach.
Studies in dogs exposed to 750 mg/kg/day Chlorothalonil chronically resulted in moderate to severe gastritis but no evidence of epithelial cell hyperplasia or neoplasia.

Chlorothalonil-Induced Renal Tumors

The registrant proposes a non-genotoxic mechanism for induction of rodent kidney tumors from Chlorothalonil. As the proposed mechanism is complex, the summary below has been broken down into a series of steps to better understand the mechanism proposed.

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

2) Following metabolism to 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 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 E-lyase. It is noted that these reactions 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 liver and pass into blood 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 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 metabolized to the cysteine-S-conjugates by gamma glutamyl transpeptidase. Mercapturic acids can also enter, be de-acetylated, and undergo bioactivation by E-lyase.

5) The "activation" of pre-mercapturic acids to nephrotoxic thiols occurs through the action of cysteine conjugate E-lyase, an enzyme found in the cytosol and mitochondria of cells of the renal proximal tubule. That the action of E-lyase is a critical step comes from work demonstrating that aminooxyacetic acid, an inhibitor of E-lyase, blocks the in vivo toxicity of S-cysteine conjugates.
6) While there is still speculation as to why the cellular damage by cysteine-S-conjugates is restricted to the $S_3$ 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 (MRID # 436536-09).

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 # 436536-08).

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 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 # 436536-11). The rate of absorption and total dose absorbed of Chlorothalonil appears similar between rats and dogs (MRID # 436536-12), but in the dog, there is less conversion to the di- and tri-methyl thio analogs of Chlorothalonil (MRID # 436536-12), which is supportive of the higher NOEL for renal toxicity in the dog vs. the rat. This difference between rats and dogs may account in part for the observed difference in renal toxicity of Chlorothalonil to rats and dogs.

9) With respect to humans, there are very limited data on the relative sensitivity to nephrotoxicity of thiol metabolites of cysteine-S-conjugates. Literature data were cited (MRID # 436536-05 and 436536-06) indicating that the activity of gamma-glutamyl transpeptidase in rat kidney is approximately 10 times greater than human. In addition, it was noted that the amount of B-lyase in human kidney is only about 10% of that in the rat kidney on a per gram basis. Based on these data, the hypothesis is put forth that more cysteine-S-conjugates are generated in the rat than human. In addition, the
kidney-to-liver ratio of gamma-glutamyl transpeptidase in the rat is 142, vs less than 5 in human. This would shift cytoxicity possibly from kidney to liver. However, it is stated that any cysteine-S-conjugates formed in the liver are likely rapidly acetylated to mercapturates and any thiols produced can be metabolized to S-methyl derivatives.

10) While the above data suggest that the human kidney may be less susceptible to the nephrotoxic effects of Chlorothalonil, this conclusion may be premature. Dr. Thomas Jones of the Biochemical Pharmacology Department at Eli Lilly Corporation was consulted by telephone regarding the proposed hypothesis that humans would be less susceptible to the nephrotoxic effects of Chlorothalonil than rats. Dr. Jones has conducted extensive research on the toxicity of cysteine conjugates and the actions of the enzymes involved in their activation to nephrotoxic metabolites, and is considered an expert in this field. While he acknowledges that the human data cited in the present submission (as related to him by the reviewer) are correct, it is his opinion that such data are misleading, and that one needs to know the activity in the target cell before one can start to draw conclusions regarding relative susceptibility. The human tissue samples used consist of a mixture of cortical and medullary elements, and the source of the tissue raises questions as to the influence of various disease states and other variables which may have been present.

Conclusions of Mechanistic Data Review

Chlorothalonil is currently listed as a Group B2 carcinogen, with a $q_1^*$ value of 0.00766 (mg/kg/day)$^{-1}$, based on the increased incidence of renal tumors in female rats observed in a two-year study. 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 the 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 the 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 Chlorothalonil to nephrotoxic metabolites within the kidney itself. Data presented in support of human susceptibility are inconclusive, as noted above. Questions regarding relative absorption of
Chlorothalonil in humans, delivered dose to the kidney, and relative susceptibility are unanswered at this time. Based on this, the Agency does not concur with the registrant's hypothesis regarding the relative susceptibility of humans to Chlorothalonil induced renal toxicity at this time.

F. Structure-Activity Considerations

4-hydroxy-2,5,6-trichloroisophthalonitrile

In the first Peer Review Document, the only analog listed was 4-hydroxy-2,5,6-trichloroisophthalonitrile (DS-3701), mentioned as the major metabolite in rats and found in meat and milk. This metabolite has been tested for carcinogenicity in both rats and mice (Accession numbers 071527, 072270, 072276, and 071531) and has been found to be negative for carcinogenicity in both species. Previous metabolism studies (HED document # 003802) indicated that oral administration of Chlorothalonil results in conversion of approximately 5% of the administered dose to DS-3701 in the gut, and approximately 0.6% in urine. However, published data on the stability of Chlorothalonil dosing solutions (Pharmacologist, 22:3, 1980) demonstrated that DS-3701 was a product of degradation of Chlorothalonil in the dosing solution. Therefore, this metabolite may be, in part, an artefact rather than a true metabolite.

Hexachlorobenzene

The Agency is in possession of older carcinogenicity studies on hexachlorobenzene in mice and hamsters. The acceptability of these studies has not been ascertained; the available data indicate an increased incidence of hepatomas when fed in the diet at 100 ppm to mice and 50 ppm to hamsters.

Pentachlorophenol

The Agency has published a registration standard on pentachlorophenol (1986). In this document (HED document # 007561), three separate carcinogenicity studies in mice indicated no significant increase in tumor incidence over control at doses up to 30 mg/kg/day.
G. Weight-of-Evidence Considerations

The Health Effects Division Carcinogenicity Peer Review Committee is asked to consider the following toxicology data in determining the carcinogenic potential of Chlorothalonil:

1) In a two-year carcinogenicity study conducted in Osborn-Mendel rats by the National Cancer Institute, dietary Chlorothalonil at a dose of 10,126 ppm resulted in a statistically significant increase in the incidence of renal adenomas and carcinomas combined for both male and female rats (males, \( p = 0.028 \); females, \( p = 0.007 \)), and a significant trend was observed for combined incidence of renal tumors in females (\( p = 0.007 \)).

2) In a 129 week study conducted in Fischer 344 rats by IRDC, dietary Chlorothalonil at 175 mg/kg/day resulted in a significant increase in incidence of renal adenoma and carcinoma in both male and female rats, with a significant trend noted at control. At a dose of 80 mg/kg/day, Chlorothalonil resulted in a significant increase in incidence of renal adenoma and carcinoma combined for both male and female rats. Male rats also showed a significant increase in renal adenoma incidence at 80 mg/kg/day. With regard to stomach tumors, the only significant observation in this study was that of a significant trend in female rats for the incidence of gastric squamous mucosal papilloma and carcinoma combined.

3) In a carcinogenicity study in B6C3F1 mice conducted by the National Cancer Institute, dietary administration of Chlorothalonil for 91-92 weeks at doses up to and including 20,000 ppm resulted in no evidence of tumorigenicity.

4) In a carcinogenicity study conducted in CD-1 mice by SDS Biotech, dietary administration of Chlorothalonil at doses of 0, 107, 214 and 428 mg/kg/day to CD-1 mice for 2 years resulted in a significant trend for the incidence of combined renal adenoma and carcinoma in male mice only. With regard to stomach tumors, the data indicated that for male mice, a significant increase in the incidence of squamous cell carcinoma was observed at 214 mg/kg/day; for female mice, a significant increase in incidence of squamous cell carcinoma at the 214 and 428 mg/kg/day dose levels was observed, as was a significant trend at control. It is noted that the incidence for squamous cell carcinoma in female mice was the same at the 214 and 428 mg/kg/day dose levels.

5) In the IRDC Fischer 344 rat study and the SDS Biotech CD-1 Mouse study, renal tumors observed 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. These observations indicate that tumor formation is observed at doses which also cause significant non-neoplastic effects.
6) In the second Peer Review of Chlorothalonil, the carcinogenicity studies presented in the first Peer Review Document were presented to the Science Advisory Panel. The SAP did not comment specifically on the Agency evaluation and classification of Chlorothalonil, although they did agree that the renal tumors in the CD-1 male mouse were biologically significant at concentrations below the maximum tolerated dose. With regard to the mutagenicity database on Chlorothalonil, the Peer Review Committee discussed the opinion of the SAP that the mode of action of tumor induction by Chlorothalonil was non-genotoxic. The committee agreed that based on the available data, it cannot be said that Chlorothalonil is devoid of genotoxic activity; however, it should be noted that all the positive responses are weak responses. The Peer Review Committee voted to retain the B2 classification of Chlorothalonil at this second meeting.

7) In a two-year carcinogenicity study conducted by IRDC, groups of Charles River CD-1 male mice (60 per group) were administered diets containing technical Chlorothalonil at doses of 0, 10, 40, 175, or 750 ppm (1.42, 5.71, 25, and 107.1 mg/kg/day) for two years. Chlorothalonil induced tubular hyperplasia and karyomegaly in the 175 and 750 ppm dose groups. Animals in the 750 ppm dose group also showed an increased incidence of tubular hypertrophy. Squamous hyperplasia and hyperkeratosis of the forestomach was also increased at 40ppm and above. There was no evidence of induction of renal or gastric neoplasms from dietary administration of Chlorothalonil in this study.

8) In a study conducted by Ricerca, Inc., Charles River Fischer 344 rats (65/sex/group) received dietary levels of 0, 2, 4, 15, and 175 mg/kg/day Chlorothalonil (98.3% a.i.) for 111 weeks (males) and 125 weeks (females). Carcinogenic potential was evidenced by a statistically significant trend and pair-wise increase in the incidence of kidney tubular adenomas, carcinomas, and adenomas and/or carcinomas combined as well as stomach papillomas in male and female rats at the 175 mg/kg/day dose level. In male rats, additional evidence of tumorigenicity was observed in the form of a significant trend and pair-wise comparison in the incidence of kidney tubular carcinomas at 175 mg/kg/day. 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. At 4 mg/kg/day, stomach squamous cell papillomas were observed in increased incidence, and this difference was statistically significant.

In female rats, a significant trend and pair-wise comparison was observed for the incidence of stomach squamous cell papillomas at 175 mg/kg/day vs control. There was also a significant trend for stomach squamous cell carcinoma in female rats.

At study termination, increased incidence of renal tubular epithelial cell hyperplasia and clear cell hyperplasia were observed in male and female rats at 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 (HED document # 008901, review by David S. Liem, Ph.D, dated 9/5/91).
At the 4.0 mg/kg/day dose level and above, hyperplasia and hyperkeratosis of the forestomach was observed in increased incidence in both male and female rats at the terminal exam. At the interim sacrifice, these lesions of the forestomach were observed at the 15 mg/kg/day dose level and above. The observation that these lesions were observed in increased incidence at lower dose levels as the length of treatment increased supports the hypothesis of chronic irritation of the forestomach by Chlorothalonil.

9) The registrant submitted additional mechanistic data in support of non-genotoxic mechanisms of action for induction of forestomach and renal tumors. In the stomach, it is 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 is proposed that certain thiol metabolites of Chlorothalonil exert a toxic effect upon the kidney mitochondria, disrupting mitochondrial respiration with eventual cell death and compensatory proliferation that if sustained, could eventually lead to neoplasia. As pointed out earlier in this document (page 24), it is the Agency's opinion that a non-genotoxic mechanism for induction of renal tumors (and stomach tumors) by Chlorothalonil is supported. However, the relevance or lack of relevance of the rat and dog model for evaluation of tumorigenic potential in humans has not been clearly established. It is proposed that consideration of the re-classification of Chlorothalonil as to carcinogenic potential should be based upon evidence in support of the proposed non-genotoxic mechanism, and should not be based at this time upon arguments suggesting that the rat is a poor model for evaluating human risk for renal tumorigenicity of Chlorothalonil.

H. Classification of Carcinogenic Potential

The Health Effects Division Carcinogenicity Peer Review Committee has reviewed the carcinogenicity database from the submitted studies on Chlorothalonil. In addition, the mechanistic data in support of the processes underlying tumor formation in the forestomach and kidney were also evaluated with respect to the request for re-classification of the carcinogenicity of Chlorothalonil. 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 suggesting that mitochondrial toxicity is linked to carcinogenicity of Chlorothalonil in the kidney was not definitive, and that other mechanisms could also be operative. Based on these considerations, the Committee voted to retain the B2 classification of Chlorothalonil.