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

SEP 4 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Chlorothalonil

FROM: Esther Rinde, Ph.D. Esther Rinde 7/27/87
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Lois Rossi
Product Manager #21
Registration Division (TS-767c)

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 oncogenic potential.

A. Individuals in Attendance:

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

Theodore M. Farber — Theodore M. Farber

William L. Burnam — William L. Burnam

Reto Engler — Reto Engler

fa Louis Kasza — Reto Engler for

Robert Beliles — Robert A. Beliles

Richard Levy — Richard Levy

Judith Hauswirth — Judith Hauswirth

Esther Rinde — Esther Rinde

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

David Ritter

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Richard Hill/Don Barnes

Diane Beal

Jack Quest

4. Other Attendees: The following individuals were also present: Lois Rossi and Robert Forrest (Registration Division (FHB) and Brian Dementi (Tox. Branch).

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Mr. 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]. The material reviewed and the above memos are attached to the file copy of this report.

C. Background Information:

Chlorothalonil (DS-2787; 2,4,5,6-tetrachloroisophthalonitrile) is a widely used agricultural fungicide and is also used as a mildewicide in paints. In a 1978 study, NCI found renal adenomas and carcinomas in both sexes of Osborne-Mendel rats; more recent studies have been performed in the Fischer 344 rat and in the B6C3F1 mouse.

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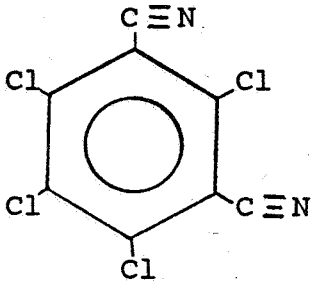
Structure of Chlorothalonil:



D. Evaluation of Oncogenicity Evidence for Chlorothalonil:

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 1
Incidence of Renal Neoplasms (%)

		Control	5,063 ppm	10,126 ppm
Carcinoma	M	0/10	1/45	3/49
	F	0/10	1/48	2/50
Adenoma	M	0/10	2/45	1/49
	F	0/10	0/48	3/50
Combined	M	0/10 (0)	3/45 (6.7)	4/49 (8.2)
	F	0/10 (0)	1/48 (2.1)	5/50 (10.0)

Historical Controls:
The in-house incidence (65/sex pooled controls used in other concurrent studies) for these neoplasms (combined) was 3/240 (1.25%) for male rats, and 0/235 (0%) for female rats.

For the statistical analysis of the NCI data, the above pooled controls from other assays run concurrently, were used. The combined incidence of renal neoplasms was significantly increased over pooled controls, in high dose males ($p=0.028$) and females ($p=0.016$), by the one-tailed Fisher exact test; in females there was also a significant trend ($p=0.007$) by the Cochran Armitage test.

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2. IRDC Rat Oncogenicity Study

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

MRID 146939-146945

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. Tables 2 and 3, and the accompanying statistical analyses were provided by B. Fisher [Memo, 7/20/87].

Historical Controls: Data supplied by the performing laboratory for male and female Fischer 344 rats, showed no occurrence of either of these forestomach tumors in six studies, representing 740 rats (370 per sex).

Additional toxicological changes produced by Chlorothalonil were compound-related effects on the kidneys which included dose-related chronic glomerulonephritis. Increased (significantly) relative liver weights were also observed in all dosed males and in mid- and high-dose females. Gross necropsy revealed a compound related effect on the kidneys and stomach. Increased hyperplasia/hyperkeratosis was observed in the squamous mucosa of the esophagus, parathyroid, duodenum and stomach. Increased mucosal hypertrophy of the duodenum and necrosis of the stomach were also observed. High dose males showed reduced survival (37%) after 27 months (mostly in the last 3 months) compared to concurrent controls (53%).

Based on the above findings, the MTD appears to have been exceeded for males at the highest dose (3500 ppm); for females, the highest dose seems to represent an MTD. Nevertheless, males showed a tumor response even at low and mid-dose, where the MTD was not exceeded; moreover, females showed a dose-related increase in tumors at all dose levels.

It was suggested that renal tumorigenesis in these rats is mediated via chlorothalonil-induced hyperplasia of the cortico-tubular epithelium of the nephron (incidences: 0/60, 32/60, 30/60, 36/60 at 0 (control), 40, 80, 175 mg/kg, respectively). It was noted that while the incidence of kidney hyperplasia reached a plateau at all dose levels, the tumor response increased with higher doses (Table 2) of Chlorothalonil.

TABLE 2

Chlorothalonil - IRDC Fischer 344 Rat Study
Incidence (%) of Renal Tumors

A. Males

	Dose				
	Control				
	0	40	80	175	mg/kg/day
	0	800	1600	3500	PPM
Renal Tumor Rates[1]					
Carcinomas	1/66(2)*	3/61(5)	1/60(2)	6/60(10)*	
Adenomas[2]	0/66(0)**	2/61(3)	5/60(8)*	12/60(20)**	
Both Carcinomas and Adenomas	1/66(2)**	5/61(8)	6/60(10)*	18/60(30)**	

B. Females

	Dose				
	Control				
	0	40	80	175	mg/kg/day
	0	800	1600	3500	PPM
Renal Tumor Rates[1]					
Carcinomas	0/60(0)**	1/60(2)	3/61(5)	12/59(20)**	
Adenomas[2]	0/60(0)**	1/60(2)	4/61(7)	7/59(12)**	
Both Carcinomas and Adenomas	0/60(0)**	2/60(3)	7/61(11)**	19/59(32)**	

[1]Number of tumor bearing animals/number of animals examined
[2]Does not include animals with Carcinoma

Cochran-Armitage Trend and Fisher Exact Test Results:

Significance of Cochran-Armitage Trend test denoted at Control. Significance of Fisher Exact test of pairwise comparison with control denoted at Dose level.

* $p < .05$, ** $p < .01$

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TABLE 3

Chlorothalonil - IRDC Fischer 344 Rat Study
Incidence (%) of Forestomach Tumors
(Gastric Squamous Mucosa - Papilloma and Carcinoma)

A. Males

Fore-Stomach Tumor Rates[1]	Dose: Control 0	40	80	175 mg/kg/day
	0	800	1600	3500 PPM
Carcinoma	1/66/(2)	0/60(0)	0/60(0)	1/60(2)

B. Females

Fore-Stomach Tumor Rates[1]	Dose: Control 0	40	80	175 mg/kg/day PPM
Carcinoma	0/60	0/60	1/61	1/59
Papilloma[2]	0/60	1/60	2/61	2/59
Both Carcinoma and Papilloma	0/60(0)*	1/60(2)	3/61(5)	3/59(5)

[1]Number of tumor bearing animals/Number of animals examined
[2]Does not include animals with Carcinoma

Cochran-Armitage Trend and Fisher Exact
Test Results:

Significance of Trend test denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p < .05$, ** $p < .01$

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 [Spencer, 1978].

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 [from Lacayo Memo, 5/17/85] and accompanying statistical analysis [Lacayo Memo and B. Fisher, personal communication] are given in Table 4.

The incidence of gastric squamous cell carcinoma of the forestomach was statistically increased over concurrent controls in both sexes at 1500 ppm, and in females at 3000 ppm, as well. A positive trend was found for squamous cell carcinoma of the forestomach in the female.

Comparison with historical controls (but not concurrent controls), revealed a dose-related trend (p=0.001) for renal tumors [Lacayo, 5/17/85]. The Committee agreed, that since these are rare tumors, which were also seen in the rat, the tumor response in these mice was convincing.

Historical Controls:
Spontaneous incidences for tumors in CD-1 mice are given in Table 5. The incidences of renal tubular tumors and gastric squamous cell carcinoma in treated male mice exceeded the upper value of the historical control range: 1.7 for renal; 1.7, 2.0 (males and females, respectively) for gastric.

The MTD appears to have been exceeded at 1500 ppm, based on decreased survival in male mice (35% vs 52% in concurrent controls).

Table 4

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TABLE 4

Chlorothalonil - CD-1 Mice Study

A. Incidence (%) of Renal Tubular Tumors

	Dose			
	Control			
	0	107	214	428 mg/kg/day
	0	750	1500	3000 PPM
	Males			
Renal Tumor Rates[1]				
Adenomas[2]	0/57	3/59 (5)	4/59 (7)	2/56 (4)
Carcinomas	0/57	3/59	0/59	2/56
Both Carcinomas and Adenomas	0/57**	6/59 (10)	4/59	4/56 (7)

B. Incidence (%) of Stomach Carcinomas

	Dose			
	Control			
	0	107	214	428 mg/kg/day
	0	750	1500	3000 PPM
	Males			
Stomach Carcinoma Rates[1]				
Squamous Cell	0/55	2/59 (3)	5/59* (9)	1/51 (2)
Glandular	0/55	1/59 (2)	2/59	0/51
	Females			
Squamous Cell	0/57*	2/60 (3)	6/58* (10)	5/58* (9)
Glandular	0/57	1/60 (2)	1/58 (2)	2/56 (4)

[1]Number of tumor bearing animals/Number of animals examined
[2]Does not include animals with Carcinoma

Cochran-Armitage Trend and Fisher Exact Test Results:
Significance of Trend test denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.

* $p < .05$, ** $p < .01$

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TABLE 5

SPONTANEOUS TUMOR INCIDENCE IN CD-1 MICE
HISTORICAL CONTROL DATA

Source*/ Tissue	Neoplasms	MALES Affected Animals	MALES Incidence % Mean	MALES Incidence % Range	FEMALES Affected Animals	FEMALES Incidence % Mean	FEMALES Incidence % Range %
A/Kidney	Adenoma	3/1490	0.2	0 - 1.3	3/1490	0.2	0 - 1.7
	Carcinoma	4/1490	0.3	0 - 1.7	0/1490	0	----
A/Stomach	Polyp	3/1490	0.2	0 - 3.3	0/1490	0	----
	Adenocarcinoma	2/1490	0.1	0 - 1.7	4/1490	0.3	0 - 2.0
	Squamous cell carcinoma	0/1490	0	----	1/1490	0.1	0 - 1.7
B/Kidney	Adenoma	1/99	1.0	----	0/102	0	----
	Carcinoma	0/99	0	----	0/102	0	----
A/Stomach	Adenocarcinoma	3/99	3.0	----	4/102	4.0	----
	Squamous papilloma	1/99	1.0	----	0/102	0	----
C/Kidney	Adenoma	0/57	0	----	0/53	0	----
	Carcinoma	0/57	0	----	0/53	0	----
C/Stomach	Polyp	0/47	0	----	0/46	0	----
	Adenocarcinoma	0/47	0	----	0/46	0	----
	Squamous papilloma	0/47	0	----	0/46	0	----
	Squamous cell carcinoma	0/47	0	----	0/46	0	----
D/Kidney	Adenoma	3/815	0.4	----	0/799	0	----
	Carcinoma	0/815	0	----	0/799	0	----
D/Stomach	Squamous papilloma	1/748	0.1	----	2/754	0.3	----
	Squamous cell carcinoma	0/748	0	----	1/754	0.1	----

*A - International Research and Development Corporation tabulation of findings from two year studies totaling 1490 CD-1 mice of each sex (R.P. Burton letter to Jacoby, 12/9/83).

B - Homburger, F., et al. Aging Changes in CD-1 Mice Reared Under Standard Laboratory Conditions. J. Natl. Cancer Inst. 55: 37-43, 1975.

C - Diamond Shamrock Study: "A Chronic Dietary Study in Mice with DS-3701." (conducted at Bio/Dynamics, Inc., 1979)

D - Bio/Dynamics, Incorporated tabulation of findings from 14 chronic studies in CD-1 mice (Burton, 12/9/83).

D. 4. SDS Biotech Mouse Study (continued)

As in the case of the rats, chronic glomerulonephritis (not statistically significant) was seen in all treated groups. There was a statistically significant increase in the incidence and severity of hyperplasia/hyperkeratosis of the esophageal squamous mucosa in treated mice of both sexes, which was dose-related and which was not seen in concurrent controls. Compound-related effects seen in the kidney included renal enlargement, discoloration, cysts, nodules and masses.

E. Additional Toxicology Data on Chlorothalonil:

1. Metabolism

Oral absorption of aqueous suspensions of Chlorothalonil is low. Total excretion in urine and bile is probably less than 20%. There is a difference in pharmacodynamics between doses of $\leq$ 20 mg/kg/day and 200 mg/kg/day; at doses $\leq$50 mg/kg/day, the majority is excreted in 24 hours, at 200 mg/kg, excretion and blood levels are prolonged. The proposed pathway for Chlorothalonil excretion is given in Figure 1. Major detoxification occurs in the liver, by conjugation with glutathione. These conjugates are mainly excreted directly into bile; some may be transported to the kidney, where they are converted to thiol metabolites, the excretion of which is rate-limited, thus may lead to nephrotoxicity (and possibly tumor formation) when overloading occurs. The major metabolite in rats and in ruminants (cow) is 4-hydroxy-2,5,6-trichloro-isophthalonitrile.

2. Mutagenicity

Chlorothalonil was tested and found to be negative in the following acceptable assays: rat, mouse and hamster micronucleus tests; rat, mouse and hamster chromosomal aberration tests; Ames tests, with and without activation; mouse and rat cytogenetics assays *in vivo*. A weak positive response was elicited with Chlorothalonil in a chinese hamster bone marrow cytogenetics assay, which did not show dose-response. A weakly positive response was also reported in an NIH Sister-Chromatid Exchange assay. None of the metabolites of Chlorothalonil have been tested in these assays, however.

PROPOSED PATHWAY

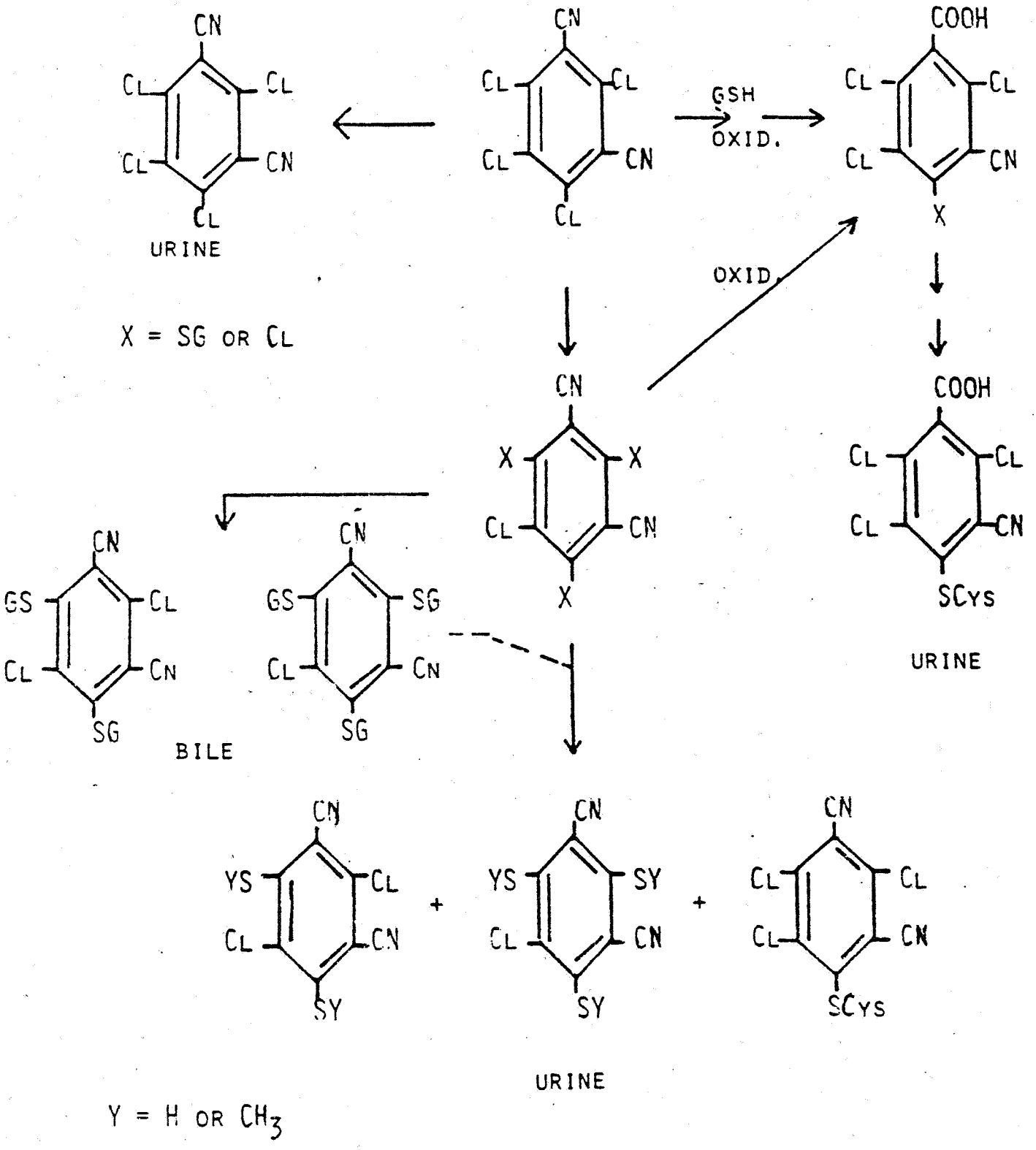


FIGURE 1

E. 3. Developmental Effects

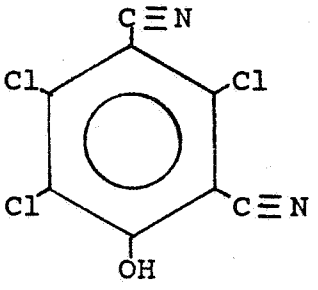
In a three-generation dietary (0, 0.15, or 3.0/2.0 % chlorothalonil) rat study (Charles River), the following were observed: growth depression in parents and offspring; pitted renal surfaces and discoloration of the kidneys in parents; gastric wall thickening in high dose P1; focal renal tubular epithelial vacuolation in mid and high dose P3; gastric and esophageal acanthosis and hyperkeratosis in low and middle dose P3 group. No increases in malformations at any dose level were reported.

In a gavage (0, 100 or 400 mg/kg/body wt.) rat teratogenicity study (Sprague-Dawley), there were a significant number of early resorptions and post-implantation losses and reduced food consumption; there were no abortions, but 2/25 dams at high dose (400 mg/kg) died. No increase in malformations at any exposure level was noted, although Chlorothalonil was embryotoxic at high exposure levels.

4. Structure-Activity Correlations

No studies were available for structural analogs of Chlorothalonil. There is data for 4-hydroxy-2,5,6-trichloroisophthalonitrile (DS-3701), which is the major metabolite in rats, and the only one found in meat and milk.

Structure of DS-3701:



DS-3701 was not oncogenic in acceptable studies in two species: Sprague-Dawley CD-1 rats (0,0.5,or 3mg/kg/day) and CD-1 mice (0,375,750, or 1500 ppm) .

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Chlorothalonil to be of importance in a weight-of-the evidence determination of oncogenic potential.

1. In an NCI study, Chlorothalonil fed in the diet to Osborne-Mendel rats produced a statistically significant increase in combined renal adenoma/carcinoma in both sexes, at the high dose (10,126 ppm) after 80 weeks; in females there was also a significant trend. There were no neoplasms in concurrent controls. The usual deficiencies in NCI protocol were noted. Tumor incidence at both dose levels (5,063 and 10,126 ppm) exceeded that of NCI historical controls.

2. In an IRDC study, dietary Chlorothalonil (up to 3500 ppm) produced a statistically significant increase in renal adenomas in treated Fischer 344 rats at 1600 and 3500 ppm in males, and at 3500 ppm in females. Carcinomas were also statistically increased in both sexes at 3500 ppm and carcinomas/adenomas, combined were significant for both sexes at 1600 and 3500 ppm. There were no renal tumors in concurrent female controls.

In female rats, a positive trend ($p<0.05$) was found for combined carcinoma/papilloma of the forestomach; there were no tumors of the forestomach in concurrent female controls. In-house controls in these rats showed no occurrence of either of these forestomach tumors in six studies (740 rats). A high incidence of renal hyperplasia which correlated with the incidence of renal neoplasms in male rats, was also found.

Additional toxicological changes included effects on the kidney (dose-related glomerulonephritis) and GI.

3. Chlorothalonil when fed in the diet to CD-1 mice up to 3000 ppm (HDT) produced renal adenomas and carcinomas in males, but not in females, and gastric carcinoma in both sexes.

The incidence of gastric squamous cell carcinoma of the forestomach was statistically increased over concurrent controls in both sexes at 1500 ppm, and in females at 3000 ppm, as well. A positive trend was found for squamous cell carcinoma of the forestomach in the female.

F. Weight of Evidence Considerations (continued)

3. CD-1 mice (continued)

The incidences of renal tumors were not statistically significant, however, there was a positive dose-related trend ($p=0.001$) when compared with that of historical controls. There were no tumors reported for concurrent controls of either sex. Furthermore, since renal tumors are rare and were also seen in the rat, the Committee agreed that the tumor response in these mice was convincing.

As with the rats, compound-related effects on the kidney, and renal glomerulonephritis were found. In addition there was a dose-related increase in hyperplasia/hyperkeratosis of the esophagus, which was not found in concurrent controls.

4. In an NCI study, Chlorothalonil, fed to B6C3F1 mice was not oncogenic up to 20,000 ppm (nominal dose).

5. Chlorothalonil was not mutagenic in several acceptable assays, however a weak positive response (not dose-related) in a chinese hamster bone marrow cytogenetics assay was noted. A weakly positive response was also reported in an NIH Sister-Chromatid Exchange assay. It was also noted that none of the metabolites of Chlorothalonil had been tested.

6. No developmental effects were noted in a 3-generation study (dietary) in Charles River rats or in a teratology study (gavage) in Sprague-Dawley rats, however effects on growth and embryotoxicity were observed.

Esophageal and gastric hyperkeratosis were seen in these Charles River rats further confirming observations in the CD-1 mice.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

Chlorothalonil was classified as Group B2 (Probable Human Carcinogen) based on increased incidence of malignant and/or combined malignant and benign tumors (both sexes) in 2 rat studies and in the mouse, 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 1.1×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents [B. Fisher, 7/20/87].

At the Committee's suggestion, the quantification of human risk for Chlorothalonil included an attempt to correlate the results in the two rodent species, based on dose per body surface area.

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