9/10/90

Duplicate 02/27/

PEER REVIEW FILES

007718

CHEMICAL NAME: Chlorothalonil
CASWELL NO.: 215B
CAS NO.: 1897-45-6
REVIEWER: Ritter/Jaeger

CURRENT AGENCY DECISION

B2; 1.1 x 10-2 (HED)

TUMOR TYPE / SPECIES

Renal adenomas & carcinomas (M & F) Forestomach papillomas (F); Fischer 344 rats; Renal adenomas/carcinoma; Osborne Mendel rats (M & F); Renal (F); Forestomach (M & F); CD-1 mice

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. 05/20/88	2. 06/09/88	2. 07/20/88	2. B2; 1.1 x 10-2
1. 05/11/87	1. 05/28/87	1. 09/04/87	1. B2; 1.1 x 10-2

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 09/23/87	1.

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. 03/04/88	1. / /
1. 07/20/87	

MISCELLANEOUS:

SAP includes: Information Concerning Chlorothalonil for the SAP 09/23/87; Revised Tables, 09/22/87; and partial transcript of SAP Meeting, 09/23/87. Miscellaneous: 10 documents, 5/17/85-11/03/88. Stamped 2/2/90; #PR-007718; 395 p.; nha.

09/10/90

PEER REVIEW FILES

CHEMICAL NAME: Chlorothalonil
CASWELL NO.: 215B
CAS NO.: 1897-45-6
REVIEWER: Ritter/Jaeger

CURRENT AGENCY DECISION

B2; 1.1 x 10-2 (HED)

TUMOR TYPE / SPECIES

Renal adenomas & carcinomas (M & F) Forestomach papillomas (F); Fischer 344 rats; Renal adenomas/carcinoma; Osborne Mendel rats (M & F); Renal (F); Forestomach (M & F); CD-1 mice

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. 05/20/88	2. 06/09/88	2. 07/20/88	2. B2; 1.1 x 10-2
1. 05/11/87	1. 05/28/87	1. 09/04/87	1. B2; 1.1 x 10-2

	SAP MEETING	SAP CLASSIFICATION
	2. / /	2.
	1. 09/23/87	1.

QUALITATIVE RISK ASSESSMENT DOCUMENT	QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
3. / /	3. / /	1. / /
2. / /	2. 03/04/88	
1. 07/20/87	1. 07/20/87	

MISCELLANEOUS:

SAP includes: Information Concerning Chlorothalonil for the SAP, 09/23/87; Revised Tables, 09/22/87; and partial transcript of SAP Meeting, 09/23/87. Miscellaneous: 10 documents, 5/17/85-11/03/88. Stamped 09/10/90; about 290 p., nha, #PR-007718.
Chlorothalonil Summary Sheet 05/40/90, 1 p., stamped 09/10/90.

Peer Review Documents
(Memo dates)

20/88

00771B

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

FILE COPY

JUL 20 1988

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Second Peer Review of Chlorothalonil - Reevaluation Following the Sept. 23, 1987 Science Advisory Panel Review.

FROM: Esther Rinde, Ph.D. E. Rinde 6/21/88
Scientific Mission Support Staff (TS-769c)

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

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: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Theodore M. Farber	Theodore M. Farber
William L. Burnam	
Robert Beliles	
Lynnard J. Slaughter	
Judith Hauswirth	
Richard Levy	
Kerry Dearfield	
Esther Rinde	

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

David Ritter _____

Bruce Jaeger _____

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 _____

Reto Engler _____

Diane Beal _____

Jack Quest _____

Marion Copley _____

4. Other Attendees:

Lois Rossi, Mario Fiol (RD) and Esther Saito (SIS) were also present.

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)).

A copy of the above material and the transcript of the SAP meeting (9/23/88) are attached to the file copy of this report.

C. Considerations:

The initial classification (B2) of Chlorothalonil by the Peer Review Committee was reconsidered. This B2 classification was based on increased incidences of malignant and/or combined malignant/benign tumors (both sexes) in two species: rat (2 strains) and in the CD-1 mouse (Tables 1-4). This evidence was presented to the SAP, as follows:

1. NCI Osborne-Mendel Rat Study (1978)

Chlorothalonil fed in the diet to Osborne-Mendel rats, resulted in a statistically significant increase in combined renal adenoma/carcinoma in both sexes, with a significant dose-related trend in females (in males the trend was not significant, since the tumor incidence at the low and high dose was 3 and 4, respectively) (Table 1).

2. IRDC Fischer 344 Rat Study (1985)

Chlorothalonil when fed in the diet to Fischer 344 rats, resulted in a statistically significant increase in the incidence of renal adenomas and carcinomas, with a significant dose-related trend, in both sexes (Tables 2).

In female rats, there was also a statistically significant increase in papilloma and combined papilloma/carcinoma of the forestomach with a significant dose-related trend (Table 3).

3. SDS Biotech CD-1 Mouse Study (1979)

Chlorothalonil when fed in the diet to CD-1 mice, resulted in a statistically significant increase in squamous cell carcinoma of the forestomach in both sexes, with a positive dose-related trend for combined papilloma/carcinoma in females (Table 4).

Increases in the incidence of renal tumors were statistically significant for combined adenoma/carcinoma in male mice only, but there was no positive trend, since these rare tumors were seen at all treatment levels. The renal tumor response in these mice was considered convincing, because of the rarity[1] of renal tumors, because renal tumors of the same type and location were seen in the adequate rat study, and because there were no tumors reported for concurrent controls of either sex (Table 4).

---

[1]Mean historical control incidence for renal adenoma and/or carcinoma: less than 1% (1490 animals for IRDC; 815 for Bio-Dynamics).

C. Considerations (Contd.):

4. NCI B6C3F1 Mouse Study (1978)

Chlorothalonil fed in the diet to B6C3F1 mice, was not oncogenic at doses up to 20,000 ppm (nominal dose).

(Table 5 summarizes the pertinent findings in all 4 studies.)

The SAP Panel 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.

All of the available data have now been reviewed and evaluated (the supplemental data are summarized in David Ritter's memos to Lois Rossi (4/7/88 and 6/7/88) and accompanying DERs). 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/d 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 epithelia 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 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 maintains that the "forestomach lesions associated with Chlorothalonil 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.

C. Considerations (Contd.):

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.

D. Classification of Oncogenic Potential:

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).

C. Considerations (Contd.):

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.

D. Classification of Oncogenic Potential:

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).

TABLE 1

CHLOROTHALONIL - NCI OSBORNE-MENDEL RAT STUDY

Incidence of RENAL NEOPLASMS (%)

		Control	253	506	mg/kg/day
		0	5063	10126	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)*	

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

TABLE 2 (Revised)

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	0/60(0)**	4/60(7)	2/60(3)	14/60(23)**
Adenomas[2]	0/60(0)*	3/60(5)	5/60(8)*	5/60(8)*
Both Carcinomas and Adenomas	0/60(0)**	7/60(12)**	7/60(12)**	19/60(32)**

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)	0/60(0)	12/60(20)**
Adenomas[2]	0/60(0)**	3/60(5)	10/60(17)**	12/60(20)**
Both Carcinomas and Adenomas	0/60(0)**	4/60(7)	10/60(17)**	24/60(40)**

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

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

TABLE 3 (Revised)

CHLOROTHALONIL - IRDC Fischer 344 Rat Study
Incidence (%) of FORESTOMACH TUMORS
(Gastric Squamous Mucosa - Papilloma and Carcinoma)

A. Males

Fore-Stomach Tumor Rates[1]	Control 0	Dose 40	80	175 mg/kg/day
	0	800	1600	3500 PPM
Sq. Carcinoma	0/60(0)	0/60(0)	0/60(0)	1/60(2)
Sq. Papilloma[2]	0/60(0)	1/60(2)	1/60(2)	2/60(3)
Both Carcinoma and Papilloma	0/60(0)	1/60(2)	1/60(2)	3/60(5)

B. Females

Fore-Stomach Tumor Rates[1]	Control 0	Dose 40	80	175 mg/kg/day PPM
Sq. Carcinoma	0/60	0/60	0/60	1/60(2)
Sq. Papilloma[2]	0/60**	1/60(2)	2/60(3)	6/60(10)*
Both Carcinoma and Papilloma	0/60(0)**	1/60(2)	2/60(3)	7/60(12)**

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

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

TABLE 4 (Revised)

CHLOROTHALONIL - CD-1 Mice Study

A. Incidence (%) of RENAL TUBULAR TUMORS

Renal Tumor Rates[1]	Dose: Control 0 / 0	107 / 750	214 / 1500	428 mg/kg/day / 3000 PPM
	Males			
Adenomas[2]	0/60	3/60(5)	3/60(5)	4/60(7)
Carcinomas	0/60	3/60(5)	1/60(2)	1/60(2)
Both Carcinomas and Adenomas	0/60	6/60(10)*	4/60(7)	5/60(8)*

B. Incidence (%) of STOMACH TUMORS

Stomach Tumor Rates[1]	Dose: Control 0 / 0	107 / 750	214 / 1500	428 mg/kg/day / 3000 PPM
	Males			
Sq.Cell Carcinoma	0/60	2/60(3)	5/60(8)*	2/60(3)
Sq.Cell Papilloma[2]	0/60	0/60	0/60	0/60
Both Carc.and Paps.	0/60	2/60(3)	5/60(8)*	2/60(3)
Glandular Carcinoma	0/60(0)	1/60(2)	2/60(3)	0/60(0)
	Females			
Sq.Cell Carcinoma	0/60	0/60(0)	6/60(10)*	2/59(3)
Sq.Cell Papilloma[2]	0/60	2/60(3)	0/60	3/59(5)
Both Carc.and Paps.	0/60*	2/60(3)	6/60(10)*	5/59(8)*
Glandular Carcinoma	0/60	1/60(2)	1/60(2)	2/59(3)

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

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

TABLE 5

		RENAL TUMORS			FORESTOMACH TUMORS Sq. Cell	
		Carcinoma	Adenoma	Combined	Carcinoma	Papilloma C
RAT						
OSBORNE-MENDEL NCI	M	+	+	+*	N/R	
	F	+	+	+*T	N/R	
FISCHER 344 IRDC	M	+*T	+*T	+*T	+	+
	F	+*T	+*T	+*T	+	+*T
MOUSE						
CD-1 SDS Biotech	M	+	+	+*	+*	+
	F	-	-	-	+*	+
B6C3F1 NCI	M	-	-	-	N/R	
	F	-	-	-		

+ Positive
- Negative
* Statistically Significant by pairwise corparison with contr
T Statistically Significant Trend by Cochran Armitage
N/R Not Reported

9/4/87

FILE COPY

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

FILE COPY

SEP 4 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBST

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, 1957 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	
William L. Burnam	
Reto Engler	
Louis Kasza	
Robert Beliles	
Richard Levy	
Judith Hauswirth	
Esther Rinde	

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).

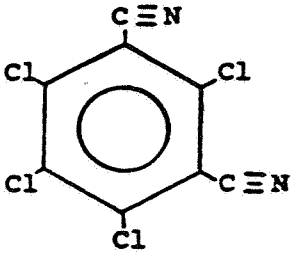
B. Material Reviewed:

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 attache to the file copy of this report.

C. Background Information:

Chlorothalonil (DS-2787; 2,4,5,6-tetrachloroisophthalonitrile) i a widely used agricultural fungicide and is also used as a mildewicide in paints. In a 1978 study, NCI found renal adenoma 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.

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.

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. 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/
	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
	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.

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

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 / 0	40 / 800	80 / 1600	175 / 3500 mg/kg/day 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

TABLE 4

Chlorothalonil - CD-1 Mice Study

A. Incidence (%) of Renal Tubular Tumors

	Dose			
	Control			
	0	107	214	428 mg/kg/d
	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/d
	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.

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

TABLE 5

007718

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 - 2.3
	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 ≤ 20 mg/kg/day and 200 mg/kg/day; at doses ≤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

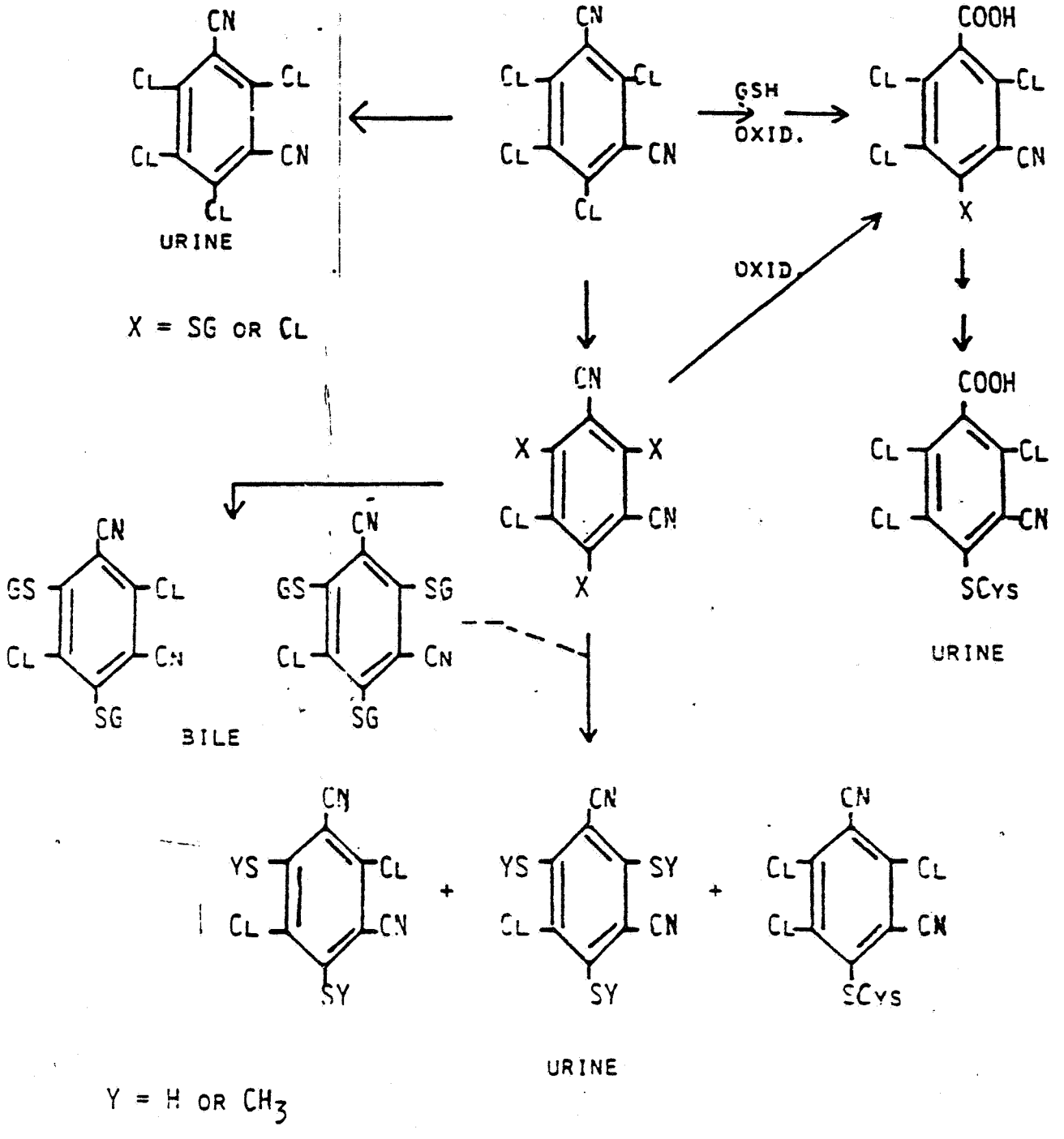


FIGURE 1

E. 3. Developmental Effects

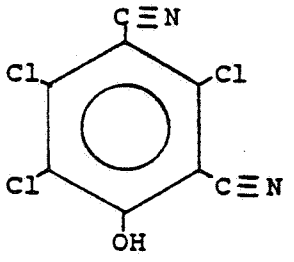
In a three-generation dietary (0, 0.15, or 3.0/2.0 % chlorothalonil) rat study (Charles River), the following were observed: growth depression in parents and offspring; pitted renal surfaces and discoloration of the kidneys in parents; gastric wall thickening in high dose P1; focal renal tubular epithelial 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 tract.

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.

SAP Executive Summary; Meeting Date(s)	9/23/87
includes: Information for the SAP	9/23/87
Revised Tables	9/22/87
SAP Transcript (partial)	9/23/87

SAP Executive Summary

10/1/87 and 9/23/87

C:718

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

October 1, 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Transmittal of the Final FIFRA Scientific Advisory Panel Reports on the September 23, 1987 Meeting

TO: Douglas D. Campt, Director
Office of Pesticide Programs (TS-766C)

The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) was an open meeting held in Arlington, Virginia to review the following topics:

1. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Assert as a Class D Oncogen;

2. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Chlorothalonil as a B-2 Oncogen;

3. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Dichlorvos (DDVP) as a Class B-2 Oncogen and Neurotoxin;

4. A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Linuron as a Class C Oncogen.

Please find attached the Panel's final reports on the four agenda items discussed at the meeting.

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel (TS-769)

Attachments

cc: Panel Members
John A. Moore
James Lamb
Al Heir
Susan H. Wayland
Anne Barton
Mary Beatty, CMO
EPA Participants

21

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Chlorothalonil as a B-2 Oncogen

---

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency in connection with the peer review classification of Chlorothalonil as a B-2 oncogen. The review was conducted in an open meeting held in Arlington, Virginia, on September 23, 1987. All Panel members, except Dr. Thomas W. Clarkson, were present for the review. In addition, the three new oncoming members to the Panel, Drs. Robert Anthony, Edward Bresnick, and Mont Juchau were also present at the meeting.

Public notice of the meeting was published in the Federal Register on September 4, 1987.

Oral statements were received from staff of the Environmental Protection Agency and from Dr. Gary Eilrich, Fermenta Plant Protection Company.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Chlorothalonil

The Agency requested the Panel to focus its attention upon the scientific issues relating to the Peer Review of chlorothalonil. There follows the issues and the Panel's response to the issues:

Issue:

Chlorothalonil was classified by the TOX Branch Peer Review Committee as B2 (Probable Human Carcinogen), based on increased incidences of malignant and/or combined malignant/benign tumors (both sexes) in two species: rat (2 strains) and in the CD-1 mouse.

The Agency requests any comments the Panel may wish to make regarding the biological significance of the renal tumors in the CD-1 male mouse, taking into consideration their rareness, and that they were of the same type and location as seen in the (adequate) Fischer 344 rat study?

Panel Response:

The Panel believes that the occurrence of renal tumors in male CD-1 mice is biologically significant in those animals exposed to chlorothalonil at concentrations below the maximum tolerated dose. However, the Panel is concerned that the data reviewed do not include all information currently available to the Agency. Furthermore, the data which were reviewed by the Agency have been incompletely analyzed since age-adjusted statistical analysis was lacking.

Issue:

Does the Panel have any specific comments regarding our overall assessment of the weight-of-the-evidence and classification of this chemical in accordance with the Agency's Guidelines for Carcinogen Risk Assessment?

Panel Response:

The Panel is concerned that the data on which the weight-of-evidence is based are incomplete. Although the Panel acknowledges that the Agency must make decisions at finite points during the process of data acquisition, the Panel believes that the additional data made available to the Agency since peer review should be assessed before a weight-of-evidence conclusion is reached.

The Panel wishes to point out that chlorothalonil illustrates the awkwardness of the present carcinogen classification scheme. The Panel believes that chlorothalonil, which is not genotoxic, should not be forced into the same category as potent genotoxic carcinogens. The Agency is encouraged to define further the Guidelines for Carcinogen Risk Assessment.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 10/1/87

9/23/87

007713

INFORMATION CONCERNING CHLOROTHALONIL

FOR THE

SCIENTIFIC ADVISORY PANEL

September 23, 1987

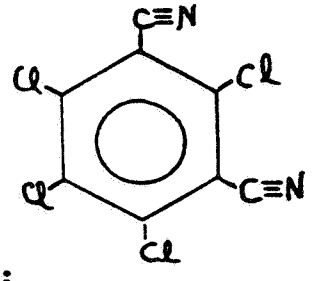
TABLE OF CONTENTS

A Set of Scientific Issues being Considered by the Agency in Connection with the Peer Review of Chlorothalonil

INTRODUCTION:

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; DS-2787) is a widely used agricultural fungicide, also used as a mildewicide in paints.

STRUCTURE:



WEIGHT OF EVIDENCE:

The relevant oncogenicity data base consists of two rat chronic/oncogenicity studies and two (one negative) mouse chronic/oncogenicity studies.

List all studies done

1. NCI Osborne-Mendel Rat Study (1978) - Table 1

Chlorothalonil fed in the diet to Osborne-Mendel rats, resulted in a statistically significant increase in combined renal adenoma/carcinoma in both sexes, with a significant dose-related trend in females.

2. IRDC Fischer 344 Rat Study (1985) - Tables 2 & 3

Chlorothalonil when fed in the diet to Fischer 344 rats, resulted in a statistically significant increase in the incidence of renal adenomas and carcinomas, with a significant dose-related trend, in both sexes.

There was also a statistically significant dose-related trend for combined papilloma/carcinoma of the forestomach in female rats.

TABLE 1

Chlorothalonil - NCI OSBORNE-MENDEL RAT STUDY

Incidence of Renal Neoplasms (%)

		Control	253	506	mg/kg/day
		0	5,063	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)^{T}	1/48(2.1)	5/50(10.0)**	

For the statistical analysis of the NCI data, pooled controls from other assays run concurrently, were used. The pooled control incidence for combined renal neoplasms was 3/240 (1.25%) for male rats, and 0/235 (0%) for females.

The combined incidence of renal neoplasms in treated rats 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 ^{T}(p=0.007) by the Cochran Armitage test.

TABLE 2

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

A. Males

	Dose				
	Control 0	40	80	175	mg/kg/da
	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(32)**	

B. Females

	Dose				
	Control 0	40	80	175	mg/kg/da
	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.

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

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.

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

TABLE 4

Chlorothalonil - CD-1 Mice Study

A. Incidence (%) of Renal Tubular Tumors

	Dose				
	Control 0 0	107 750	214 1500	428 3000	mg/kg/d 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 0	107 750	214 1500	428 3000	mg/kg/d 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.

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

WEIGHT OF EVIDENCE (Continued)

3. SDS Biotech CD-1 Mouse Study (1979) - Table 4

Chlorothalonil when fed in the diet to CD-1 mice, resulted in a statistically significant increase in squamous cell carcinoma of the forestomach in both sexes with a positive dose-related trend in females.

Increases in the incidence of renal tumors were not statistically significant (by pairwise comparison), however there was a positive dose-related trend (p=0.001) for combined renal adenomas/carcinomas in male mice only. The renal tumor response in these mice was considered convincing, because of the rarity[1] of renal tumors, because renal tumors of the same type and location were seen in the adequate rat study, and because there were no tumors reported for concurrent controls of either sex.

4. NCI B6C3F1 Mouse Study (1978)

Chlorothalonil fed in the diet to B6C3F1 mice, was not oncogenic up to 20,000 ppm (nominal dose).

5. Ancillary Information

Chlorothalonil was not mutagenic in several acceptable assays (which included point mutation, chromosomal aberration, and cytogenetic assays); however, in the chinese hamster bone marrow cytogenetics assay, a weak positive (not dose-related) response was noted. A weakly positive response was also reported in an NIH Sister-Chromatid Exchange assay. None of the metabolites of Chlorothalonil have been tested for mutagenic activity.

No studies were available for structural analogs, however DS-T (4-hydroxy-2,5,6-trichloroisophthalonitrile) (a major metabolite in rats) was not oncogenic in Sprague-Dawley CD-1 rats (0,0.5,or 3mg/kg/day) or in CD-1 mice (0,375,750 or 1500 ppm).
0, 54, 107 214 mg/kg/day

---

[1]Mean historical control incidence: less than 1% (1490 animals .

ISSUES:

Chlorothalonil was classified by the TOX Branch Peer Review Committee as B2 (Probable Human Carcinogen), based on increased incidences of malignant and/or combined malignant/benign tumors (both sexes) in two species: rat (2 strains) and in the CD-1 mouse.

1. The Agency requests any comments the Panel may wish to make regarding the biological significance of the renal tumors in the CD-1 male mouse, taking into consideration their rareness, and that they were of the same type and location as seen in the (adequate) Fischer 344 rat study?

2. Does the Panel have any specific comments regarding our overall assessment of the weight of evidence and classification of this chemical in accordance with the Agency's Guidelines for Carcinogen Risk Assessment?

9/22/87

Reto

007718

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

SEP 22 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTA

SUBJECT: Revised Tables 2,3, and 4 on [redacted] for Sept. 23 Open Meeting

FROM: Peer Review Committee

TO: Scientific Advisory Panel Members

At the time of the Peer Review Committee Meeting (6/28/87), the Committee was presented one set of data for tumor incidences. Subsequently, these data were re-analyzed and Tables 2-4 edited accordingly (the tables originally presented to you).

We have since learned that the original data, upon which the Committee based its evaluation and conclusion, was correct; the enclosed set of "Revised Tables" should therefore be used instead of the ones in your issue paper.

TABLE 2 (Revised)

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	0/60(0)**	4/60(7)	2/60(3)	14/60(23)**
Adenomas[2]	0/60(0)*	3/60(5)	5/60(8)*	5/60(8)*
Both Carcinomas and Adenomas	0/60(0)**	7/60(12)**	7/60(12)**	19/60(32)**

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)	0/60(0)	12/60(20)**
Adenomas[2]	0/60(0)**	3/60(5)	10/60(17)**	12/60(20)**
Both Carcinomas and Adenomas	0/60(0)**	4/60(7)	10/60(17)**	24/60(40)**

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

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

TABLE 3 (Revised)

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 / 0	40 / 800	80 / 1600	175 mg/kg//day / 3500 PPM
Sq. Carcinoma	0/60(0)	0/60(0)	0/60(0)	1/60(2)
Sq. Papilloma[2]	0/60(0)	1/60(2)	1/60(2)	2/60(3)
Both Carcinoma and Papilloma	0/60(0)	1/60(2)	1/60(2)	3/60(5)

B. Females

Fore-Stomach Tumor Rates[1]	Dose: Control 0	40	80	175 mg/kg//day PPM
Sq. Carcinoma	0/60	0/60	0/60	1/60(2)
Sq. Papilloma[2]	0/60**	1/60(2)	2/60(3)	6/60(10)*
Both Carcinoma and Papilloma	0/60(0)**	1/60(2)	2/60(3)	7/60(12)**

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

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

TABLE 4 (Revised)

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
Renal Tumor Rates[1]	Males			
Adenomas[2]	0/60	3/60(5)	3/60(5)	4/60(7)
Carcinomas	0/60	3/60(5)	1/60(2)	1/60(2)
Both Carcinomas and Adenomas	0/60	6/60(10)*	4/60(7)	5/60(8)*

B. Incidence (%) of STOMACH TUMORS

	Dose			
	Control			
	0	107	214	428 mg/kg/day
	0	750	1500	3000 PPM
Stomach Tumor Rates[1]	Males			
Sq.Cell Carcinoma	0/60	2/60(3)	5/60(8)*	2/60(3)
Sq.Cell Papilloma[2]	0/60	0/60	0/60	0/60
Both Carc.and Paps.	0/60	2/60(3)	5/60(8)*	2/60(3)
Glandular Carcinoma	0/60(0)	1/60(2)	2/60(3)	0/60(0)
	Females			
Sq.Cell Carcinoma	0/60	0/60(0)	6/60(10)*	2/59(3)
Sq.Cell Papilloma[2]	0/60	2/60(3)	0/60	3/59(5)
Both Carc.and Paps.	0/60*	2/60(3)	6/60(10)*	5/59(8)*
Glandular Carcinoma	0/60	1/60(2)	1/60(2)	2/59(3)

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

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

9/23/87 Attached

C.77.8

ENVIRONMENTAL PROTECTION AGENCY

* * *

SCIENTIFIC ADVISORY PANEL

Open Meeting

* * *

Environmental Protection Agency
Crystal Mall, Building No. 2
Room 1112
1921 Jefferson Davis Highway
Arlington, Virginia

September 23, 1987
8:30 a.m.

MILLER REPORTING CO., INC.
507 C Street, N.E.
Washington, D.C. 20002
(202) 546-6666

47

4-hydroxy compound, but that there was no unequivocal identification. What we had said at the time was that it was possible that up to possibly 5 percent of what was excreted in urine could have been 3701, based strictly on chromatography.

The more recent studies have stated very clearly that we have looked for, have tried to find the 4-hydroxy compound using GCMS methods. We have not been able to find it. We have stated it very clearly in at least three reports on metabolite identification. In those same reports, we have identified the thiol metabolites of Chlorothalonil in the urine.

DR. EILRICH: I am Gary Eilrich again. I think some of those studies have also shown that the 4-thiol and the 4-hydroxy co-chromatograph, so I think what we probably saw before was more the 4-thiol rather than 4-hydroxy. It was just not properly identified.

CHAIRMAN KILGORE: Dr. Swenberg?

DR. SWENBERG: I would just like to pursue my questioning once more. I understand the agency's position on having to stop at some point and make decisions, but it would seem that we have some fairly sizable submission of data here that should be incorporated in the report before we come to

that decision. Do you agree with that? Do you think there is a reason to proceed on and formalize this category without incorporating that data?

DR. JAEGER: The question is whether or not there is a no-effect level for this oncogenic event. I am not prepared to address that. I would like to draw the Committee's attention to a report of a symposia that was again chaired by Dr. Hook and Dr. Hewlitt, where NTP data were examined. This was 1984 and published in Fundamental and Applied Tox, where Chlorothalonil was one of the compounds, and it is listed as a organo-halgide compound.

They indicate here that whether a causal relationship exists between nephrotoxic potential and nephrocarcinogenic effects is presently unknown. This is a panel of experts. They go on to say that little is known about the mechanism of chemically induced renal neoplasia, and they go on to address the data for organo-halgide induced cancers.

Certainly, in view of this report by a body of experts in nephrotoxicity, I would be reluctant to say that yes, we are prepared to review this data and change our opinion. I think when the community does that, then certainly we would like to be involved in that. But the data that they

are talking about, qualitatively, isn't going to really change what we have already looked at, in my opinion. My opinion is we were aware of the glutathione conjugate, aware of the di-thiol and the tri-thiol metabolites. The no-effect level information has not been presented with regard to long-term studies, and as I understand it, this short-term study, which demonstrates a no-effect level for the oncogenic events.

The recent data that we have looked at by WHO and by EPA, clearly showed effects at all levels in both rats and mice except for the B6C3F1 mouse. The Committee here says that the response of renal lesions produced in mice is often more diffuse than it is in the rat, so therefore, maybe the response in mice is not going to be as clear as in the rat studies.

Early studies submitted prior to 1974, of which there are about four or five long-term studies, I believe, by Hazelton -- I think Dr. Busey was part of those, and then Dr. Paynter was -- they showed clearly that there was no oncogenic response, although they showed clear nephrotoxicity in all the studies. I don't know the extent of the pathology in those reports, but the most recent data we have seen shows

are talking about, qualitatively, isn't going to really change what we have already looked at, in my opinion. My opinion is we were aware of the glutathione conjugate, aware of the di-thiol and the tri-thiol metabolites. The no-effect level information has not been presented with regard to long-term studies, and as I understand it, this short-term study, which demonstrates a no-effect level for the oncogenic events.

The recent data that we have looked at by WHO and by EPA, clearly showed effects at all levels in both rats and mice except for the B6C3F1 mouse. The Committee here says that the response, renal lesions produced in mice is often more diffuse than it is in the rat, so therefore, maybe the response in mice is not going to be as clear as in the rat studies.

Early studies submitted prior to 1974, of which there are about four or five long-term studies, I believe, by Hazelton -- I think Dr. Busey was part of those, and then Dr. Paynter was -- they showed clearly that there was no oncogenic response, although they showed clear nephrotoxicity in all the studies. I don't know the extent of the pathology in those reports, but the most recent data we have seen shows

that there is an oncogenic event at every dose, and there is none in the control group.

DR. SWENBERG: It appears rather clear that there is an oncogenic response to this compound across sexes and across species that has to be dealt with. I appreciate that. On the other hand, it appears that we have a fairly large body of data on negative genotoxicity testing and on metabolism, and there is a possible explanation for this data. I think the company raises a good issue on redoing the classification to better deal with these agents.

I understand where you are coming from, as well, and that you have a classification now that you have to work within.

DR. EILRICH: Yes. I want to comment that the 90-day staging study, which I referred to, should shed some new light on a mechanism of action of cellular toxicity or subcellular toxicity that is demonstrated by one of the matabolites.

Also, I would like to comment that the no-effect level study, which has been submitted, was a two-year study on male mice.

DR. SWENBERG: I think it is also worth pointing

out to contrast the response here with genotoxic renal carcinogens, where you can easily get 100 percent incidences in rats and mice, here, you run into this similar situation. This is not a hyalin droplet nephropathy issue, I don't believe, because of the lack of sex and species specificity, but you still run into that plateauing somewhere around 20 percent incidence of renal tumors.

CHAIRMAN KILGORE: Any additional questions of either side here from the panel?

DR. MIZENS: Maija Mizens. I just wanted to make the comment on the mouse study that was recently submitted, that we can appreciate has not been reviewed, is that in that study, it has been no-effect levels have been established for the preneoplastic lesions, both in the forestomach and in the kidney, so there are no-effect levels in that study that were established.

DR. SWENBERG: It would be important to utilize that data in any risk assessment if it maintains the B-2 categorization.

CHAIRMAN KILGORE: Any other comments from the panel?

(No response.)

CHAIRMAN KILGORE: Any other comments from the floor

Qualitative/Quantitative Risk Assessment

3/4/88

007718

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

FILE COPY

MAR 4 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Risk Characterization

Caswell No. 215B

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 2/26/88

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Richard Levy, M.P.H.
Leader - Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

3-3-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

The risk characterization of worker exposure to chlorothalonil is based upon three reports. Two contain worker exposure data prepared by Exposure Assessment Branch (memorandum on Chlorothalonil Exposure Assessment, K.E. Warkentien, January 19, 1988 and Chlorothalonil Exposure Assessment, M.P. Firestone, November 23, 1987). The other specifies the unit risk, Q_1^*, 1.1×10^{-2} in $(mg/kg/day)^{-1}$ in human equivalents (memorandum on Chlorothalonil - Rat Study, Qualitative and Quantitative Risk Assessment, B. Fisher, July 20, 1987).

54

3/4/88

C27718

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

FILE COPY

MAR 4 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Risk Characterization

Caswell No. 215B

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 2/26/88

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Richard Levy, M.P.H.
Leader - Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

3-3-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

The risk characterization of worker exposure to chlorothalonil is based upon three reports. Two contain worker exposure data prepared by Exposure Assessment Branch (memorandum on Chlorothalonil Exposure Assessment, K.E. Warkentien, January 19, 1988 and Chlorothalonil Exposure Assessment, M.P. Firestone, November 23, 1987). The other specifies the unit risk, Q_1^*, 1.1×10^{-2} in $(mg/kg/day)^{-1}$ in human equivalents (memorandum on Chlorothalonil - Rat Study, Qualitative and Quantitative Risk Assessment, B. Fisher, July 20, 1987).

54

The worker exposure calculations are based upon the following assumptions.

1. An average worker has a weight of 70 kg.
2. Exposure is not adjusted for dermal absorption.
3. For ground applications, the mixer/loader and applicator are the same person.
4. Respiratory exposure is negligible compared to dermal exposure.

Table 1 presents detailed calculation of worker exposure for mg/kg/day for a average lifetime and also the range of exposure for selected agricultural products. Also presented is the average and range of environmental risks.

Table 2 presents the rounded estimate of these risks.

When assessing risk of specific Public Health Hazards, EPA takes a conservative posture. Therefore, when risks are expressed by the order of magnitude or the nearest exponent (to the base 10), it is rounded upwards by adjusting risks upward from zero to one-half order of magnitude.

The lifetime risk estimate of chlorothalonil in the worst-case would be 10^{-3}.

Table 1

Chlorothalonil - Risk Characterization

	Exposure [1.] mg/kg/yr		Exposure [2.] mg/kg/day (Lifetime) $\left([1.] \times \frac{1}{365} \times \frac{35}{70}\right)$		Risk $[2.] \times Q_1^* \text{ (mg/kg/day)}^{-1}$ $([2.] \times 1.06 \times 10^{-2})$	
	Mean	Range	Mean	Range	Mean	Range
1. Ground Boom Application						
Tomatoes - CA	5.32	3.44 - 6.89	.007288	.004712 - .009438	7.7×10^{-5}	$5.0 \times 10^{-5} - 1.0 \times 10^{-4}$
" - FL	16.73	10.82 - 21.65	.022918	.014822 - .029658	2.4×10^{-4}	$1.6 \times 10^{-4} - 3.1 \times 10^{-4}$
Potatoes	0.89	0.71 - 1.05	.001219	.000973 - .001438	1.3×10^{-5}	$1.0 \times 10^{-5} - 1.5 \times 10^{-5}$
Onions	2.33	1.65 - 3.02	.003192	.002260 - .004137	3.4×10^{-5}	$2.4 \times 10^{-5} - 4.4 \times 10^{-5}$
Peanuts	1.12	0.89 - 1.34	.001534	.001219 - .001836	1.6×10^{-5}	$1.3 \times 10^{-5} - 1.9 \times 10^{-5}$
Turf - Fairways	4.15	1.85 - 6.45	.005685	.002534 - .008836	6.0×10^{-5}	$2.7 \times 10^{-5} - 9.4 \times 10^{-5}$
" - Greens & Tees	0.06	0.03 - 0.09	.000082	.000041 - .000123	8.7×10^{-7}	$4.4 \times 10^{-7} - 1.3 \times 10^{-6}$
2. Aerial Application						
Mixer/Loader						
Tomatoes	22.03	14.26 - 28.51	.030178	.019534 - .039055	3.2×10^{-4}	$2.1 \times 10^{-4} - 4.1 \times 10^{-4}$
Potatoes	9.14	7.29 - 10.94	.012521	.009986 - .014986	1.3×10^{-4}	$1.1 \times 10^{-4} - 1.6 \times 10^{-4}$
Onions	14.16	10.00 - 18.33	.019397	.013699 - .025110	2.1×10^{-4}	$1.5 \times 10^{-4} - 2.7 \times 10^{-4}$
Peanuts	5.22	4.17 - 6.25	.007151	.005712 - .008562	7.6×10^{-5}	$6.1 \times 10^{-5} - 9.1 \times 10^{-5}$
Pilot						
Tomatoes	1.58	1.02 - 2.04	.002164	.001397 - .002795	2.3×10^{-5}	$1.5 \times 10^{-5} - 3.0 \times 10^{-5}$
Potatoes	0.65	0.52 - 0.78	.000890	.000712 - .001068	9.4×10^{-6}	$7.5 \times 10^{-6} - 1.1 \times 10^{-5}$
Onions	1.01	0.72 - 1.31	.001384	.000986 - .001795	1.5×10^{-5}	$1.0 \times 10^{-5} - 1.9 \times 10^{-5}$
Peanuts	0.37	0.30 - 0.45	.000507	.000411 - .000616	5.4×10^{-6}	$4.4 \times 10^{-6} - 6.5 \times 10^{-6}$
Flagger						
Tomatoes	8.70	5.63 - 11.26	.011918	.007712 - .015425	1.3×10^{-4}	$8.2 \times 10^{-5} - 1.6 \times 10^{-4}$
Potatoes	3.61	2.88 - 4.32	.004945	.003945 - .005918	5.2×10^{-5}	$4.2 \times 10^{-5} - 6.3 \times 10^{-5}$
Onions	5.60	3.95 - 7.24	.007671	.005411 - .009918	8.1×10^{-5}	$5.7 \times 10^{-5} - 1.1 \times 10^{-4}$
Peanuts	2.06	1.65 - 2.47	.002822	.002260 - .003384	3.0×10^{-5}	$2.4 \times 10^{-5} - 3.6 \times 10^{-5}$
3. Spray Gun Application						
Turf - Greens & Tees	70.93	35.05 - 105.97	.097164	.048014 - .145164	1.0×10^{-3}	$5.1 \times 10^{-4} - 1.5 \times 10^{-3}$

Table 2

Chlorothalonil - Mean Estimate of Public Health Risk

	Estimate of Risk (Rounded)
1. Ground Boom Application	
Tomatoes - CA	10^{-5} to 10^{-4}
" - FL	10^{-4}
Potatoes	10^{-5}
Onions	10^{-5} to 10^{-4}
Peanuts	10^{-5}
Turf - Fairways	10^{-5} to 10^{-4}
" - Greens & Tees	10^{-6}
2. Aerial Application	
Mixer/Loader	
Tomatoes	10^{-4} to 10^{-3}
Potatoes	10^{-4}
Onions	10^{-4}
Peanuts	10^{-5} to 10^{-4}
Pilot	
Tomatoes	10^{-5}
Potatoes	10^{-5}
Onions	10^{-5}
Peanuts	10^{-6} to 10^{-5}
Flagger	
Tomatoes	10^{-4}
Potatoes	10^{-5} to 10^{-4}
Onions	10^{-4}
Peanuts	10^{-5} to 10^{-4}
3. Spray Gun Application	
Turf - Greens & Tees	10^{-3}

7/20/87

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

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Rat Study, Qualitative and Quantitative Risk Assessment caswell no. 21

FROM: Bernice Fisher, Biostatistician Bernice Fisher 7/20/87
Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C)

TO: David Ritter, Toxicologist
Section I, Toxicology Branch
Health and Evaluation Division (TS-769C)

THRU: for Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C)

and

for Reto Engler, Ph.D
Chief, Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C)

SUMMARY

The potency estimate, Q_1^* of Chlorothalonil is 1.1×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents [B_2]. This estimate is based upon female rat renal tumors (carcinomas and adenomas).

In female rats there was a significant survival disparity in the pairwise comparison of controls with the mid dose group.

In males rats, there was a significant increase in mortality with dose increments of the chemical, primarily due to the significant increase of deaths in the high dose group as compared with controls.

Background

The May 28, 1987 Peer Review Committee for Chlorothalonil decided that a qualitative and quantitative Risk Assessment was needed and should be based upon the renal tumor formations in rats of the SDS Biotect study of Fisher 344 strain, dosed with 0, 40, 80 and 175 mg/kg of the chemical.

Qualitative Review

Survival analysis was prepared by the use of the D.G. Thomas, H. Breslow and J.J. Gart computer program. The results of the analysis indicated that mortality did not significantly increase with increasing doses of Chlorothalonil in female rats. However, in the pairwise comparison of conrols with the mid dose (80 mg/kg) group, there was a significant ($p = .02$) difference.

In male rats, survival was significantly ($p<.02$) decreased with dose increments of Chlorothalonil. In addition the pair wise comparison of control with the highest dose (175 mg/kg) was also statistically significant ($p=.03$). See Table 1. for details.

In spite of the fact that survival was a problem in the study, the renal tumor formations only started to appear at the beginning of the 79th week of the study and most of the tumors were found in the final kill of the study in both sexes. In addition deaths on the study began about one year after it started.

Because of the late appearance of both deaths and also renal tumors, the use of the Cochran-Armitage Trend test and Fisher's Exact pairwise comparisons with controls were deemed most appropriate+ for the qualitative evaluation of the data.

The Cochran-Armitage Trend test on renal carcinomas, renal adenomas, and combined renal carcinomas and adenomas for both sexes, were all highly significant ($p<.02$). Also, all of the aforementioned groups for both sexes showed consistently significant diferences in tumor rates in the pairwise comparisons (Fisher Exact test) of controls with the highest dose (175 mg/kg) group. See Table II. for details.

+ There is no appropriate way to adjust for the survival disparties since the Peto Prevalence test would be collapsed onto too few time intervals.

Dose- Response Review

On the basis of the qualitative evaluation of renal tumors in rats, the potency estimate, Q_1^* of Chlorothalonil was based upon the proportions in females, which were the most sensitive to the chemical. This estimate was obtained from the Multi-Stage (K. Crump's computer program) Model in terms of rat mg/kg/day doses and then converted to human equivalents by the interspecies surface area adjustments as recommended by EPA Cancer Guidelines. See Table IV. for details.

Table I. Chlorothalonil - Rat Study, Mortality Rates+ and Life Table Analysis Resulta

A. Males

Dose	Weeks				
mg/kg	0-52	53-78	79-104	105-115[a]	Total
0	0/66	3/66	10/63	15/53	28/66 (42)*
40	0/61	1/66	10/60	16/50	27/61 (44)
80	2/60	1/58	14/57	9/43	26/60 (43)
175	0/60	1/60	16/59	21/43	38/60 (63)*

B. Females

Dose	Weeks				
mg/kg	0-52	53-78	79-104	105-128[b]	Total
0	0/60	1/60	10/59	18/49	29/60 (48)
40	0/60	0/60	11/60	28/49	39/60 (65)
80	1/61	3/60	6/57	33/51	43/61 (70)*
175	0/59	1/59	11/58	22/47	34/59 (58)

+ Number of animals died/ Number of live animals at beginning of interval

( ) percent
a final sacrifice at 115 weeks.
b final sacrifice at 128 weeks.

Note: The above time intervals were selected for display only. Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level.

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

Table II - Chlorothalonil - Rat Study, Renal Tumor Rates Cochran-Armitage Trend test and Fisher Exact test Results

A. Males

Dose mg/kg Renal Tumor Rates[1]	0	40	80	175
Carcinomas	1/66(2)*	3/61(5)	1/60(2)	6/60(10)*
Adenomas	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(32)**

B. Females

Dose mg/kg Renal Tumor Rates[1]	0	40	80	175
Carcinomas	0/60(0)**	1/60(2)	3/61(5)	12/59(20)**
Adenomas	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
( ) per cent

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

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

Table III. Chlorothalonil - Rat Study, Stomach Tumor Rates+ (Gastric Squamous Mucosa - Papilloma and Carcinoma) Cochran-Armitage Trend test and Fisher Exact test Results

A. Males

Tumor	Dose - mg/kg 0	40	80	175
Stomach Gastric Squamous Mucosa				
Carcinoma	1/66/(2)	0/60(0)	0/60(0)	1/60(2)

B. Females

Tumor				
Stomach Gastric Squamous Mucosa				
Carcinoma	0/60	0/60	1/61	1/59
Papilloma	0/60	1/60	2/61	2/59
Both	0/60(0)*	1/60(2)	3/61(5)	3/59(5)

+ Number of tumor bearing animals/Number of animals examined

( ) Percent

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

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

Table IV. Chlorothalonil - Rat Study - Potency Estimate, Q_1^* $(mg/kg/day)^{-1}$

	Rat	Human Equivalents
Female	2.0×10^{-3}	1.1×10^{-2}
Male	2.3×10^{-3}	1.2×10^{-2}

References

Armitage, P. (1955) Tests for Linear Trends in Proportious, Biometrics 11, 375-386.

Cochran, W.G. (1954) Some Methods for Strengthening the Common X^2 test, Biometrics 10, 417-451.

Cox, D.r. (1972) Regression Models and Life Tables (with discussion) J. Roy. Stat. Soc. Ser. B. 34, 187-220.

Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogenity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381.

Reviewer's Peer Review Package for 2nd Meeting 5/20/88

5/20/88

007718

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

FILE COPY

MAY 20 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Follow-Up Peer Review of Chlorothalonil

FROM: Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Chlorothalonil was previously reviewed and classified in Cagtegory B_2. The SAP has recommended that the Agency review of auxiliary data and has not specifically commented on the evaluation and classification on Chlorothalonil. For the FRSTR all the available data has now been evaluated and considered. The peer-review committee is requested to determine what changes, if any, in its previous evaluation are necessary based on the completed evaluation of Chlorothalonil.

A meeting to discuss these issues is scheduled for Thursday, June 9, 1988, at 2:00 in Dr. Farber's office (Room 821, CM-2).

Attachment

ADDRESSEES

T. Farber
W. Burnam
J. Quest
J. Hauswirth
E. Rinde
L. Slaughter
K. Dearfield
R. Levy
R. Heliles
A. Barton
R. Hill
D. Beal
M. Copley
D. Ritter
B. Jaeger

cc: Lois Rossi PM #21

#26:5/17/88:sp

4/7/88

007718

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

006651

APR - 7 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

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

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

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

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

Subject: Chlorothalonil; submission of supplemantal data.

Sponsor: Fermenta Plant Protection Co., Painesville, OH.

Caswell #: 215B

TOX Project #: 7-0704

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

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

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

Summary:

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

Results:

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

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

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

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

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

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

Results:

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

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

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

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

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

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

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

Summary:

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

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

Summary:

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

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

Summary:

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

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

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

Summary:

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

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

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

Rated Unacceptable due to lack of stability data.

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

Rated unacceptable due to lakc of stability data.

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

Rated Acceptable.

TABLE I

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN MICE WITH TECHNICAL CHLOROTHALONIL

Histopathologic Finding	Dietary Concentration, ppm				
	0	10/15	40	175	750
Tubular Hyperplasia					
- minimal	7	6	7	4	1
- slight	0	1	0	5	6
- moderate	1	2	1	1	5
- moderately severe	1	0	0	0	2
- severe	0	0	0	0	0
Total	9/13	9/17	8/12	10/14	14/16
Tubular Hypertrophy	1/13	0/17	1/12	0/14	6/16
Karyomegaly	0/13	4/17	2/12	8/14	8/16
Malignant Lymphoma	0/13	3/17	0/12	2/14	2/16

[a] incidence = (affected animals) / (animals from one year interim necropsy + animals which died or were killed in extremis during first year of study)

TABLE II

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE STOMACH AT ONE YEAR IN THE TUMORIGENICITY STUDY IN MICE WITH TECHNICAL CHLOROTHALONIL

Histopathologic Finding	Dietary Concentration, ppm				
	0	10/15	40	175	750
Squamous hyperplasia/ hyperkeratosis[b]	0/13	0/17	1/12	2/14	8/16
Glandular hyperplasia	0/13	2/17	2/12	1/14	4/16

[a] $\text{incidence} = \dfrac{\text{(affected animals)}}{\text{(animals from one year interim necropsy)} + \text{(animals which died or were killed } \underline{\text{in extremis}} \text{ during first year of study)}}$

[b] Number of animals in which hyperplasia or hyperkeratosis or both findings were observed in the forestomach.

Reviewer: D. Ritter, Toxicologist 4-7-88 Caswell #: 215B
Rev. Sec. # I/Toxicology Branch
Secondary Reviewer: R. Bruce Jaeger, Section Head 4/7/88
Rev. Sec. # I/Toxicology Branch

DATA EVALUATION RECORD

Study: 14-C Chlorothalonil Incubation With Stomach and Intestinal Cells

MRID: 40122911

Performing Laboratory: SDS Biotech Corp., Dept. of Safety Assessment, Painesville, OH.

Author(s): M. C. Savides, P. Marcinizyn, C. Kileen.

Study ID Number: 1172-85-0081-AM-002

Date of Study: 5/29/86

Title: II. IN VITRO Incubations Of 14C-Chlorothalonil With Stomach and Intestinal Mucosal Cells.

CORE Rating: NA; Acceptable Study.

QA Statement: Acceptable.

CONCLUSIONS:

"Cells and/or bacteria lining the stomach and small intestine are capable of metabolizing Chlorothalonil to more polar compounds. The chromatographic behavior of these compounds by HPLC suggest that they may be glutathione conjugates of Chlorothalonil".

METHODS:

Test Material: Radiolabeled 14C-Chlorothalonil with a specific activity of 25.6 mCi/mole, equivalent to 25300 DPM or 118.4 ng Chlorothalonil/ml in physiological saline.

Animals: Three Sprague-Dawley rats, fasted overnight, were killed and their stomachs and small intestines slit open. Cells lining the mucosa were scraped from the glandular and squamous areas. The small gut was everted along piece of wire and cells were again scraped off.

Procedures:

The scrapings were placed in 1 - 2 ml of the radiolabel solution (118 - 237 ng 14C-Chlorothalonil). The test tubes were centrifuged at 2000 RPM for two minutes and 100 ul supernatant samples were taken for HPLC and LSC.

The test tubes' contents were resuspended and the tubes were incubated at 37 degrees C for 6 hours. The tubes were again centrifuged and the supernants analyzed using HPLC and LSC. The results before incubation and after incubation were compared. An aliquot of the 14C-Chlorothalonil saline solution was left at room temperature overnight and served as the control.

RESULTS:

The overall radioconcentration was essentially the same for the before incubation and after incubation. Two peaks were eluted from the Before Incubation samples: the major peak was Chlorothalonil and the smaller peak was SDS-3701, the 4-hydroxymetabolite of Chlorothalonil, which represented an impurity of about 2.7 %.

The LC/LSC profile from the stomach squamous cell preparations showed two metabolites in addition to Chlorothalonil. These eluted at 16 and 23 minutes. About 30 % of radiolabeled material was Chlorothalonil.

The LC/LSC profile from the stomach glandular cell preparations likewise demonstrated two metabolites that eluted similarly to the squamous preparation above. No Chlorothalonil was detected in this preparation.

The LC/LSC profile from the intestinal cell preparations showed that all the Chlorothalonil was metabolized to 5 other compounds, eluting at 5, 12, 17, 20 and 22 minutes.

DISCUSSION:

The authors have concluded that in all three preparations Chlorothalonil degraded to more polar compounds. Three of these eluted in the 16 minute and 22-23 minute range. These elution times correspond to those seen with the mono- and di-glutathione conjugates of Chlorothalonil. They did not run standard preparations containing these moieties.

September 16, 1987

Subject: Review of Three Mutagenicity Studies with Chlorothalonil
Part of Package 7-0704 (Ritter)

From: John Chen
Review Section #1 JC 9/16/87
TB/HED

To: Bruce Jaeger, Section Head
Review Section #1 BJ 9/16/87
TB/HED

Recommendation:

1. The Registrant should be apprised of the deficiencies noted in the following mutagenicity studies which are identified in the detailed review:

A. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66471 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5079.1505, December 19, 1986. Unacceptable (lack of information related to the stability of test compound);

B. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66473 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5081.1505, December 19, 1986. Unacceptable (lack of information related to the stability of test compound).

2. The following mutagencity study is acceptable in support of the data requirements for Chlorothalonil:

A. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66474 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5080.1505, January 19, 1987. Negative response at 100 to 10000 ug/plate with or without renal metabolic activation. Acceptable

Attachment: One Liner

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen John H.S. Chen 9/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.H. Jaeger RHJ 9/14/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Gene mutation in bacteria

TOX. CHEM. No.: 215B

Accession No.:

MRID No.: 401229-07

Test Material: 5-chloro-2,4,6-trimercaptoisophthalonitrile, SDS-66471 (96.2% Purity by HPLC)

Study Number(s): T5079.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with 5-chloro-2,4,6-trimercaptoisophthalonitrile (SDS-66471)

Author(s): M. Mizuno, J.C. Killeen and R.A. Baxter

Report Issued: December 19, 1986

Conclusions:

SDS-66471 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate.

Deficiency: lack of appropriate information related to the stability of the test compound in this study.

Classification of Data: Unacceptable

34-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen 9/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger 9/16/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Gene Mutation in Bacteria — TOX. CHEM. No.: 2158

Accession No.: — MRID No.: 401229-08

Test Material: S,S'-(2,4-dicyano-3,6-dichlorophenyl)dicysteine SDS-66474 (95% Purity by HPLC)

Study Number(s): T5080.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with S,S'-(2,4-dicyano-3,6-dichlorophenyl)dicysteine (SDS-66474)

Author(s): M. Mizuno, J.C. Killeen and R.A. Baxter

Report Issued: January 19, 1987

Conclusions:

SDS-66474 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate

Classification of Data: Acceptable

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen 9/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger
Section I, Toxicology Branch (TS-769C) 9/16/87

DATA EVALUATION REPORT

Study Type: Gene mutation in bacteria — TOX. CHEM. No.: 215B

Accession No.: — MRID No.: 401229-09

Test Material: S,S',S"-(2,4-dicyano-6-chlorophenyl)tricysteine SDS-66473 (95% Purity by HPLC)

Study Number(s): T5081.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with S,S',S"-(2,4-dicyano-6-chlorophenyl)tricysteine (SDS-66473)

Author(s): M. Mizens, J.O. Killeen and R.A. Baxter

Report Issued: December 19, 1986

Conclusions:

SDS-66473 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate.

Deficiency: lack of appropriate information related to the stability of the test compound in this study.

Classification of Data: Unacceptable

5/6/88

Dow KIIIe

713

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

MAY 6 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cover Memo for Chlorothalonil (FRSTR)

FROM: Esther Saito, Chemist Esther Saito
Science Integration and Policy Staff
Hazard Evaluation Division (TS-769C)

TO: Lois Rossi, PM
Herbicide/Fungicide Branch
Registration Division (TS-767C)

THRU: for Millie Vanney
Amy Rispin, Chief
Science Integration and Policy Staff
Hazard Evaluation Division (TS-769C)

Introduction

Chlorothalonil is registered as a broad spectrum non-systemic protective fungicide. A registration standard for chlorothalonil was issued in August, 1984. Studies required by that registration standard have been submitted and reviewed. I will discuss changes that seem to be appropriate as a result of these new studies.

Product Chemistry

Chlorothalonil, as manufactured, is contaminated with hexachlorobenzene (HCB) and pentachlorobenzonitrile (PCBN) at levels that might accumulate in tissues of plants and animals due to the repeated application of chlorothalonil.

The original Registration Standard stated that technical chlorothalonil could not contain more than 0.05% of hexachlorobenzene (HCB) as a manufacturing impurity and that a validated method of analysis for HCB be available. Fermenta has submitted data that is adequate to demonstrate this condition has been met. Data submitted for the Griffin technical is not adequate because validation data for the accuracy of the analytical methods were not submitted.

30

Toxicology

Sensitization

Based on human sensitization incident reports and the results of an invalid study by Industrial Biotest Laboratories (IBT) that demonstrated chlorothalonil as a skin sensitizer, the original Registration Standard required precautionary labeling as an interim measure until a new study could be performed. A valid animal sensitization study has been submitted and reviewed, in response to the Registration Standard, and the results of the study demonstrate that chlorothalonil is not a strong sensitizer. Therefore, the precautionary labeling required in the original Registration Standard is no longer necessary.

Oncogenicity

Technical Grade Chlorothalonil

In the original Registration Standard, the Agency identified a potential oncognic concern with chlorothalonil. However, some of the studies evaluated at that time were flawed and some were inconclusive, so the Agency delayed making a decision concerning oncogenicity until an additional rat study could be submitted and evaluated. The Agency has now classified chlorothalonil as a B_2 oncogen based on an increased incidence of malignant and/or combined malignant and benign tumors in both sexes in two rat studies and a mouse study. The salient oncogenic effect was an increase in renal adenomas and carcinomas in both sexes of Fischer 344 and Osborne Mendel rats and in male CD-1 mice. In addition, increases of carcinomas of the forestomach were observed in female Fischer 344 rats and both sexes of CD-1 mice.

The oncogenic potency (Q_1^*) of chlorothalonil, as estimated from the renal adenomas and carcinomas found in the female Fischer 344 rats, was 1.1×10^{-2} $(mg/kg/day)^{-1}$. This value compares favorably with the Q_1^* of 2.2×10^{-2} $(mg/kg/day)^{-1}$ estimated from the CD-1 mouse study. Using the Q_1^* from the rat study and the exposure assessment performed by EAB, an oncogenic risk assessment was performed for workers. The risks ranged from 10^{-8}-10^{-7} for ground boom application on turf (greens and tees) to 10^{-5}-10^{-4} for spray gun application on turf (greens and tees).

A dietary risk assessment was performed using the Tolerance Assessment System (TAS). The oncogenic risk based on residues present at tolerance levels and 100 percent of the crops being treated was 10^{-4}. To obtain a more reasonable estimate of risk, a correction was made for percent of crop treated and for anticipated residues when the data were available. The oncogenic risk was then calculated as 10^{-5}. The majority of the theoretical exposure and oncogenic risk is due to tomatoes ($2.8X10^{-6}$) and celery ($1.3X10^{-6}$). These two crops account for 45 percent of the total risk.

Oncogenicity of HCB

The Agency has classified HCB as a Probable Human Carcinogen (Group B_2) based on an increased incidence of malignant tumors of the in two species, haemangioendothelioma in hamsters and hepatocellular carcinoma in rats as well as confirmed reports of hepatoma in both of these species. A Q_1^* of 1.7 $(mg/kg/day)^{-1}$ was derived using data regarding the incidence of hepatocellular carcinoma in female rats.

A risk assessment has not been performed for workers, but if it is assumed that workers would be exposed to HCB in the same ratio as to that present in the technical, the risk would be lower than the risk calculated for chlorothalonil.

In the original Registration Standard, the Agency required crop residue data be collected for HCB. Using this data and the TAS, a dietary risk of 10^{-5} has been calculated for HCB. (see attached May 4, 1988 memo.) As noted in this memo, it is likely that this analysis overestimates the dietary exposure to HCB that results from the use of chlorothalonil. The analytical limit of detection (3ppb) was assigned to all food crops for which no measurable residues were found or for which no data were available. Therefore, when the analytical method for HCB is refined to detect 1 ppb, as required in the FRSTR, it is likely that the theoretical oncogenic risk will be significantly lower.

Metabolism

There is a difference in pharmaco-dynamics depending on the dose, at doses equal to or less than 20 mg/kg/day, the majority is excreted in the feces as chlorothalonil within 24 hours, at 200 mg/kg/day excretion and blood levels are prolonged. Major detoxification occurs in the liver, by conjugation with glutathione. These conjugates are excreted directly into the bile; some may be transported to the kidneys where they are converted to thiol metabolites, the excretion of which is rate limited, and thus may lead to nephrotoxicity (and possible tumor formation) when overloading occurs.

Residue Chemistry

The general metabolism for chlorothalonil in plants and animals is still not adequately understood. The available data, although incomplete, indicate that the major residues of chlorothalonil in or on plants are the parent compound and the 4-hydroxy metabolite, both of which are contained in the tolerance expression. Data submitted in response to the original Registration Standard for plant metabolism were considered inadequate. No new animal metabolism studies were submitted. Residue data was also required for the impurities HCB and PCBN in the original Registration Standard. Adequate data has been

submitted for some crops. The Residue Chemistry chapter identifies those crops for which additional data are required. As stated in that chapter residue, levels for HCB using an analytical method with a limit of detection of at least 1 ppb, are still required. However, because the residues of PCBN on crops are in approximately the same proportion as they are in two formulations of technical chlorothalonil, and because these products were used for all the toxicity testing of the technical product, we can infer that the toxicological profile of the technical product reflects the toxicity of PCBN as an impurity. Therefore, no additional residue data for PCBN is required and the residue chemistry data tables need to be corrected to reflect this.

Ecological Effects

The only new study reviewed for this FRSTR was the oyster 96-hour shell deposition study. This study showed that chlorothalonil is very highly toxic to mollusks (EC_{50}=3.6 ppb). The avian reproduction study on the degradate and the parent at levels greater than 50 ppm and a simulated or actual field testing for aquatic organisms as required in the original Registration Standard have not been received. These studies are still required. In addition, studies for acute toxicity to freshwater fish and invertebrates are required for a typical end use product for cranberry use and a mysid shrimp life cycle test and an aquatic organism accumulation test with species other than fish are required for antifouling paint use. Because of generally heightened concerns for nontarget plants, Tier one data for plant protection testing is also being required in the FRSTR.

Environmental Fate

Chlorothalonil and its degradates are relatively persistent in soil. Chlorothalonil is stable to hydrolysis in acidic and neutral water. Its two major degradates 3-cyano-2,4,5,6-tetrachlorobenzamide and 4-hydroxy-2,5,6-trichloroisophthalonitrile are stable in acidic, neutral and alkaline solutions.

Field studies indicate that chlorothalonil residues are present in rotational crops. A petition for tolerances for rotational crops should be required. RCB will provide the data requirements necessary for establishing rotational crop tolerances.

Chlorothalonil, itself has been demonstrated to be relatively immobile. However, the 4-hydroxy degradate is mobile and the other degradates are mobile to a lesser extent. Chlorothalonil and its degradates have been found in groundwater. Therefore we are recommending a small-scale retrospective study and a field leaching study. (See ground-water statement for chlorothalonil, attached).

The Agency required the laboratory volatility study in the original Registration Standard. We granted a waiver request made by the registrant in response to the original Registration Standard. However, since then it has been determined that chlorothalonil is a B_2 oncogen and has greenhouse use. Therefore, we believe that the laboratory volatibility study should be required.

A 24-hour interim re-entry interval was established in the original Registration Standard while re-entry studies were being performed. The Agency has not received the studies, but they are still required.

The Agency also requires additional data on photodegradation in water and soil, accumulation studies on irrigated crops and in fish and further identification of degradates in the aerobic soil metabolism study.

Tolerance Assessment

Inadequate data exist for plant and animal metabolism, storage stability and some field residue studies. Therefore, the final conclusions regarding the adequacy of established tolerances can not be made now.

The Toxicology Branch/Agency ADI Committee has established an RfD of 0.015 mg/kg/day based on a two year dog feeding NOEL of 60 ppm (renal lesions). An uncertainty factor of 100 was used because of the uncertainties asociated with extrapolating from laboratory animals. The dog study was selected because it yielded the most sensititve value. The dog study appears to be of good quality and therefore is given a high confidence rating. Because the NOEL for the 13-week rat feeding study is similar to the 2-year dog study, the RfD is also given high confidence.

The TAS Routine Chronic Analysis estimates was performed. In this case the Theoretical Maximum Reisude Contribution (TMRC) for the U.S. population was calculated to be 0.013 mg/kg/day, corresponding to approximately 85 percent of the RfD. The most highly exposed subgroup were children 1-6 years of age with a TMRC of 0.022 mg/kg/day or about 150 percent of the RfD. When percent of crop treated and anticipated residues were used in place of tolerances and one hundred percent of crop treated the exposure to U.S. population was reduced to 0.00084 mg/kg/day, corresponding to 5 percent of the RfD. For children 1-6 years the exposure was reduced to 0.0014 mg/kg/day or 10 percent of the RfD.

attachment

4/7/88

CC/713

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

006651

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

APR - 7 1988

MEMORANDUM:

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

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

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

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

Subject: Chlorothalonil; submission of supplemantal data.

Sponsor: Fermenta Plant Protection Co., Painesville, OH.

Caswell #: 215B

TOX Project #: 7-0704

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

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

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

Summary:

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

Results:

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

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

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

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

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

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

Results:

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

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

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

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

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

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

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

Summary:

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

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

Summary:

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

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

Summary:

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

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

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

Summary:

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

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

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

Rated Unacceptable due to lack of stability data.

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

Rated unacceptable due to lakc of stability data.

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

Rated Acceptable.

TABLE I

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN MICE WITH TECHNICAL CHLOROTHALONIL

Histopathologic Finding	Dietary Concentration, ppm				
	0	10/15	40	175	750
Tubular Hyperplasia					
- minimal	7	6	7	4	1
- slight	0	1	0	5	6
- moderate	1	2	1	1	5
- moderately severe	1	0	0	0	2
- severe	0	0	0	0	0
Total	9/13	9/17	8/12	10/14	14/16
Tubular Hypertrophy	1/13	0/17	1/12	0/14	6/16
Karyomegaly	0/13	4/17	2/12	8/14	8/16
Malignant Lymphoma	0/13	3/17	0/12	2/14	2/16

[a] incidence = $\frac{\text{(affected animals)}}{\text{(animals from one year interim necropsy)} + \text{(animals which died or were killed in extremis during first year of study)}}$

TABLE II

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE STOMACH AT ONE YEAR IN THE TUMORIGENICITY STUDY IN MICE WITH TECHNICAL CHLOROTHALONIL

Histopathologic Finding	Dietary Concentration, ppm				
	0	10/15	40	175	750
Squamous hyperplasia/ hyperkeratosis[b]	0/13	0/17	1/12	2/14	8/16
Glandular hyperplasia	0/13	2/17	2/12	1/14	4/16

[a] $\text{incidence} = \dfrac{\text{(affected animals)}}{\text{(animals from one year interim necropsy)} + \text{(animals which died or were killed } \underline{\text{in extremis}} \text{ during first year of study)}}$

[b] Number of animals in which hyperplasia or hyperkeratosis or both findings were observed in the forestomach.

Reviewer: D. Ritter, Toxicologist Caswell #: 215B
Rev. Sec. # I/Toxicology Branch
Secondary Reviewer: R. Bruce Jaeger, Section Head
Rev. Sec. # I/Toxicology Branch

DATA EVALUATION RECORD

Study: 14-C Chlorothalonil Incubation With Stomach and Intestinal Cells

MRID: 40122911

Performing Laboratory: SDS Biotech Corp., Dept. of Safety Assessment, Painesville, OH.

Author(s): M. C. Savides, P. Marcinizyn, C. Kileen.

Study ID Number: 1172-85-0081-AM-002

Date of Study: 5/29/86

Title: II. IN VITRO Incubations Of 14C-Chlorothalonil With Stomach and Intestinal Mucosal Cells.

CORE Rating: NA; Acceptable Study.

QA Statement: Acceptable.

CONCLUSIONS:

"Cells and/or bacteria lining the stomach and small intestine are capable of metabolizing Chlorothalonil to more polar compounds. The chromatographic behavior of these compounds by HPLC suggest that they may be glutathione conjugates of Chlorothalonil".

METHODS:

Test Material: Radiolabeled 14C-Chlorothalonil with a specific activity of 25.6 mCi/mole, equivalent to 25300 DPM or 118.4 ng Chlorothalonil/ml in physiological saline.

Animals: Three Sprague-Dawley rats, fasted overnight, were killed and their stomachs and small intestines slit open. Cells lining the mucosa were scraped from the glandular and squamous areas. The small gut was everted along piece of wire and cells were again scraped off.

Procedures:

The scrapings were placed in 1 - 2 ml of the radiolabel solution (118 - 237 ng 14C-Chlorothalonil). The test tubes were centrifuged at 2000 RPM for two minutes and 100 ul supernatant samples were taken for HPLC and LSC.

92

The test tubes' contents were resuspended and the tubes were incubated at 37 degrees C for 6 hours. The tubes were again centrifuged and the supernants analyzed using HPLC and LSC. The results before incubation and after incubation were compared. An aliquot of the 14C-Chlorothalonil saline solution was left at room temperature overnight and served as the control.

RESULTS:

The overall radioccncentration was essentially the same for the before incubation and after incubation. Two peaks were eluted from the Before Incubation samples: the major peak was Chlorothalonil and the smaller peak was SDS-3701, the 4-hydroxymetabolite of Chlorothalonil, which represented an impurity of about 2.7 %.

The LC/LSC profile from the stomach squamous cell preparations showed two metabolites in addition to Chlorothalonil. These eluted at 16 and 23 minutes. About 30 % of radiolabeled material was Chlorothalonil.

The LC/LSC profile from the stomach glandular cell preparations likewise demonstrated two metabolites that eluted similarly to the squamous preparation above. No Chlorothalonil was detected in this preparation.

The LC/LSC profile from the intestinal cell preparations showed that all the Chlorothalonil was metabolized to 5 other compounds, eluting at 5, 12, 17, 20 and 22 minutes.

DISCUSSION:

The authors have concluded that in all three preparations Chlorothalonil degraded to more polar compounds. Three of these eluted in the 16 minute and 22-23 minute range. These elution times correspond to those seen with the mono- and di-glutathione conjugates of Chlorothalonil. They did not run standard preparations containing these moieties.

00665

September 16, 1987

Subject: Review of Three Mutagenicity Studies with Chlorothalonil
Part of Package 7-0704 (Ritter)

From: John Chen
Review Section #1 JC 9/16/87
TB/HED

To: Bruce Jaeger, Section Head
Review Section #1 BJ 9/16/87
TB/HED

Recommendation:

1. The Registrant should be apprised of the deficiencies noted in the following mutagenicity studies which are identified in the detailed review:

A. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66471 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5079.1505, December 19, 1986. Unacceptable (lack of information related to the stability of test compound);

B. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66473 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5081.1505, December 19, 1986. Unacceptable (lack of information related to the stability of test compound).

2. The following mutagencity study is acceptable in support of the data requirements for Chlorothalonil:

A. Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay with SDS-66474 in the presence or absence of renal metabolic activation. Microbiological Associates, Inc. Study No. T-5080.1505, January 19, 1987. Negative response at 100 to 10000 ug/plate with or without renal metabolic activation. Acceptable

Attachment: One Liner

006651

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen John H Chen 1/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger RBJ 9/16/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Gene mutation in bacteria TOX. CHEM. No.: 2158

Accession No.: MRID No.: 401229-07

Test Material: 5-chloro-2,4,6-trimercaptoisophthalonitrile, SDS-66471 (96.2% Purity by HPLC)

Study Number(s): T5079.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with 5-chloro-2,4,6-trimercaptoisophthalonitrile (SDS-66471)

Author(s): M. Mizans, J.C. Killeen and R.A. Baxter

Report Issued: December 19, 1986

Conclusions:

SDS-66471 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate.

Deficiency: lack of appropriate information related to the stability of the test compound in this study.

Classification of Data: Unacceptable

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen 9/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger 9/16/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Gene Mutation in Bacteria TOX. CHEM. No.: 215B

Accession No.: MRID No.: 401229-08

Test Material: S,S'-(2,4-dicyano-3,6-dichlorophenyl)dicysteine
SDS-66474 (95% Purity by HPLC)

Study Number(s): T5080.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with S,S'-(2,4-dicyano-3,6-dichlorophenyl)dicysteine (SDS-66474)

Author(s): M. Mizans, J.J. Killeen and R.A. Baxter

Report Issued: January 19, 1987

Conclusions:

SDS-66474 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate

Classification of Data: Acceptable

84-2 - Salmonella Mutagenicity Test

Reviewed by: John H.S. Chen 9/15/87
Section I, Toxicology Branch (TS-769C)
Secondary reviewer: R.B. Jaeger
Section I, Toxicology Branch (TS-769C) 9/16/87

DATA EVALUATION REPORT

Study Type: Gene mutation in bacteria TOX. CHEM. No.: 215

Accession No.: MRID No.: 401229-09

Test Material: S,S',S"-(2,4-dicyano-6-chlorophenyl)tricysteine
SDS-66473 (95% Purity by HPLC)

Study Number(s): T5081.1505

Sponsor: Fermenta Plant Protection Company, Painesville, Ohio 44077

Test Facility: Microbiological Associates, Inc., Bethesda, MD 20816

Title of Report: Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) with and without Renal Activation with S,S',S"-(2,4-dicyano-6-chlorophenyl)tricysteine (SDS-66473)

Author(s): M. Mizens, J.C. Killeen and R.A. Baxter

Report Issued: December 19, 1986

Conclusions:

SDS-66473 is not mutagenic in Ames Test either with or without renal metabolic activation at the concentrations tested (100 through 10000 ug/plate).

Concentrations tested: 100, 500, 2500, 5000 and 10000 ug/plate.

Deficiency: lack of appropriate information related to the stability of the test compound in this study.

Classification of Data: Unacceptable

3/4/88

00771E

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

MAR 4 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Risk Characterization

Caswell No. 215B

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Bernice Fisher 2/26/88

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Richard Levy, M.P.H.
Leader - Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

3-3-88

and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

The risk characterization of worker exposure to chlorothalonil is based upon three reports. Two contain worker exposure data prepared by Exposure Assessment Branch (memorandum on Chlorothalonil Exposure Assessment, K.E. Warkentien, January 19, 1988 and Chlorothalonil Exposure Assessment, M.P. Firestone, November 23, 1987). The other specifies the unit risk, Q_1^*, 1.1×10^{-2} in $(mg/kg/day)^{-1}$ in human equivalents (memorandum on Chlorothalonil - Rat Study, Qualitative and Quantitative Risk Assessment, B. Fisher, July 20, 1987).

98

The worker exposure calculations are based upon the following assumptions.

1. An average worker has a weight of 70 kg.
2. Exposure is not adjusted for dermal absorption.
3. For ground applications, the mixer/loader and applicator are the same person.
4. Respiratory exposure is negligible compared to dermal exposure.

Table 1 presents detailed calculation of worker exposure for mg/kg/day for a average lifetime and also the range of exposure for selected agricultural products. Also presented is the average and range of environmental risks.

Table 2 presents the rounded estimate of these risks.

When assessing risk of specific Public Health Hazards, EPA takes a conservative posture. Therefore, when risks are expressed by the order of magnitude or the nearest exponent (to the base 10), it is rounded upwards by adjusting risks upward from zero to one-half order of magnitude.

The lifetime risk estimate of chlorothalonil in the worst-case would be 10^{-3}.

Table 1

Chlorothalonil - Risk Characterization

	[1.] mg/kg/yr Exposure		[2.] mg/kg/day (Lifetime) $\left([1.] \times \frac{1}{365} \times \frac{35}{70}\right)$		Risk $[2.] \times Q_1^* \text{ (mg/kg/day)}^{-1}$ $([2.] \times 1.06 \times 10^{-2})$	
	Mean	Range	Mean	Range	Mean	Range
1. Ground Boom Application						
Tomatoes - CA	5.32	3.44 - 6.89	.007288	.004712 - .009438	7.7×10^{-5}	$5.0 \times 10^{-5} - 1.0 \times 10^{-4}$
" - FL	16.73	10.82 - 21.65	.022918	.014822 - .029658	2.4×10^{-4}	$1.6 \times 10^{-4} - 3.1 \times 10^{-4}$
Potatoes	0.89	0.71 - 1.05	.001219	.000973 - .001438	1.3×10^{-5}	$1.0 \times 10^{-5} - 1.5 \times 10^{-5}$
Onions	2.33	1.65 - 3.02	.003192	.002260 - .004137	3.4×10^{-5}	$2.4 \times 10^{-5} - 4.4 \times 10^{-5}$
Peanuts	1.12	0.89 - 1.34	.001534	.001219 - .001836	1.6×10^{-5}	$1.3 \times 10^{-5} - 1.9 \times 10^{-5}$
Turf - Fairways	4.15	1.85 - 6.45	.005685	.002534 - .008836	6.0×10^{-5}	$2.7 \times 10^{-5} - 9.4 \times 10^{-5}$
" - Greens & Tees	0.06	0.03 - 0.09	.000082	.000041 - .000123	8.7×10^{-7}	$4.4 \times 10^{-7} - 1.3 \times 10^{-6}$
2. Aerial Application						
Mixer/Loader						
Tomatoes	22.03	14.26 - 28.51	.030178	.019534 - .039055	3.2×10^{-4}	$2.1 \times 10^{-4} - 4.1 \times 10^{-4}$
Potatoes	9.14	7.29 - 10.94	.012521	.009986 - .014986	1.3×10^{-4}	$1.1 \times 10^{-4} - 1.6 \times 10^{-4}$
Onions	14.16	10.00 - 18.33	.019397	.013699 - .025110	2.1×10^{-4}	$1.5 \times 10^{-4} - 2.7 \times 10^{-4}$
Peanuts	5.22	4.17 - 6.25	.007151	.005712 - .008562	7.6×10^{-5}	$6.1 \times 10^{-5} - 9.1 \times 10^{-5}$
Pilot						
Tomatoes	1.58	1.02 - 2.04	.002164	.001397 - .002795	2.3×10^{-5}	$1.5 \times 10^{-5} - 3.0 \times 10^{-5}$
Potatoes	0.65	0.52 - 0.78	.000890	.000712 - .001068	9.4×10^{-6}	$7.5 \times 10^{-6} - 1.1 \times 10^{-5}$
Onions	1.01	0.72 - 1.31	.001384	.000986 - .001795	1.5×10^{-5}	$1.0 \times 10^{-5} - 1.9 \times 10^{-5}$
Peanuts	0.37	0.30 - 0.45	.000507	.000411 - .000616	5.4×10^{-6}	$4.4 \times 10^{-6} - 6.5 \times 10^{-6}$
Flagger						
Tomatoes	8.70	5.63 - 11.26	.011918	.007712 - .015425	1.3×10^{-4}	$8.2 \times 10^{-5} - 1.6 \times 10^{-4}$
Potatoes	3.61	2.88 - 4.32	.004945	.003945 - .005918	5.2×10^{-5}	$4.2 \times 10^{-5} - 6.3 \times 10^{-5}$
Onions	5.60	3.95 - 7.24	.007671	.005411 - .009918	8.1×10^{-5}	$5.7 \times 10^{-5} - 1.1 \times 10^{-4}$
Peanuts	2.06	1.65 - 2.47	.002822	.002260 - .003384	3.0×10^{-5}	$2.4 \times 10^{-5} - 3.6 \times 10^{-5}$
3. Spray Gun Application						
Turf - Greens & Tees	70.93	35.05 - 105.97	.097164	.048014 - .145164	1.0×10^{-3}	$5.1 \times 10^{-4} - 1.5 \times 10^{-3}$

00778

10-11

Table 2

Chlorothalonil - Mean Estimate of Public Health Risk

	Estimate of Risk (Rounded)
1. Ground Boom Application	
Tomatoes - CA	10^{-5} to 10^{-4}
" - FL	10^{-4}
Potatoes	10^{-5}
Onions	10^{-5} to 10^{-4}
Peanuts	10^{-5}
Turf - Fairways	10^{-5} to 10^{-4}
" - Greens & Tees	10^{-6}
2. Aerial Application	
Mixer/Loader	
Tomatoes	10^{-4} to 10^{-3}
Potatoes	10^{-4}
Onions	10^{-4}
Peanuts	10^{-5} to 10^{-4}
Pilot	
Tomatoes	10^{-5}
Potatoes	10^{-5}
Onions	10^{-5}
Peanuts	10^{-6} to 10^{-5}
Flagger	
Tomatoes	10^{-4}
Potatoes	10^{-5} to 10^{-4}
Onions	10^{-4}
Peanuts	10^{-5} to 10^{-4}
3. Spray Gun Application	
Turf - Greens & Tees	10^{-3}

3/2/88

REVIEWER

00718

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

MAR -

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: D. Stubbs, PM # 41
RSERB
Registration Division (TS-767C)

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. 1/Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

FROM: D. Ritter, Toxcologist
Rev. Sec. 1/Toxicology Branch
Hazard Evaluation Division (TS-769C)

Subject: 88-MI-01, Chlorothalonil, Section 18 request for use on sour cherries in Michigan.

Caswell #: 215B.

Registrant: Michigan State Department of Agriculture.

Recommendation:

We recommend that no further new uses be allowed for products containing Chlorothalonil pending resolution of questions concerning its oncogenicity.

Bases for the Conclusion:

All toxicity data requirements have been fulfilled for technical CTN. We noted in the Toxicology Chapter of the Final Registration Standard and Tolerance Reassessment for Chlorothalonil that review of additional toxicity and metabolism data did not alter our previous conclusions that CTN was a B2 oncogen (probable human carcinogen; D. Ritter to Lois Rossi, PM # 21, 2/24/88). We further recommended that CTN be sent for Special Review. Accordingly, any further regulatory actions for CTN- containing products should be defered pending outcome of the Special Review.

2/24/88

007718

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

FEB 24 1988

MEMORANDOM

Subject: Chlorothalonil, Toxicology Chapter of the Registration Standard

To: Lois Rossi PM-21
Registration Division (TS-767C)

From: David Ritter
Toxicologist
Review Section I
Toxicology Branch, HED (TS-769)

Through: Robert P. Zendzian PhD 2/24 88
Registration Standard Coordinator
Toxicology Branch

William Burnam, Deputy Chief
Toxicology Branch

Attached is the Toxicology Chapter of the Registration Standard for Chlorothalonil. The following portions of this chapter are available on Word Perfect. You may obtain a copy from this reviewer.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
D. ADI Reassessment
E. Toxicological Issues
F. Toxicology Summary Tables
H. One Liners

cc
Rispin, SIS
Zendzian
Coberly

-103

Toxicology Chapter

of the

Chlorthalonil

Registration Standard

Prepared by

David Ritter
Toxicologist
Review Section I
Toxicology Branch, HED

Table of Contents

A. Toxicology Summary

Chlorothalonil (CTN; DS-2787; 2,4,5,6-tetrachloroisophthalonitrile) is a widely used agricultural fungicide with numerous tolerances published under 40 CFR 180.275. Chlorothalonil is also used as a mildewicide in paints.

The principle crop residues consist of the parent compound and its metabolite, DS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile). DS-3701 is the only detectable residue in meat and milk (9/30/84, NTIS # PB 85-247245/AS). DS-3701 was not oncogenic in rats and mice. These findings were discussed in the original Registration Standard and will not be considered further in this document.

CTN is not acutely toxic by the oral, or dermal routes of exposure, but is a severe inhalation toxicant (Toxicity Category I) and eye irritant (Toxicity Category I). It does not produce maternal toxicity, is not fetotoxic and is not teratogenic. Apart from inducing a weak clastogenic response in Chinese Hamster Ovary cell assays, the material is not genotoxic, as evidenced by numerous mutagenicity assays performed using not only CTN but a number of related chemical moieties. CTN is not a dermal sensitizing agent.

NCI found that Chlorothalonil possessed oncogenic properties, inducing renal adenomas and carcinomas in rats but not in mice. A more recent oncogenic study in mice revealed that the same lesions were induced in males but not in females; A Q*1 of 2.4 x 10-2 was calculated for these effects. A repeat rat oncogenic study showed that Chlorothalonil induced renal adenomas and carcinomas in males and females with the incidence increasing with increasing dose. This study also showed that Chlorothalonil may induce papillomas and squamous cell carcinomas of the gastric mucosa.

Chlorothalonil induced non-neoplastic changes in the renal tubular tissues and in the gastric mucosa in these rats.

Chronic toxic effects in earlier dog and rat feeding studies were largely limited to renal and gastric effects, similar to those reported in the later studies.

Metabolism studies in rats suggest that Chlorothalonil is excreted mainly via the GI tract, with ca 6 - 11 % excreted in the urine; there may be biliary excretion; and there may be an excretion rate-limiting active transport mechanism in the renal tubule. Other data show that the urinary metabolites of Chlorothalonil are di- and trithiochloro-phthalonitrile compounds. Available evidence suggests that GSH conjugation of certain metabolites may be involved in the renal response to Chlorothalonil.

3. Toxicology Profile

81 Series Acute toxicity and Irritation Studies

81-1 Acute Oral

An acceptable Acute Oral toxicity study was performed using rats. The LD50 was > 10,000 mg/kg bw. CTN was classified as TOX Category IV (MRID 00094941). The requirement for an Acute oral toxicity study is satisfied.

81-2 Acute Dermal

An acceptable Acute Dermal toxicity study was performed using New Zealand White rabbits. The Dermal LD50 was > 10,000 mg/kg bw. CTN was classified as TOX Category III (MRID 00094940). The requirement for an Acute Dermal toxicity study is satisfied.

81-3 Acute Inhalation

An Acceptable Acute Inhalation was performed using rats. The LC50 for males was 96 ugm/L and 92.5 ugm/L for females. CTN was classified as TOX Category I (MRID 00094942). The requirement for an Acute Inhalation toxicity study is satisfied.

81-4 Primary Eye Irritation

An acceptable Primary Eye Irritation was performed using New Zealand White rabbits. The maximum eye irritancy score was 92.7/110. The product is a severe eye irritant with Toxicity Category Rating of I. (MRID 00030352). The requirement for a Primary Eye Irritation study is satisfied.

81-5 Primary Dermal Irritation

An acceptable Primary Dermal Irritation study was performed using rabbits. The primary irritancy score was less than 1/8. The material is rated non-irritating with a Toxicity Category Rating of IV. (MRID 00094939). The requirement for a Primary Dermal Irritation study is satisfied.

81-6 Dermal Sensitization

An acceptable "Closed Patch" dermal sensitization study was performed in Guinea Pigs. Chlorothalonil was not sensitizing according to this method. The Toxicity Category for CTN in this study is "Non-sensitizing" (MRID00144112). The data requirement for a dermal sensitization study is satisfied.

81-7 Acute Delayed Neurotoxicity 107

No data are available on the acute neurotoxic effects of CTN. This test is only required for compounds which are organophosphate inhibitor of cholinesterase, or are related to such inhibitors or metabolites of such inhibitors. CTN is not an organophosphate; therefore, a study is not required.

82 Series Subchronic Testing

82-1 Subchronic Oral

Rat

An acceptable 13 Week Feeding study was performed using rats at doses of 0, 1.5, 3, 10 and 40 mg/kg/day. The NOEL was determined to be 3.0 mg/kg bw/day based on microscopic changes in the kidney. (MRID 00147943). Histopathological reevaluation of the kidneys was performed using light and electron microscopy. The previous NOEL of 3.0 mg/kg bw/day was lowered to 1.5 mg/kg bw/day. (MRID 00127852). The requirement for a Subchronic Oral study in a rodent species (rat) is satisfied.

Mouse

An acceptable Subchronic Oral feeding study was performed using CD-1 Charles River Mice at doses of 0, 7.5, 15, 50, 270 and 750 ppm. The NOEL in this study based on effects in the kidney was determined to be 15 ppm (ca. 2.5 -3.0 mg/kg bw/day). (MRID 00138148). Histopathological reevaluation of the kidneys was performed using light and electron microscopy. The previous NOEL of 15 ppm was confirmed (MRID 00147945). The requirement for a Subchronic Oral study in a rodent species (mouse) is satisfied.

Dog

No data are available on the subchronic oral toxicity of CTN in the dog. An acceptable chronic study in the dog is available; therefore, a subchronic study in the dog is not required.

82-2 Subchronic Dermal (21-day)

An acceptable 21 Day Dermal study was performed using rabbits. Animals demonstrated only mild erythema/edema. There were no systemic effects reported. The overall NOEL was 0.1 mg/kg bw/daybased on local irritiation. (MRID 00158254). The requirement for a Sub-chronic dermal study is satisfied.

82-3 Subchronic Dermal (90-day)

No data are available on the 90 day subchronic dermal toxicity of CTN. A study is not required under the present use pattern.

82-4 Subchronic Inhalation

No data are available on the suchronic inhalation toxicity of CTN. A study is not required because the existing acceptable end-uses should not result in repeated inhalation exposure.

82-5 Subchronic Neurotoxicity

No data are available on the subchronic neurotoxicity of CTN. Since an acute neurotoxicity study is not required, and there is no evidence of neurotoxicity in mammalian species, this study is not required.

83 Series Chronic and Long Term Studies.

83-1 Chronic Toxicity

Dog 1.5 mg/kg/day / 3.0 mg/kg/day

An acceptable two year dog feeding study was performed using Beagle Dogs at 0, 60 and 120 ppm. A No-Observable-Effect-Level (NOEL) was determined to be 60 ppm based the induction of renal tubular vacuolization and pigmentation (00114034).

Rat

In an acceptable chronic feeding study in the rat, Fischer 344 rats were offered diets containing 0, 800, 1600 or 3500 ppm (equivalent to 40, 80 and 175 mg/kg bw/day) CTN (98.1 % purity) for 116 weeks (males) or for 129 weeks (females). Non-neoplastic changes in the rat kidney included: chronic glomerulo- nephritis which increased in severity in a dose-related manner in all groups; dose-related increase in cortical tubular hyperplasia in dosed rats; increased incidence of tubular cysts in dosed rats; and increased incidence in dosed males only of hyperplasia of the papillary/pelvic epithelium (MRID 00146945).

The requirement for chronic studies in a rodent and non-rodent species is satisfied.

83-2 Oncogenicity

Mouse 1424 or 2895 mg/kg/day

In an acceptable oncogenicity study using B6C3F1 Mice, CTN was offered in the diet to groups of 50 males and 50 females (B6C3F1) for 91-92 weeks at 10,000 or 20,000 ppm. No significant increase in tumor incidence was reported (MRID 00030286).

A second acceptable oncogenicity study using CD-1 mice was performed which showed that Chlorothalonil, when offered in the diet at 0, 750, 1500 or 3000 ppm (equivalent to 0, 107, 214 and 428 mg/kg bw/day, respectively) for two years, induced renal tubular adenomas and carcinomas in males but not in females. No tumors were reported for the concurrent controls of either sex. Data from historical control files showed that these lesions are exceedingly rare in this strain (p = 0.002). Risk Assessment of this study determined that the oncogenic potency factor, Q1*, is 2.4 x 10-2 in mg/kg bw/day (Lacayo, 1985) 1/ Treated males also demonstrated squamous carcinomas and glandular carcinomas of the gastric mucosa. Control mice did not exhibit these lesions (MRID 00127858).

1/ H. Lacayo Memo to D. Beavers, 5/17/85.

Rat

In an oncogenic study in Osborne-Mendel rats, NCI found that Chlorothalonil possessed oncogenic properties, inducing renal adenomas and carcinomas in rats when offered in the diet at levels of 0, 5,063 or 10,126 ppm (TWA). The combined incidence of renal neoplasms was significantly increased over pooled controls in both males (p=0.028) and females (p=0.016) using the Fisher exact test. The in-house incidence of these neoplasms was 3/240 (1.25%) for males and 0/235 (0%) for females (MRID 00030286).

A second acceptable oncogenicity study was performed as a chronic rat study as noted above (MRID 00146945). Fischer 344 rats were offered diets containing 0, 800, 1600 or 3500 ppm (equivalent to 40, 80 and 175 mg/kg bw/day) CTN (98.1 % purity) for 116 weeks (males) or for 129 weeks (females). Chlorothalonil induced neoplastic changes in the renal tubular epithelium in treated male and female animals but not in the corresponding controls. Chlorothalonil also induced papillomas and carcinomas of the squamous epithelium of the forestomach in these rats, with a significant dose-related trend for females. Control males and females had no such neoplasia.

Historical control data supplied by the performing laboratory on male and female Fischer 344 rats showed no occurence of either of these tumors in six studies, representing 740 rats (370 rats per sex). A Q1* of 1.1 x10-2 was calculated based on tumor data derived from this rat study (Fischer, 1987) 2/.

The data suggest that renal tumorigenesis in these rats is mediated via chlorothalonil-induced hyperplasia of the cortico- tubular nephron. Taken together, the above findings suggest that the Maximum Tolerated Dose (MTD) is 175 mg/kg bw/day based upon effects on the kidney such as increased organ/body weight ratios, increased BUN and creatinine, and histopathological alterations of renal structures and reduced survival in the high dose males (MRID 00146945).

The requirement for oncogenicity studies in two species is satisfied.

83-3 Teratogenic Effects

An acceptable teratology study was performed using rabbits at doses of 0, 5.0 or 50.0 mg/kg bw/day on gestation days 6 - 18. Four does on 50 mg/kg aborted. The overall rate-of-weight gain was less than controls for the 50 mg/kg group. The maternal toxicity NOEL was 5.0 mg/kg bw/day. The fetotoxicity and teratogenic NOEL was 50 mg/kg bw/day. (00127855). The requirement for a teratology study in a non-rodent species is satisfied.

2/ B. Fisher Memo to D. Ritter, 7/20/87.

An acceptable teratology study was performed using Sprague-Dawley rats given oral doses of CTN at 0, 25, 100 or 400 mg/kg bw/day during days 6 through 15 of gestation. It was concluded that CTN was fetotoxic and maternally toxic at 400 mg/kg/day but did not induce terata at any level tested. The fetotoxic and maternal toxicity NOEL was 100 mg/kg bw day. The teratogenic NOEL was 400 mg/kg bw/day.(MRID 00130733). The requirement for a teratology study in a rodent species is satisfied.

83-4 Reproductive Effects

In an acceptable multi-generation reproduction study, CTN was administered in the diet to three generations of Charles River rats at 0, 0.15, 1.5, and 3.0/2.0 percent. Growth depression was reported in all control and parental groups and in all offspring. No increase in malformations was reported for any level tested. There was no effect on the reproductive indices. (00038913). The requirement for a multi-generation reproduction study is satisfied.

84 Series Mutagenicity

84-2 Mutagenicity

The acceptable mutagenicity data using CTN are summarized as follows:

STUDY	RESPONSE	MRID #
In Vivo Mouse Bone Marrow (CTN)	Negative	00147946
In Vivo Rat Bone Marrow (CTN)	Negative	00147947
In Vivo Bone Marrow Chin. Hams. (CTN)	Weakly clastogenic	00147948
Salmonella Gene Mutation (CTN)	Negative	00147949

85 Series Special Studies

85-1 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 equal to or less than 20 mg/kg/day and 200 mg/kg/day; at doses equal to or less than 50 mg/kg/day, the majority is excreted in 24 hours, at 200 mg/kg/day excretion and blood levels are prolonged. The proposed pathway for Chlorothalonil is given in figure I. Major detoxification occurs in the liver, by conjugation with glutathione. These conjugates are excreted directly into the bile; some may be transported to the kidneys where they are converted to thiol metabolites, the excretion of which is rate limited, and thus may lead to nephrotoxicity (and

metabolite in rats and ruminants (cow) is 4-hydroxy-2,5,6-trichloroisophthalonitrile. [DLR001, DLR002, DLR003, DLR004, DLR005, DLR006, 00147970, 00147969, 00162383, 40122915]. The requirement for metabolism studies is satisfied.

Structure-Activity Relationships

There are no studies available on compounds that possess a chemical structure similar to that of Chlorothalonil; however, there is a considerable body of data on the major animal metabolite, DS-3701, which was discussed in the original Registration Standard.

85-2 Domestic Animal Safety.

No data are available on the safety of CTN to domestic animals. Such studies are not required under the present use patterns.

85-3 Dermal Absorption

An acceptable dermal absorption study was performed in male rats using 14C-chlorothalonil. The test material was administered in acetone at a dosage rate of 5 mg applied to an area of 25 cm2 or 0.20 mg/cm2. The rate of absorption from the skin was relatively constant (6.3%) from 24 to 120 hours after application. Animals exposed for 120 hours had absorbed 27.7% of the dose and excreted 18% of the dose in the feces, 6% in the urine, with 20% lost at the time of application due to evaporation. Approximately 4% of the dose remained in the carcasses of animals exposed for 120 hours. The data suggest that the rate of dermal absorption of chlorothalonil was constant and that the amount of the dose absorbed was dependent upon the exposure time (DLR007). The requirement for a dermal absorption study is satisfied.

C. Dat Gaps

CTN is registered for use on crops and the following Guideline studies are required for registration:

81-1 Acute Oral
81-2 Acute Dermal
81-3 Acute Inhalation
81-4 Promary Eye Irritiation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization
81-7 Acute Delayed Neurotoxicity

82-1 Subchronic Oral, two species, rodent and non-rodent.
82-2 Subchronic Dermal (21 Day)
82-3 Subchronic Dermal (90 Day)
82-4 Subchronic Inhalation
82-5 Subchronic Neurotoxicity

83-1 Chronic Toxicity, two species, rodent and non-rodent.
83-2 Oncogenicity, two species.
83-3 Teratogenicity, two species.
83-4 Reproduction

85-1 Metabolism
85-2 Domestic Animal Safety
85-3 Dermal Absorption

Based on this assessment of the toxicology data base the following Guideline toxicology studies have been identified as data gaps and are required: No Guideline studies are required.

D. ADI Reassessment

The Toxicology Branch/Agency ADI Committee has established an RfD of 0.015 mg/kg bw/day based on a two year dog feeding NOEL of 60 ppm (renal lesions) (MRID00114034).

Additional data considered were a two year feeding/oncogenic study in rats and a two year oncogenicity study in mice; each study was positive for neoplasms in the kidneys.

An uncertainty factor of 100 was used because of the uncertainties associated with extrapolating from laboratory animals. The dog study was selected because it yielded the most sensitive value.

No new data have been submitted that would change the above RfD value (renal lesions) (MRID00114034). (R. Engler, 1986). 1/.

E. Toxicological Issues

All toxicity data submitted to the Agency in support of registration of CTN have been reviewed. There is nothing contained in these data that persuades the Agency to alter its previous finding that CTN is a B2 oncogen as was classified in the Toxicology Peer Review of 9/10/87 (Rinde, 9/10/87)2/ based on increased incidence of malignant and/or combined malignant and benign tumors in both sexes in two rat studies and a mouse study as follows:

1. Statistically significant increases in the incidence of renal adenomas and carcinomas in both sexes of Fischer 344 rats, and a dose-related increase in the incidence of papillomas and carcinomas of the forestomach in females.

2. A statisically significant increase in the incidence of renal adenomas and carcinomas in both sexes of Osborne-Mendel rats .

3. Chlorothalonil produced statistically significant increases in the incidence of carcinomas of the forestomach in both sexes of CD-1 mice, with a positive dose-related trend for females. In addition, there was a positive dose-related trend for combined renal adenomas and carcinomas in the males. The Committee considered that these tumors were important because of their rarity, and because they were of the same type and involved the same organs and tissues as seen the rat studies cited above.

1/ RfD document, R. Engler, 3/12/86.
2/ E. Rinde Peer Review Memo to Lois Rossi, PM # 21, 10/4/87.

The oncogenic potency (Q1*) of Chlorothalonil was estimated from the renal adenomas and carcinomas found in the female Fischer 344 rats to be 1.1 x10-2 in human equivalents (Fisher, 7/20/87). This value compares favorably with the Q1* of 2.2 x 10-2 estimated for the CD-1 mouse study (Lacayo, 1985).

Chlorothalonil, a number of metabolites and related chemicals were not genotoxic when tested in a wide array of mutagenicity assays. Nonetheless, there is sufficent evidence, in accordance with the Cancer Assessment Guidelines (FR 9/24/86), to conclude that Chlorothalonil is a probable human oncogen (B2), and that this trigger puts the chemical into Special Review as provided in 40 CFR

Reviewer's Peer Review Package for 1st Meeting

5/11/87

00713

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

[redacted]

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Chlorothalonil

FROM: for E. Rinde
Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review is a data package on Chlorothalonil. prepared by Mr. David Ritter. A meeting to discuss and evaluate the weight-of-the-evidence is scheduled for Thursday, May 28, 1987, at 10:00 AM in Dr. Farber's office (Room 821 of CM-2).

ADDRESSEES

T. Farber
W. Burnam
J. Quest
E. Rinde
J. Hauswirth
L. Kasza
R. Levy
B. Jaeger
D. Ritter
A. Barton
D. Beal
D. Barnes
R. Beliles

cc: L. Rossi

#18 5/11/87 sp

113

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: R. Engler, Ph.D.
Peer Review Committee

FROM: D. Ritter, Toxicologist
Rev. Sec. # 1
Toxicology Branch DR 4-22-87

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1
Toxicology Branch 5/4/87

Subject:

Peer review for Chlorothalonil.

Attached find the Toxicology Branch Peer Review chapter for the Fungicide, Chlorothalonil. We are not appending a Risk Analysis on the most recent rat oncogenicity study in accordance with your memo of 4/2/87. This will proceed on a parallel track.

Summary of Issues

The Toxicology Branch Peer Review Group is asked to review, evaluate and comment on the following findings and issues in order to determine the appropriate oncogenic classification of the agricultural fungicide, Chlorothalonil (2,4,5,6-tetrachloroisopthalonitrile; DS-2787; Bravo 500):

a. The significance of the occurence of renal tubular adenomas and carcinomas in male CD-1 male mice and male and female Fischer 344 rats in studies conducted under contract to SDS Biotech;

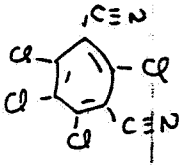
b. The significance of the occurence of esophageal and gastric papillomas and carcinomas in high dose female Fischer 344 rats in studies conducted under contract to SDS Biotech.

c. Depending on whether the Committee finds that Chlorothalonil is an animal oncogen, an assessment of the appropriate method of determining Risk.

d. Depending on the oncogenic Risk whether to consider some or all of the pending requests for temporary tolerances in racs and/or whether to cancel any and/or all agricultural uses of Chlorothalonil.

TABLE OF CONTENTS

CHLOROTHALONIL



DS-2787

SUMMARY

Chlorothalonil (DS-2787; 2,4,5,6-tetrachloroisophthalonitrile) is a proprietary product of the SDS Biotech Corporation (formerly Diamond Shamrock) of Painesville, OH. It is a widely used agricultural fungicide with numerous tolerances published under 40 CFR 180.275. The principle crop residues consist of the parent compound and its metabolite, DS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile). DS-3701 is the only detectable residue in meat and milk. Chlorothalonil is also used as a mildewicide in paints.

NCI found in 1978 that Chlorothalonil possessed oncogenic properties, inducing renal adenomas and carcinomas in rats. A more recent feeding study in mice revealed that the same lesions were induced in males but not in females; A Q^*_1 of 2.4×10^{-2} was calculated for these effects. A new rat feeding study showed that Chlorothalonil induced renal adenomas and carcinomas in males and females with the incidence increasing with increasing dose. This study also showed that Chlorothalonil may induce papillomas and squamous cell carcinomas of the gastric mucosa*.

As discussed below, Chlorothalonil induces non-neoplastic changes in the renal tubular tissues and in the gastric mucosa in these rats. Chlorothalonil did not induce genetic damage in any of a large number of mutagenicity studies; was not teratogenic, fetotoxic or especially toxic to nursing dams. Chronic toxic effects in earlier dog and rat feeding studies were largely limited to renal and gastric effects, similar to those reported in the later studies.

Metabolism studies in rats suggest that Chlorothalonil is excreted mainly via the GI tract, with ca 6 - 11 % excreted in the urine: there may be biliary excretion; and there may be an excretion rate-limiting active transport mechanism in the renal tubule. Other data show that the urinary metabolites of Chlorothalonil are di- and trithiochlorophthalonitrile compounds. Available evidence suggests that GSH conjugation of certain metabolites may be involved in the renal response to Chlorothalonil.

* A Risk Analysis is being calculated on this study and it will be appended per the memo of R. Engler, 4/2/87.

DETAILED CONSIDERATIONS

Oncogenicity

Rat

The National Cancer Institute (NCI) studied the oncogenic potential of Chlorothalonil in male and female Osborne-Mendel rats. They found that the material induced renal adenomas and carcinomas after 80 weeks dietary exposure to 5,063 and 10,126 ppm (time weighted average). No neoplasms were reported for the concurrent controls. This study was subsequently found to contain serious deficiencies in design and execution (Spencer, 1978), and was therefore given a CORE rating of "Supplemental" for the purpose of evaluating oncogenicity (Ritter, 1984). Nevertheless, the study contained valuable information on the renal tumorigenicity of CTN:

RENAL NEOPLASMS IN THE OSBORNE-MENDEL RAT[1]

	Males			Females		
	Control	Low Dose	High Dose	Control	Low dose	High dose
Carcinoma	0/10	1/45	3/49	0/10	1/48	2/50
Adenoma	0/10	2/45	1/49	0/10	1/48	3/50
Combined	0/10	3/45	4/49	0/10	1/48	5/50

There were 65 pooled control males and females used in other assays run concurrently. These were used in the statistical analyses of these data. Neoplasms in males were statistically increased above those of the pooled controls ($p = 0.030$)[2]. Historical control data showed an in-house incidence for these lesions of 3/240 (1.25%) for male rats. In the females, the incidence of renal tumors was likewise significantly increased over the pooled controls ($p = 0.007$).

SDS Biotech submitted a rat oncogenicity study in response to the Data Call-In associated with the Registration Standard for Chlorothalonil. Fischer 344 rats were offered diets containing 0, 800, 1600 or 3500 ppm for 116 weeks (males) or for 129 weeks (females). These levels are equivalent to 40, 80 and 175 mg/kg bw/day, respectively. Chlorothalonil induced neoplastic changes in the renal tubular epithelium in treated male and female animals but not in the corresponding controls.

[1] From Spencer, 1978.
[2] Cochran-Armitage test.

Incidence of Renal Tumors of Epithelial Origin, Independent Evaluation*

Tumor Type	Control M	Control F	40 mg/kg/day M	40 mg/kg/day F	80 mg/kg/day M	80 mg/kg/day F	175 mg/kg/day M	175 mg/kg/day F
Tubular Adenoma (per 60 animals)	0	0	3	3	5	10	7[a]	15[b]
Tubular Carcinoma (per 60 animals)	0	0	4	1	2	0	14[a]	12[b]
Total Animals with tumors (per 60 animals)	0	0	7	4[d]	7	10[e]	19	24[c]

a. Includes 2 males with combined incidence of tubular adenoma and tubular carcinoma.
b. Includes 3 females with combined incidence of tubular adenoma and tubular carcinoma.
c. Includes one female with a tubular carcinoma, originally diagnosed as invasive lipomatous tumor.
d. Includes one female with a tubular adenoma, originally diagnosed as negative.
e. Includes 4 females with a tubular adenoma, originally diagnosed as negative.

Chlorothalonil also induced papillomas and carcinomas of the squamous epithelium of the forestomach in these rats, but only the high dose females had an incidence for these lesions that was significantly different from that of the corresponding controls, which had no such neoplasia. Historical control data supplied by the performing laboratory (IRDC) on male and female Fischer 344 rats showed no occurence of either of these tumors in six studies, representing 740 rats (370 rats per sex).

Non-oncogenic effects--

Non-neoplastic changes in the rat kidney included: chronic glomerulonephritis which increased in severity in a dose-related manner in all groups; dose-related increase in cortical tubular hyperplasia in dosed rats; increased incidence of tubular cysts in dosed rats; and increased incidence in dosed males only of hyperplasia of the papillary/pelvic epithelium. The data suggest that renal tumorigenesis in these rats is mediated via chlorothalonil-induced hyperplasia of the cortico-tubular nephron.

Histopathological Re-evaluation of Renal Tissue. #764-5TX-85-001-002. 3/7/85. Submitted to the WHO/JMPR for 1985 review.

	MALE RATS			
	Control	40 mg/kg bw	80 mg/kg bw	175 mg/kg bw
Epithelial Hyperplasia (Prox. Conv. Tub.)	0/60	32/60	30/60	36/60
Kidney Adenoma or carcinoma	0/60	7/60	7/60	19/60
Number of Tumor-bearing rats	0/0	6/7	7/7	19/19

Parameters measured which were compound related and associated with the effects on the kidneys included increased BUN and serum creatinine in high dose males and females, decreased serum albumin and serum glucose in high dose males and females, increased urine volume and decreased specific gravity in all treated males throughout the study, and in all treated females initially (first year), but in high dose females only, after the first year. The relative kidney weights were significantly increased in all treated males and in mid-dose and high dose females only. Relative liver weight was affected in the same groups, being significantly increased in all dosed males, and mid- and high-dose females only. Gross necropsy of all animals demonstrated a compound related effect on the kidneys and stomach. In all dosed male groups and the high dose female group there were kidney masses and/or nodules as well as increased granularity of the surface of the kidneys (the latter observed in all dose groups). There were increased incidences of erosions and ulcerations in the non-glandular stomach of all dosed rats as well as a significant increase in discoloration of the mucosa in high dose males.

Other changes included increased hyperplasia/hyperkeratosis of the squamous mucosa of the esophagus (all dose groups); increased mucosal hypertrophy of the duodenum (all dose groups); hyperplasia of the parathyroid (all dosed male and high dose female groups, considered a secondary lesion as a result of severe chronic renal disease); increased hyperplasia/hyperkeratosis of the squamous mucosa of the stomach of all dose groups; increased incidences of foci of necrosis or ulcers in the glandular stomach of all dose groups; increased incidence of suppurative prostatitis in all male dosed groups (considered associated with treatment related to renal lesions). Complete involution of the thymus was increased in high dose males and all female dose groups.

High dose males showed reduced survival after 24 months when compared to that of the corresponding controls.

Taken together, the above findings suggest that the Maximum Tolerated Dose (MTD) is 175 mg/kg bw/day based upon effects on the kidney such as increased organ/body weight ratios, increased BUN and creatinine, and histopathological alterations of renal structures and reduced survival in the high dose males (Ritter, 1986/Busey, 1985).

Note: A Risk analysis will be appended to the final document.

Mouse

In the NCI study noted above, 50 males and 50 female B6C3F1 mice per group were offered diets containing 10,000 or 20,000 ppm for 91 - 92 weeks. 10 animals per sex served as concurrent conttrols. After two weeks these doses were reduced to 5,000 and 10,000 ppm respectively. No significant tumors were reported (Spencer, 1978).

SDS Biotech submitted an oncogenicity study using CD-1 mice which showed that Chlorothalonil when offered diets containing 0, 750, 1500 or 3000 ppm (equivalent to 0, 107, 214 and 428 mg/kg bw/day, respectively) in the diet for two years induced renal tubular adenomas and carcinomas in males but not in females. No tumors were reported for the concurrent controls of either sex. Data from historical control files showed that these lesions are exceedingly rare in this strain ($p = 0.002$). Risk Assessment of this study determined that the oncogenic potency factor is Q_1^* of 2.4×10^{-2} in mg/kg bw/day (Lacayo, 1985). The Agency review of this study found no dose-dependent relationship for the induction of these neoplasms, and concluded that an additional supporting study in rats was needed to more fully evaluate the oncogenic potential of Chlorothalonil in rodents. Treated males also demonstrated squamous carcinomas and glandular carcinomas of the gastric mucosa. Control mice did not exhibit the lesions. (Ritter, 1984).

NEOPLASMS IN CD-1 MICE OFFERED DIETARY CHLOROTHALONIL FOR TWO YEARS

	Kidney			
Lesion	Control	750 ppm	1500 ppm	3000 ppm
Tubular Adenoma	0/60	3/60	4/60	2/60
Tubular Carcinoma	0/60	3/60	0/60	2/60
Combined	0/60	6/60	4/60	4/60

	Gastric			
Lesion	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	1/60	5/60	2/60
Glandular Carcinoma	0/60	1/60	2/60	0/60

Absorption, distribution and excretion

Data from a multiple dose study at 1.5, 5, 50 or 160 mg/kg, each administered five times at 24 hour intervals to male Sprague Dawley rats, indicated that there were shifts in the times to peak blood concentrations with increasing single and multiple doses of chlorothalonil for both sexes. Significant depletion ($\geq$ 50%) of radiolabel from blood occurred by 24 hours post-dose for both sexes at dose levels less than or equal to 50 mg/kg. At 160 mg/kg, an apparent plateau in radiolabel concentration in blood was reached after a single dose, suggesting saturation of blood between 50 and 160 mg/kg. The concentrations of radiolabel in kidneys after single dose administration showed no apparent sex-related differences, but the times to peak kidney concentrations did appear to increase with increased dose level for both sexes. With multiple doses, the maximum kidney concentration was found 2 hours after the fifth dose at all dose levels. As with blood levels, peak kidney concentrations may have reached a plateau by the final 160 mg/kg dose. The maximum kidney concentration after five doses is proportional to the total administered dose at 1.5 mg/kg (3.12 ug equiv/g) and 5 mg/kg (8.03 ug equiv/g); and at 50 mg/kg (31.5 ug equiv/g) and 160 mg/kg (105 ug equiv/g), but are not proportional between the two lower and two higher doses. In this multiple dose study, kidney concentrations at 1.5, 5 and 160 mg/kg decrease 50% by 24 hours, but decreased only 20% by 27 hours at 50 mg/kg. By 7 days after the fifth dose, kidneys contained 14, 16, 23 and 25 percent of their maximum concentrations at 1.5, 5, 50 and 160 mg/kg, respectively. The authors suggest that the data demonstrate apparent saturation of blood, plateau of radiolabel in kidneys, and a trend toward slower depletion (or greater retention) of radiolabel from the kidney caused by increased and/or repeated doses of chlorothalonil. The authors further suggest that shifts in metabolism occur between doses of 5 and 50 mg/kg/day (Ritter, 1986/Savides et al., 1985).

In a similar second study, the author proposed a mathematical model for chlorothalonil kinetics:

$$V_A = V_T + V_B + V_U$$

where

V_A = rate of absorption in blood;
V_T = rate of absorption into tissues;
V_B = rate of elimination in the bile;
V_U = rate of elimination in urine.

(Dementi, 1987/Savides, 1986).

Male Sprague-Dawley rats were administered ^{14}C-labeled DS-2787 at levels of 5, 50 or 200 mg/kg by gavage. Urine and feces were collected at 2, 9, 24, 96 and 168 hours. Blood was collected at termination. These samples and representative tissues and organs were assayed for activity. 83% of the administered activity appeared in the feces, most during the first 48 hours in all dose groups. 5 -7% of the administered dose appeared in the urine. Blood levels of activity were dose-dependent with the 5 mg/kg groups peaking at 2 - 9 hours, then falling off to one fourth that by the 24th hour. 50 mg/kg groups showed a similar pattern, peaking at 2 - 9 hours, then dropping to one fourth by 24 hours. The 200 mg/kg group showed peak blood levels at 9 hours, falling to half that by 24 hours.

0.55% and 0.72% of the activity was found in the kidneys and liver respectively, and the kidney retained activity longer than any other tissue. Other tissues did not retain activity (Ritter, 1986/ Marciniszyn, 1984).

An identical study was performed in female Sprague-Dawley rats. Again, the major route of elimination was via the feces, with 79% of the 5 mg/kg dose eliminated in the first 48 hours; 85% of the 50 mg/kg dose was eliminated during the first 72 hours and 85% of the 200 mg/kg dose was eliminated by 72 hours. At 5 mg/kg, 11% of the administered dose was excreted in the urine over the test period with 92% of this being lost in the first 24 hours. At 50 mg/kg about 9% was lost with 80% being accounted for during the first 24 hours, and the 200 mg/kg animals excreted a total of 5.4% with 57% lost by 24 hours; 85% lost by 48 hours and 95% being excreted by 72 hours. The authors suggest the rate of excretion at this dosage level was not dose-dependent, and the urinary excretion mechanism could have been saturated. Blood peak concentrations showed a pattern similar to those of the males; maximum levels for the 5 mg/kg and 50 mg/kg animals being reached by 9 hours and the 200 mg/kg groups reaching maximum concentration between 9 and 24 hours. The later peak time in the high dose group could be due to delayed stomach emptying time.

Kidney and liver again showed a pattern similar to that of the males for retention of the adminsitered dose, maximum activity at 5 mg/kg occuring at 2 hours; at 50 mg/kg, 9 hours, and 200 mg/kg at 24 hours (Ritter, 1986/Marciniszyn, 1985).

Together with the corresponding male study, this study supports a tentative conclusion the renal excretory mechanism is rate-limiting for chlorothalonil; that the bulk of activity remains in the gut, and that there is reason to believe that stomach emptying time is delayed at the 200 mg/kg level.

The absorption of ^{14}C-chlorothalonil (purity 99.7%) through the skin was assessed in male Sprague-Dawley rats. A dose of 5 mg/kg was applied (46.7 ug/cm^2) to the clipped back (25 cm^2) of each rat. Twenty seven animals were treated and groups of three rats were subsequently killed at 2, 4, 8, 12, 24, 48, 72, 96 and 120 hours after application. The treated skin, blood, kidneys, liver, intestinal contents, remaining carcass, urine, feces and cage washes were analyzed for radioactivity. The rate of absorption from the skin was relatively constant (6.3%) from 24 to 120 hours after application. Animals exposed for 120 hours had absorbed 27.7% of the dose and excreted 18% of the dose in the feces, 6% in the urine, with 20% lost at the time of application due to evaporation. Approximately 4% of the dose remained in the carcasses of animals exposed for 120 hours. Mean concentration of radioactivity in blood, liver and kidney appeared to plateau after 72 hours. Excretion of radioactivity in feces appeared to be related to the blood concentrations, but urinary excretion appeared to be independent of blood concentrations. The urinary excretion pattern, attaining constancy of 1.2% of the applied dose per day, suggested that the renal excretory mechanism for chlorothalonil and/or its metabolites becomes saturated and is an active, rather than passive, form of excretion. Surface residues, nonetheless, constituted the bulk of activity. Data suggest that the rate of absorption of chlorothalonil was constant and that the amount of the dose absorbed was dependent upon the exposure time (Ritter, 1986/Marciniszyn, 1985).

Biliary excretion of ring labeled ^{14}C-chlorothalonil (purity 99.7%) was examined in Sprague-Dawley rats orally gavaged with 5 mg/kg. Animals (8 males, 4 females) were fasted, except for water, 16 hours prior to bile duct cannulation. Fifty percent of the males and females had sodium taurocholate (a choleretic substance) infused at a rate of 25 mg/hour. Animals were restrained and bile samples collected at hourly intervals from 0 to 48 hours after dosing. Blood was sampled at 6 and 24 hours and at termination. Urine and fecal samples were also collected periodically. Levels of radioactivity were determined in each bile, blood, urine and fecal sample and in the GI tracts, carcasses and cage washings.

Approximately 91.2% of the administered radioactivity was recovered. The presence of activity in the blood, urine and bile demonstrate that absorption via the gut occurs. The data indicate that approximately 34% of the administered dose was absorbed, with the remainder (67%) found in the feces and G.I. tract and represented non absorbed material. Biliary excretion accounted for 17-21% of the administered dose, with maximum concentrations eliminated within

2 hours of dosing. Urinary excretion, of about 8-12% of the labeled dose, shows this to be a significant route of elimination, but not a major one. No appreciable tissue binding is demonstrated as evidenced by low residual carcass levels, approximately 2% of the administered dose. Absorption via blood was also minimal, with maximum concentration less than 0.4% of the labeled dose (Ritter, 1985/Ignatoski, 1985).

Orally administered radiolabeled chlorothalonil to bile duct-cannulated male rats was excreted in the bile at a fairly constant rate as a percentage of adminstration levels of 1.5 5 and 50 mg/kg bw. At 200 mg/kg bw. the percent of dose excreted was significantly lower, suggesting saturation of the biliary excretory mechanism. The saturation limit for urinary exretion lies between 5 and 50 mg/kg bw. (Dementi, 1987/Savides, 1986).

Metabolism

Groups of Sprague-Dawley male rats (5 per dose) were administered 5000 mg/kg chlorothalonil (purity 97.8%) via oral gavage to measure the time course of the acute effect of a single dose on body weight, liver and kidney weights and liver and kidney GSH concentrations. Rats were sacrificed at 1, 3, 9, 18, 24 or 48 hours post-dosing. The data demonstrated significantly increased relative liver and kidney weights, reduced hepatic GSH concentration up to 24 hours post-dosing, and significantly increased renal GSH concentration up to 48 hours after treatment. The authors suggest that the hepatic GSH changes are related to its conjugation with chlorothalonil but were inconclusive regarding the renal GSH changes (Ritter, 1986. Sadler et al., 1985b).

The effect of a single administration of chlorothalonil (purity 97.3%) on liver and kidney glutathione (GSH) concentrations was assessed in male Sprague-Dawley rats, administered 5 mg/kg chlorothalonil via i.p., or 5000 mg/kg via oral gavage. Concentrations of GSH in liver and kidney determined 2 hours after i.p., or 24 hours after oral gavage demonstrated no differences between control and i.p. groups regarding GSH levels. However, chlorothalonil administered orally caused lower hepatic GSH and higher renal GSH concentrations. The authors suggest this supports the proposed metabolic pathway which includes a GSH conjugate formed in the liver which is subsequently metabolized in the kidney to a sulfur-containing, potentially nephrotoxic, compound (Ritter, 1986/Ignatoski, 1985).

Male Sprague-Dawley rats were dosed orally or i.p. with radio-labeled monoglutathione conjugate of DS-2787 at a level of 115 mg/kg bw. Urine was collected during the six hour test period over dry ice. The animals were then killed and the kidneys removed. A blood sample was obtained at termination. These tissues were prepared and counted using LSC. IP animals and excreted radiolabel in the urine at a rate approximately 10 times that of the orally dosed animals. IP animals showed blood levels at a rate approximately 10 times that of the orally dosed animals. Chlorothalonil-monogluthione derivative appears to have a metabolic pathway in the kidney similar to that of chlorothalonil (Dementi, 1987/Savides, 1986).

Male rats were dosed orally with ^{14}-C ring-labeled DS-2787 at 200 mg/kg bw. The pooled urines, taken at 24 and 48 hours were analyzed for metabolites by GC/MS. 2.4 % of the administered dose appeared in the urine. The urinary metabolites were tentatively identified as dithiodichlorophthalonitrile and trithiochlorophthalonitrile in approximately a 1:1 ratio. The authors postulate that chlorothalonil metabolism proceeds via hepatic conjugation with glutathione followed enzymatic degradation. These metabolites are then transported to the kidneys where they converted to thiol derivatives and excreted. (Interim Report; Ritter, 1986/Marciniszyn, 1985). The two metabolites were later identified as the methyl derivatives of dithiodichlorophthalonitrile and trithiochlorophthalonitrile (Dementi, 1987/Savides, 1986).

Discussion

Absorption of chlorothalonil into the mammalian bloodstream occurs via the skin and the gut. The amount absorbed is roughly proportional to the dose employed; the maximum blood level attained being when the oral dose reaches 160 mg/kg bw. The data suggest that the liver is the principle site of metabolism, conjugation with GSH being the major detoxifying process. These conjugates then are excreted directly into the bile or are transported to a lesser extent to the kidney, where they are converted to thiol metabolites that may be nephrotoxic. The excretion of these appears to be dependent on a rate-limiting step, (probably an active transport mechanism) followed by accumulation of them, and the subsequent development of damage to the renal epithelial tissues. Additional studies are underway to more fully elicit the renal response to chlorothalonil.

Developmental Effects

Chlorothalonil was offered in the diet to three generations of rats at 0, 0.15, and 3.0/2.0 %. Growth depression was reported in all parents. Pitted renal surfaces and gross discoloration of the kidneys was reported. Gastric wall thickening was reported in the P1 generation in the high dose groups. Growth depression was reported in all offspring. Focal renal tubular epithelial vacuolation at the middle and high dose P3 rats. In addition, gastric and esophageal acanthosis and hyperkeratosis was reported in the low and middle dose P3 groups. No increase in malformations was reported for any level, however (Long, 1969).

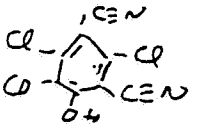
A rat teratology study using chlorothalonil by gavage at 0, 25, 100 or 400 mg/kg bw/day on days 6 through 15 resulted in no abortions, although 3/25 dams on the 400 mg/kg bw dose level died. Animals receiving this dose showed reduced food consumption and a significant number of early resorptions and post-implantation losses. No malformations were reported at any exposure level. (Jaeger, 1983).

Mutagenicity

The mutagenicity data are summarized in the attached tables. Chlorothalonil was not clastogenic in In Vivo bone marrow abberration assays in rats and mice; was weakly clastogenic in Chinese hamsters; was negative for mutagenic effects in numerous activated and non--activated Ames assays.

Structure-Activity Relationships

There are no studies available on compounds that possess a chemical structure similar to that of Chlorothalonil; however, there is a considerable body of data on DS-3701 (4-hydroxy-2,5,6-trichlore - phthalonitrile), the major metabolite in rats, and the only metabol. in meat and milk. Therefore, dietary exposure to this material is potentially significant.



DS-3701

Chronic Toxicity of DS-3701

Rat

75 male and female Sprague-Dawley CD-1 rats per group received 0, 0.5 or 3.0 mg DS-3701 per kg bw/day for two years. Evidence of microcytic anemia was present during the study and at termination, including decreased hematocrit, hemoglobin and mean cell volume. Histopathologic examination of the full range of tissues and organs failed to reveal evidence of neoplastic alterations at any exposure level (Ritter, 1983).

Groups of 30 male and 30 female Sprague-Dawley rats were offered diets containing 0, 10, 20, 50, 100 or 200 ppm DS-3701 for 76 weeks. No neoplasia was reported. Ulceration of the cornea was reported. Systemic effects were limited to reduced body weights and reduced testicular weights at levels above 50 ppm (Long, 1978).

Mouse

60 CD-1 mice per sex per dose level were offered diets containing 0, 375, 750 or 1500 ppm DS-3701 for 105 weeks (equivalent to 0, 53.6, 107.1 and 214.3 mg/kg bw day, respectively). Although no tumor increases were reported that could be related to exposure, there was an inverse relationship for the appearance of neoplasms with increased dose. The systemic NOEL was less than 375 ppm in the diet based on significant reduction in red cell count in the treated female groups. The material is not considered to be an oncogen (Ritter, 1984).

~~CASWELL FILE~~

CC/713

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

MAY 17 1985

004455

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Risk Assessment for Chlorothalonil Based on Diamond Shamrock's Two Year Chronic Mouse Feeding Study. Accession No. 071541.

Caswell No. 215B

FROM: Herbert Lacayo, Statistician
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Dianne Beavers, Product Manager Team #21
Herbicide Fungicide Branch
Registration Division (TS-767)

THRU: Bertram Litt, Leader
Statistics Team, Mission Support Staff
Toxicology Branch/HED (TS-769)

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

Summary:

The study data analyzed below indicate that chlorothalonil (CTN) is a renal carcinogen in male CD-1 mice. The weight of evidence determination with respect to human carcinogenicity will be made by the Toxicology Branch Cancer Review Committee.

Chlorothalonil has a potency factor Q_1^* of 2.4×10^{-2} for exposure expressed in mg/kg body weight/day.

Background:

The Registrant submitted their own risk assessment. Sufficient methodological detail was not given in their submission to determine precisely why the Diamond Shamrock results were two orders of magnitude lower than that obtained by Crump's multi-stage model (Ref. 1), where this latter model was implemented is accordance to procedures recommended by the EPA draft guidelines.

Study Description:

The National Cancer Institute Study (NCI-CG-TR-41, 1978) contains evidence that CTN induces renal neoplasm in Osborne-Mendel male and female rats. This prompted Diamond Shamrock Corporation to perform a second study in mice ("a Chronic Dietary Study in Mice with Technical Chlorothalonil," dated April, 1983) to test the null hypothesis that chlorothalonil does not cause kidney tumors. Their two year feeding study used 97.7% CTN, CD-1 mice and was carried out by Bio/Dynamics.

Test mice were assigned randomly to four groups of 60 males and 60 females per treatment. The treatment groups consisted of control, low, medium, and high dose respectively as shown below.

TABLE 1

Experimental Design for the Chlorothalonil Feeding Study

Group	Dose (ppm)	Number of Males	Number of Females
I	0	60	60
II	750	60	60
III	1500	60	60
IV	3000	60	60

The study was initiated February, 1980 and terminated after 24 months. All surviving mice were sacrificed at the end of the study period. Animals dying or sacrificed during the study or at termination were necropsied.

Qualatative Analysis:

The Registrant and D. Ritter, EPA Toxicologist, note average survival in all groups except high dose males; and "food consumption and weight gain were comparable among groups." They both summarize the results by noting that there is nothing in the study which would either cause the tumor data to be excluded or cause difficulties in its interpertation.

Statistical review indicates no discernable strong dose related trends in the mortality of the test animals. However, as noted by the Registrant, mortality is significantly higher for high dose males when compared to controls ($p = .07$ by Fischer's Exact test). Second, female mortality by 18 months was significantly higher than male mortality for corresponding study groups ($p \leq .01$ by Fischer's Exact test). These mortality data are summarized below in Table 2.

TABLE 2

Cummalative Mortality At Six Month Intervals

	MALES				FEMALES			
DOSE (ppm)	6	12	18	24	6	12	18	24
0	1	3	8	29	4	8	20	42
750	0	2	10	35	2	3	18	38
1500	5	7	8	26	3	6	17	37
3000	2	10	13	38	3	9	20	41

Body weights for both male and female for all treatment groups means were comparable to controls for both sexes. Although significant differences were not noted within either sex, the female mice appeared to exhibit greater variability for both within and between group variances.

The tumors of greatest interest were renal tumors in male mice. The data are summarized in Table 3.

TABLE 3

Dose (ppm)	0	750	1500	3000
Response	0/57	6/59	4/59	4/56

Because the tumor rate rises then flattens out by 1500 ppm, it is clear that the departure from linearity explains the lack of a statistically significant dose-response trend ($p = .14$ by the Peto or Armitage-Cochran tests). However, when historical data are utilized (Ref. 2,3) it may be shown that the effect is dose related. This is done by reasoning similar to that given in Ref. 2. Using a background tumor rate of $p = .002$ (estimated from data in Ref. 3), binomial distribution theory implies that the probability of having 14 or more male mice with renal tumors in a group of 231 is less than .0001. Stated more formally, the dose effect of chlorothalonil is statistically significant at the $p = .0001$ level, compared to the referenced historical controls under the binomial distribution assumption.

Quantitative Risk Assessment:

In addition to the renal tumors noted above, all treatment groups (in both sexes) exhibited gastric carcinomas. These are summarized below.

TABLE 4

Gastric
(Number of Tumors/Number of Animals at Risk)

	0 ppm	750 ppm	1500 ppm	3000 ppm
Female				
Squamous cell Carcinoma	0/57	2/60	6/58	5/58
Glandular	0/57	1/60	1/58	2/56
Total	0/57	3/60	7/58	7/58
Male				
Squamous cell Carcinoma	0/55	2/59	5/59	1/51
Glandular	0/55	1/59	2/59	0/51
Total	0/55	3/59	7/59	1/102

Squamous cell and Glandular carcinomas are not normally additive. However, in this case Dr. L. Kasza, Staff Pathologist, suggests that there may be evidence of multiple tissue tumors that may be due to the same causative agent or mechanism.

For risk assessment purposes we will use the rare renal tumors rather than gastric tumors because that effect is detected at a lower dose. The problem of the non monotonicity of the dose response with the renal tumors can be dealt with by eliminating the 1500 and 3000 ppm dose groups as recommended by the Crump multi-stage procedure and the Mantel/Tukey paper (Ref. 6). This approach is consistent with EPA policy (see Ref. 4) that tends to select the data groups giving the highest potency (Q_1^*).

Crump's multi-stage procedure was applied to the following renal-tumor-data set where human equivalent dose is expressed in mg/kg/day.

TABLE 5

Renal Tumors

Human Equivalent Dose (mg/kg/day)	0	8.2
Response	0/57	6/59

The human equivalent dose (in the absence of experimental data) was calculated by standard methods (see Appendix for formulas).

The results of the multi-stage modeling are given below.

MLE of Q_1	Est of Q_1^*
1.31×10^{-2}	2.4×10^{-2}

Note that the Chi Square value is not shown, as it is not relevant because there are only two dose groups to fit. Note that the MLE (maximum likelihood estimate) of Q and Q_1^* are close. Hence, there is a close correspondence between the point estimate of the slope based on the data, and the 95% upper bound on this slope.

Diamond Shamrock carried out their own independent risk assessment producing results which differ from ours by about two orders of magnitude. This discrepancy might be reconciled as follows:

1. If the Registrant used all four groups without surface area adjustment of the dose and if they used the maximum likelihood estimate for potency (instead of $Q_1^* = 2.4 \times 10^{-2}$), their estimate would be 2.8×10^{-3}.

2. If the Registrant also performed a surface-area correction of say $(6000/40)^{1/3} = 11.4$, they would find a potency, Q_1^*, of about 2.45×10^{-4}.

3. By working backwards from the Registrant's risk data we have found that their potency was about 2.28×10^{-4} to 2.46×10^{-4}. This includes the 2.45×10^{-4} value calculated above. That possibly clarifies the two orders of magnitude differences between the results.

For completeness, we list two other possible sources of error:

1. The Registrant appears to count all animals on test while Toxicology Branch reviewers count only non-autolyzed mice.

2. The Registrant appears to over estimate the "Annualized Daily Exposure" by not taking into consideration that a worker will generally be exposed for only 1/2 his(her) life time.

Characterization of Risk:

The risk for the TMRC and some of the published tolerances (see Appendix for complete list) are given below where the risk are based on a $Q_1^* = 2.4 \times 10^{-2}$.

TABLE 6

	Exposure (mg/kg/day)	Risk
Celery	.001073	10^{-5}
Cucumber	.000907	10^{-5}
Melons	.002504	10^{-5} to 10^{-4}
Beans (snap)	.001226	10^{-5}
Tomatoes	.00359	10^{-4}
Cabbage	.0009198	10^{-5}
TMRC	.011905	10^{-4}

Worker risks were obtained from S.E. Noren's memo to R. Engler dated December 17, 1984 (Ref. 5), the basic data and risks are given below.

TABLE 7

Worker Risks Based on $Q_1^* = 2.4 \times 10^{-2}$ and 100% Dermal Penetration

Ground Application	LADD[a]	Risk[b]
Sprayer Mixer	.0415	10^{-3}
Aerial Application		
Mixer	.029	10^{-4} to 10^{-3}
Flagman	.011	10^{-4}
Pilot	.005	10^{-4}

[a] LADD = Lifetime Average Daily Dose (see Appendix for detail).

[b] Risk = $Q_1^* \times$ LADD

APPENDIX

I. Reference

II. Formulas

III. Published Tolerances

I. REFERENCE

1. Crump, K. S. (1982) An improved procedure for low-dose carcinogenic risk assessment from animal data. Journal of Environmental Pathology and Toxicology Vol. 5, No. 2, 675-684.

2. Memorandum, H. Lacayo to R. Engler. Subject: Use of Historical Data..... dated Feb. 29, 1985.

3. Letter. R. P. Burton of Biotech to H. M. Jacoby of EPA dated Dec. 19, 1983.

4. Water Quality Criteria Documents, Federal Register, Vol. 45. No. 231, Friday, Nov. 28, 1980.

5. Memorandum. S. E. Noren to R. Engler, Subject: Applicator Exposure for Chlorothalonal.

6. N. Mantel, N. R. Bohidar, C. C. Brown, J. L. Ciminera, and J. W. Tukey. An improved Mantel-Bryan procedure for "safety" testing of carcinogens. Cancer Research 345, 865-872 (1975).

II. FORMULAS

A. LADD Formula

The Lifetime Average Daily Dose (mg/kg/day is approximated by:

LADD = (Dose acquired in one working day in mg/kg/day)

x (No. of working days per year with the chemical)/365

x (35 years of working)/(70 years lifetime)

$$= (\text{One day exposure}) \times \frac{\text{days exposed/yr}}{365} \times \frac{35}{70}$$

B. Conversion of ppm to mg/kg/day

1 ppm in mouse diet = .150 mg/kg/day

Quick Conversion (for ppm only)

$$1 \text{ ppm in diet for animal} = \frac{\text{(Wt of diet in grams)}}{\text{(Wt of animal in grams)}}$$

= mg/kg/day for animal

C. Interspecies Conversion Factor

Let SA = Surface Area

W_h = body weight of humahn
W_a = body weight of animal
d_h = dose for human (mg/kg/day)
d_a = dose for animal (mg/kg/day)

If we assume the surface ares is proportional to $W^{2/3}$ and that equivalent doses (in mg/day) are proporational to surface areas, then $d_h = d_a \times (W_a/W_h)^{1/3}$.

For example extrapolation of mouse to an "equivalent" human dose can be done as follows:

1. Convert mouse dose which is usually in ppm to mg/kg/day.

   .15 x (mouse dose in ppm) = mouse dose in mg/kg/day.

2. Therefore,

   Human Equiv. Dose = (mouse dose in mg/kg/day) $\times (25/55000)^{1/3}$

DAR 2-20-86 (a)

C. 713

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Tumorigenicity Study in Rats.

LABORATORY: IRDC.

STUDY NUMBER & DATE: 099-5TX-80-234-008 5/28/85 W. D. Busey.

ACCESSION NUMBER: 258759. MRID 146939 - 146945

MATERIAL TESTED: Chlorothalonil 98.1 % (containing [redacted] % HCB or less).

ANIMALS: Fischer 344 rats, 60 per sex per group.

METHODS:

Dosing:

Rats were offered diets containing 0, 800, 1600 or 3500 ppm for 116 weeks (males) and for 129 weeks (females). These levels are equivalent to 40, 80 and 175 mg/kg/day, respectively.

Husbandry, Food Consumption, Body Weights and Observations for Effects:

Standard GLP.

Necropsy:

Standard GLP.

RESULTS: (Jaeger, R.B., 1985 WHO/JMPR monograph, 11/20/85)

"Survival was comparable in all groups, both sexes, for the first 24 months. Continuation on study decreased survival in high dose males resulting in all males sacrificed at 27 months. Females were terminated on schedule at 30 months. The major cageside clinical observation included dark yellow urine in high dose males and females from weeks 27-91. An increased brown, red staining around the anogenital region of mid- and high dose females was also observed. There was a significant body weight decrease (10-29%) in high dose males and females throughout study, as well as a 5-12% body weight decrease in both sexes at the mid dose. There was no body weight reduction in low dose animals. Food consumption was unaffected, except for an increase in high dose animals, generally towards the last half of the study.

"Mononuclear cell leukemia is a common finding (approx. 20%) in Fischer 344 rats at an average age of 2 years (so-called "Fischer rat leukemia"). In this particular study there was an inverse relationship with dose in that this finding was most pronounced in controls. This was supported by numerous hematological, clinical chemistry and micropathological findings. These effects were most noticeable in controls males. They included: decreased RBC, Hgb, Hct, and platelet counts, with increased MCV, MCH, reticulocytes, nucleated RBC and segmented

neutrophils. These changes were accompanied by enlarged spleen at 0 and 40 mg/kg, and are suggestive of a macrocytic normochromic regenerative anemia. Also, in control males, there were increases in total bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase levels; findings which are common in the Fischer rats in later stages of this disease.

"Parameters measured which were compound related and associated with the effects on the kidneys included increased BUN and serum creatinine in high dose males and females, decreased serum albumin and serum glucose in high dose males and females, increased urine volume and decreased specific gravity in all treated males throughout the study, and in all treated females initially (first year), but in high dose females only, after the first year. The relative kidney weights were significantly increased in all treated males and in mid- dose and high dose females only. Relative liver weight was effected in the same groups, being significantly increased in all dosed males, and mid- and high-dose females only. Gross necropsy of all animals demonstrated a compound related effect on the kidneys and stomach. In all dosed male groups and the high dose female group there were kidney masses and/or nodules as well as increased granularity of the surface of the kidneys (the latter observed in all dose groups). There were increased incidences of erosions and ulcerations in the non-glandular stomach of all dosed rats as well as a significant increase in discoloration of the mucosa in high dose males.

"Histologically there was evidence of compound related effects on the kidneys, esophagus, stomach and duodenum. Non-neoplastic changes in the kidney included: chronic glomerulonephritis which increased in severity in a dose-related manner in all groups; dose related increase in cortical tubular hyperlasia in all dosed rats; increased incidence of tubular cysts in all dosed rats; and increased incidence in dosed males only of hyperplasia of the papillary/pelvic epithelium. Other changes included increased hyperplasia/hyperkeratosis of the squamous mucosa of the esophagus (all dose groups); increased mucosal hypertrophy of the duodenum (all dose groups); hyperplasia/ hyperkeratosis of the parathyroid (all dosed male and high dose female groups, considered a secondary lesion as a result of severe chronic renal disease); increased hyperplasia/hyperkeratosis of the squamous mucosa of all dose groups; increased incidences of foci of necrosis or ulcers in the glandular stomach of all dose groups; increased incidence of suppurative prostatitis in all male dose groups (considered associated with treatment related renal lesions); complete involution of the thymus was increased in high dose males and all female dose groups. Interesting inverse dose related changes included: chronic interstitial prostatitis (increased in control and low dose male groups); increased incidence of medullary tumors of the adrenal (Control and low dose female groups); increased incidences of osteoschlerosis of the femur and sternum (control females); and an increased incidence of basophilic cell focus/foci of the liver (control females - a common finding in aging Fischer 344 rats).

"Neoplastic changes associated with treatment were observed in kidneys and stomach (forestomach). Tubular adenomas and carcinomas, anaplastic renal carcinomas and transitional cell carcinomas were observed in the kidney of treated rats only, being statistically significant in all dosed rats except low dose females. There was also a possible decrease in time to tumor in high dose rats for renal adenomas and carcinomas. There was no evidence however, that the occurrence of

14

cortical tubular hyperplasia or tubular cysts predisposed animals to such tumors since only 1/11 tumor bearing low dose rats also had a tubular cyst. This was also true at the higher doses as well.

"Papillomas and carcinomas of the squamous mucosa of the stomach were present in treated rats only, but statistically significant in high dose females only. Again, there was no evidence that hyperplasia or hyperkeratosis predisposed the animals to such tumors as the degree or severity of hyperplasia/hyperkeratosis varied from slight to marked in these animals, and was equally prevalent in non-tumor bearing rats, although the severity of response increased in a dose-related manner."

CONCLUSIONS:

Results of this study demonstrate that chlorothalonil produced renal adenomas and carcinomas in Fischer 344 rats (both sexes) at $\geq$ 40 mg/kg b.wt. (see Tables 1 - 5, appended). Secondary to this response was a dose-related increase in papillomas of the stomach (0/60, 1/60, 1/60, 2/60 for 0, 40, 80 and 175 mg/kg males; and 0/60, 1/60, 2/60 and 6/60 for 0, 40, 80 and 175 mg/kg females; see Table 6, appended).

CORE RATING:

Guideline.

TABLE 1

Incidence[a] of Renal Tumors of Epithelial Origin, Original Report

Tumor Type	Control		40 mg/kg/day		80 mg/kg/day		175 mg/kg/day	
	M	F	M	F	M	F	M	F
Tubular Adenoma	0	0	2	2	4	4	**11[b]	**9
Tubular Carcinoma	0	0	5	1	2	2	*7	**11
Transitional-cell Carcinoma	0	0	0	0	0	0	2[b]	0
Anaplastic Renal Carcinoma	0	0	0	0	1	0	0	3
Total Animals with These Tumors	0	0	*7	3	*7	*6	**19	**23

[a]Kidneys from 60 animals of each sex were examined for all groups.
[b]Includes one male with tubular adenoma and transitional cell carcinoma.
*Statistically different from control - $p<0.05$ (Fisher's exact test).
**Statistically different from control - $p<0.01$ (Fisher's exact test).

TABLE 2

Incidence of Renal Tumors of Epithelial Origin, Independent Evaluation

Tumor Type	Control		40 mg/kg/day		80 mg/kg/day		175 mg/kg/day	
	M	F	M	F	M	F	M	F
Tubular Adenoma	0	0	3	3	5	10	7[a]	15[b]
Tubular Carcinoma	0	0	4	1	2	0	14[a]	12[b]
Total Animals with tumors	0	0	7	4[d]	7	10[e]	19	24[c]

a. Includes 2 males with combined incidence of tubular adenoma and tubular carcinoma.

b. Includes 3 females with combined incidence of tubular adenoma and tubular carcinoma.

c. Includes one female with a tubular carcinoma, originally diagnosed as invasive lipomatous tumor.

d. Includes one female with a tubular adenoma, originally diagnosed as negative.

e. Includes 4 females with a tubular adenoma, originally diagnosed as negative.

TABLE 3

Correlation of Renal Hyperplasia with Tubular Adenoma and Carcinoma, Original Report (Males)

Pathological Finding	Control	40 mg/kg/day	80 mg/kg/day	175 mg/kg/day
Glomerulo - nephritis	39/60	56/60	56/60	60/60
Cortical Tubular hyperplasia	0/60	7/60	9/60	22/60
Kidney Adenoma or Carcinoma	0/60	7/60	7/60	19/60
Number of Tumor bearing rats with renal hyperplasia	0/0	0/7	0/7	3/19

TABLE 4

Correlation of Renal Hyperplasia with Tubular Adenoma and Carcinoma, Independent Evaluation (Males)

Pathological Finding	Control	40 mg/kg/day	80 mg/kg/day	175 mg/kg/day
Chronic progressive nephropathy	47/60	52/60	54/60	57/60
Focal Epithelial Hyperplasia (Prox. Conv. Tub.)	0/60	6/60	20/60	6/60
Epithelial Hyperplasia (Prox. Conv. Tub.)	0/60	32/60	30/60	36/60
Kidney Adenoma or carcinoma	0/60	7/60	7/60	19/60
Number of Tumor bearing rats with renal hyperplasia	0/0	6/7	7/7	19/19

TABLE 5

Correlation of Renal Hyperplasia with Tubular Adenoma and Carcinoma, Independent Evaluation (Females)

Patholigical Finding	Control	40 mg/kg/day	80 mg/kg/day	175 mg/kg/day
Chronic Progressive Nephropathy	45/60	49/60	47/60	51/60
Focal Epithelial Hyperplasia (Prox. Conv. Tub.)	6/60	22/60	34/60	42/60
Epithelial Hyperplasia (Prox. Conv. Tub.)	5/60	35/60	39/60	48/60
Kidney Adenoma or Carcinoma	0/60	4/60	10/60	24/60
Number of Tumor bearing rats with renal hyperplasia	0/0	4/4	10/10	21/24

The incidence of papillomas and carcinomas of the stomach were dose-related, but statistically significant only in high dose females (0/60, 1/60, 1/60, 2/60 for males and 0/60, 1/60, 2/60, and 6/60 for females at 0, 40, 80 and 175 mg/kg dose levels, respectively) [See Table 6]. Although there was no apparent correlation between forestomach tumors and the incidence of hyperplasia or hyperkeratosis, the non-neoplastic changes may have been obscured by the progression to tumor. Nonetheless, there was a dose-related increase in the severity of hyperplasia/hyperkeratosis in the forestomach.

TABLE 6

Incidence[a] of Tumors in the Gastric Mucosa

Site/ Tumor Type	Control M	Control F	40 mg/kg/day M	40 mg/kg/day F	80 mg/kg/day M	80 mg/kg/day F	175 mg/kg/day M	175 mg/kg/day F
Forestomach/ Papilloma:	0	0	1	1	1	2	2	6*
Squamous Carcinoma:	0	0	0	0	0	0	1	1
Total number of animals with forestomach tumors:	0	0	1	1	1	2	3	7*
Fundal stomach/ Mucosal polyp:	1	0	0	0	0	0	0	0
Adenocarcinoma:	0	0	0	0	0	1	0	0

[a]Stomachs from 60 animals of each sex were examined.
*Statistically different from control - $p < 0.05$ (Fisher's exact test)

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

DOSE LEVEL (MG/KG/DAY)	NO. OF ANIMALS PER SEX
0	60
40	60
80	60
175	60

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

DOSE LEVEL (MG/KG/DAY)	SURVIVAL				
	12 MO.	18 MO.	24 MO.	TERMINATION	
				27 MO.	30 MO.
MALE					
0	59/60	57/60	47/60	32/60	---
40	60/60	59/60	49/60	34/60	---
80	58/60	57/60	43/60	34/60	---
175	60/60	59/60	42/60	22/60	---
FEMALE					
0	60/60	59/60	48/60	---	31/60
40	60/60	60/60	49/60	---	21/60
80	59/60	56/60	49/60	---	17/60
175	60/60	59/60	47/60	---	25/60

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

DOSE LEVEL (mg/kg/day)	INCIDENCE OF PRIMARY RENAL TUMORS OF EPITHELIAL ORIGIN	
	MALES	**FEMALES**
0	0/60	0/60
40	7/60*	3/60
80	7/60*	6/60*
175	19/60**	23/60**

(lipomatous tumor ...)

* P<0.05 (FISHER'S EXACT TEST)
** P<0.01 (FISHER'S EXACT TEST)

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES* OF GLOMERULONEPHRITIS, TUBULAR CYSTS, AND HYPERPLASIA IN MALES

	DOSE LEVEL (mg/kg/day)			
MICROSCOPIC FINDING	0	40	80	175
GLOMERULONEPHRITIS				
VERY SLIGHT	15	4	5	0
SLIGHT	24	19	8	2
MODERATE	11	28	21	15
MARKED	1	5	23	43
TOTAL	51	56	57	60
TUBULAR CYSTS	4	15	19	34
CORTICAL HYPERPLASIA	0	7	8	22
PAPILLARY/PELVIC HYPERPLASIA	0	6	11	10

*KIDNEYS FROM 60 RATS WERE EXAMINED FOR EACH GROUP

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES* OF GLOMERULONEPHRITIS, TUBULAR CYSTS, AND HYPERPLASIA IN FEMALES

	DOSE LEVEL (mg/kg/day)			
MICROSCOPIC FINDING	0	40	80	175
GLOMERULONEPHRITIS				
VERY SLIGHT	12	18	9	3
SLIGHT	21	14	8	5
MODERATE	8	19	26	23
MARKED	3	2	9	25
TOTAL	44	53	52	56
TUBULAR CYSTS	2	14	26	37
CORTICAL HYPERPLASIA	0	8	21	17
PAPILLARY/PELVIC HYPERPLASIA	1	1	2	2

*KIDNEYS FROM 60 RATS WERE EXAMINED FOR EACH GROUP

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCE OF POLYP OR ADENOCARCINOMA OF GLANDULAR MUCOSA IN THE STOMACH

DOSE LEVEL (mg/kg/day)	MALES	FEMALES
0	1/60	0/60
40	0/60	0/60
80	0/60	1/60
175	0/60	0/60

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

DOSE LEVEL (mg/kg/day)	INCIDENCE OF SQUAMOUS CELL PAPILLOMA AND CARCINOMA IN THE STOMACH	
	MALES	FEMALES
0	0/60	0/60
40	1/60	1/60
80	1/60	2/60
175	3/60	7/60*

* $P < 0.05$ (FISHER'S EXACT TEST)

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES* OF HYPERPLASIA/HYPERKERATOSIS IN THE FORESTOMACH IN MALES

	DOSE LEVEL (mg/kg/day)			
DEGREE OF HYPERPLASIA /HYPERKERATOSIS	0	40	80	175
VERY SLIGHT	0	2	1	0
SLIGHT	2	15	6	3
MODERATE	1	34	33	20
MARKED	0	9	18	37
TOTAL INCIDENCE	3	58	58	60

*STOMACHS FROM 60 RATS WERE EXAMINED FOR EACH GROUP

TUMORIGENICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES* OF HYPERPLASIA/HYPERKERATOSIS IN THE FORESTOMACH IN FEMALES

	DOSE LEVEL (mg/kg/day)			
DEGREE OF HYPERPLASIA /HYPERKERATOSIS	0	40	80	175
VERY SLIGHT	2	3	2	0
SLIGHT	3	26	14	3
MODERATE	0	21	30	26
MARKED	0	10	14	31
TOTAL INCIDENCE	5	60	60	60

*STOMACHS FROM 60 RATS WERE EXAMINED FOR EACH GROUP

WTOL 1-31-84
CC:718
(a)

DATA EVALUATION REPORT

STUDY: Chronic Mouse Feeding Study

LABORATORY: Biodynamics Laboratory, East Millstone, NJ

STUDY NUMBER & DATE: DTX-79-0102

ACCESSION NUMBER: 071541

MRID:

MATERIAL TESTED: Technical Chlorothalonil 97.7%

ANIMALS: CD-1 Mice males and females

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

ROUTE OF ADMINSTRATION: Dietary, prepared fresh weekly with samples of test material taken for analysis.

LEVELS OFFERED: 0, 750, 1500 and 3000 ppm.

SCHEME OF ADMINISTRATION: 60 mice/sex/group. Control and treated diets offered ad libitum.

OBSERVATIONS: Daily for mortality and gross signs of toxicity. Weekly complete physical exam. Body weight and food consumption - pretest, then weekly through week 14; biweekly on weeks 14 through 25, then monthly thereafter until completion of the experiment.

BIOLIGICAL MEASUREMENTS:

Blood samples were obtained by orbital puncture, 10/sex/group, at 12, 18 and 24 months. Parameters measured were:

Hemoglobin	Hemaotcrit	Red cells
Total leukocytes	Differential leukocytes	Red cell morphology

POST-MORTEM EXAMINATION:

Gross examination was made of all animals dead or dying during the study and on all survivors of the 24 month test period. Survivors were killed by exsanguination under ether anesthesia. The following tissues were reserved in 10% neutral buffered formalin for subsequent histopathological examination: (*) organ weights obtained.

Adrenal*	Aorta	Bone
Bone marrow	Brain*	Esophagus
Eyes (in Bouin's Solution)	Gallbladder	Gonads* (in Bouin's Solution)
Heart*	Intestine	Kidneys*
Liver*	Lungs	Lymph nodes
Mammary gland	Pancreas	Nerve
Parathyroid	Pituitary	Preputial Gland
Prostate	Salivary	Seminal vesicle
Skeletal muscle	Skin	Spinal Cord
Spleen*	Stomach	Thymus
Thyroid	Tongue	Trachea
Urinary bladder	Uterus	Gross lesions

RESULTS:

OBSERVATIONS:

Mortality - Average survival in all groups was good except for the 3000 ppm males which showed a decreased average survival time when compared to that of the controls:

AVERAGE DAYS SURVIVAL TABLE

	MALES		FEMALES	
DOSE	DAYS*	FULL SURVIVORS†	DAYS	FULL SURVIVORS
Control	660.6	31/60	585.8	18/60
750 ppm	660.3	25/60	609.0	21/60
1500 ppm	675.1	33/60	624.0	23/60
3000 ppm	610.2	21/60	590.6	19/60

* Average number of days on test per group. Does not included animals dying by accident.

† Numerator = total animals alive at 735 days or more. Denominator = total animals begun on test.

Food consumption and weight gain were comparable among the groups.

Decreased hemoglobin, hematocrit and red cell values were reported in high dose males at 24 months and in high dose females at 12 and 24 months. Hyperplastic bone marrow was reported in all treatment group males and females. Hyperplasia of the splenic red pulp was noted in the male treatment groups. Hemosiderosis was not a prominent finding in this study.

POST MORTEM FINDINGS:

GROSS NECROPSY

Relative spleen weights were reported in the high-dose females; no significant pathology was reported, however. Spleen enlargement occured in the mid- and high-dose males. Ovarian weight ratios were decreased in all treatment groups; no histopathological findings were associated with this, however. The same was true for the relative testes weights in the high-dose males. Kidney weights were significantly increased ($p < 0.01$) in all treatment groups. We consider this finding to be dose-related.

Compound related effects in the kidney were described as renal enlargement, discoloration, surface irregularities, pelvic dilation, cysts, nodules and masses in all treatment groups. No other compound-related effects in other organs or tissues were reported.

MICROSCOPIC EXAMINATION

Stomach

The incidence and severity of hyperplasia and hyperkeratosis of the esophageal squamous mucosa in treated males and females was significant and was considered to be dose-related. This was not seen control animals. There was a significant increase in gastric squamous cell tumors in the 1500 ppm females but this was not dose related. Glandular epithelial tumors were present in the treated groups but not in statistically significant numbers. See Table I.

Kidney

Chronic glomerulonephritis was seen in all groups, but the incidence was not significantly different among them, although it was higher in the 3000 ppm males. Increased tubular degeneration was noted in 750 and 1500 ppm males and in the 1500 ppm females. Increased incidences of cortical cysts were seen in all treated males and in the high dose females.

Adenomas and carcinomas of the cortical tubules were increased in all treatment-group males but not in females. See Table I for the incidence of these lesions. The only neoplasm seen in the females was one renal hemangiosarcoma in a low-dose female.

DISCUSSION[1]:

"Chlorothalonil has presented evidence of nephrotoxicity in earlier studies in rats, mice and dogs, predominantly in males. In an NCI rat study [(NCI, etc)] there was presumptive evidence of adenomas and carcinomas of the renal tubular epithelium. Although primary renal tumors are rare in rodents, there was no positive trend for elicitation of adenomas and carcinomas in the renal cortical tubules of male mice in this study, and therefore the evidence for tumorigenicity of chlorothalonil in the kidney remains elusive. The effects on the kidney, in this study, are nonetheless considered compound related ...".

CONCLUSIONS:

CTN produces evidence of hyperplasia and/or tumorigenesis in the squamous cell and epithelial cell layers of the esophagus and stomach in males and females. In addition, renal neoplasms not seen in control animals were reported in males only; their incidence, although statistically significantly increased over that of the controls, did not appear to be dose-related.

A no-effect level for chronic effects has not been demonstrated in this study.

Overall, we conclude that this study presents evidence that CTN can induce gastric and renal neoplasms in CD-1 mice.

CORE RATING:

For Chronic effects: Supplemental; not repairable.

For Oncogenic effects: Guideline.

---

[1] From: Jaeger, R.B., et al. WHO/FAO Report, 1983, Geneva.

TABLE I

NEOPLASMS IN MALE CD-1 MICE FED CHLOROTHALONIL IN THE DIET FOR TWO YEARS

STUDY # DTX-79-0102

KIDNEY

	Control	750 ppm	1500 ppm	3000 ppm
Tubular Adenoma	0/60	3/60	4/60	2/60
Tubular Carcinoma	0/60	3/60	0/60	2/60
Total Neoplasms	0/60	6/60	4/60	4/60

The One-Hit Slope Coefficient $B^{(1)}$ =9 .37 x 10^{-4} mg/kg/day^{-1}

Based on the response of the 750 ppm mice Risk = B x $12.6^{(2)}$ Exposure = 0.0118 mg/kg/day^{-1} x 0.0.01305 mg/kg/day (the TMRC) = 1.54 x 10^{-4}.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	1/60	5/60	2/60
Glandular Carcinoma	0/60	1/60	2/60	0/60
Total Neoplasms	0/60	2/60	7/60	2/60

The One-Hit Slope Coefficient B = 4.06 x 10^{-5} mg/kg/day^{-1}

Based on the Squamous Carcinoma response of the 1500 ppm mice, Risk = B x $12.6^{(2)}$ x Exposure = 5.12 mg/kg/day^{-4} x 0.01305 mg/kg/day (the TMRC) = 6.68 x 10^{-6}.

---

(1) Slope Coefficient calculated by Roger Gardner, 1/30/84.

(2) Cube root of the ratio of human body weight to mouse body weight.

NEOPLASMS IN FEMALE CD-1 MICE FED CHLOROTHALOPNIL IN THE DIET FOR TWO YEARS

Study # DTX 79-0102

KIDNEY

No lesions were reported in this organ.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	2/60	6/60	5/59
Glandular Carcinoma	0/60	1/60	1/60	2/59

The One Hit Slope Coefficient $B^{(1)} = 7.09 \times 10^{-5}$ mg/kg/day^{-1} is based on the Squamous Carcinoma response of the 1500 ppm females. Risk = B x $12.6^{(2)}$ x Exposure.

Risk = $8.93 \times 10^{-4} \times 0.01305$ mg/kg/day (the TMRC) = 1.75×10^{-5}.

---

(1) B value calculated by Roger Gardner, 1/30/84.

(2) Cube root of the ratio of human body weight to mouse body weight.

TUMORIGENICITY STUDY WITH DS-2787 IN MICE

DOSE (PPM)	NO. OF ANIMALS INITIATED ON STUDY	
	MALES	FEMALES
0	60	60
750	60	60
1500	60	60
3000	60	60

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

SURVIVAL

DOSE LEVEL (PPM)	18 MONTHS		24 MONTHS	
	MALES	FEMALES	MALES	FEMALES
[illegible]	52/60	40/60	31/60	18/60
750	50/60	42/60	25/60	21/60
1500	52/60	43/60	31/60	23/60
3000	47/60	40/60	20/60	19/60

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

CC/713

INCIDENCE OF TUBULAR ADENOMAS AND CARCINOMAS

	DOSAGE		
0 PPM	750 PPM	1500 PPM	3000 PPM
MALES			
0/60	6/60	4/60	4/60
FEMALES			
0/60	0/60	0/60	0/60

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF GLOMERULONEPHRITIS, TUBULAR DEGENERATION AND CORTICAL CYSTS

	DOSAGE (PPM)			
	0	750	1500	3000
MALE				
GLOMERULONEPHRITIS:				
VERY SMALL AMOUNT	10	7	2	5
SMALL AMOUNT	4	5	2	11
MODERATE AMOUNT	5	4	6	15
MARKED AMOUNT	1	1	0	2
TOTAL	20	17	10	33
TUBULAR DEGENERATION:	4	13	23	3
CORTICAL CYSTS:	22	33	36	33
FEMALE				
GLOMERULONEPHRITIS:				
VERY SMALL AMOUNT	0	1	0	3
SMALL AMOUNT	2	3	0	4
MODERATE AMOUNT	3	6	4	7
MARKED AMOUNT	1	3	1	1
TOTAL	6	13	5	15
TUBULAR DEGENERATION:	2	2	10	4
CORTICAL CYSTS:	9	8	8	22

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF ANIMALS WITH GASTRIC TUMORS OF ALL TYPES

DOSAGE

0 PPM	750 PPM	1500 PPM	3000 PPM
MALES			
1/60	5/60	7/60	4/60
FEMALES			
0/60	3/60	7/60	8/59

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF ANIMALS WITH GASTRIC TUMORS OF ALL TYPES

DOSAGE

0 PPM	750 PPM	1500 PPM	3000 PPM
MALES			
1/60	5/60	7/60	4/60
FEMALES			
0/60	3/60	7/60	8/59

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

CC/713

INCIDENCE OF ADENOMATOUS DIVERTICULUM OR POLYP AND CARCINOMA OF GLANDULAR MUCOSA

DOSAGE			
0 PPM	750 PPM	1500 PPM	3000 PPM
MALES			
1/60	2/60	2/60	2/60
FEMALES			
0/60	1/60	3/60	3/59

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF ADENOMATOUS DIVERTICULUM OR POLYP AND CARCINOMA OF GLANDULAR MUCOSA

DOSAGE

0 PPM	750 PPM	1500 PPM	3000 PPM
MALES			
1/60	2/60	2/60	2/60
FEMALES			
0/60	1/60	3/60	3/59

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF SQUAMOUS-CELL PAPILLOMA AND CARCINOMA

DOSAGE

	0 PPM	750 PPM	1500 PPM	3000 PPM
MALES	0/60	2/60	5/60	2/60
FEMALES	0/60	2/60	6/60	5/59

CHRONIC MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF HYPERPLASIA AND HYPERKERATOSIS OF THE SQUAMOUS MUCOSA IN THE FORESTOMACH

	DOSAGE (PPM)			
	0	750	1500	3000
HYPERPLASIA				
MALE	1/60	21/60	42/60	39/60
FEMALE	2/60	21/60	41/60	43/59
HYPERKERATOSIS				
MALE	0/60	44/60	50/60	53/60
FEMALE	3/60	45/60	43/60	49/59

SPONTANEOUS TUMOR INCIDENCE IN CD-1 MICE
HISTORICAL CONTROL DATA

Source*/ Tissue	Neoplasm	MALES Effected Animals	MALES Mean Incidence(%)	MALES Range of Incidences(%)	FEMALES Effected Animals	FEMALES Mean Incidence(%)	FEMALES Range of Incidences
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	---
B/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 totalling 1490 CD-1 mice of each sex

B - Homburger, F. et. al., Aging changes in CD-1 Ham/ICR mice reared under standard laboratory conditions, J. Natl. Canc. Inst. 55, 37, 1975

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

D - Bio/dynamics, Incorporated tabulation of findings from 14 chronic studies in CD-1 mice

JNBORK

00713

TOXICITY STUDIES WITH CHLOROTHALONIL IN RATS

70 M/70 F control
35 M/35 F Test Groups

1. TWO YEAR RAT STUDY (1964-1966)

DOSE LEVELS: 0. 1500. 15000 PPM 30,000 (resumed after 2 wk) (food refusal)

No Tumors
irreversible Kidney effect

2. TWO YEAR RAT STUDY (1964-1966) 35 M/35 F

DOSE LEVELS: 0. 5000 PPM ↓ growth cathartic

Kidney - tub. hypertrophy; ep. Alterations

3. EIGHTEEN MONTH RAT STUDY (1966-1967) 15 M/15 F

DOSE LEVELS: 0. 500. 1000 PPM, 5000 ppm (food refusal)

Kidney effects - hypertrophy, ep. hyp., vacuolation in males - dose related. No other effects reported

4. TWENTY-TWO WEEK RAT STUDY (1967) 35 M/35 F

DOSE LEVELS: 0. 250. 500. 750. 1500 PPM

All Test Groups - Kidney - irregular swelling of tub ep.; ep degen.; tub. dilatation - mostly males

5. TWO YEAR RAT STUDY (1968-1970) 50 M/50 F

DOSE LEVELS: 0. 4. 10. 20. 30. 40. 60 PPM

NOEL = 60 ppm

[illegible] necrosis of ep lining of pr. conv. tub. in cortex at [illegible] and [illegible] at high dose

6. FOUR MONTH RAT STUDY (1975) 15 M/15 F

DOSE LEVELS: 0. 1. 2. 4. 15. 30. 60. 120 PPM

Gross most Kidneys had fat-like nodules

Histo no dose related changes

REPORTED RENAL LESIONS IN TOXICITY STUDIES WITH CHLOROTHALONIL

1. ALTERATIONS IN THE DEEP PROXIMAL TUBULES OF HYPERTROPHIC AND HYPERPLASTIC NATURE

   - CHARACTERIZED BY FAIRLY LARGE POLYGONAL CELLS WITH PALE VACUOLATED CYTOPLASM OFTEN CONTAINING GRANULAR YELLOW GREEN PIGMENT

   - DILATED TUBULES

2. DEGENERATIVE CHANGES IN TUBULAR EPITHELIUM

   - PROTEIN IMBIBITION, PIGMENTATION, CELLULAR DISSOLUTION

3. HYPERPLASIA OF THE CAPSULAR EPITHELIUM OF THE GLOMERULUS

4. DILATATION OF THE LOOP OF HENLE

5. ZONE BETWEEN CORTEX AND PAPILLA MARKED INCREASE WITH A LOOSE LACE-LIKE APPEARANCE

6. CHANGES CHARACTERIZED BY PROTEIN IMBIBITION, TUBULAR HYPERTROPHY, IRREGULARITY AND VACUOLATION OF THE EPITHELIAL CELLS OF PROXIMAL CONVOLUTED TUBULE

7. RENAL CYSTS

8. VACUOLATION OF EPITHELIUM IN COLLECTING TUBULES

CORRELATION BETWEEN THE OCCURRENCE OF TUMORS AND TUBULAR HYPERPLASIA IN THE KIDNEY (IN RATS)

DOSE LEVEL (mg/kg/day)	MALES		FEMALES	
	TUMORS (chronic)	HYPERPLASIA (subchronic)	TUMORS (chronic)	HYPERPLASIA (subchronic)
1.5		-		-
3.0		-		-
10		-		-
40	+	+	+	-
80	+	+	+	-
175	+	+	+	+
375		+		+
750		+		+
1500		+		+

CORRELATION BETWEEN THE OCCURRENCE OF TUMORS AND TUBULAR HYPERPLASIA IN THE KIDNEY (IN MICE)

DOSE LEVEL (ppm)	MALES		FEMALES	
	TUMORS (chronic)	HYPERPLASIA (subchronic)	TUMORS (chronic)	HYPERPLASIA (subchronic)
7.5		-		-
15		-		-
50		-		-
275		-		-
750	+	+/-	-	-
1500	+		-	
3000	+		-	

215 E

DATE: November 28, 1978

SUBJECT: Review of NCI Carcinogenesis Report on Chlorothalonil. Caswell#477

FROM: H.W. Spencer, Ph.D.
Toxicology Branch/HED

TO: R. Gessert, DVM
Acting Deputy Branch Chief
Toxicology Branch/HED

Carcinogenesis Review of NCI Report.

Material tested: Chlorothalonil, (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile)

(1) batch of 98.5% purity also consisting of 1.24% pentachlorobenzenonitrile, 0.06% other tetrachlorodicyanobenzene isomers and smaller quantities of other partially chlorinated dicyanobenzenes.

(1) batch of 98% purity also consisting of 0.6% pentachlorobenzonitrile, 1.2% other tetrachlorodicyanobenzene isomers and smaller quantities of other partially chlorinated dicyanobenzenes.

(1) batch of analytical grade (99.7%) chlorothalonil.

The test materials were stored in their original glass containers at 4°C.

Animals tested:

(1) Osborne-Mendel rats obtained from Battelle Memorial Institute, Columbus, Ohio.

(2) B6C3F1, hybrid mice obtained from A.R. Schmidt, Madison, Wisconsin.

The rats were quarantined for (7) days and the mice were held for (15) days prior to use in the studies. Both species were 35 days old when placed on study.

Methods:

Diets were of finely ground Wayne Lab. Blox. to which appropriate amounts of the test material were added. A pre-mix was made by hand-mixing and was further diluted to the appropriate concentration. Two percent (2%) of the feed weight consisted of corn oil which was added after the test pre-mix was diluted into the diet. The final addition of 2% by weight of acetone was made and the final diet was mechanically mixed for at least 25 minutes.

177

EPA FORM 1320-6 REV. 3-76)

Mixed feed was kept no longer than (1) week. The test material mixed in the diet was found to be stable at ambient tempertures for that period.

Treatment:

Rats were treated initially for (1) week at 20,000 ppm and 10,000 ppm for high-dosed and low-dosed groups respectively. After the (1) week period, the 50/sex groups were exposed to one-half the previous dosages for the succeeding 79 weeks. After 80 weeks of treatment the animals were observed for from 30-31 weeks prior to sacrifice.

Mice were on study for 91-92 weeks. 50/sex/dose were treated with diets containing 20,000 ppm and 10,000 ppm in the high and low dosed groups respectively for 2 weeks.

Males' treatments were then reduced to 5000 ppm and 2500 ppm for the two dosages for the following 78 weeks. A final 11-12 week observation period was included prior to sacrifice.

Females were treated like the males in the first 2 weeks but then respective 20,000 and 10,000 ppm groups were given diets of 10,000 and 5000 ppm for the next 10 weeks. The dosages were subsequently reduced respectively to 5000 and 2500 ppm for the remaining 68 weeks of treated diet. An 11-12 week observation period was also employed prior to necropsy of these animals.

Both rat and mouse studies contained control groups of only 10/sex. Control groups of on-going studies which over-lapped these two particular studies in time were grouped together for statistical analysis of the present studies. Pooled controls number 65/sex for rats and 60/sex for mice.

Only control groups of mice were not obtained from the same supplier.

Observations

Both studies were treated essentially the same for observation purposes. Animals were observed 2X daily and weighed at regular intervals with palpation for masses at the weighings.

Animals moribund at clinical observation were also sacrificed and necropsied as well as those surviving the treatment. Microscopic examination included the following tissues: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gall bladder (mice only), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain.

Staining was routinely H & E but different if need indicated.

Results in Rats

Observations were analyzed to check for survival rates in treated animals. Growth curves showing a depression of growth rate when compared to the matched controls in each sex were produced.

Only estimated male survival rate probabilities were reduced for treated animals ($p = 0.002$).

Neoplasms in the tubular epithelium of the rat kidneys were observed histologically.

	MALE			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Carcinoma	0/10	1/45	3/49	0/10	1/48	2/50
Adenoma	0/10	2/45	1/49	0/10	0/48	3/50
Total	0/10	3/45	4/49	0/10	1/48	5/50

Renal tumors are rarely observed in other control Osborne-Mendel rats at this laboratory. The relatively high incidence in this bioassay of tubular-cell renal tumors in dosed rats indicates a compound-related effect. In addition, a transitional-cell carcinoma, a carcinosarcoma, a liposarcoma, and a hamartoma were recorded in low-dose male rat kidneys.

Based on the histopathologic evaluation, the results indicate that phthalonil induced renal neoplasms in the rats under the conditions of this bioassay.

The results of the Cochran-Armitage test on the incidence of male rats with carcinoma, tubular-cell adenocarcinoma, adenoma, adenocarcinoma, or papillary adenoma of the kidney are significant ($P = 0.030$) using the pooled controls. The Fisher exact comparison of the incidence in the high-dose group with that in the pooled-control group shows a P value of 0.035, which is above the 0.025 level for significance when the Bonferroni inequality criterion is used for multiple comparison. Historical records of this bioassay program at this laboratory indicate an incidence of tubular-cell adenomas in male rats of 3/240 (1.25) with no other renal tumors occurring.

In females the Cochran-Armitage test on the incidence of female rats with adenomas, carcinomas, tubular-cell adenomas or tubular cell adeno-carcinomas are significant ($P = 0.007$) and the Fishers' exact test shows that the incidence of these lesions in the high-dose group is significantly greater than that in the pooled controls ($P = 0.016$).

Data from the laboratory's one large number of animals indicate the controls to be virtually free of the type tumors seen in the kidney of the test animals.

Mice

Growth rate inhibition in females was not evident. However, male mean body weights were lower than those of controls over most of the study.

Clinical signs in all dosed groups in the second year included alopecia, loss of weight, rough coats, nodular masses - some appeared intermittently in males thoughout the last year.

At week 62 and until the end of the study, a majority of the males were hyperexcitable.

In mice, no tumors were found to occur at a significantly greater incidence in dosed animals than in controls.

Comments

(1) In the review of Table A 1, male rats: Neoplasms.

This reviewer finds the summary disposition of animals is incomplete. This problem is also present in Table A 2, female rats: neoplasms.

(2) This reviewer considers the numbers of tissues and organs omitted in the histopathology report of such a small number of control animals as a major deficiency of the study.

(3) The individual impurities in each test material batch should have been enumerated since such high dosages were used.

(4) Why are statements as to animals being missing not substantiated in the summary disposition tables?

Toxicology Branch considers this study adequate to indicate the material tested chlorothalonil, technical, was carcinogenic to the renal tissues of the Osborne-Mendel rat under the conditions of the study.

In the concurrent mouse study - no obvious carcinogenic activity was noted.

Study No. NCI-GG-TR-41 carried out by Gulf South Research Institute for Tracor Jitco for NCI, 1978.

TOX/HED:ss:RGessert:11-28-78

1/29/79

NCI RAT BIOASSAY

INCIDENCE OF TUBULAR ADENOMAS AND CARCINOMAS

	DOSAGE		
	0 PPM	5063 PPM	10126 PPM
MALES	0/10	3/46	4/49
FEMALES	0/10	1/48	5/50

INCIDENCES OF TUMORS IN THE NCI MOUSE STUDY

TISSUE/TUMOR TYPE	SEX	DOSE LEVELS 0	5000 PPM	10,000 PPM
STOMACH:				
SQUAMOUS CELL CARCINOMA	MALE	0/8	1/46	0/50
	FEMALE	0/10	0/49	1/46
SQUAMOUS CELL PAPILLOMA	MALE	0/8	1/46	0/50
	FEMALE	0/10	0/49	0/46
PAPILLOMA, NOS	MALE	0/8	0/46	0/50
	FEMALE	0/10	1/49	0/46
ALL GASTRIC TUMORS	MALE	0/8	2/46	0/50
	FEMALE	0/10	1/49	1/46
KIDNEY:				
TUBULAR CELL ADENOMA	MALE	0/10	0/46	0/49
	FEMALE	0/10	0/49	1/46

Tox Chem No. 215 B CHLOROTHALONIL

File Last Updated 4/21/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Registration standard					003925
Teratology - rabbit; Inst. Env. Toxicology; 75-2077; 5/30/75	DS-2787 99.3% pure	071539	Teratogenic NOEL > 50 mg/kg (HDT) Maternal NOEL = 5 mg/kg. Maternal LEL = 50 mg/kg (four spontaneous abortions). Feto toxic NOEL = not established, additional information needed. Levels tested by gavage in Japanese White (Funabashi) strain- 0, 5.0 and 50 mg/kg.		Supplementary Supply individual pup data examination details of aborted embryos in the 50 mg test group. 003725
Teratology - rat; Wil Laboratories; 517-5TX-0011-003; 5/13/83	Tech. Chlorothalonil	250855	Teratogenic NOEL > 400 mg/kg/day (HDT) Fetotoxic NOEL > 400 mg/kg/day Maternal NOEL = 100 Maternal LEL = 400 mg/kg/day (mortality reduced body weight, increased resorptions and post implantation bases Levels tested by gavage in Sprague Dawley strain - 0, 25, 100 and 400 mg/kg/day		Guidelir 003797
1 Generation Reprod. - rabbit; Hazleton Lab	Daconil 2787		reproductive NOEL = 0.25% (LDT) reproductive LEL = 0.5% decreased pup survival maternal NOEL = 0.25% (LDT) maternal LEL = 0.5% decreased food consumption terata NOEL = 0.5%		001101
3 Generation reprod. - rat; Hazleton Lab; #200-150	Mixture (93.6% chlorothalonil)		Reproductive LEL < 0.15% (LDT) depressed pup weights, gastric and esophageal acanthosis in offspring Maternal NOEL < 0.15% depressed body weight		001101

Tox Chem No. 215B Chlorothalonil

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
3 Generation reprod. - rat; Hazleton Lab;#200-155	Mixture (93.6% chlorothalonil)		Reproductive NOEL < 0.5% (single dose tested) decreased fetal weight Maternal NOEL < 0.5% body weight depression		001101
21 Day inhalation - rat; IBT;#663-03477; 8/27/73	Bravo 6F				Invalid 001064
104 Week feeding - dog; Hazleton Lab;#200-206;	chlorothalonil technical		systemic NOEL = 60 ppm systemic LEL = 120 ppm (histopathological changes in kidneys) Levels tested = 0, 60 or 120 ppm		001101
16 Week feeding - dog; Hazleton Lab	Mixture (93.6% chlorothalonil)		systemic NOEL < 250 ppm (LDT) increased PBI		001109 001101
2 Year feeding - dog; Hazleton Lab	Mixture (93.6% chlorothalonil)		systemic NOEL < 0.15% (LDT) kidney and liver pigmentation		001101 001109
90 Day feeding - mice; Concord Woods An. Fac.; 618-5TX-83-0007-004; 9/2/83	98% Technical	072269	NOEL = 15 ppm LEL = 50 ppm - hyperplasia and hyperkeratosis of gastric mucosa. Also see study #5TX-79-0102		Minimum 003802 004950

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
13 week feeding - rat; Huntingdon Res. Center; #5TX-81-0213; 2/23/83	DS-2787 Tech	071537	NOEL = 3 mg/kg/day. LEL = 10 mg/kg/day (increased incidence of dilated renal tublues and increased hyperkeratosis in gastric epithelium). Levels tested by diet to Charles River strain - 0, 1.5, 3.0, 10, and 40 mg/kg/day. RE-EVALUATION NOEL = 1.5 mg/kg/day LOEL = 3.0 mg/kg/day(increased number of irregular intracytoplasic inclusion bodies in the proximal convoluted tubules of all males) See study # 562-5TX-81-0213-004-001		Guideline 003725 004950

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90 Day feeding - rat; TR Evans Res. Cen.; #5TX-80-0200; 10/19/81	DS-2787 (Tech. 98% with [redacted] HCB)	071535	NOEL < 40 mg/kg/day (relative kidney weights increased at all test levels;urinary vol. and Specific Gravity affected at all test levels.).Levels tested - 0, 40, 80, 175, 375, 750 and 1500 mg/kg/day in Charles River CD strain.		Minimum 003725
4 Month feeding - rat; Bio-Tox; #24-201	Chlorotahlonil (DAC 2787)		Systemic NOEL = 120 ppm (HDT)		001097
2 Year feeding/oncogenic - rat; Hazleton Lab; #200-148	Mixture (93.6% chlorothalonil)		systemic NOEL = 0.15% (LDT) systemic LEL = 1.5% depression of growth, kidney nephritis		001108 001101
2 Year feeding/oncogenic - rat; Hazleton Lab; #200-205	chlorothalonil technical		systemic NOEL = 60 ppm (HDT) oncogenic NOEL > 60 ppm Levels tested = 0, 4, 10, 20, 30, 40 and 60 ppm		001101 001108
2 Year feeding/oncogenic - rat; Hazleton Lab; #200-154	Mixture (93.6% chlorothalonil)		systemic NOEL < 0.5% (single dose tested) kidney hypertrophy		001108 001101
18 Month feeding - rat; Hazleton Lab;#200-175	Mixture (93.6% chlorothalonil)		systemic NOEL < 0.05% (LDT) growth depression, tubilar hypertrophy		001107 001101
4 Month feeding - rat; Hazleton Lab;#200-198	technical		Systemic NOEL < 250 ppm (LDT) Systemic LEL = 250 ppm swelling of tubular epithelial cells, tubular dilatation and cast formation		001108

(INERT INGREDIENT INFORMATION IS NOT INCLUDED)

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Oncogenic - rat; NCI - CAS;1897-45-6	technical		neoplasms of the renal tubular epithelium in both males and females		NCI # 41 1978
Oncogenic - mice; NCI - CAS;1897-45-6	technical		oncogenic potential negative		NCI # 41 1978
2 Year feeding/oncogenic - mice; Biodynamics; DTX-79-0102; 2/8/79	Tech. 97.7%	071541	Oncogenic NOEL < 750 ppm (LDT) (renal neoplasms in males and evidence of hyperplasia and/or tumorigenesis in the squamous cell and epithelial layer of the esophagus and stomach in both sexes. Systemic NOEL <750ppm (LDT) (decreased ovary wt, hyperplastic bone marrow hyperplasia of splenic red pulp in males, increased kidney wt. with surface irregularities, pelvic dilation, cysts,nodules, masses, tubular degeneration). Levels tested by diet in CD-1 strain 0, 750, 1500, and 3000 ppm.		Supplementary for chronic effects; no NOEL demonstrated. Guideline for oncogenic effects 003725
Radiotracer ^{14}C in mice; Concord Woods An. Fac,; 613-4AM-82-0178-001; 8/19/83	^{14}Chlorothalonil	072277	Major route of elimination is via feces. 5 - 10% in urine. (M)		Acceptable 003802
Radiotracer ^{14}C in rat; Concord Woods An. Fac.; 000-4AM-82-0052-001; 9/2/83	^{14}Chlorothalonil	072277	Chlorothalonil converts to 4-OH metabolite in gut at ca 5% of dose. Urinary conversion only 0.6% (M)		Acceptable 003802
Dose-Response biliary excretion of ^{14}C given intraduodenally in rats; Mem. U. of Newfoundland; 342-4M-79-0005-002; 8/25/83	^{14}Chlorothalonil	072277	@ 0.5 mg/kg bw excreted 35.8% in bile; 5.0 mg/kg excreted 31.2% in bile; 50 mg/kg excreted only 11% in bile.		Acceptable 003802

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Excretion of ^{14}C in rat Bile After Intraduodenal Administration;Mem. U. of Newfoundland; 429-4BE-80-0163-003; 10/16/83	^{14}Chlorothal-onil	072277	Material dose intraduodenally @ 0.5, 5, 10, 50, 100 or 200 mg/kg resulted in biliary excretion pattern that suggested a dose response. May be pharmacokinetic overloads at higher doses.		Acceptable 003802
Cell transformation - newborn rat; Microbiol. Assoc.; DTX 77-0037; 10/6/78	DS-2787 tech, 96%	099243	negative for phenotypu transformations in F1706 and H4536p+2 cell lines		001110
Mutagenic DNA repair; Microbiol. Assoc.; DTX 77-0033; 6/29/77	DAC 2787	099243	interferes with DNA repair in TA-1538. Tested at 2 - 20 ug/plate		001110
Mutagenic-mammalian cell - gene point mutation; Microbiol Assoc.; DTX 77-0034; 6/29/77	DAC 2787	099243	negative for chinese hamster cells V-79 and BALB/3 t3 mouse fibroblasts dose = .3 ugm/ml for 2 hours		001110
Mutagenic - Ames; Microbiol. Assoc.; DTX 77-0035; 6/29/77	DAC 2787	099243	negative for TA-1535; TA-100; TA-1537 and TA-1538 (his) status of ST DAC-2787 plated at .33, .66, 1, 3.3, 6.6 ug/plate		001110
Mutagenic - Ames; Inst. Environ. Tox. Jpn; DTX 61-0002	DAC 2787	099243	negative for DNA repair synthesis in B. Subutis #M44.		001110

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - micronucleus test - rat; Lab. D'Histpath. & Cytopharm., Paris; #000-5TX-81-0024-000(#576); 11/3/81	DS-2787	071539	No induction of Wistar strain rat bone marrow erythrocyte nuclei at levels up to and including 5000 mg/kg (HDT). Positive control was MMS at 65 mg/kg.		Acceptable 003725
Mutagenic micronucleus test - mice; Lab. D'Histopath. & Cytopharm., Paris; #000-5TX-81-0024-000(#505); 5/12/81	DS-2787	071539	Does not induce mouse bone marrow erythrocyte micronuclei in Swiss CFLP strain at levels up to and including 5000 mg/kg (HDT). Positive control was MMS at 65 mg/kg.		Acceptable 003725
Mutagenic micronucleus test - hamster; Lab. D'-Histopath. & Cytopharm., Paris; #000-5TX-81-0024-000 (#591); 12/22/81	DS-2787	071539	No significant increase in Chinese hamster bone marrow erythrocyte micronuclei at levels up to and including 5000 mg/kg (HDT). Positive control was MMS at 65 mg/kg/day		Acceptable 003725
Mutagenic - chromosomal abber. - rat; Lab. d' Histopath. & Cytopharm., Paris; #000-5TX-81-0025-000(#590); 5/12/81	DS-2787	071539	Significant numbers of chromosomal abnormalities not induced in Wistar rats at up to 5000 mg/kg (HDT). Positive control was MMS at 65 mg/kg/day.		Acceptable 003725
Mutagenic - chromosomal abber. - mice; Lab. d' Histopath. & Cytopharm., Paris; #000-5TX-81-0025-000(#542); 7/28/81	DS-2787	071539	Bone marrow chromosomal anomolies not increased at levels up to 2500 mg/kg (HDT) in Swiss CFLP strain. Positive control was urethan at 2000 mg/kg.		Acceptable 003725
Mutagenic - chromosomal abber. - hamster; Lab. d'Histopath & Cytopharm, Paris; #000-5TX-81-0025-000(#525); 7/2/81	DS-2787	071539	Bone marrow chromosomal anomalies not increased at up to 1000 mg/kg (HDT) in Chinese hamster. Positive control was MMS at 65 mg/kg.		Acceptable 003725

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Category	Core Grade/ Doc. No.
Dermal sensitization - human	Daconil B		Delayed skin irritation reaction Considered a sensitizer		001067
Dermal patch test - human; IBT #8537-08862; 8/27/76	Daconil B				Invalid 003817
Acute oral LD_{50} - rat; Biodynamics; #77-0060; 9/19/77	chlorothalonil 40.76%		LD_{50} = 4.2 g/kg ataxia, nasal discharge, lethargy, piloerection and prostration	III	Minimum 001063
Acute dermal LD_{50} - rabbit; Biodynamics; #77-0063; 10/24/77	chlorothalonil 40.76%		LD_{50} > 20 g/kg moderate to severe erythema, slight edema	IV	Minimum 001063
Primary dermal Irrit. - rabbit; Biodynamics; #77-0061; 9/19/77	chlorothalonil 40.76%		PI Index = 1.3/8.0 slighty irritating but non-corrosive	IV	Minimum 001063
Primary eye irritation - rabbit; Biodynamics; #77-0062; 9/19/77	chlorothalonil 40.76%		corneal opacity, conjunctival irritation and ulceration	I	Minimum 001063
Acute inhalation LC_{50} - rat; Biodynamics; #77-0064; 12/6/77	chlorothalonil 40.76%		LC_{50} > 7.16 mg/L/4 hr	III	001063
Primary eye irritation - rabbit; MB Res. Lab.; 11/30/77	Bravo 500		corneal opacity persists by day 7	I	Minimum 001063
Primary eye irritation - rabbit; IRDC; #293-024; 10/30/73	Technical		corneal opacity, bulge on the corneal surface were still in evidence at 14 days	I	Minimum 001065
Primary dermal irrit. - rabbit	Daconil B 10% suspension		PI Index = 1.8/8.0 mildly irritating	III	001067

Study/Lab/Study #/Date	Material	No.	LD_{50}, LC_{50}, PIS, NOEL, LEL	Category	Doc. No.
Primary dermal irrit. - rabbit	Daconil B 1% suspension in PEG		PI Index = 1.9/8.0 mildly irritating	III	001067
Primary dermal irrit. - rabbit	Daconil B 0.1% suspen. in PEG		PI Index = 0.9/8.0 slightly irritating	III	001067
Primary eye irritation - rabbit; IBT; #601-03816; 9/7/73	2.88% Flowable		corneal vascularization, pannus and conjunctival swelling at 14 days	I	Minimum 001068
Primary eye irritation - rabbit; IRDC; #293-025; 10/30/73	2.88% Flowable		conjunctival redness, chemosis and discharge at 14 days	II	Minimum 001068
Acute oral LD_{50} - dog; Hazleton Lab	Technical		LD_{50} > 5000 mg/kg	IV	001109 001101
Acute oral LD_{50} - rat; Hazleton Lab	Technical		LD_{50} > 10,000 mg/kg (female)	IV	001109 001101
Acute oral LD_{50} - rat; Hill Top Res. Lab	Technical		LD_{50} > 10,000 mg/kg (male)	IV	001109 001101
Acute inhalation LC_{50} - rat; Hazleton Lab	Mixture (93.6% chlorothalonil)		LC_{50} > 4.7 mg/L/1 hr	IV	001109 001101
Primary eye irritation - rabbit; Hill Top Res. Lab	Technical		Transient conjunctivitis	IV	001109 001101
Acute dermal LD_{50} - rabbit; Hill Top Res Lab	technical		LD_{50} > 10,000 mg/kg	III	001109 001101

Study/Lab/Study #/Date	Material	Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit; IRDC; # DTX 77-0069; 11/22/77	tech. DAC-2787	099244	severe irritant; 14 days 5/6 eyes showed corneal opacity	I	Guideline 001110
Primary eye irritation - rabbit; SIBI; #DTX 77-0121; 1/25/78	DAC 2787	099244	severe irritant	I	Guideline 001110
Primary eye irritation - rabbit; SIBI; #DTX 77-x0125; 2/14/78	Bravo W-75	099244	severe irritant	I	Guideline 001110
Acute oral LD_{50} - rat; Biodynam.;# DTX 78-0001; 6/30/78	DAC 2787	099244	LD_{50} > 28.2 g/kg	III	Guideline 001110
Acute inhalation LC_{50} - rat; Biodynam.;# DTX 78-0019	DAC W-75	099244	LC_{50} = 0.54 mg/L	II	Guideline 001110
Acute dermal LD_{50} - rabbit; Biodynam.; #DTX 78-0002; 5/12/78	DAC 2787	099244	some edema and erythema no deaths	III	Minimum 001110
Acute oral LD_{50} - rat; Biodynam.;# DTX 78-0005; 6/30/78	Bravo 75W	099244	LD_{50} = 19.0 g/kg ataxia, lethargy, staining	IV	Minimum 001110
Acute inhalation LC_{50} - rat; Biodynam.; #DTX 78-0008; 2/14/79	Bravo 75W	099244	Contractor LC_{50} = 0.54 mg/L Petitioner LC_{50} = 0.90 mg/L	II	Supplement 001110
Acute dermal LD_{50} - rabbit; Biodynam.; #DTX 78-0006; 5/22/78	Bravo 75W	099244	LD_{50} > 20 g/kg	IV	Minimum 001110
Primary dermal irrit - rabbit; Bio[illegible] DTX 78-0007	Bravo 75W	099244	PI Index = 2.0/8.0	II	Minimum 001110

Tox Chem No. 215 B Chlorothalonil

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Biodynamics Systems Corp.; #10230; 7/2/80	TBTO .... 0.5% Tetrachloro-isophthalo-nitrile 0.7% (liquid)		LD_{50} = 198 mg/kg (male) LD_{50} = 223 mg/kg (female) lacrimation, diarrhea, lethargy, paralysis of hind limbs	II	Minimum 000441
Primary eye irritation - rabbit; Raltech Scientific; #80771; 11/25/80	chlorothalonil 11.25%		At 24 hours unable to score corneal opacity and irritation due to severe chemosis. Corneal opacity was observed with increasing severity over 21 day period.	I	Guideline 000796
Acute oral LD_{50} - rat; Borriston Lab; #209C; 10/1/79	chlorothalonil 48%	243157	LD_{50} = 8.6 g/kg (male) LD_{50} = 7.0 g/kg (female)	IV	Guideline 000864
Acute dermal LD_{50} - rabbit; Borriston Lab; #209D; 8/3/79	chlorothalonil 48%	243157	LD_{50} > 10 g/kg (single dose tested)	III	Minimum 000864
Primary eye irritation - rabbit; Borriston Lab; #209B; 6/15/79	chlorothalonil 48%	243157	corneal opacity severe through day 14	I	Guideline 000864
Primary dermal irrit. - rabbit; Borriston Lab; #209E; 8/2/79	chlorothalonil 48%	243157	PIS = 5.0/8.0 severe erythema observed in all animals by day 7	II	Guideline 000864
Primary eye irritation- rabbit; Raltech;#806243; 10/31/80	Chlorothalonil 9.5% (538-114)	Not Accessioned	Corneal opacity persisting thru 21 days	I	Guideline 001235
Primary eye irritation - monkey; Bio/dynamics; project #6436-80;3/17/81	Chlorothalonil 40.4%	245574	At 24 hours 6/9 monkeys had corneal opacity (1/6=10, 4/6 = 20, 1/6 = 40) Conjunctive irritation present At day 21, no corneal opacity or any other irritation	II	Guideline 002037

Tox Chem No. 215B

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit; Bio/dynamics; project #6504-80; 3/17/81	Chlorothalonil 40.4%	245399	At 24 hours 6/9 had corneal opacity (2/6 = 10, 4/6 = 20) Iris and conjunctive irritation present All irritation had cleared by day 14	II	Guideline 002037
Acute oral LD_{50} - rat; Bio-Research Lab.; project #12761; 2/27/81	Chlorothalonil (tetrachloro-isophthalonitrile) 96%	246843	LD_{50} greater than 10,000 mg/kg(HDT)	IV	Guideline 002798
Acute dermal LD_{50} - rabbit; Bio-Research Lab ; project #12762; 12/15/80	Chlorothalonil (tetrachloro-isophthalonitrile) 96%	246843	LD_{50} greater than 10,000 mg/kg	III	Guideline 002798
Acute inhalation LC_{50} rat; Bio-Research Lab; project #9383; 2/18/81	Chlorothalonil (tetrachloro-isophthalonitrile) 96%	246843	LC_{50} 94 ug/L (male) LC_{50} 92.5 ug/L (female)	I	Guideline 002798
Primary dermal irritation - rabbit; Bio-Research Lab.; project #12763	Chlorothalonil (tetrachloro-isophthalonitrile) 96%	246843	No irritation at 24 hrs. Very slight erythema in 2/6 at 72 hrs. but had cleared by day 4.	IV	Guideline 002798
Acute oral LD_{50} - rat; Applied Biological Sciences Lab; ABSL #17480; 3/26/81	chlorothalonil (tetrachloro-isophthalonitrile) 12.5%		LD_{50} > 5 g/kg	IV	Guideline 002818
Primary dermal irritation - rabbit; Applied Biological Sciences Lab; ABSL #18538 ; 12/21/81	chlorothalonil (tetrachloro-isophthalonitrile) 12.5%		At 24 hrs and 72 hrs. severe erythema in all animals. Edema at 24 hrs, clear at 72 hrs. PIS = 5.1	II	Guideline 002818

Tox Chem No. 215B

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation rabbit; Applied Biological Sciences Lab; ABSL #10548; 12/21/81	chlorothalonil (tetrachloro-isophthalonitrile) 12.5%		No corneal opacity. At 24 hrs., 2/9 had iris irritation (2/9 = 5); 9/9 conjunctive redness (1/9 = 1, 7/9 = 2, 1/9 = 3); chemosis (1/9 = 1, 2/9 = 2, 3/9 = 3, 1/9 = 4); 9/9 discharge (1/9 = 1, 8/9 = 3). All irritation clear by day 7.	III	Guideline 002818
Acute inhalation, LC_{50} rat; Bio-Research Lab.; Lab. report #9451; 5/4/81	Chlorothalonil (tetrachloro-isophthalonitrile) 96.0%	247442	LC_{50} (M) = 0.220 mg/L (0.189 and 0.257 mg/L). LC_{50} (F) = 0.259 mg/L (0.193 and 0.347 mg/L). Combined male and female LC_{50} = 0.225 mg/L (0.190 and 0.267 mg/L).	II	Guidelin 002837
Acute oral, LD_{50} - rat; Diamond Shamrock; report #DS-2787; 2/7/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LD_{50} > 16.24 g/kg. Toxic signs were lethargy chromdacryorrhea and chromorhimarrhea	IV	Guideline 002805
Acute dermal, LD_{50} rabbit; Diamond Shamrock; #DS-2787; 2/6/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LD_{50} > 14.13 g/kg. Slight to severe erythema and edema from day 1 to day 14	III	Guideline 002805
Acute inhalation, LC_{50} Diamond Shamrock; report #DS-2787; 4/28/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LC_{50} was 0.11 mg/L (0.09 - 0.14). Labored heating excessive ocular, nasal tral servtions	I	Guideline 002805
Primary eye irritation rabbit; Diamond Shamrock; report #DS-2787; 2/7/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	3/12 corneal opacity; 3/12 iris irritation; conjunctive irritation in 12/12 animals; 4/12 could not be scored due to severe chemosis and corneal opacity. Corneal opacity persisted in 7/12 through day 14; conjunctive irritation persisted through day 14.	I	Guideline 002805

Tox Chem No. 215B

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit; Bio/dynamics; project #6504-80; 3/17/81	Chlorothalonil 40.4%	245399	At 24 hours 6/9 had corneal opacity (2/6 = 10, 4/6 = 20) Iris and conjunctive irritation present All irritation had cleared by day 14	II	Guideline 002037
Acute oral LD_{50} - rat; Bio-Research Lab.; project #12761; 2/27/81	Chlorothalonil (tetrachloro-isophthalonit-rile) 96%	246843	LD_{50} greater than 10,000 mg/kg(HDT)	IV	Guideline 002798
Acute dermal LD_{50} - rabbit; Bio-Research Lab ; project #12762; 12/15/80	Chlorothalonil (tetrachloro-isophthalonit-rile) 96%	246843	LD_{50} greater than 10,000 mg/kg	III	Guideline 002798
Acute inhalation LC_{50} rat; Bio-Research Lab; project #9383; 2/18/81	Chlorothalonil (tetrachloro-isophthalonit-rile) 96%	246843	LC_{50} 94 ug/L (male) LC_{50} 92.5 ug/L (female)	I	Guideline 002798
Primary dermal irrita-tion - rabbit; Bio-Research Lab.; project #12763	Chlorothalonil (tetrachloro-isophthalonit-rile) 96%	246843	No irritation at 24 hrs. Very slight erythema in 2/6 at 72 hrs. but had cleared by day 4.	IV	Guideline 002798
Acute oral LD_{50} - rat; Applied Biological Sciences Lab; ABSL #17480; 3/26/81	chlorothalonil (tetrachloro-isophthaloni-trile) 12.5%		LD_{50} > 5 g/kg	IV	Guideline 002818
Primary dermal irritation - rabbit; Applied Biological Sciences Lab; ABSL #18538 ; 12/21/81	chlorothalonil (tetrachloro-isophthaloni-trile) 12.5%		At 24 hrs and 72 hrs. severe erythema in all animals. Edema at 24 hrs, clear at 72 hrs. PIS = 5.1	II	Guideline 002818

Tox Chem No. 215B

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation rabbit; Applied Biological Sciences Lab; ABSL #18538; 12/21/81	chlorothalonil (tetrachloro-isophthalonitrile) 12.5%		No corneal opacity. At 24 hrs., 2/9 had iris irritation (2/9 = 5); 9/9 conjunctive redness (1/9 = 1, 7/9 = 2, 1/9 = 3); chemosis (1/9 = 1, 2/9 = 2, 3/9 = 3, 1/9 = 4); 9/9 discharge (1/9 = 1, 8/9 = 3). All irritation clear by day 7.	III	Guideline 002818
Acute inhalation, LC_{50} rat; Bio-Research Lab.; Lab. report #9451; 5/4/81	Chlorothalonil (tetrachloro-isophthalonitrile) 96.0%	247442	LC_{50} (M) = 0.220 mg/L (0.189 and 0.257 mg/L). LC_{50} (F) = 0.259 mg/L (0.193 and 0.347 mg/L). Combined male and female LC_{50} = 0.225 mg/L (0.190 and 0.267 mg/L).	II	Guidelin 002837
Acute oral, LD_{50} - rat; Diamond Shamrock; report #DS-2787; 2/7/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LD_{50} > 16.24 g/kg. Toxic signs were lothargy chromdacryorrhea and chromorhinarrhea	IV	Guideline 002805
Acute dermal, LD_{50} rabbit; Diamond Shamrock; #DS-2787; 2/6/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LD_{50} > 14.13 g/kg. Slight to severe erythema and edema from day 1 to day 14	III	Guideline 002805
Acute inhalation, LC_{50} Diamond Shamrock; report #DS-2787; 4/28/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	LC_{50} was 0.11 mg/L (0.09 - 0.14). Labored heating excessive ocular, nasal tral servtions	I	Guideline 002805
Primary eye irritation rabbit; Diamond Shamrock; report #DS-2787; 2/7/80	Chlorothalonil (tetrachloro-isophthale-nitrile) 96.0%	246769	3/12 corneal opacity; 3/12 iris irritation; conjunctive irritation in 12/12 animals; 4/12 could not be scored due to severe chemosis and corneal opacity. Corneal opacity persisted in 7/12 through day 14; conjunctive irritation persisted through day 14.	I	Guideline 002805

Tox Chem No. 215B

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No. Guideline
Primary dermal irritation - rabbit; Diamond Shamrock; #DS-2787; 2/6/80	Chlorothalonil (tetrachloro-isophthalo-nitrile) 96.0%	246769	At 24 and 72 hrs., slight to well-defined erythema and edema. PIS = 1.12	IV	002805
Dissimilation chemicals metabolite or impurity or contaminant or salt or photodegradent or etc			Caswell #496D		
Risk Assessment Chronic - mice; Diamond Shamrock; April 1983	Technical	071541	Based on renal tumors a Q_1^* of 2.4×10^{-2}		004455
Dermal Sensitization - guinea pig; 5TX-81-0132; April 15, 1982	Technical Chlorothalonil 97%	253856	PIS for PCNB pos. Control 1.0/8.0. PIS for Technical 0.0/8.0 upon challenge dose. Not a gross sensitizer.		Minimum 004479

Tox Chem No. 215B (& 812)

File Last Updated 05/08/86

Current Date 04/22/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Stillmeadow, Inc.; #2781-82; 11/5/82	Sulphur 27.25% Chlorothalonil 19.15%	249039	LD_{50} > 5020 mg/kg	IV	Guideline 005043
Acute dermal LD_{50} - rabbit; Stillmeadow, Inc.; #2781-82; 11/8/82	Sulphur 27.25% Chlorothalonil 19.15%	249041	LD_{50} > 2010 mg/kg	III	Guideline 005043
Acute inhalation LC_{50} - rat; #2785-82; 11/24/82	Sulphur 27.25% Chlorothalonil 19.15%	249043	LC_{50} > 72.03 mg/l	III	Guideline 005043
Primary eye irritation - rabbit; Stillmeadow, Inc.; #2783-92; 10/14/82	Sulphur 27.25% Chlorothalonil 19.15%	249042	5/6 & 3/3 redness (sc.1 & 2); 4/6 & 1/3 chemosis (sc.1 & 2); Day 4: irritation clear	III	Guideline 005043
Primary dermal irritation - rabbit; Stillmeadow, Inc.; #2784-82; 11/8/82	Sulphur 27.25% Chlorothalonil 19.15%	249040	24 hrs: 6/6 erythema and edema (sc. 2 & 3); 72 hrs: 6/6 erythema and edema (sc. 1 to 3); Primary Irritation Score: 3.73	III	Guideline 005043

Tox Chem No. 215B --- File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenicity - Ames test Salmonella typhimurium SDS Biotech. Corp. #694-STX-85-0042-002 10/22/85	SDS-3939 (90.5% purity)	260841	Not a mutagen in the Ames test (TA-98, TA100, TA1535, TA1537, and TA-1538) either with or without renal metabolic activation at the concentrations tested. Concentrations tested: 100, 500, 2500, 5000, and 10,000 ug/plate under the activated assay system and 40, 200, 1000, 2000, and 4000 ug/plate under the nonactivated assay system.		Acceptable 005040
Mutagenicity - Ames test Salmonella typhimurium SDS Biotech Corp. #694-STX-85-0043-002; 6/24/85	SDS-66382 (97.5% purity)	260841	Not a mutagen in the Ames test (TA98, TA100, TA1535, TA1537, and TA1538) either with or without renal metabolic activation at the concentrations tested. Concentrations tested: 100, 500, 2500, 5000, and 10,000 ug/plate with and without activation.		Acceptable 005040

Tox Chem No. 215B (Chlorothalonil) File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acceptable Daily Intake- EPA/ OPP/ HED TOx.	TECH		PADI= 0.015 mg/kg/day Safety Factor = 100 Dated: Updated: Study: 2-Year Feeding Dog Study NOEL: 1.5 mg/kg Lab.: Hazelton Laboratories Study No.: 200-206 Study Date: 5/06/70 Doc.No.: 004994 Comments: Neoplasms of the renal tubular epithelium in rats (both sexes) and mice (male). Final evaluation pending.		004994

Tox Chem No. 215B (Chlorothalonil) File Last Updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acceptable Daily Intake- EPA/ OPP/ HED TOx.	TECH		PADI= 0.015 mg/kg/day Safety Factor = 100 Dated: Updated: Study: 2-Year Feeding Dog Study NOEL: 1.5 mg/kg Lab.: Hazelton Laboratories Study No.: 200-206 Study Date: 5/06/70 Doc.No.: 004994 Comments: Neoplasms of the renal tubular epithelium in rats (both sexes) and mice (male). Final evaluation pending.		004994

Tox Chem No. 215B (& 101) File Last Updated _____ Current Date 10/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Bioassay System Corp.; #10656; 5/7/81	Bis(tributyltin) oxide .30% chlorothalonil .70%	252618	No mortalities, clinical signs, or abnormalities		Supplementary 005475
Acute dermal LD_{50} - rabbit; Bioassay System Corp.; #10656; 5/3/81	Bis(tributyltin) oxide .30% chlorothalonil .70%	252617	No mortalities or abnormalities		Supplementary 005475
Primary dermal irritation - rabbit; Bioassay System Corp; #10656; 6/1/81	Bis(tributyltin) oxide .30% chlorothalonil .70%	252616	24 hrs. 6/6 slight erythema (scores of 1); 1/6 slight edema (sc. 1); 72 hrs. irrition ceased. Primary Irritation Score: 0.418	IV	Guideline 005475
Primary eye irritation - rabbit; Bioassay System Corp; #10656; 5/29/81 5/29/81	Bis(tributyltin) oxide .30% chlorothalonil .70%	252615	24 hrs.: 5/6 (unwashed) and 1/3 (washed) had hyperemia (5/6=1)(1/3=1) and chemosis (5/6=1) (1/3=2); 1/6 discharge (1/6=2); 72 hrs: irritation cleared. No corneal opacity or iris irritation.	III	Guideline 005475
Acute inhalation LC_{50}- rat; Diamond Shamrock; Report #DS-2787; April 28, 1980	Chlorothalonil (tetrachloroisophthalonitrile) 96%		LC_{50} was 0.11 mg/l (0.09 and 0.14)	II	Guideline 005384

Tox Chem No. 2158 (Chlorothjalonil) | File Last Updated _____ | Current Date: 10/30/86

Study/Lab/Study #/Date	Material	EPA Accession/ MRID No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
30-Month oncogenic-rat; IRDC;5TX-80-234;5-28-85	DS-2787; 98.1% [redacted] HCB	258759	Levels tested in Fischer 344 strain -0, 800, 1600 and 3500 ppm for 116 weeks in males & 129 weeks in females. Oncogenic NOEL <800 ppm(LTD)(renal adenomas and carcinomas & a dose related increase in papillomas of the stomach		Guideline 004950
13-Week feeding - rat; Huntington Res. Ctr.; 562-5TX-81-0213-004-001;06/28/83	DS-2787; 98.1%	258768	Histopathologic re-evaluation of renal tissues - New NOEL = 1.5 mg/kg/day. EM and light microscopy. See also study # 5TX-81-0213		Acceptable 004950
90-Day feeding - mice; Exp. Path. Labs; 5TX-79-0102;9-2-83.	DS-2787 Tech.	258769	Histopathologic re-evaluation of renal tissues. No undetected toxic effects noted. NOEL remains at 15 ppm(study #618-5TX-83-0007-004		Acceptable 004950
Hepatic & Renal GSH - male rat; Safety Asses. Animal Facility; 751-5TX-85-0032-001; 6/27/85	DS-2787 Tech. 97.8%	258776	5000 mg/kg reduces liver GSH but increases renal GSH @ 48 hrs.		Acceptable 004950
Metabolism - rat; Concorde Woods Lab.; 621-4AM-83-0061-001; 6/28/85	99.7 % DS-2787 w/radiolable	258776	200 mg/kg produced dithiolichloro- and trithiochloro-metabolite (2.4% of AD). Interim Report. Males tested only		Acceptable 004950
Dermal absorption, male rat - Concord Woods; 649-4AM-84-0010-001; 12/26/84	99.7 % pure DAC-2787 14-C-label	258774	6.3% applied dose absorbed dermally daily. 18% excreted in feces; 6% in urine (total dose). Suggests bile metabolism and saturated urinary excretory mechanism at low levels.		Guideline 004950

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Tox Chem No. 215B Chlorothalonil | File Last Updated _____ | Current Date: 10/30/86

Study/Lab/Study #/Date	Material	EPA Accession/ MRID No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute effect on hepatic & renal GSH, rats; Safety Assess. Animal Fac.; 732-5TX-85-0006-001; 6/19/85	97.8 % pure DAC-2787 14-C-label	258776	5 mg/kg bw IP produces no effect on renal or hepatic GSH. 5000 mg/kg bw orally reduces hepatic GSH but increases renal GSH @ 24 hours.		Acceptable 004950
Metabolism - rat; Huntingdon Res. Ctre.; 631-4AM-83-0011-002; 7/2/84	99.7 % pure DAC-2787 14-C-label	258775	Most activity excreted by GI tract; ca 5-7% excreted in urine. Tissues per se did not retain activity.		Acceptable 004950
Metabolism - rat; Huntingdon Res. Ctre.; 633-4AM-83-0062-002; 1/3/85	99.7 % pure DAC-2787 14-C-label	258775	5 mg/kg single po dose. 34% AD absorbed by gut; remainder lost in feces and GI tract. 8-10% AD excreted in urine. No tissue residues.		Guideline 004950
Metabolism - rat; Huntingdon Res. Ctre.; 631-4AM-84-0078-002; 7/10/85	99.7 % pure DS-2787 14-C-label	258775	80 - 90% AD excreted in feces. ca. 11% excreted in urine; 96% in first 24 hours(female)		Guideline 004950
Metabolism - rat; Huntingdon Res. Ctre.; 631-4AM-84-0079-001; 7/15/85. INTERIM REPORT	99.7 % pure DS-2787 14-C-label	258776	Results tentative; conclude support saturation hypothesis of kidney excretory mechanisms. INTERIM REPORT		Supple-mentary 004950
Dissimilation chemicals metabolites or impurity or contaminant or salt or photodegradent or etc.			Caswell # 506A 825 A, B, C 833 D, E 874 C, D, E 876 C, D		

Tox Chem No 215B (and 501A) File Last Updated _____ Current Date 4/15/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
[redacted]					

PENDING REGISTRATION INFORMATION IS NOT INCLUDED

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

1-31-84

MEMORANDUM

Subject:

Chlorothalonil Registration Standard.

Weight of Evidence review for oncogenicity.

TO: Dianne Beavers, PM Team # 21
Herbicide-Fungicide Branch
Registration Division TS-767

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769

FROM: David L. Ritter, Toxicologist
Rev. Sec.# 1/Toxicology Branch
Hazard Evaluation Division TS-769

Summary:

Results of animal experiments provide some evidence that Chlorothalonil (CTN) is carcinogenic in experimental animals[1],[2].

Discussion:

The National Cancer Institute (NCI) Study

CTN was assayed for oncogenicity by the NCI in 19[illegible]. Their published report (NCI-CG-TR-41, 19[illegible]) contained some evidence that CTN induced renal neoplasms in Osborne-Mendel male and female rats. No neoplasms were reported in concurrent control animals of either sex. The rats were offered diets containing 5063 ppm (time weighted average) or 10,126 ppm (time weighted average) for 80 weeks; then were returned to the basal diet for an observation period of 30 - 31 weeks. (Table I).

Serious deficiencies were noted in this study in the Toxicology Branch review of H. Spencer, Ph.D., 11/28/78. These included:

- Rats were not exposed to the test material for the full length of the study; after 80 weeks the animals were placed on basal diets for another 30 - 31 weeks.
- Only 10 rats/sex were used as concurrent controls.
- Control animals were not obtained from the same supplier.

Overall, we conclude that this study, while containing some evidence suggestive of oncogenicity in rats, is not of sufficient quality to make a definitive conclusio

A similar study in mice was negative for evidence of oncogenic potential.

The Diamond Shamrock Study

STUDY: Chronic Mouse Feeding Study

LABORATORY: Biodynamics Laboratory, East Millstone, NJ

STUDY NUMBER & DATE: DTX-79-0102 2/[illegible]81

DS-2787[4] was offered to groups of 60 male or female CD-1 mice at 0, 750, 1500 or 300 ppm in the diet for two years. Tubular adenomas and tubular carcinomas were induced in male mice only in treated groups with no incidences of these lesions being report in control animals. Treated male mice also demonstrated squamous carcinomas and glandular carcinomas of the gastric mucosa while the control males did not exhibit such lesions. However, there was no dose-dependant relationship in the occurrance of these lesions. (See Tables II and III).

In order to properly evaluate the occurrance of these lesions in mice we have asked the Petitioner to provide us with additional histopathologic profiles on control mi of this strain D. Ritter memorandum to Diane Beavers, 10/[illegible]81. These data have not been received.

Overall, we consider that this study was properly conducted and reported. Therefore, the Weight of Evidence for the demonstrated oncogenicity of CTN rests mostly upon it, with the NCI study occupying a supplemental role.

The 4-hydroxy metabolite, a major crop residue, did not induce hec, asms (Study # 0024-001, 2/17/82) in mice. In order to clarify the metabolism of CTN and its 4-hydroxy metabolite, we are asking that animal metabolism studies be done that demonstrate the metabolic pathways and metabolic products at both high and low levels.

Recommendations:

1. That the NCI study be considered Supplementary only, due to deficiencies noted above;

2. That all pending temporary tolerances for residues of CTN in racs be renewed as requested.

3. That all pending new tolerances be reconsidered on a case-by-case basis with respect to incremental risk and/or incremental exposure.

4. That a preliminary Risk Analysis be utilized in the interim until the following additional data are received and evaluated:

   - data on the incidence of renal neoplasms in additional CD-1 mice concurrent control groups (historical controls);
   - a new rat feeding/oncogenicity study using Technical CTN;
   - new animal metabolism studies demonstrating metabolic pathways and metabolic byproducts at high and low levels of exposure.

(NOTE: We emphasize "preliminary" to indicate the Risk Analysis is incomplete at present, but nonetheless provides a basis which should serve to augment regulatory decisions.)

FOOTNOTES

[1] See also IARC Monograph, Volume 30, March, 1983.

[2] Memo from Dr. Huff, NTP, 5/30/83, appended

[3] The NCI analysis of the test material showed: CTN - 98.0 to 98.5%; Pentachlorobenzonitrile (PCBN) - 0.6 to 1.24%; Hexachlorobenzene (HCB) - < 0.03%.

[4] Technical product 97.7% CTN; less than [redacted] HCB. No estimate of PCBN.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

TABLE I

NCI-CG-TR-41, 1978 Dietary Chlorothalonil in the Osborne-Mendel Rat

INCIDENCE OF RENAL NEOPLASIA[1]

	MALES			FEMALES		
	Controls	5063 ppm	10,126 ppm	Controls	5063 ppm	10,126 ppm
CARCINOMA	0/10[2]	1/45	3/49	0/10[2]	1/48	2/50
ADENOMA	0/10	2/45	1/49	0/10	0/48	3/50
TOTAL	0/10	3/45	4/49	0/10	1/48	5/50

The One-Hit Slope Coefficient B is 1.69×10^{-4} for males and 2.08×10^{-4} for females (memorandum from Bill Burnam to Cara Jablon, 4/17/81).

Using the most sensitive sex, the females, Risk = B x 5.5^{3} x 0.01305 (the TMRC) = 1.49×10^{-5}.

---

[1] Copied from the H. Spencer review of 11/13/78.

[2] Concurrent control were 10 animals per sex. Pooled controls represented 62 males and 62 females.

[3] Cube root of the ratio of human body weight-to rat body weight = 5.5.

TABLE II

NEOPLASMS IN MALE CD-1 MICE FED CHLOROTHALONIL IN THE DIET FOR TWO YEARS

STUDY # DTX-79-0102

KIDNEY

	Control	750 ppm	1500 ppm	3000 ppm
Tubular Adenoma	0/60	3/60	4/60	2/60
Tubular Carcinoma	0/60	3/60	0/60	2/60
Total Neoplasms	0/60	6/60	4/60	4/60

The One-Hit Slope Coefficient $B^{(1)} = 9.37 \times 10^{-4}$ mg/kg/day^{-1}

Based on the response of the 750 ppm mice Risk = B x 12.6[2] Exposure = 0.0118 mg/kg/day^{-1} x 0.0.01305 mg/kg/day (the TMRC) = 1.54×10^{-4}.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	1/60	5/60	2/60
Glandular Carcinoma	0/60	1/60	2/60	0/60
Total Neoplasms	0/60	2/60	7/60	2/60

The One-Hit Slope Coefficient B = 4.06×10^{-5} mg/kg/day^{-1}

Based on the Squamous Carcinoma response of the 1500 ppm mice, Risk = B x 12.6[2] x Exposure = 5.12 mg/kg/day^{-4} x 0.01305 mg/kg/day (the TMRC) = 6.68×10^{-6}.

---

(1) Slope Coefficient calculated by Roger Gardner, 1/30/84.
(2) Cube root of the ratio of human body weight to mouse body weight.

TABLE III

NEOPLASMS IN FEMALE CD-1 MICE FED CHLOROTHALOPNIL IN THE DIET FOR TWO YEARS

Study # DTX 79-0102

KIDNEY

No lesions were reported in this organ.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	2/60	6/60	5/59
Glandular Carcinoma	0/60	1/60	1/60	2/59

The One Hit Slope Coefficient $B^{(1)} = 7.09 \times 10^{-5}$ mg/kg/day^{-1} is based on the Squamous Carcinoma response of the 1500 ppm females. Risk = B x $12.6^{(2)}$ x Exposure.

Risk = $8.93 \times 10^{-4} \times 0.01305$ mg/kg/day (the TMRC) - 1.75×10^{-5}.

(1) B value calculated by Roger Gardner, 1/30/84.
(2) Cube root of the ratio of human body weight to mouse body weight.

Although several absolute and relative organ weight changes were determined, only the kidney weight changes are considered compound related. Relative kidney weights were increased in both sexes at all treatment levels, but gross and histopathological evaluations revealed no correlative compound related effects. Gross necropsy findings were unremarkable among all groups. The only dose related histologic effect of treatment, which was inversely related to dose, was a finding of acute gastritis in the non-glandular portion of the stomach in all treatment groups. Based upon the relative kidney weight changes at all levels with compound related effects on specific gravity and urine volume at $\geq$ 375 mg/kg a clear no adverse effect level has not been demonstrated. The depressed SGPT activity at all treatment levels in both sexes, considered compound related, is difficult to interpret particularly since relative liver weights were increased at $\geq$ 750 mg/kg for both sexes.

NOEL < 40 mg/kg/day

CORE RATING:

Supplemental. A clearcut no effect level was not demonstrated.
Not repairable.

OTHER PERTINENT STUDIES

TERATOLOGY

RABBIT (1966) **NEGATIVE**

RABBIT (1975, JAPAN) **NEGATIVE**

0, 1, 2.5, 5 mg/kg NOEL = 2.5
MATERNALLY TOXIC AT 5
NOT TERATOGENIC

RAT (1983) 0, 25, 100, 400 mg/kg **NEGATIVE**

Maternally toxic at 400 mg/kg
NOT TERATOGENIC

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MAY 3 1984

MEMORANDUM:

TO: Henry Jacoby, PM # 21
Herbicides/Fungicides Branch
Registration Division TS-767C

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

FROM: David L. Ritter, Toxicologist
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C

Subject: EPA Reg. # 677-313 - Review of miscellaneous Toxicity Data.

Caswell #: 215B

Sponsor: SDS Biotech. (Formerly Diamond Shamrock Corp., Cleveland, OH.)

This Rat Teratology Study, # 517-5TX-0011-003, is reviewed under the attached DER.

We find that the study is acceptable for regulatory purposes.

DATA EVALUATION REPORT

STUDY: Teratology Study in Rats EPA # 677-313

LABORATORY: WIL Research Laboratories

DATE: 5/13/83

STUDY NUMBER: # 517-5TX-0011-003

ACCESSION NUMBER: 250855

MATERIAL TESTED: Technical Chlorothalonil

ANIMALS: Sprague-Dawley gravid female rats

METHODS:

"Groups of Sprague-Dawley rats (25 females/group) were administered chlorothalonil orally, via gavage, doses of 0, 25, 100 and 400 mg/kg/day from day 6 through 15 of gestation. Surviving females were necropsied on day 20 and fetuses delivered by hysterotomy. The number and position of viable/nonviable fetuses, early/later resorptions, mean number of corpora lutea and total number of implantations were recorded. External, internal and skeletal examinations of fetuses were performed for evidence of abnormalities and anomalies. Half of the fetuses were evaluated for soft tissue anomalies and the other half for skeletal effects.

[RESULTS]:

There was no dose related mortality in the 25 and 100 mg/kg/day groups. However, three dams in the 400 mg/kg which died during treatment were considered related to compound ingestion. There were no abortions in any group. General appearance and behavior were unremarkable except for evidence of cathartic action at 400 mg/kg (e.g. loose feces, matting of urogenital fur). Mean maternal body weights were significantly different (less) than control at the high dose. Food consumption was significantly reduced in all treatment groups initially (days 6-9), and in the high dose group throughout the dosing period (days 6-15). There were no differences compared to control for mean number of viable fetuses, implantation sites, corpora lutea or fetal weights. There was a significant increase in the number of early resorptions in the high dose group, as well as post implantation losses, when compared to controls. There were no reported effects on number or percentage of fetuses/litters with external, internal or skeletal malformations or developmental variations at any dose level administered.

[CONCLUSION]:

Chlorothalonil was considered maternally toxic to rats at 400 mg/kg but there was no evidence of teratogenicity at any level tested (Rodwell et al., 1983)."

CORE RATING:

Guideline.

REFERENCE

Jaeger, R.B. "The Toxicity of Chlorothalonil". Report to the Joint Committee on Pesticide Residues. FAO/WHO. Geneva. 1983. (Draft).

New

007713

TOXICITY STUDIES WITH CHLOROTHALONIL IN DOGS

1. TWO YEAR DOG STUDY (1964-1966)

DOSE LEVELS:

0, 1500, 15000, 30000 PPM

2. SIXTEEN WEEK DOG STUDY (1967)

DOSE LEVELS:

0, 250, 500, 750 PPM

3. TWO YEAR DOG STUDY (1968-1970)

DOSE LEVELS:

0, 60, 120 PPM

NOEL = 120 (3 mg/kg)

OTHER PERTINENT STUDIES
MUTAGENICITY

AMES ASSAY (HEPATIC ACTIVATION)	NEGATIVE
CHINESE HAMSTER V-79 ASSAY	NEGATIVE
BALB/3T ASSAY	NEGATIVE
HOST MEDIATED ASSAY (MICE)	NEGATIVE
MICRONUCLEUS TEST	
RAT	NEGATIVE
MOUSE	NEGATIVE
HAMSTER	NEGATIVE
CHROMOSOMAL ABERRATION TEST (single and multiple dose)	
RAT	NEGATIVE
MOUSE	NEGATIVE
HAMSTER	EQUIVOCAL RESULTS
DOMINANT LETHAL ASSAY (MICE)	NEGATIVE
DNA REPAIR	
S. TYPHIMURIUM	POSITIVE
B. SUBTILIS	NEGATIVE
CELL TRANSFORMATION	NEGATIVE
AMES ASSAY (RENAL ACTIVATION)	NEGATIVE

OTHER MUTAGENICITY STUDIES

NIH STUDIES

AMES	NEGATIVE
SISTER CHROMATID EXCHANGE ("in vitro")	WEAK POSITIVE
CHROMOSOMAL ABERRATION ("in vitro")	POSITIVE
SEX-LINKED RECESSIVE LETHAL	NEGATIVE

DPR
2-20-86

C. 713

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Time Course of the Acute Effect of Technical Chlorothalonil on Hepatic and Renal Glutathione (GSH) Content in Male Rats.

LABORATORY: Safety Assessment Animal Facility, Painesville, OH.

STUDY NUMBER & DATE: 751-5TX-85-0032-001; E. M. Sadler, 6/27/85.

ACCESSION NUMBER: # 258776.

MATERIAL TESTED: SD-2787 Technical; 97.8% pure.

ANIMALS: Male Sprague-Dawley Rats, 41 days old at initiation of the study.

METHODS:

Husbandry: Standard GLP.

Feed: Withheld for sixteen hours prior to dosing, then offered ad libitum from four hours post-dosing.

Dosing: Animals were assigned 5/group in twelve groups. Six groups received vehicle (0.5% in methylcellulose) and six received 5000 mg/kg test material in 0.5% methylcellulose in a single oral dose.

One vehicle control group and one treatment group per time interval was killed at 1, 3, 9, 18, 24 or 48 hours post-dosing.

Animals were observed twice daily for signs of toxicity. Body weights were determined intially and at termination.

Determination of GSH:

At the appropriate time interval rats were killed and liver and kidneys were obtained, weighed, homogenized and analyzed for GSH content by an acceptable method. Data were statistically analyzed using Student's T-tes

RESULTS:

Observations:

Administration of vehicle did not induce toxic sequelae, nor did those animals whose exposure time was less than nine hours. Animals sacrificed nine to forty-eight hours post-dosing showed soft stools, ano-genital staining and red nasal discharge.

Body weights:

Treated animals sacrificed at the 24 and 48 hour interval had reduced terminal body weights.

Liver Weights:

24 and 48 hour liver to body weight ratios were reduced in treated animals.

Kidney Weights:

Kidney to body weight ratios were increased in the 9, 18 and 48 hour groups.

GSH Content:

Hepatic GSH content was significantly decreased by 20% in the 9 hour group; 40% in the 18 hour group, and 25% in the 24 hour rats. No reduction was seen in the 48 hour rats.

Renal GSH was significantly increased by 21% at 9 hours; 45% at 18 hours; 38% at 24 hours and by 101% at 48 hours.

CONCLUSIONS:

Chlorothalonil given by gavage to male rats at a single oral dose of 5000 mg/kg. induces significantly increased liver and kidney-body weight ratios, reduces hepatic GSH content up to twenty-four hours following challenge and increases renal GSH content significantly at up to 48 hours after treatment.

The investigators suggest that the hepatic GSH changes are related to its conjugation with chlorothalonil, but that the mechanism for renal reduction in GSH content is not known.

CORE RATING:

Guideline.

2-20-86

CC,713

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Acute Effect of Technical Chlorothalonil on Hepatic and Renal Glutathione (GSH) Content in Rats.

LABORATORY: Safety Assessment Animal Facility, Painesville, OH.

STUDY NUMBER & DATE: 732-5TX-85-0006-001; 6/19/85. J. A. Ignatoski.

ACCESSION NUMBER: #258776.

MATERIAL TESTED: Technical Chlorothalonil; 2,4,5,6-tetrachloroisophthalonitrile 97.8 % pure.

ANIMALS: Young Sprague Dawley male rats.

METHODS:

Husbandry: Standard GLP.

Dosing: 3 rats per group were selected. Group I received 1 mg/ml corn oil I.P. Group II received 5 mg/kg DS-2787 I.P. Group III received 1 mg/kg in 0.5 % aqueous methylcellulose P.O. Group IV received 5000 mg/kg P.O. in 0.5 % methylcellulose. Groups V^a and VI^a received the same treatments as Groups III and IV except each of these contained 5 rats/group.

Observations:

Animals were checked once daily prior to dosing and twice daily thereafter.

Body Weights:

Obtained initially only.

GSH Content Determination:

Groups I and II were killed at 2 hours post-dosing; the remaining groups were killed at 24 hours post-dosing.

At the pre-determined times, animals were killed and the livers and kidneys were prepared and analyzed for GSH content using standard laboratory wet-tissue procedures. GSH content was determined using a spectrophotometer.

Tissue GSH content values were analyzed using Student's "t" test.

RESULTS:

Observations for gross overt effects were negative in all groups.

No significant differences were reported in renal or hepatic GSH in rats dosed with 5 mg/kg i.p. of chlorothalonil in corn oil (Groups I and II).

Renal content of GSH was significantly increased in chlorothalonil - treated rats (Groups III vs. IV) at 24 hours following intubation with 5000 mg/kg in methyl cellulose. The increase was about 25 % more than the level of the corresponding control group. Hepatic GSH levels were reduced, but not significantly so.

Renal GSH content was significantly increased in the duplicate groups (Groups V and VI) but the hepatic GSH content was significantly reduced.

CONCLUSIONS:

1. 5 mg/kg BW of chlorothalonil given i.p. affects neither the renal nor hepatic GSH content when measured 2 hours after treatment.

2. 5000 mg/kg BW given by gavage reduces hepatic GSH content when measured at 24 hours following administration; the same dose increases renal GSH content.

   It was suggested that this supports the proposed metabolic pathway which includes a GSH conjugate formed in the liver which is subsequently metabolized in the kidney to a sulfur containing, potentially nephrotoxic compound.

CORE RATING:

Acceptable.

Letter

C.713

2-20-87

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Identification of Metabolites in Urine and Blood Following Oral Administration of ^{14}C-labeled Chlorothalonil to Male Rats: The Thiol metabolites in Urine (Interim Report).

LABORATORY: Concord Woods Laboratories, Painesville, OH.

STUDY NUMBER & DATE: # 621-4AM-83-0061-001; J.P. Marciniszyn, 6/28/85.

ACCESSION NUMBER: # 258776

MATERIAL TESTED: 99.7 % pure ^{14}C-DS-2787 with specific activity of 124.7 mCi/mmole.

ANIMALS: CD Sprague-Dawley male rats.

METHODS:

One group of 8 males (group I) and one group of 5 males (group II) each received 200 mg test material/ kg body weight on different days. Three males were undosed and served as controls.

Group I rats had urine collected at 24 and 48 hours. Group II had samples collected at 17 hours (termination).

Blood samples were taken just prior to necropsy for group I at 48 hours and for group II at 17 hours (to be analyzed later).

Only four group I urine samples could be used because of fecal contamination. These were pooled and subjected to extraction and LSC and MS analyses for urinary metabolites (procedures attached).

No group II urines were used for analysis because of the time difference.

RESULTS:

The authors calculated that each of the four remaining animals received 55.1 mg of radio-labeled DS-2787 in 0.75 % methylcellulose. The combined urines contained 2.4 % of the total administered dose. Ethyl acetate extration removed 15.4 % of this or 0.35 % of the administered dose. 54.5 % of the labeled DS-2787 or 1.3 % of the administered dose was removed by subsequent acidification/ethyl acetate extraction. The remainder was deemed to be unextractable (30 % of label or 0.78 % of administered dose).

Two metabolites were subsequently identified by GC/MS analyses: dithiodichloroisophthalonitrile and trithiochloroisophthalonitrile. These were present in about a 1:1 ratio. They may exist as the free sulfhydryl and as the methylated derivative.

CONCLUSIONS:

Male rats administered oral DS-2787 ring-labeled with ^{14}C at 200 mg/kg produced urinary metabolites at 2.4 % of the administered dose. The metabolites were determined to be dithiodichlorophthalonitrile and trithiochlorophthalonitrile in an approximate ratio of 1:1. These may have existed as the free sulfhydryl and as the methylated form. The authors postulate that hepatic metabolism proceeds through conjugation with glutathione (GSH) followed by enzymatic degradation. The smaller conjugates are then transported via the bloodstream to the kidney where they are converted to thoil metabolites and excreted in the urine.

CORE RATING:

Minimum Data. Only four animals could be used instead of the original eight, and the urine samples were pooled.

DHR 2-20-86

00,718

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Biliary Excretion of Radio-labeled ^{14}C-DS-2787 to Rats Following Oral Adminstration.

LABORATORY: Huntington Research Centre, Cambridgeshire, England

STUDY NUMBER & DATE: 633-4AM-83-0062-002; 1/3/85; J. A. Ignatoski.

ACCESSION NUMBER: #258775

MATERIAL TESTED: Mixture of ^{14}C-ring labeled and cold DS-2787, 99.7% pure; 27.9 uCi//mg in 0.75% methylcellulose suspending medium.

ANIMALS: 8 male and 4 female Sprague-Dawley rats (ave. 260 gm).

METHODS:

Husbandry:

Standard GLP.

Feed and Water:

Standard ad libitum.

Dosing:

Fasted except for water for 16 hours prior to bile duct cannulation procedure. 2-four male groups and 1-four female groups were used. Of these, two in each group had an additional cannula inserted into the duodenal bile tract; sodium tauracholate (a choleretic substance) was infused at a rate of 25 mg/hour into this fixture.

Animals were observed for a short time to insure adequate bile flow, then the rats were intubated with ^{14}C-DS-2787 at 5 mg/kg in a single dose.

Sample Collection:

Animals were restrained and bile samples were collected at hourly intervals from 0 to 48 hours after dosing. Blood was sampled at 6 and 24 hours and at termination. Urine was collected in CO_2-chilled containers during the 0 - 6 hour period, the 6 - 24 hour period and the 24 - 48 hour period. Feces was collected during the 0 - 24 hour and the 24 - 48 hour periods.

Termination:

The animals were killed and the GI tract, stomach, large intestine and small intestine were excised, tied off and stored at -20° C. Cages were washed and the washings measured for activity.

Measurements:

Samples of bile, urine, cage washings, methanolic extracts of the carcasses were diluted with appropriate scintillator fluid and counted. Feces samples were homogenized and mixed with cellulose, combusted and counted. Whole blood samples also were combusted and counted.

The GI tract portions were separately minced, homogenized in acetone 50%, combusted in oxygen and counted. Carcasses were minced with rat chow and homogenized with methanol. The resultant supernatants were directly counted; the solids were air-dried, combusted and counted.

RESULTS:

Excretion of Radioactivity:

50.3 and 61.1% of the administered dose was excreted in the feces of the males and females respectively. 21.1 and 16.7% was excreted in the bile, males and females respectively. Urine, GI tract and carcasses contained 9.6% (males and females combined), 6.4 and 2.2% respectively, of the administered dose. Overall recovery was said to be 91.2%.

No increase or decrease in the amount excreted in the bile was reported for those rats receiving taurocholate. The bulk of activity was excreted during the first 12 hours (e.g., 70 - 80%) in all groups. Of this, most was excreted during the 1 - 2 hour period for males and females irrespective of taurocholate administration.

The urine was next in order of magnitude of excretion, amounting to about 10% of the administered dose in both sexes. Taurocholate administration did not effect the renal excretion rate in either sex.

Blood concentrations were variable but were highest during the first 24 hours (ca. 200 ng-eq/ml at 6 hours and ca. 90 ng/eq/ml at 24 hours. Maximum blood concentration was 0.4% of administered dose. Taurocholate administration did not appear to effect these findings.

Fecal and residual GI tract content of activity accounted for approximately 60 % of the administered dose.

CONCLUSIONS:

The presence of activity in the blood, urine and bile clearly demonstrates that gut absorption occurs, and to a significant extent. Overall, the gut absorbed approximately 24 % of administered dose, with the remainder (67%) found in the feces and GI tract and represented non-absorbed material. Biliary excretion accounted for 17 -21 % of the administered dose, with maximum concentration eliminated within 2 hours of dosing.

Urinary excretion, at about 8 - 10 % of the labeled dose, shows this to be a significant route of elimination, but not a major one. No appreciable tissue binding is demonstrated as evidenced by low residual carcass levels, ca. 2 % of administered dose. Absorption via blood was also minimal, with maximum concentration less than 0.4% of the labeled dose.

CORE RATING:

Guideline.

042
2-20-86

DATA EVALUATION REPORT

CC/713

CHLOROTHALONIL

STUDY: Dermal Absorption Study in Male Rats

LABORATORY: Concord Woods Laboratories, Painesville, OH.

STUDY NUMBER & DATE: 649-4AM-34-0010-001 12/26/84 MARCH 15TH, 1984

ACCESSION NUMBER: 258774

MRID: NA

MATERIAL TESTED: ^{14}-C-Chlorothalonil, 99.7 % pure (117.4 mCi/mmole)

ANIMALS: Sprague-Dawley CD male rats, ca. 234 gm.

METHODS:

Husbandry: Standard GLP.

Diet and Feeding: Standard rat lab chow, fresh weekly.

Dosing:

Rats were assigned exposure groups and received 5 mg cold and "hot" CTN in 4 ml acetone, distributed over 25 cm^2 shaven skin, or an average dose of 46.7 ugm/cm^2 skin; this was approximately equal to 112 uCi/rat. The treated area was covered with a non-occlusive patch to prevent grooming of the application site.

Three rats per group were exposed for 2, 4, 8, 12, 24, 48, 72, 96 or 120 hours. Non-treated rats served as controls.

Sampling:

Blood was collected at termination and the amount of radioactivity was determined for blood and plasma by liquid scintillation chromatography (LSC).

Urine was collected at termination and analyzed by LSC on animals all exposed up to 24 hours, then at 24 hour periods thereafter from those remaining.

Fecal samples were collected along with the urine samples, but were frozen with dry ice, ground up and combusted for radioactive CO_2.

The protective patch was removed, extracted with acetone and the activity counted by LSC.

The treated and adjacent skin was removed and washed with acetone for counting for surface residues.

The skin was then chopped into small pieces, dry-frozen and homogenized and extracted twice with methanol and acetone for separate LSC determination of unbound residues.

The extracted skin was air-dried and combusted for determination of bound residues.

The intestinal tract less contents was assayed for radioactivity at termination as were the liver, kidneys and carcass.

RESULTS:

Blood:

Activity in the blood plateaued at ca. 72 hours, reaching a level of about 0.18% of the administered dose or approximately 140 ng-eq/ml. About 89 % of total blood activity was located in the plasma.

Liver and kidneys:

Concentration of activity in the liver plateaued similarly to that for the blood; the kidneys plateaued later (between 72 and 120 hours) and was somewhat higher in magnitude.

Carcass:

No apparent pattern was discernable for the carcass; only about 4 % of the administered dose was found there. This included all soft tissues and blood.

Urine:

Urinary excretion was determined to be a total of 6.04% of the total dose. The authors calculated that a constant rate of ca. 1.2 % of the total dose was excreted daily in the urine.

Feces:

Fecal radioactivity (plus gut contents) accounted for the greatest amount of material excreted. There was a close parallel between fecal excretion and blood concentration with time; whereas urinary excretion was independent of blood concentrations. This was attributed to dermal absorption and excretion into the bile, thence into the feces.

Absorbed dose:

The authors observed that the rate of dermal absorption at 2 and four hours exposure was essentially the same (15.1 and 16.4 ug-equivalents, respectively), with the average daily absorption becoming constant after 24 hours and thereafter at a mean rate of 73.2 ± 15.3 ug ^{14}C-Chlorothalonil per day.

Skin Residues:

Skin residues, i.e., those washed off and those recovered from the dressing, dropped from 70.6 % at 24 hours to 44.5 % at 120 hours of the total applied dose. Residues penetrating the skin dropped from 60.3 to 19.6 % of the applied dose. Bound residues increased from 8.4 to 22.5% during this period, and the extractable activity remained at 2.5 % of the applied dose throughout the exposure period. Calculations indicated that 20% of the entire dose was lost at the time of application through evaporation.

CONCLUSIONS:

The rate of absorption from the skin is relatively constant (6.3 %) from 24 to 120 hours following a single dermal application in acetone of 5 mg/kg body weight. The principle route of exretion is via the feces (18 % of the total dose) with excretion in the urine (6 % of the total dose) being the secondary route of elimination. Fecal levels paralleled those for blood. There was substantial loss of activity during the application phase, indicating loss by evaporation.

The urinary excretion pattern, attaining constancy of 1.2 % of applied dose per day, suggested that the renal excretory mechanism for CTN and/or its metabolites is quickly saturated.

Surface residues constitute the bulk of activity, however.

DISCUSSION:

The above evidence suggesting that the renal excretory system for CTN is saturated at relatively low blood/plasma levels (e.g., 140 ng-equivalents) following dermal exposure may have relevance to the chronic renal toxicity of this material in light of the comparatively low oral doses used in earlier feeding studies (NOEL = 60 ppm in the diet of rats). Chronic effects on the kidneys included renal tubular necrosis and chronic glomerulonephritis (Tierney, 1981), and hyperplasia and tubular epithelial dilation, glomerulosclerosis and pigmentation (Paynter, 1976). This finding could also have implications for oncogenic effects on renal tubules reported in laboratory rodents (Campbell, 1978, and Tierney, ibid.).

The appearance of substantial activity in fecal matter strongly supports the conclusion that there is metabolism/secretion in the bile.

CORE RATING:

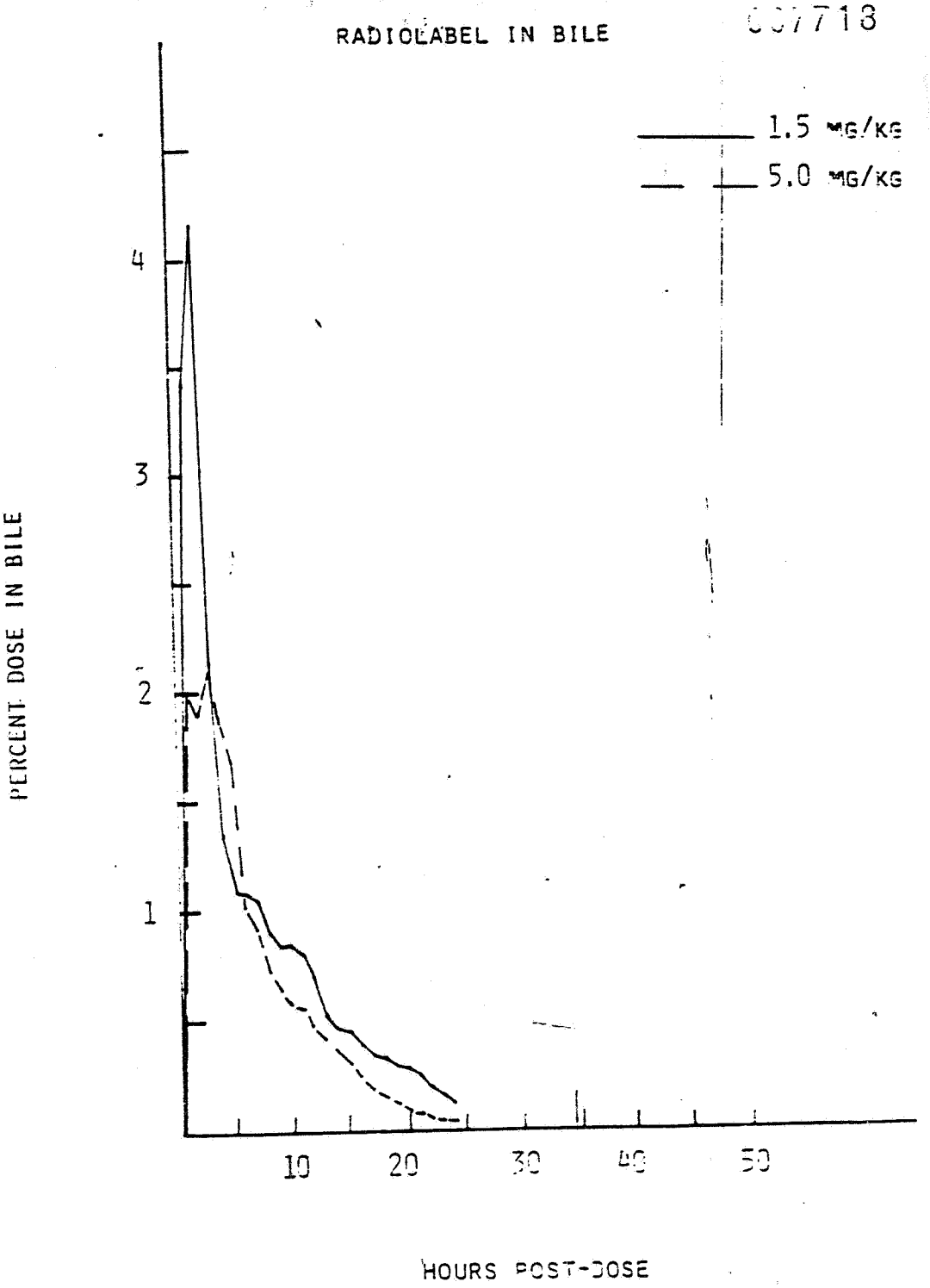
Guideline.

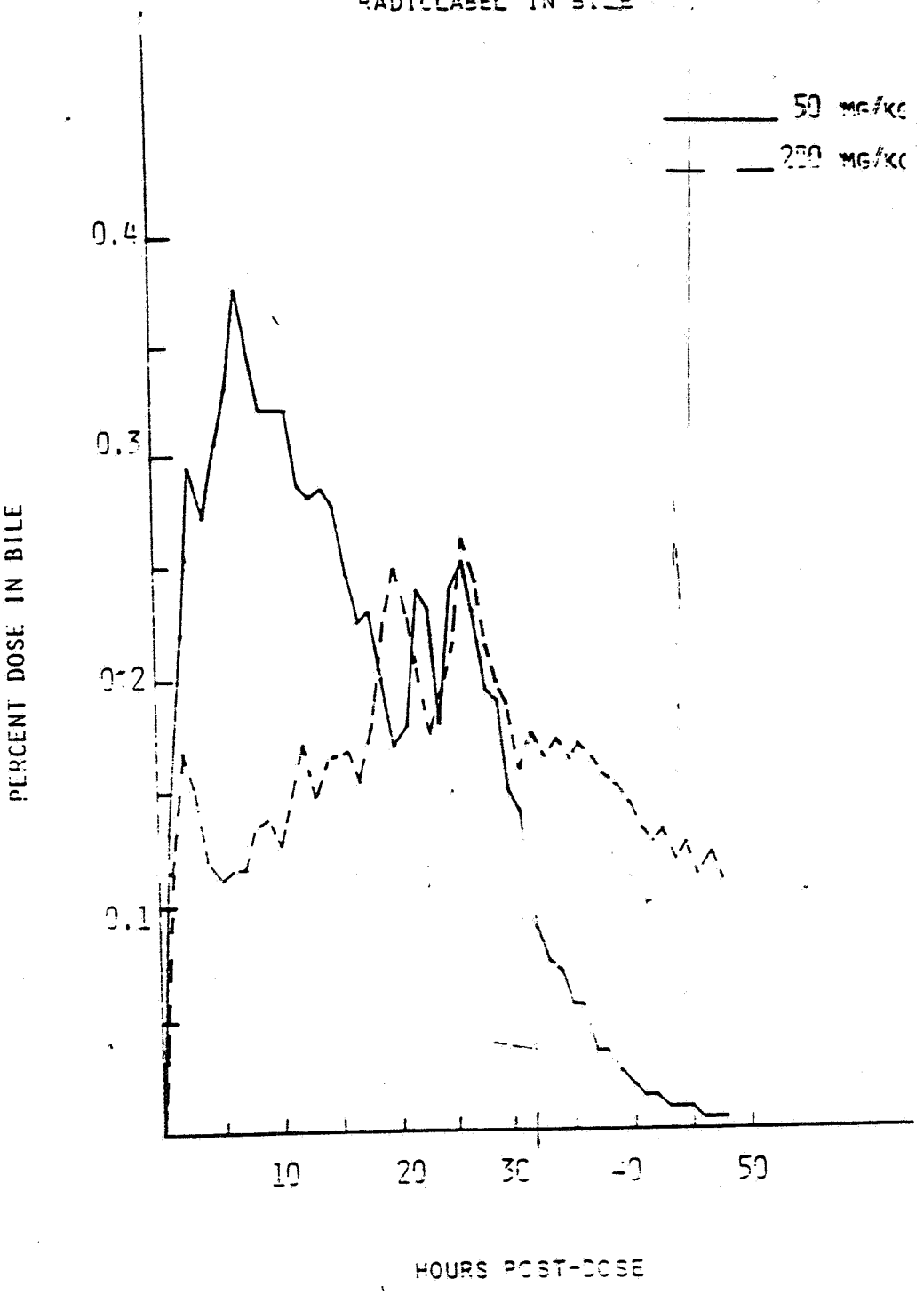
Reviewer: D. Ritter # 47

TECH: 20 hours

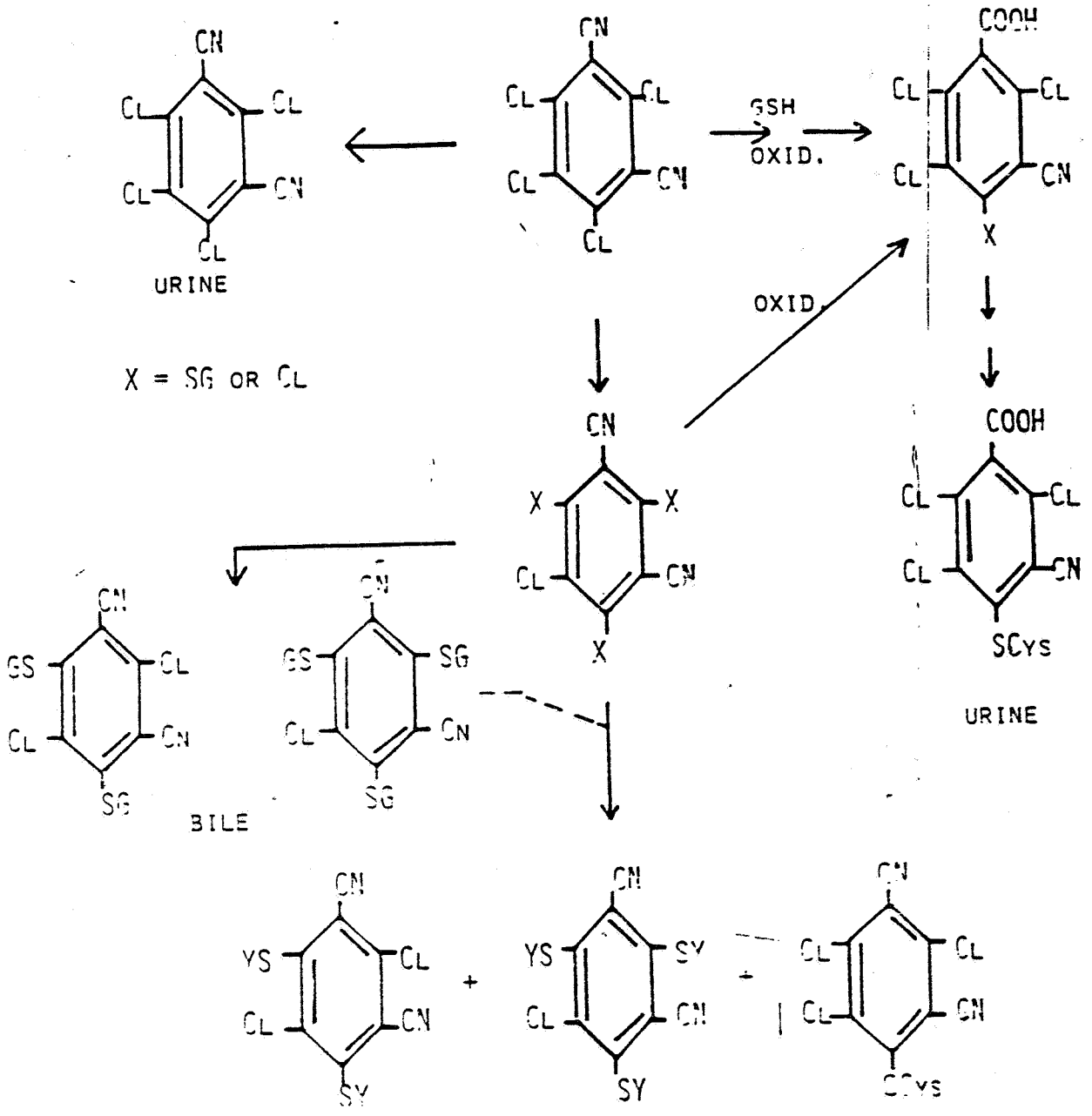
BILIARY DATA

	DOSE LEVEL (mg/kg)			
	1.5	5	50	200
% AD IN BILE	19.23 ± 3.76			7.80 ± 0.88
% AD ABSORBED	29.81 ± 5.66			16.89 ± 2.90

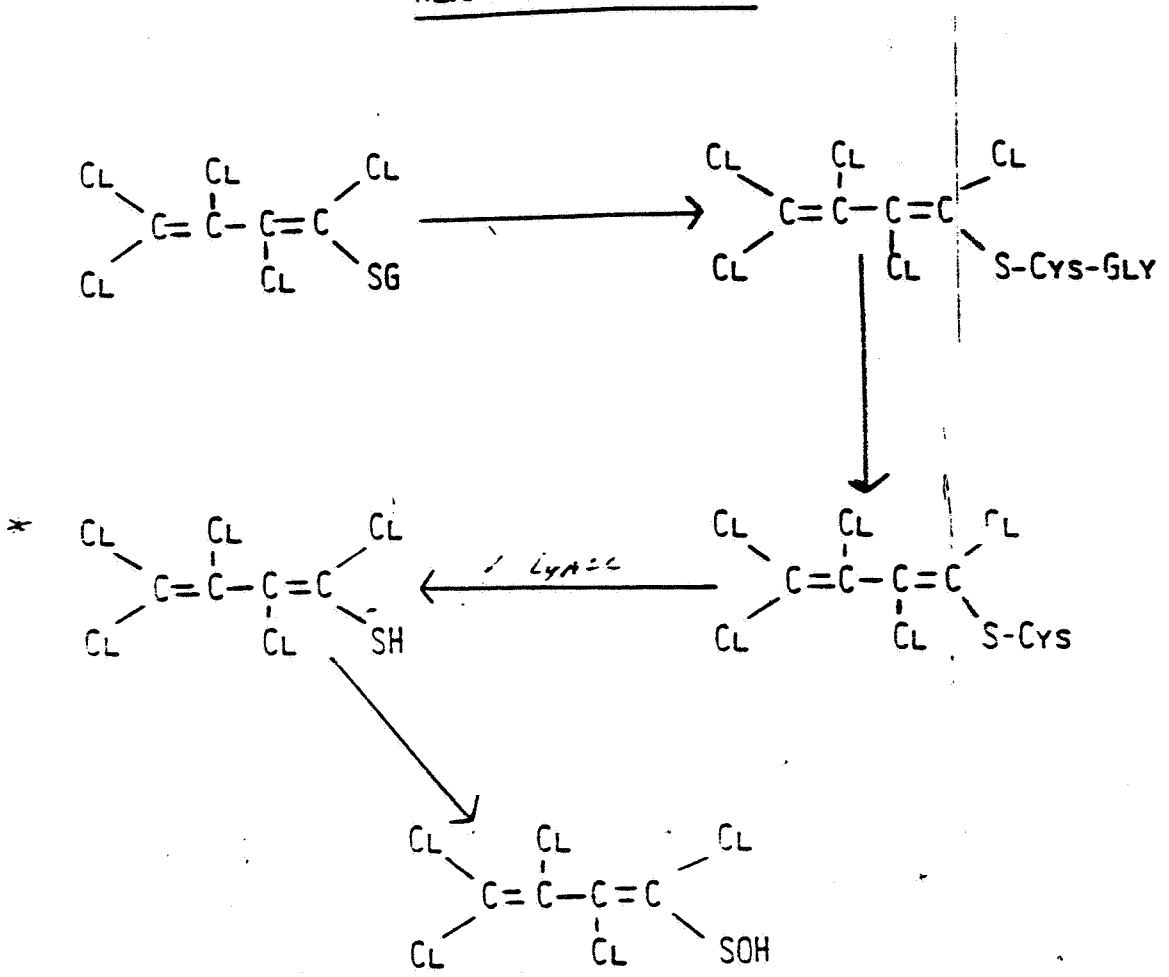

RADIOLABEL IN BILE
1.5 MG/KG
5.0 MG/KG
PERCENT DOSE IN BILE
4
3
2
1
10
20
30
40
50
HOURS POST-DOSE

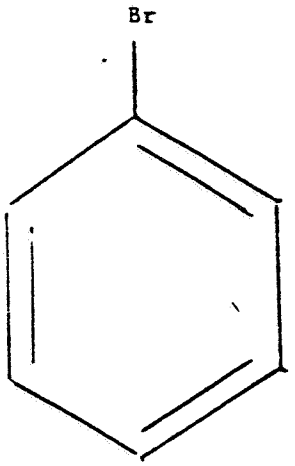

RADICLABEL IN BILE
50 MG/KG
200 MG/KG
PERCENT DOSE IN BILE
0.4
0.3
0.2
0.1
10
20
30
40
50
HOURS POST-DOSE

PROPOSED PATHWAY

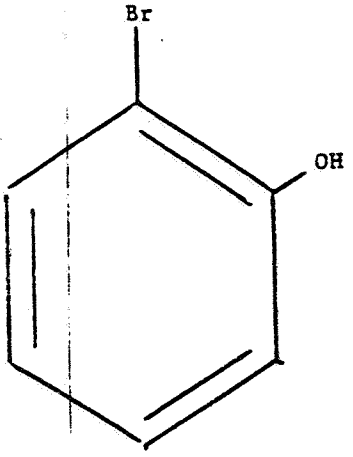


HEXACHLOROBUTADIENE

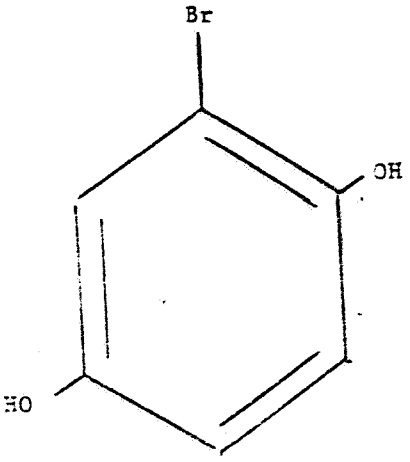




Bromobenzene



O-Bromophenol



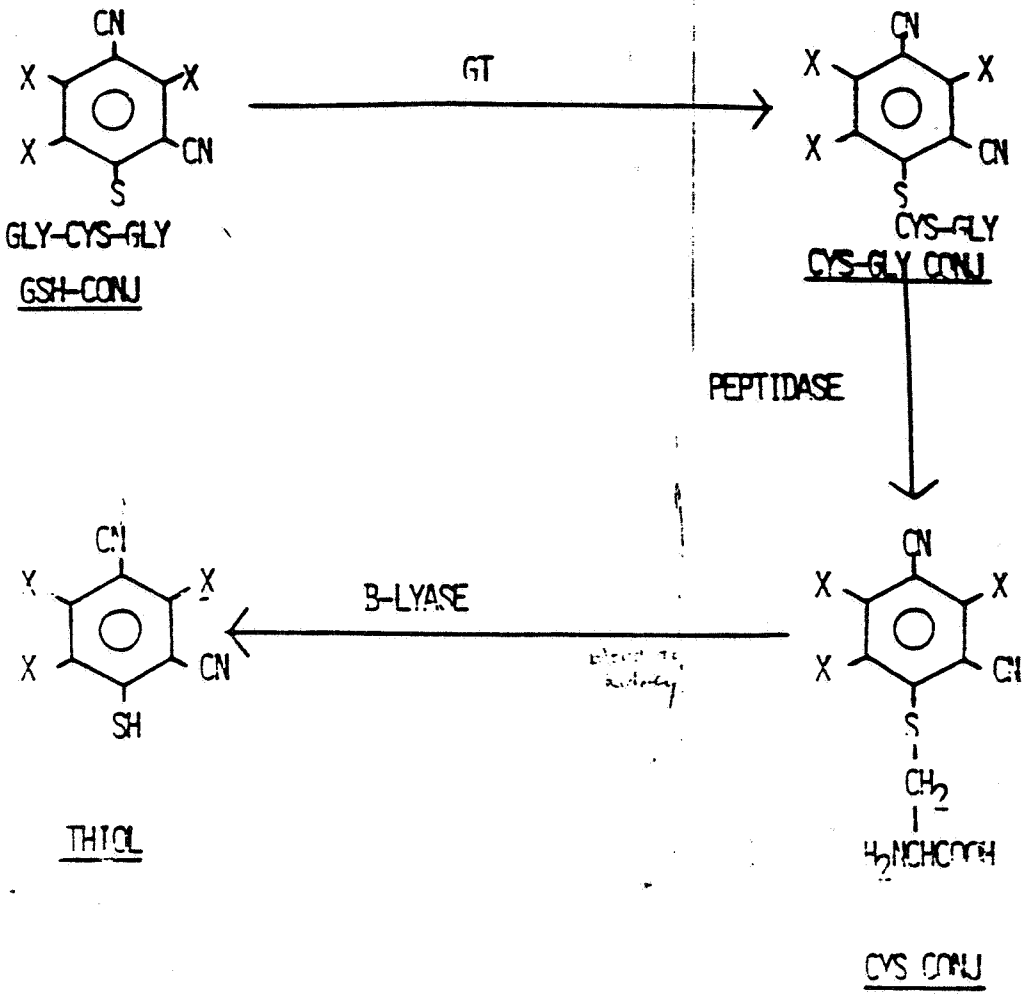
2-Bromohydroquinone

S.S. Lau, et.al., NCI/NIH

EFFECT ON GSH LEVELS

	% OF CONTROL		
HEPATIC	9 HRS.	18 HRS.	48 HRS.
HCBD	60	100	140
SDS-2787	80	60	100
RENAL			
HCBD	120	121	250
SDS-2787	120	145	200

PROPOSED PATHWAY



ADDITIONAL STUDIES

1. DNA/PROTEIN BINDING
2. KIDNEY FRACTIONATION
3. METABOLITE IDENTIFICATION
4. GSH CONJUGATE
5. ENZYME INHIBITORS
6. FEMALE BILE
7. FEMALE MULTIPLE DOSE
8. FURTHER STUDIES

SUMMARY OF METABOLISM - CHLOROTHALONIL

1. ORAL ABSORPTION OF AQUEOUS SUSPENSION IS LOW. TOTAL EXCRETION (C14) IN URINE AND BILE IS PROBABLY <20%.

2. DIFFERENCE IN PHARMACO-DYNAMICS BETWEEN DOSES OF ≤50 AND 200 MG/KG/DAY

3. AT DOSES ≤50 MG/KG/DAY MAJORITY IS EXCRETED IN 24 HOURS. AT 200 MG/KG EXCRETION AND BLOOD LEVELS ARE PROLONGED.

4. IN MOUSE AND PROBABLY RAT, AT HIGH DOSE (I.E. 200 MG/KG) EFFECT ON GI TRACT.

SUMMARY OF METABOLISM - CHLOROTHALONIL

1. ORAL ABSORPTION OF AQUEOUS SUSPENSION IS LOW. TOTAL EXCRETION (C14) IN URINE AND BILE IS PROBABLY <20%.

2. DIFFERENCE IN PHARMACO-DYNAMICS BETWEEN DOSES OF ≤50 AND 200 MG/KG/DAY

3. AT DOSES ≤50 MG/KG/DAY MAJORITY IS EXCRETED IN 24 HOURS. AT 200 MG/KG EXCRETION AND BLOOD LEVELS ARE PROLONGED.

4. IN MOUSE AND PROBABLY RAT, AT HIGH DOSE (I.E. 200 MG/KG) EFFECT ON GI TRACT.

90 DAY TOXICITY STUDY WITH DS 2787 IN RATS

DOSE (MG/KG/DAY)	NO. OF ANIMALS INITIATED ON STUDY	
	MALES	FEMALES
0	20	20
40	20	20
80	20	20
175	20	20
375	20	20
750	20	20
1500	20	20

(JANUARY 1981 THRU APRIL 1981)

90-DAY TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

RESULTS

1. BODY WEIGHT DEPRESSION AT DOSES ≥375 MG/KG/DAY

2. CLINICAL PATHOLOGY EFFECTS AT 30 AND 90 DAY EVALUATIONS, EG. SGPT DEPRESSIONS AT ALL DOSE LEVELS, DECREASED URINE VOLUMES AT DOSE LEVELS ≥375 MG/KG/DAY

3. PHYSICAL OBSERVATION EFFECTS AT DOSES ≥750 MG/KG/DAY, EG. SOFT STOOL

4. ORGAN WEIGHT EFFECTS, EG. KIDNEY WEIGHT INCREASES AT ALL DOSE LEVELS

5. HISTOPATHOLOGY EFFECT, I.E. ACUTE GASTRITIS

6. NO TREATMENT-RELATED RENAL HISTOPATHOLOGY

90-DAY TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES OF HYPERPLASIA AND AND INCREASED SIZE OF THE PROXIMAL TUBULE IN THE KIDNEY

	DOSE LEVEL (mg/kg/day)						
LESION/SEX	0	40	80	175	375	750	1500
TUBULAR HYPERPLASIA/							
MALES:	0/20	16/20	15/20	18/20	16/20	20/20	19/20
FEMALES:	0/20	1/20	1/20	12/20	6/20	14/20	18/20
INCREASED TUBULE SIZE/							
MALES:	0/20	6/20	14/20	17/20	15/20	18/20	19/20
FEMALES:	0/20	0/20	0/20	3/20	0/20	2/20	12/20

SUBCHRONIC TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

STUDY DESIGN

Dose (mg/kg/day)	No. of Animals per Sex			
	Initiation	6 Week Necropsy	13 Week Necropsy	Recovery Period
0	25	5	10	10
1.5	25	5	10	10
3.0	25	5	10	10
10.0	25	5	10	10
40.0	25	5	10	10

- Treatment initiated - March 1982
- End of treatment - June 1982
- End of recovery - September 1982
- Scheduled report date - June 1983

SUBCHRONIC TOXICITY STUDY WITH

CHLOROTHALONIL IN RATS

INCIDENCE OF HYPERPLASIA AND HYPERKERATOSIS OF THE NON-GLANDULAR STOMACH

SEX/NECROPSY INTERVAL	DOSE LEVEL (MG/KG/DAY) 0	1.5	3.0	10	40
MALES					
6 WEEK	0/5	0/5	1/5	2/5	5/5
13 WEEK	1/10	0/10	1/10	6/10	10/10
26 WEEK	1/7	0/10	2/10	0/10	3/10
FEMALES					
6 WEEK	0/5	0/5	0/5	3/5	5/5
13 WEEK	0/10	0/10	1/10	6/10	10/10
26 WEEK	3/10	2/9	1/9	0/9	2/10

SUBCHRONIC TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

INCIDENCES OF EPITHELIAL HYPERPLASIA AND INCREASED SIZE OF THE PROXIMAL CONVOLUTED TUBULES IN MALES

	DOSE LEVEL (mg/kg/day)				
HISTOPATHOLOGIC CHANGE/NECROPSY INTERVAL	0	1.5	3.0	10	40
TUBULAR HYPERPLASIA/					
6 WEEK	0/5	1/5	0/5	2/5	4/5
13 WEEK	0/12	0/10	0/10	0/10	10/10
26 WEEK	0/8	0/10	0/10	0/10	9/10
INCREASED TUBULE SIZE/					
6 WEEK	0/5	0/5	0/5	1/5	1/5
13 WEEK	0/12	0/10	0/10	0/10	1/10
26 WEEK	0/8	0/10	0/10	0/10	1/10

SUBCHRONIC TOXICITY STUDY WITH T-117-11 IN RATS

BLOOD CHEMISTRY AND URINALYSIS PARAMETERS

BLOOD CHEMISTRY

GLUCOSE
ALKALINE PHOSPHATASE
GLUTAMIC-PYRUVIC TRANSAMINASE
GLUTAMIC-OXALOACETIC TRANSAMINASE
TOTAL LACTIC DEHYDROGENASE
UREA NITROGEN
TOTAL PROTEINS
ALBUMIN
GLOBULIN
A/G RATIO
SODIUM
POTASSIUM
CHLORIDE
CALCIUM
INORGANIC PHOSPHORUS
CREATININE
CHOLESTEROL
TOTAL BILIRUBIN

URINALYSIS

COLOR
APPEARANCE
VOLUME
PH
SPECIFIC GRAVITY
PROTEIN
REDUCING SUBSTANCE
GLUCOSE
KETONES
BILIUBIN
UROBILINOGEN
OCCULT BLOOD
NITRITES
URINE CREATININE
MICROSCOPIC EXAM
URINE CONCENTRATING AND DILUTING ABILITY

SUBCHRONIC TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

RESULTS

1. DECREASED SGPT ACTIVITY AT ≥3.0 MG/KG/DAY
   - REVERSIBLE BY 13 WEEKS WITHDRAWAL

2. HYPERPLASIA AND HYPERKERATOSIS OF SQUAMOUS MUCOSA OF FORESTOMACH AT ≥10 MG/KG/DAY
   - REVERSIBLE BY 13 WEEKS WITHDRAWAL

3. INCREASED KIDNEY WEIGHTS AT ≥3.0 MG/KG/DAY
   - REVERSIBLE BY 13 WEEKS WITHDRAWAL

SUBCHRONIC TOXICITY STUDY WITH CHLOROTHALONIL IN RATS

RESULTS CONT'D

4. KIDNEY MICROSCOPY

- H & E: NO COMPOUND-RELATED EFFECTS

- EM: INCREASED NUMBER OF ELECTRON-DENSE INCLUSION BODIES AT ALL DOSE LEVELS IN MALES ONLY /SOME REVERSIBILITY BY 13 WEEKS WITHDRAWAL

- SPECIAL STAIN (NEUTRAL RED): INTRACELLULAR INCLUSION BODIES APPEAR TO CORRELATE WITH EM

90-DAY MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

STUDY DESIGN

DIETARY CONCENTRATION PPM	COMPOUND CONSUMPTION MG/KG MALE/FEMALE	NUMBER OF ANIMALS PER SEX		
		INITIATION	6-WEEK NECROPSY	90-DAY NECROPSY
0	-/-	15	5	A. S.
7.5	1.2/1.4	15	5	A. S.
15	2.5/3.0	15	5	A. S.
50	8.5/9.8	15	5	A. S.
275	47.7/51.4	15	5	A. S.
750	123.6/141.2	15	5	A. S.

A. S. -- ALL SURVIVORS

90-DAY MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

RESULTS OF HISTOPATHOLOGIC EVALUATION

1. NO TREATMENT-RELATED RENAL EFFECTS WERE OBSERVED FOLLOWING EXAMINATIONS USING H & E, PAS, TRICHROME, MALLORY-HEIDENHAIN AND NEUTRAL RED STAINS

2. TREATMENT-RELATED HYPERPLASIA AND HYPERKERATOSIS OF THE SQUAMOUS MUCOSA OF THE FORESTOMACH WERE OBSERVED AT DOSAGES ≥50 PPM (8.5 AND 9.8 MG/KG/DAY FOR MALES AND FEMALES, RESPECTIVELY)

90-DAY MOUSE STUDY WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF HYPERPLASIA AND HYPERKERATOSIS OF THE SQUAMOUS MUCOSA IN THE FORESTOMACH

	DOSAGE (PPM)					
	0	7.5	15	50	275	750
6 WEEKS:						
MALE	0/5	0/5	0/5	0/5	5/5	5/5
FEMALE	0/5	0/6[a]	0/6[a]	0/6[a]	6/6[a]	5/5
13 WEEKS:						
MALE	0/10	0/10	0/10	3/10	10/10	10/10
FEMALE	0/10	0/9	0/9	4/9	8/9	10/10

[a] INCLUDES ONE MOUSE WHICH DIED OR WAS SACRIFICED PRIOR TO 6 WEEKS

dose-related increase in severity as well as incidence.

90-DAY MOUSE STUDY
WITH TECHNICAL CHLOROTHALONIL

INCIDENCE OF TUBULAR HYPERPLASIA IN THE KIDNEY

NECROPSY INTERVAL/SEX	DIETARY CONCENTRATION (ppm) 0.0	7.5	15	50	275	750
SIX WEEK/						
MALES:	0/5	0/5	0/5	0/5	1/5	2/5
FEMALES:	0/5	0/6	0/6	0/6	0/6	0/5
THIRTEEN WEEK/						
MALES:	0/10	0/10	0/10	0/10	0/10	2/10
FEMALES:	0/10	0/9	0/9	0/9	0/9	1/10

Veltch 2-20-86

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Distribution of Radioactivity Following Repeated Oral Administration of ^{14}C-DS-2787 to Male Sprague Dawley Rats. Interim Report # I.

LABORATORY: Huntingdon Research Centre, Huntingdon, England.

STUDY NUMBER & DATE: 631-4AM-84-0079-001; M.C. Savides, 7/15/85.

ACCESSION NUMBER: # 258776.

MATERIAL TESTED: ^{14}C-labeled DS-2787 85.9 mCi/mmole; 124.7 mCi/mmole or 62.4 mCi/mmole; 99.7 % purity.

ANIMALS: Young Male Sprague-Dawley male rats.

METHODS:

NOTE: This is a single-versus multiple dose study utilizing data reported in this Petition (See attached Bibliography) and certain data not yet officially submitted. Acordingly, We will only reiterate the Sponsor's summary here. Full and independent review awaits submission of all data pertaining to this analysis.

"Comparisons have been made of the data obtained from male and female rats after a single administration of ^{14}C-chlorothalonil at dose levels of 5, 50, or 200 mg/kg with data obtained from male rats administered ^{14}C-chlorothalonil at dose levels of 1.5, 5, 50 or 160 mg/kg/day for five consecutive days.

"Data from blood indicated that there were probable shifts in the times to peak blood concentrations with increasing single and multiple doses of chlorothalonil for both sexes. Significant depletion (≥ 50%) of radiolabel from blood occured by 24 hours post-dose for both sexes at dose levels less than or equal to 50 mg/kg. At 160 mg/kg/day, an apparent plateau in the concentration of radioactivity in the blood was reached after a single dose, which suggested that saturation of blood occurred between 50 and 160 mg/kg.

"The concentrations of radiolabel in kidneys after single dose administration showed no apparent sex-related differences, but the times to peak kidney concentrations did appear to increase with increased dose level for both sexes. With multiple doses, maximum kidney concentrations were found 2 hours after the fifth dose at all levels. The shift in time to peak concentrations, especially at 160 mg/kg/day, suggested that a plateau may have been reached by the final 160 mg/kg/day dose.

"After cessation of multiple dosing, the decreases in radiolabel in kidneys with time suggested that there was a trend toward slower depletion (or greater retention) in the kidneys with increased dose levels.

" Urinary data suggested a decreased rate of excretion for both sexes as single dose levels increased and a possible trend toward decreased urinary excretion (as a percent of the dose) as single or multiple dose levels increased. A possible change in urinary excretion may have occured between doses of 5 and 50 mg/kg/day.

"It is suggested from the data that the apparent saturation of blood, the apparent plateau of radiolabel in kidneys, the trend toward slower depletion (or greater retention) of radiolabel from kidney and, possibly, the slight trend toward decreased urinary excretion are caused by increased and/or repeated doses of chlorothalonil. The effects on some of these parameters appear to occur at a dose between 50 and 160 mg/kg (blood saturation and kidney plateau) whereas the others appear to occur at a dose between 5 and 50 mg/kg. The data indicate that the effects are accompanied by an inability of the rat to respond to high doses of chlorothalonil in a manner similar as it would respond to low doses."

CORE RATING

Supplemental

REFERENCES

1. Study of the Distribution of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats. Document Number: 631-4AM-83-0011-002.

2. Study of the Distribution of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Female Sprague-Dawley Rats. Document Number: 631-4AM-84-0078-002.

3. Study of the Distribution of Radioactivity Following Repeated Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats. (Report in Preparation).

4. Levels of Radioactivity in Blood Following Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Rats. Document Number: 621-4AM-83-0013-002.

5. Identification of Metabolites in Urine and Blood Following Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Rats: The Thiol Metabolites in Urine. (Interim Report).

6. Pilot Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Sprague-Dawley Rats. Document Number: 633-4AM-83-0062-002.

7. Study of the Dermal Absorption of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) by Male Rats. Document Number: 649-4AM-84-0010-001.

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Oral Distribution Metabolism in the Male Rat.

LABORATORY: Huntington Research Centre, Cambridgeshire, England.

STUDY NUMBER & DATE: 631-4AM-83-0011-002, Marciniszyn, J.P., 7/2/84.

ACCESSION NUMBER: # 258775

MATERIAL TESTED: Mixture of ^{14}-C-ring labeled and cold DS-2787, 99.7 % pure, specific activity = 85.9 mCi/mmole in 0.75 % methylcellulose.

ANIMALS: 4 male Sprague-Dawley rats per dose level, 5, 50 or 200 mg/kg administered initially by intubation; termination at 2, 9, 24, 96 and 168 hours post-dosing.

METHODS:

Dosing:

Animals were intubated with test material at the stated dose levels. Urine and feces were collected from each animal at 24 hour intervals except those terminated at 2 and 9 hours. Blood was collected at termination. Urine, feces and blood samples were assayed for radioactivity. The following organs and tissues were removed and assayed for radioactivity:

Liver	Kidneys	Muscle
Fat	Muscle	Heart
Lungs	Stomach	Sm. Intestine
Lge. Intestines	Stomach contents	Intestinal Contents

RESULTS:

Animals receiving 200 mg/kg test material had loose stools which contaminated the urine samples to an undetermined degree.

Excretion of Radioactivity:

GI Tract

The major route of excretion was in the feces (ca. 83%). Three quarters of this occured during the first 48 hours at the low and mid dose level; the high dose animals excreted about 60 % during this interval.

43 % of the low dose was found in the small gut at 2 hours, with 15 % in the stomach. By 9 hours the stomach had emptied and 57 % of the administered dose was found the small gut.

At 2 hours the mid-dose group retained 30 % of the administered dose in the stomach; this had not changed significantly at 9 hours. At 24 hours the stomach had emptied and half the AD was found in the large gut.

56 % of the high dose remained in the stomach at 2 hours and 52 % remained at 9 hours. 13 % remained in the stomach at 24 hours.

Urine

Only 5 -7 % of AD appeared in the urine. Fecal contamination and reduced sample size resulted in equivocal results and cannot be further interpreted.

Blood

5 mg/kg groups showed their highest level at 2 hours (0.3 ug-eq/ml). This level persisted through the 9 hour period, then dropped to one fourth by 24 hours.

50 mg/kg groups showed their highest concentration at 9 hours (4.9 ug-eq/ml); these were essentially depleted to one fourth this level at 24 hours.

200 mg/kg groups showed peak blood levels at 9 hours (13.4 ug-eq/ml) with only half that at 24 hours.

Kidney and Liver

Low dose groups showed 0.55 % of AD in the kidneys and 0.72 % of AD in the livers. Renal levels expressed as ug-eq/gm were 3 to 30 times greater than those for the livers. Kidneys retained their activity longer than any other tissue. Renal and hepatic levels were not shown to be proportional to dose at any time.

Other Tissues

The investigators consider that tissue levels of radioactivity were not significant at any time; those for the stomach and large and small intestine were dependant on the activity of the their contents.

CONCLUSIONS:

The major route of excretion in this study is via the GI tract; of this, most is eliminated during the first 9 - 24 hours. Urinary excretion occurs at a low but continuous rate, indicating saturation of the renal excretory mechanism(s). Blood levels are low following dosing; these are dose-dependant with the highest levels attained at up to 9 hours, decreasing rapidly thereafter. Renal retention lasted longer than liver; tissues did not store activity.

DATA EVALUATION REPORT

CHLOROTHALONIL

TUDY: Oral Distribution/Metabolism in the Female Rat.

ABORATORY: Huntington Research Centre, Cambridgeshire, England.

TUDY NUMBER & DATE: 631-4AM-84-0078-002, Marciniszyn, J.P., 7/10/85.

CCESSION NUMBER: # 258775

ATERIAL TESTED: Mixture of ^{14}C-ring labeled and cold DS-2787, 99.7 % pure, specific activity = 124.7 mCi/mmole in 0.75 % methylcellulose.

NIMALS: 4 female Sprague-Dawley rats per dose level, 5, 50 or 200 mg/kg administered initially by intubation; termination at 2, 9, 24, 96 and 168 hours post-dosing.

METHODS:

Dosing:

Animals were intubated with test material at the stated dose levels. Urine and feces were collected from each animal at 24 hour intervals except those terminated at 2 and 9 hours. Blood was collected at termination. Urine, feces and blood samples were assayed for radioactivity. The following organs and tissues were removed and assayed for radioactivity:

Liver	Kidneys	Muscle
Fat	Muscle	Heart
Lungs	Stomach	Sm. Intestine
Lge. Intestines	Stomach contents	Intestinal Contents

RESULTS:

Physical effects were limited to a finding of loose and watery stools in rats receiving 200 mg/kg during the first 24 hours, causing some contamination of urine.

Excretion of Radioactivity:

GI Tract

The major route of excretion was in the feces for all doses. At 5 mg/kg 79 % was eliminated during the first 48 hours and constituted 96 % of the total administered dose. 85 % of the 50 mg/kg dose was excreted during the first 72 hours, constituting 97 % of the administered dose. Animals receiving 200 mg/kg excreted 85 % in the feces, accounting for 93 % of the administered dose.

The stomach was essentially empty of radioactivity at 24 hours.

Urine

At 5 mg/kg about 11 % of the administered dose was excreted in the urine over the 7 day course of the study, with 92 % of this being lost during the first 24 hours. Those animals receiving 50 mg/kg excreted abou 9 % of the administered dose with 80 percent of this gone by the end of the first 24 hours. Animals dosed with 200 mg/kg excreted 5.4 % of th AD; of this, 57 % was excreted in 24 hours, 85 % in 48 hours and 95 % in 72 hours. The increase in rate of excretion was not entirely dose-dependant at this level, suggesting that the urinary excretion mechanism was saturated.

Blood

5 mg/kg animals showed peak blood concentrations at 2 hours and 9 hours (630 and 616 ng-equivalents respectively); 50 mg/kg animals showed highest blood concentration at 9 hours (8190 ng-equivalents). Animals receiving 200 mg/kg showed peak blood concentrations at 9 and 24 hours (11,400 and 15,400 ng-equivalents). The authors consider that these data support a conclusion that the peak blood concentrations, seen at different times, could have been due to delayed stomach emptying.

Kidney

At the 5 mg/kg dose level the kidneys had the highest percentage of AD (0.71 %) with the bulk of this appearing at 2 hours (0.41 % AD/gm). At 50 mg/kg renal concentration was greatest at 9 hours (0.17 AD/gm). At 200 mg/kg the peak renal concentration occured at 24 hours (0.07 % AD/gm). The authors consider that the delay in peak renal concentrations is due to delayed emptying time from the stomach as dose increased.

Liver

A similar pattern was seen in the liver. 5 mg/kg animals showed peak liver concentration at 2 hours (1.17 ug/gm), 50 mg/kg rats showed peak hepatic concentration at 9 hours (5.54 ug/gm) and at 200 mg/kg, the peak liver content occured at 24 hours (3.25 ug/gm).

Other Tissues

Radioactivity remaining in these tissues was not considered to be remarkable.

CONCLUSIONS:

As in the male study, the major route of excretion in this study is via the GI tract; of this, most is eliminated during the first 9 - 24 hours. Urinary excretion occurs at a low but continuous rate, indicating saturation of the renal excretory mechanism(s). Blood levels are low following dosing; these are fairly dose-

dependant for the low and middle dose. The highest blood levels were attained at up to 9 to 24 hours for the high dose animals, decreasing rapidly thereafter.

Renal retention was low and lasted longer than liver; tissues did not store activity.

Taken together with the results of the Male study (631-4AM-83-0011-002, Marciniszyn, J.P., 7/2/85), these results support a tentative conclusion that the renal excretory machanism is rate-limiting for elimination of Chlorothalonil absorbed into the blood-stream; that the bulk of activity remains in the gut or is re-excreted via the biliary apparatus into the feces, and that there is reason to believe that stomach evacuation is somewhat delayed at the 200 mg/kg dose level.

CORE Rating:

Guideline

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 8/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264351

Test Material: Mono-Glutathione Conjugate of ^{14}C-Chlorothalonil

Syncnyms: ^{14}C-SDS-66382

Study Number: 631-4AM-85-0064-001
Report/SDS-66382

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Physical Sciences Laboratories,
SDS Biotech Corp., P.O. Box 348
Painesville, OH

Title of Report: Pilot Study to Determine the Concentration of Radiolabel in Kidneys Following Administration of the Mono-Glutathione Conjugate of ^{14}C-Chlorothalonil to Male Rats

Authors: M.C. Savides, J.P. Marciniszyn, J.C. Killeen, Jr., and J.A. Ignatoski

Report Issued: April 23, 1986

Purpose of Study:

The purposes of this pilot study appear to have been threefold: 1) to further characterize the route of metabolism of chlorothalonil, 2) ". . . to determine if radiolabel from a dose of a monoglutathione conjugate of chlorothalonil would be found in the kidney in the same relative amount as previously reported for an equimolar dose of chlorothalonil, and 3) to compare oral and intraperitoneal doses of the monoglutathione conjugate with respect to the presence of thiol conjugates of chlorothalonil in the urine" (p. 2).

Conclusions:

The glutathione pathway plays an important role in the metabolism of chlorothalonil as evidenced by the finding

that similar thiol metabolites result whether chlorothalonil or monoglutathione conjugate is administered to the rat. Radiolabeled chlorothalonil and the conjugate yield approximately equivalent percentages of radiolabel in the urine. The oral route of administration results in much higher urinary levels of thiols of chlorothalonil than does the intraperitoneal route, suggesting a role of the gastrointestinal tract in glutathione metabolism.

These are pilot study findings and are only indicative of the involvement of the one metabolic pathway. Additional study further characterizing the metabolism by the glutathione route will be necessary, as well as investigations into the potential role of other pathways.

Critical Review Criteria:

A. Materials:

1. Test Compound: Monoglutathione conjugate of ^{14}C-chlorothalonil.

   Description: The specific activity of the radiolabeled material was 0.576 mCi/mMole. The test material was stored in the dark at -8 °C.

   Purity: The nonlabeled starting material was chlorothalonil of 99.7% purity. The radiochemical purity of the final test material (i.e., the glutathione conjugate) was 91.3%. There was uniform labeling of the benzene ring.

   Contaminants: Not indicated.

2. Test Animals: Species: Rat, male; Strain: CD Sprague-Dawley; Weight: 287 to 332 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

Testing was performed using a mixture of radiolabeled and nonradiolabeled monoglutathione conjugate of chlorothalonil in a 0.75 percent methylcellulose/water suspension.

"Eight rats were assigned to each of three groups, Group I (oral), Group II (intraperitoneal), and Group III (intraperitoneal pilot). Untreated rats in Group III were used for control tissue.

"Food was removed from the cages of control and experimental rats at approximately 4 P.M. the night prior to dosing. These cages contained a water source but no

food. Just prior to dosing, experimental animals were placed individually in metabolism cages which contained a water source but no source of food. The cages were placed over containers of dry ice to freeze any collected urine. The rats were dosed as close as posible to 8 A.M. Each experimental rat received a single dose of 115 mg SDS-66382/kg in 0.75 percent methylcellulose (115 mg SDS-66382/kg body weight/10 mL suspension). Rats in Group I were dosed orally and those in Group II were dosed intraperitoneally. Control rats (Group III) were not dosed.

"Six hours after administration of SDS-66382, control and experimental rats were sacrificed by exsanguination under ether anesthesia. Kidneys were removed from all animals, and carcasses were stored frozen for future disposal. Prior to termination, blood samples were collected from animals under ether anesthesia by orbital sinus puncture. Blood samples were assayed for radioactivity by combusting aliquots of blood and counting the trapped CO_2 by Liquid Scintillation Counting (LSC).

"Urine samples were collected over dry ice. Cages were rinsed with 50 percent methanol in water to collect any urine which did not flow into the collection cup. The total volume was measured and duplicate 0.1 mL aliquots were assayed for radioactivity by LSC. The urine was stored frozen and subsequently analyzed for sulfhydryl metabolites.

"Kidneys were removed at termination, rinsed twice in a 50 percent methanol/water solution, and then stored in plastic bags. Th kidneys were rinsed a third time in 50 percent methanol/water when they were removed from the bags for analysis. These solutions (15 mL each) were subsequently analyzed for radioactivity by LSC. The capsules of the kidneys were removed, and the kidneys were minced with scissors. Aliquots of the kidney tissue were weighed for subsequent biological oxidation and LSC. The remainder of the kidneys were stored frozen. The capsules were analyzed separately for the presence of radiolabel by biological oxidation and subsequent LSC (pp. 11-13)."

Results:

There were no external adverse effects noted for the dosed animals. Necropsy revealed the presence of some fluid (< 1 to 2 mL) in the peritoneal cavities of animals dosed via this route. Via oral administration, the monoglutathione is

much less toxic than chlorothalonil on an equimolar basis, suggesting that glucuronide conjugation of chlorothalonil is probably detoxifying (p. 19).

With respect to blood concentrations of radiolabel it was found that 6 hours postdosing the average blood concentrations were 13.3 mMole-equiv/mL for orally dosed rats and 132.1 mMole-equiv/mL for those dosed intraperitoneally (i.p.) (table 2, p. 27). It is speculated that the evidently rapid absorption by the i.p. route can be attributed to the abundant blood supply and large surface area of the peritoneal cavity.

As was true in the case of blood, levels of radiolabel in the kidney were considerably higher for those rats dosed i.p. (705 nMole-equiv/gram) than for those dosed orally (49.5 nMole-equiv/gram) (table 3, p. 28). The average percentages of the administered doses found in the kidney were 3.22 percent for the i.p. dosed group and 0.20 percent for the group administered orally.

Animals dosed i.p. excreted via the urine much higher percentages of the administered dose than did the orally dosed group. The percentages were 5.35 $\pm$ 4.25 and 0.64 $\pm$ 0.31 percent, respectively (table 4, p. 29).

In a previous study cited by the authors (p. 19), which involved the administration of essentially equimolar doses of radiolabeled chlorothalonil, the percent of the administered dose located in the kidney 6 hours postdosing was close to the percentage found in the present study. Comparisons between percentages of dose found in kidney, blood, and urine for the two studies are tabulated below as taken from the study report (p. 20).

	Oral Chloro-thalonil	Oral Chlorothalonil, Monoglutathione	Intraperitoneal Chlorothalonil, Monoglutathione
Kidney (% administered dose)	0.26	0.20	3.22
Blood (% administered dose)	0.24*	0.40	3.96

*This is an estimate, as plasma was assayed rather than whole blood.

food. Just prior to dosing, experimental animals were placed individually in metabolism cages which contained a water source but no source of food. The cages were placed over containers of dry ice to freeze any collected urine. The rats were dosed as close as posible to 8 A.M. Each experimental rat received a single dose of 115 mg SDS-66382/kg in 0.75 percent methylcellulose (115 mg SDS-66382/kg body weight/10 mL suspension). Rats in Group I were dosed orally and those in Group II were dosed intraperitoneally. Control rats (Group III) were not dosed.

"Six hours after administration of SDS-66382, control and experimental rats were sacrificed by exsanguination under ether anesthesia. Kidneys were removed from all animals, and carcasses were stored frozen for future disposal. Prior to termination, blood samples were collected from animals under ether anesthesia by orbital sinus puncture. Blood samples were assayed for radioactivity by combusting aliquots of blood and counting the trapped CO_2 by Liquid Scintillation Counting (LSC).

"Urine samples were collected over dry ice. Cages were rinsed with 50 percent methanol in water to collect any urine which did not flow into the collection cup. The total volume was measured and duplicate 0.1 mL aliquots were assayed for radioactivity by LSC. The urine was stored frozen and subsequently analyzed for sulfhydryl metabolites.

"Kidneys were removed at termination, rinsed twice in a 50 percent methanol/water solution, and then stored in plastic bags. Th kidneys were rinsed a third time in 50 percent methanol/water when they were removed from the bags for analysis. These solutions (15 mL each) were subsequently analyzed for radioactivity by LSC. The capsules of the kidneys were removed, and the kidneys were minced with scissors. Aliquots of the kidney tissue were weighed for subsequent biological oxidation and LSC. The remainder of the kidneys were stored frozen. The capsules were analyzed separately for the presence of radiolabel by biological oxidation and subsequent LSC (pp. 11-13)."

Results:

There were no external adverse effects noted for the dosed animals. Necropsy revealed the presence of some fluid (< 1 to 2 mL) in the peritoneal cavities of animals dosed via this route. Via oral administration, the monoglutathione is

much less toxic than chlorothalonil on an equimolar basis, suggesting that glucuronide conjugation of chlorothalonil is probably detoxifying (p. 19).

With respect to blood concentrations of radiolabel it was found that 6 hours postdosing the average blood concentrations were 13.3 mMole-equiv/mL for orally dosed rats and 132.1 mMole-equiv/mL for those dosed intraperitoneally (i.p.) (table 2, p. 27). It is speculated that the evidently rapid absorption by the i.p. route can be attributed to the abundant blood supply and large surface area of the peritoneal cavity.

As was true in the case of blood, levels of radiolabel in the kidney were considerably higher for those rats dosed i.p. (705 nMole-equiv/gram) than for those dosed orally (49.5 nMole-equiv/gram) (table 3, p. 28). The average percentages of the administered doses found in the kidney were 3.22 percent for the i.p. dosed group and 0.20 percent for the group administered orally.

Animals dosed i.p. excreted via the urine much higher percentages of the administered dose than did the orally dosed group. The percentages were 5.35 $\pm$ 4.25 and 0.64 $\pm$ 0.31 percent, respectively (table 4, p. 29).

In a previous study cited by the authors (p. 19), which involved the administration of essentially equimolar doses of radiolabeled chlorothalonil, the percent of the administered dose located in the kidney 6 hours postdosing was close to the percentage found in the present study. Comparisons between percentages of dose found in kidney, blood, and urine for the two studies are tabulated below as taken from the study report (p. 20).

	Oral Chloro-thalonil	Oral Chlorothalonil, Monoglutathione	Intraperitoneal Chlorothalonil, Monoglutathione
Kidney (% administered dose)	0.26	0.20	3.22
Blood (% administered dose)	0.24*	0.40	3.96

*This is an estimate, as plasma was assayed rather than whole blood.

	Oral Chloro-thalonil	Oral Chlorothalonil, Monoglutathione	Intraperitoneal Chlorothalonil, Monoglutathione
Urine (% administered dose)	1.19	0.64	5.35
Thiols (% in urine)	8.3	14.1	< 1

Thus, when radiolabeled chlorothalonil or its monoglutathione derivative are administered orally in separate studies at equimolar doses, the percentages of radiolabel located in kidney are very similar, suggesting similar routes of metabolism.

As to the characterization of urinary metabolites resulting from oral dosing with the glutathione derivative, the di- and trithiols of glutathione were identified among other unidentified substances (metabolites). The dithiol derivative accounted for 9 percent of the extractables and the trithiol approximately 5 percent (p. 18). Urine from animals dosed i.p. contained the dithiol (1 percent of extractables) and no trithiol.

Based upon the above limited information, the authors develop the concept that an essential route of metabolism for orally administered chlorothalonil includes glucuronide formation (mono-, di-, and triglucuronides) in the gastrointestinal tract, followed by cleavage to smaller fragments which are absorbed into the portal circulation. The fragments in question are theorized to be cleaved in the kidney to the thiol metabolites (nephrotoxins, p. 23). In support of this, the authors cite a recent in vitro study in which it was shown that mucosal cells from the stomach and small intestine will affect these conjugation reactions. Such reactions in the gastrointestinal tract prior to absorption into the portal circulation would help explain the greater abundance of thiols in the urine following oral dosing as opposed to intraperitoneal dosing.

Evidence in support of this proposed sequence of metabolic events for chlorothalonil include the finding of similar metabolites in urine following dosing with either chlorothalonil or the monoglutathione metabolite. The authors conclude that the glutathione pathway is intimately involved in the metabolism of chlorothalonil. This appears to be a reasonable, but limited, conclusion. Additional study would be necessary to adequately characterize the various aspects of the glutathione and possibly other metabolic pathways.

Core Classification: Minimum.

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264351

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 633-4AM-85-0012-002
Report/SDS-2787

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Huntingdon Research Centre,
Huntingdon, Cambridgeshire, England

Title of Report: Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats

Authors: M.C. Savides, J.P. Marciniszyn, J.C. Killeen, Jr., and J.A. Ignatoski

Report Issued: May 13, 1986

Conclusions:

At low doses (1.5 mg/kg) of orally administered radiolabeled chlorothalonil, cannulation of the bile duct had little or no effect on blood levels of radiolabel for at least 24 hours postdosing. However, at higher doses (50 and 200 mg/kg) blood levels of radiolabel were substantially higher in noncannulated animals, perhaps due to reabsorption in the intact animal.

In the dose range 1.5 to 50 mg/kg, biliary excretion, in terms of the percentage of dose administered 48 hours postdosing, was essentially constant. However, at the higher dose, 200 mg/kg, the comparable biliary excretion figure was significantly reduced, suggesting saturation of the overall biliary excretion pathway.

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head 4/27/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264351

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 633-4AM-85-0012-002
Report/SDS-2787

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Huntingdon Research Centre,
Huntingdon, Cambridgeshire, England

Title of Report: Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C-SDS-2787) to Male Sprague-Dawley Rats

Authors: M.C. Savides, J.P. Marciniszyn, J.C. Killeen, Jr., and J.A. Ignatoski

Report Issued: May 13, 1986

Conclusions:

At low doses (1.5 mg/kg) of orally administered radiolabeled chlorothalonil, cannulation of the bile duct had little or no effect on blood levels of radiolabel for at least 24 hours postdosing. However, at higher doses (50 and 200 mg/kg) blood levels of radiolabel were substantially higher in noncannulated animals, perhaps due to reabsorption in the intact animal.

In the dose range 1.5 to 50 mg/kg, biliary excretion, in terms of the percentage of dose administered 48 hours postdosing, was essentially constant. However, at the higher dose, 200 mg/kg, the comparable biliary excretion figure was significantly reduced, suggesting saturation of the overall biliary excretion pathway.

Data on urinary excretion of radiolabel indicate that saturation via this route of elimination lies somewhere in the dosage range 5 to 50 mg/kg.

With respect to the fecal excretion there appeared to be no relationship between fecal elimination and biliary excretion.

Kidney concentrations did not appear to be affected by bile duct cannulation suggesting a lack of meaningful biliary reabsorption from the GI tract did not influence kidney levels.

Special Review Criteria:

A. Materials:

1. Test Compound: ^{14}C-Chlorothalonil.

   Description: Mixture of nonlabeled and ^{14}C-labeled chlorothalonil suspended in 0.75% methylcellulose.

   Batch No.: N/A.

   Purity: The nonlabeled chlorothalonil was analytical grade; 99.7% purity. The ^{14}C-chlorothalonil had a specific activity of 78.8 mCi/mMole and radiochemical purity of 97.7 to 98.4 percent.

   Contaminants: N/A.

2. Test Animals: Species: Rate, male; Strain: CD Sprague-Dawley; Weight: 258 to 281 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

"Twenty-four male Sprague-Dawley CD rats, 258 to 281 grams in body weight at time of dosing, were obtained from Charles River Breeding Labs and were allocated at random to dosing groups. The bile ducts of all rats were cannulated immediately prior to dose administration. Six rats were dosed at each of four dose levels: 1.5, 5, 50, or 200 mg/kg. The ^{14}C-chlorothalonil was orally administered as a microparticulate suspension in 0.75 percent methylcellulose.

"Bile was collected continuously in 60 minute fractions from dose administration until 48 hours post-dosing. Blood samples were collected at various times after dosing and at termination. The choice of sampling times was based upon A) the times to peak blood concentration found in a pharmacokinetic study (1) which for

5 mg/kg was 6.1 ± 1.1 hours, for 50 mg/kg was 8.9 ± 0.7 hours and for 200 mg/kg 15.9 ± 5.8 hours; B) the sampling times used in a previously conducted bile study (2) at 5 mg/kg which were 6, 24, and 48 hours and C) based upon the data from the pharmacokinetic study, which showed that the time to peak blood concentrations increased with increasing dose level, three of the sampling times at 1.5 mg/kg were chosen to be less than 6 hours after dosing.

"For animals dosed at the 1.5 mg/kg dose level, blood samples were collected from three of the rats at 2, 5, 8, and 48 hours postdose and from the other three rats at 4, 6, 24, and 48 hours postdose. At 5 and 50 mg/kg, blood samples were collected from three rats at each dose level at 2, 6, 10, and 48 hours postdose and from the other three rats at each dose level at 4, 8, 24, and 48 hours. At 200 mg/kg, blood samples were collected from three of the rats at 6, 10, 16, and 48 hours postdose and from the other three rats at 8, 12, 24, and 48 hours.

"Urine and fecal samples were collected 6, 24, and 48 hours after dosing. At termination, the kidneys were removed for separate analyses and the gastrointestinal tract was separated from each carcass. Levels of radioactivity were determined in each bile, blood, urine, fecal and kidney sample and in the tissues and contents of the gastrointestinal tract, remaining carcass and cage washes (pp. 2-4)."

Results:

1. Concentrations of Radiolabel in Blood

At the 1.5 mg/kg dose, the mean blood concentration of radiolabel for the first 2 to 6 hours was essenticonstant at 74.3 ng equiv/mL, and declined steadily beyond the 6-hour time point. As compared with a similar study, cited by the petitioner, involving noncannulated animals, blood levels of radiolabel were essentially the same for periods up to 6 hours, suggesting that cannulation had no effect upon radiolabeled uptake into blood at this low dose for a 6-hour period. After 24 hours, the blood level for cannulated animals (28 ng equiv/mL) was still essentially the same as for uncannulated rats (19 ng equiv/mL). This parallel in blood levels between cannulated and uncannulated animals at the 1.5 mg/kg dose level did not prevail at higher doses, where noncannulated animals exhibited higher blood level, as one might expect from the point of view of the

likelihood of reabsorption of radiolabeled consequent to enterohepatic circulation. Thus at 4 hours postadministration of 5 mg/kg, the time point of maximum blood concentration, the mean blood level was 264 ng equiv/mL as compared with 489 ng equiv/mL in noncannulated rats, i.e., in cannulated rats, blood levels were approximately 54 percent that in noncannulated animals.

Following the 50 mg/kg dose, the maximum blood concentration was reached in 6 hours where the mean concentration was 3180 ng equiv/mL. In non-cannulated rats, the blood concentrations at 6 hours were 2 to 2.5 times that of cannulated rats.

At the highest dose level, 200 mg/kg, there were two peaks in blood concentration, but such levels in the noncannulated rats of the present study were reported as being at least twice as high as in cannulated rats.

2. Biliary Extraction

Percentages of the administered doses excreted in bile within 48 hours of administration were reported as follows:

Dose, mg/kg	Mean Dose Percent Excreted in Bile, 48 Hours	Mean Time to Peak Bile Conc., Hours
1.5	22.5	2
5	16.4 (19.07)*	3
50	16.2	7
200	7.3	25-26

*Results obtained when additional experimental results included in the average.

At all doses administered, radioactivity was measurable in bile within 1 hour. The biliary excretion of 22.5 percent of the administered dose as observed at the 1.5 mg/kg dose level was significantly different from the 16.4 percent figure following the 5 mg/kg dose. However, the study directors invoke an argument that the difference between these doses is actually not significant when data from another experiment at 5 mg/kg is averaged with data of this experiment, yielding 19.07 percent, as indicated above. The petitioner claims that for the first three doses there are no significant differences in the 48-hour excretion percentages. The percentage figure at the high dose

is significantly different from the other three. While there was not a significant difference between the percentage excretion figures at the 5 and 50 mg/kg doses, the profiles of excretion were different. Prolonged excretion of radiolabel was observed at 50 mg/kg. Also, at 50 mg/kg there was a multipeak excretion profile. Prolonged excretion was also observed, to an enhanced degree, at the 200 mg/kg dose. While the number of ug equivalents excreted in 48 hours at the 200 mg/kg dose was approximately twice the number excreted at 50 mg/kg (tables 30 and 42), the quantities excreted within 17 hours was the same for both doses, namely, 1389 ug equivalents (p. 9). The most logical explanations for this finding would be that there was a saturation of the major metabolic pathway (or of active secretion) for chlorothalonil, or saturation of intestinal absorption, a phenomenon which occurred at a dose somewhere between 5 and 50 mg/kg. Since animals with vastly different amounts of radiolabel in gastrointestinal contents showed essentially no difference in biliary excretion, as reported by the study director (p. 9), it is reasonable to conclude that saturation of biliary secretion accounts for the finding of equal amounts of radiolabel in bile at 17 hours postadministration of either the 50 or 200 mg/kg doses.

3. Urinary Excretion

The percent of the administered dose appearing in urine (combined urine and cage washings) 48 hours postadministration was esentially the same for the 1.5, 5, and 50 mg/kg doses, and averaged 3.08 percent for the three doses combined. For the 200 mg/kg dose, the percent of administered dose appearing in urine was substantially less, 4.73 percent (table 2, p. 33). The study director notes that excretion at the 1.5 and 5 mg/kg doses was rapid. Essentially 94 and 88 percent of the quantities excreted were eliminated within 24 hours. However, for the 50 mg/kg dose, excretion was only 67 percent complete by 24 hours. Excretion as a percentage of dose was still lower at the 200 mg/kg dose. These observations lead to the logical conclusion that, as with biliary excretion, absorption or excretion mechanisms were saturated at the high dose and that saturation probably occurs at a dose somewhere between 5 and 50 mg/kg.

4. Fecal Excretion

Again, as percentages of the administered dose, radiolabel contained in feces 48 hours postadministration was essentially constant for the 1.5, 5, and 50 mg/kg doses, when for the three dose groups combined the mean value for radiolabel excreted in feces was 61 percent. On the average, an additional 3 percent of the administered dose was found in the GI tract, thus 64 percent of the total dose was accounted for in the feces and GI tract at all but the highest dose level. However, at the high dose 32.5 percent was in feces and 26.1 percent in the GI tract, accounting for a combined 58.6 percent of the administered dose. Just why so much more was located in the GI tract at the higher dose is unclear, but may be attributable to, or a reflection of, the high variability among animals at this dose. It was concluded by the study director that no direct relationship existed between fecal elimination, or content of radiation in the GI tract, and biliary excretion (p. 13). This appears to be a reasonable conclusion.

5. Kidney Concentrations

In cannulated rats kidney concentrations of radiolabel 48 hours postadministration of chlorothalonil did not increase in direct proportion to dose. There were progressive deficits with respect to a linear increase with increasing dose, i.e., the response was nonlinear.

When kidney concentration data from cannulated rats obtained at three doses (5, 50, and 200 mg/kg) 48 hours postadministration are included with comparable data from noncannulated rats at the 24-, 96-, and 168-hour time points on a semilog plot of kidney concentration vs. time plot, a continuous linear plot covering 24 to 168 hours is obtained, i.e., data from cannulated rats appears to be superimposable with data from noncannulated rats. This leads to the reasonable conclusion that kidney concentrations were not affected by bile duct cannulation. Hence, enterohepatic circulation or biliary readsorption from the GI tract did not play a significant role in kidney levels (p. 14, Figure 4, p. 38).

6. Recovery of Radioactivity

At the three lower doses, percent recoveries of radiolabel were essentially the same, averaging 92.4 percent. Recovery was significantly less at

the highest dose, namely 74.1 percent. The lower recovery figure for the high dose group may be attributable to the relatively high levels in feces and GI contents and difficulties inherent in measuring such levels in feces and contents.

7. Kinetic Model for Chlorothalonil

A kinetic model is presented which is identical to the model described in the accompanying metabolism study, 1173-84-0079-AM-003, and has been commented upon in the review of the latter study.

Core Rating: Minimum.

Reviewed by: Brian Dementi, Ph.D. Brian Dementi, 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head 3/23/87
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264350

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 1173-84-0079-AM-003

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: Huntingdon Research Centre,
Huntingdon, Cambridgeshire, England
October 19, 1984 to May 1985

Title of Report: Study of the Distribution of Radioactivity Following Repeated Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Sprague-Dawley Rats

Authors: M.C. Savides, J.P. Marciniszyn, J.C. Killeen, Jr., and J.A. Ignatoski

Report Issued: July 3, 1986

Special Review Criteria

A. Materials:

1. Test Compound: A mixture of nonlabeled chlorothalonil and ^{14}C-labeled chlorothalonil suspended in 0.75% methylcellulose.

   Description and Purity: The nonlabeled material was analytical grade chlorothalonil of 99.7% purity. ^{14}C-Chlorothalonil was of specific activity 62.4 mCi/mMole having radiochemical purity of 98.4%.

   Batch No.: N/A.

   Contaminants: N/A.

2. Test Animals: Species: Rat, male; Strain: CD Sprague-Dawley; Age: 9 to 10 weeks; Weight: Approximately 300 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

The purpose of the study is to assess the absorption, tissue distribution, and excretion of radioactivity during 7 days following repeated oral administration of radiolabeled chlorothalonil.

1. Animal Assignment: "Eighty-eight male Sprague-Dawley rats were obtained from Charles River Breeding Labs. Eighty of the rats were allocated at random to four dose groups of 20 animals each and eight rats were allocated to supplemental groups to determine blood concentrations during the dosing regimen. The four groups of 20 animals each were dosed at levels of 1.5, 5, 50, or 160 mg/kg/day for 5 days at 24 hour intervals. Animals were dosed orally using a plastic syringe fitted with an animal feeding needle. The exact weight of the < 2.0 mL volume administered was determined by weighing the syringe before and after compound administration. Four rats at each dose level were terminated 2, 9, 24, 96, and 168 hours after the fifth dose administration. Urine and feces were collected at 24-hour intervals during the dosing regimen, after the fifth dose and at necropsy.

"All eighty animals were killed by exsanguination under halothane/oxygen anesthesia. Liver, kidneys, fat, muscle, heart, lungs, stomach, small and large intestines, stomach contents, small intestinal contents and large intestinal contents were removed from each animal. Blood, tissues and gastrointestinal tract contents were assayed for radioactivity.

"The supplemental group of eight animals was divided into four subgroups of two rats, each representing one dose level. The two rats in each subgroup were dosed on 5 consecutive days at 1.5, 5, 50, or 160 mg/kg/day. Blood samples were collected at 6 and 24 hours after the first, third, and fifth dose administrations and the samples were assayed for radioactivity. The eight rats were sacrificed 24 hours after the final dose in the same manner discussed above but no tissues were collected (pp. 2-3)."

2. Diet Preparation: Animals were fed ad libitum using Spratt's Laboratory Diet No. 1. Animals were also allowed fresh water ad libitum. The test compound was not administered via diet or drinking water, hence no specific diet preparation or water formula was necesary.

3. Statistics: The following procedures were utilized in analyzing the numerical data: N/A.

4. Quality Assurance was affirmed in a statement by J.A. Ignatoski, Ph.D., Director, Department of Safety Assessment, July 3, 1986.

C. Results:

(The following information is paraphrased and follows the outline of results as presented in the study on pages indicated.)

1. Observations: Animals treated at the high dose, 160 mg/kg/day, had loose stools primarily during the 24 hours immediately following the first of five doses in the series. While fecal consistency was essentially normal beyond the initial 24-hour period, many fecal pellets contained a white, mucosal material throughout the study (p. 3).

2. Radioactivity in Feces and Gastrointestinal Tract: Based upon data presented in Appendix A, it is evident that the principal route of elimination of radiolabel is via the feces, where the radiolabel presence accounted for 82 to 85 percent of the total dose administered at the various dose levels. Elimination via this route was rapid and essentially 90 percent complete within 24 hours following the fifth dose administered in all of the various dosing regimens.

   Evacuation time (or rate) of radiolabel from the stomach was dose dependent when evacuation was essentially complete within 9 hours following the final (5th) dose for the 50 mg/kg/day regimen, but only within 24 hours following the 160 mg/kg/day regimen (p. 4).

3. Radioactivity in Urine: The percent of the total radiolabel excreted via urine within 7 days after the fifth dose was dose dependent. For the 1.5 and 5 mg/kg/day doses 6.65 and 6.55 percent, respectively, were eliminated, whereas for the

50 and 160 mg/kg/day doses 4.36 and 4.96 percent were eliminated under like circumstances. The average amount of radiolabel excreted in urine during 24 hours following each dose was a constant for each dose level. The data suggest a different mechanism of elimination via the urine for the low dose as opposed to the high dose groups (pp. 4-6).

4. Radioactivity in Blood: Data for all dose levels reveal that less than 1 percent of any dose level administered was present in the blood at any moment of sampling. Peak blood concentrations at the various doses occurred at the indicated time points after the final dose.

Dose, mg/kg/day	Peak Blood Concentration, Hours	Blood Level, ng equiv/mL
1.5	2	185
5	2	519
50	9	4300
160	2, 9	12,950

At all dose levels, blood concentrations at the 6-hour time point after the first dose were equivalent to those concentrations evident 6 hours after the final dose, suggesting to this reviewer that a steady state or equilibrium is established quickly and is well maintained (pp. 6-7).

5. Radioactivity in Kidneys: When assayed for radiolabel at various time points during 2 to 168 hours after the fifth dose, the highest concentrations in kidneys occurred at the 2-hour time point, regardless of dose level. These peak concentrations were 3.12, 8.03, 31.1, and 105 ug equiv/g at the respective doses of 1.5, 5, 50, and 160 mg/kg/day.

Kidney concentrations were proportional to dose for the two lower doses (~ 0.1 percent of administered dose/g) and also for the two higher doses (~ 0.05 percent of administered dose/g), but proportionalities did not hold between the second and third dose groups.

Plots of kidney depletion rates obtained for 1.5, 5, and 160 mg/kg/day dose groups indicate depletion is biphasic, with the phase change occurring at 24 hours after the final dose. For the 50 mg/kg/day dose, elimination was not biphasic (pp. 7-9).

Dose, mg/kg/day	Rate Constant, Hour^{-1}		
	2-24 hrs	24-168 hrs	2-168 hrs
1.5	.0369	.0078	
5			.0091
150	.0298	.0078	
160	.0300	.0047	

We are unable to offer a reliable explanation for the single rate constant observed at the 5 mg/kg/day dose.

6. Radioactivity in Other Tissues: Concentrations of radiolabel in fat, heart, liver, lungs, and muscle were unremarkable. GI tissues, apart from contents containing radiolabel, also appeared unremarkable. Kidney tissue had the highest radiolabel concentration and was five- to sevenfold that of the liver, the tissue incorporating the next highest concentration of radiolabel. Radiolabel depletion rate for liver was threefold that of the kidneys (p. 9).

D. Chlorothalonil Kinetics Model:

The petitioner has developed a theoretical model for chlorothalonil kinetics. This model is based upon the assumption that ". . . chlorothalonil absorption and excretion may be described by a one-component model, where chlorothalonil and/or its metabolites are absorbed into the bloodstream and eliminated from the blood compartment by distribution to tissues, by excretion into bile and by excretion into urine" (p. 10). The mathematical expression of this model as derived would be:

$$V_A = V_T + V_B + V_U$$

where

V_A = rate of absorption into blood;
V_T = rate of absorption into tissues from blood;
V_B = rate of elimination via bile; and
V_U = rate of elimination via urine.

This is a simple model not taking into consideration a number of factors which could influence rates of distribution. Nevertheless, when values for V_T, V_B, and V_U, arrived at by deductive application of more fundamental information pertaining to these three parameters as obtained in ancillary studies, are introduced into the

model equation, values for V_A can be calculated or estimated at various doses of chlorothalonil. For example, employing this technique, the petitioner obtains V_A = 143.63 ug/equiv/hr at a dose of 50 mg/kg and 258.41 ug/equiv/hr at the 200 mg/kg dose. The ratio of these, 258.41/143.63 = 1.8, indicates that the rate of absorption nearly doubles when the dose increases fourfold, within the indicated dose range.

In attempting to establish the validity of the model equation, the petitioner also employs actual biliary and urinary excretion data obtained in the adjunct biliary study (633-4AM-85-0012-002) to determine actual amounts and ratios of chlorothalonil absorbed within 24- and 48-hour time periods at doses of 50 and 200 mg/kg. Thus within 24 hours post-administration of 50 mg/kg and 200 mg/kg, 2780.8 ug and 5389.8 ug equivalent were absorbed, the ratio being 1.94.

The same ratio calculated in like manner at 48 hours was 2.12 (pp. 10-19). Both ratios (1.94 and 2.12) compare favorably with the 1.8 ratio derived from more fundamental principles. Hence, the petitioner considers these findings to support the one-component model as proposed to describe the kinetics of chlorothalonil absorption and excretion. To a first approximation, this appears to be reasonable.

Conclusions:

1. The relative rate of absorption of chlorothalonil following a 200 mg/kg dose is only approximately twice that following administration of a 50 mg/kg dose.

2. Less than 1 percent of any single dose of chlorothalonil administered is present in the blood at any moment. Peak blood concentrations following single doses were approximately dose related. An apparent saturation of blood occurs during multiple dosing between the dosing rates of 5 and 50 mg/kg/day and is indicative of a steady state.

3. Radiolabel depletion from kidney was biphasic at doses of 1.5, 5, and 160 mg/kg/day, but not so at 50 mg/kg/day. There is no satisfactory explanation for this difference.

4. The data suggest a different mechanism of elimination via the urine for the two low dose groups as opposed to the two high dose groups.

5. The principal route of elimination of radiolabel is fecal, accounting for 82 to 85 percent of administered dose. Elimination via this route was rapid and essentially 90 percent complete within 24 hours following the final dose. These findings were independent of dose level under study and indicate most chlorothalonil is excreted unchanged in the feces. Furthermore, this is consistent with the low percentage of the administered dose present in the blood at any moment post-administration.

6. A theoretical model for chlorothalonil kinetics ($V_A = V_T + V_B + V_U$) was proposed where the rate of absorption into the blood (V_A) is equivalent to the sum of the rates of transfer into tissues (V_T) and elimination via bile (V_B) and urine (V_U). To a first approximation the model appears reasonable.

Core Rating: Minimum.

Reviewed by: Brian Dementi, Ph.D. Brian Dementi 3/3/87
Section I, Toxicology Branch (TS-769C)
Secondary Reviewer: R. Bruce Jaeger, Section Head
Section I, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Metabolism, Rat Tox. Chem. No.: 081901

Accession No.: 264350

Test Material: ^{14}C-Chlorothalonil

Synonyms: ^{14}C-SDS-2787

Study Number: 621-4AM-83-0061-002
Report/SDS-2787

Sponsor: SDS Biotech Corporation
Painesville, OH

Testing Facility: SDS Biotech Corp., Department of Safety Assessment, Painesville, OH

Title of Report: Identification of Metabolites in Urine and Blood Following Oral Administration of ^{14}C-Chlorothalonil (^{14}C-SDS-2787) to Male Rats: II. Effects of Multiple Dose Administration on the Excretion of Thiol Metabolites in Urine

Authors: M.C. Savides, J.P. Marciniszyn, J.C. Killeen, Jr., and J.A. Ignatoski

Report Issued: May 23, 1986

Purpose of Study:

To identify chlorothalonil metabolites in urine and to assess the effects of multiple dosing on excretion of thiol metabolites in urine.

Conclusions:

1. The pH of urine excreted by male rats was observed to increase in response to repeated administration of chlorothalonil at all doses employed. During 4 days of dosing, urine pH rose approximately 1 pH unit at doses of 50 mg/kg and less, and increased 1.7 pH units at the high dose of 160 mg/kg.

2a. Dithiodichloroisophthalonitrile and trithiochloroisophthalonitrile were positively identified as methyl derivatives in the extractable urine fraction. On day 1 of dosing, these metabolites constituted 20.9, 24.9, and 32.2 percent of the total radiolabel in urine at the 5, 50, and 160 mg/kg dose levels, respectively. These percentages decreased with repeated administration of a given dose. Hence, > 70 to 80 percent radiolabeled material in urine was not well characterized. Involvement of the glutathione pathway is considered likely.

2b. Cysteinyltrichloroisophthalonitrile and cysteinyltrichlorocyanobenzoic acid are speculated to be among those metabolites counted in the nonextractable urine fraction. Qualitative evidence for this is inadequate.

Special Review Criteria:

A. Materials:

1. Test Compound: ^{14}C-Chlorothalonil.

Description: Radiochemical purity, 97%; uniformly labeled in benzene ring.

Batch No.: N/A.

Purity: Analytical grade chlorothalonil of 99.7% purity.

Contaminants: N/A.

2. Test Animals: Species: Rat; Strain: CD Sprague-Dawley; Age: 11 to 12 weeks; Weight: 300 to 350 grams; Source: Charles River Breeding Laboratories, Portage, MI.

B. Study Design:

"Eighty male rats were assigned at random to four dosing groups. Twenty rats were dosed at each of four dose levels: 1.5, 5, 50, or 160 mg/kg on 5 consecutive days. Four rats from each dose level were sacrificed at 2, 9, 24, 96, and 168 hours after the final (5th) dose administration. Urine samples were collected from each dose group at 24-hour intervals after each dose administration.

"To obtain sufficient material for subsequent analyses, urine samples collected from several animals

on a given collection day at a given dose level were pooled. Urine which was found to be contaminated with feces was excluded from the pools. Pooled samples were appropriately labeled as to time of collection, dose level, and animal numbers.

"The volume of the pooled urine samples was measured and aliquots were taken for measurement of the amount of radiolabel by liquid scintillation counting (LSC) according to SOP #04-T106-00. The total amount of radioactivity in the filtrate was determined by multiplying the amount of radioactivity in the aliquots (as DPM per mL) by the total volume of the filtrate. The amounts of radiolabel in urine and in the filtrate were converted to microgram equivalents per sample using the total DPM/sample and the specific activity of the appropriate dosing suspension (DPM/ug).

"B. EXTRACTION

Preliminary experiments had shown that radiolabel was not effectively extracted from urine at either neutral (pH 7) or alkaline (pH 10) pH. Extraction under acidic (pH 2) conditions was found to be effective; therefore the pH of each urine filtrate was adjusted to pH 2 with 1 N HCl prior to extraction. The acidified urine filtrate was extracted three times with four volumes of ethyl acetate per volume of filtrate. (The ethyl acetate used had been saturated with 1 N HCl.) The quantity of radiolabel extracted into the ethyl acetate phase was determined by LSC and the extractability was calculated as a percent of the total radioactivity extracted into the organic phase. The three ethyl acetate extracts were combined and the number of microgram equivalents in the extracts was calculated using the amount of radiolabel (DPM) present in each extract and the specific activity of the appropriate dosing suspension (DPM/ug).

"The acidic, aqueous phase, which remained after extraction with pH 2 ethyl acetate, was designated as the nonextractable phase of urine. The volume of the nonextractable phase was measured and the amount of radiolabel was determined by LSC.

"C. SEP PAK® TREATMEMT

The combined extract was rotovapped to remove the ethyl acetate. The residue was dissolved in a known, small volume of methanol, introduced into a Water's® C-18 Sep Pak® cartridge and eluted from

the cartridge with methanol. The amount of radiolabel eluted with methnol was quantified by LSC and converted to microgram equivalents of chlorothalonil. Approximately 97 to 99% of the radiolabel eluting from the Sep Pak® was recovered in the methanol eluate.

"D. DERIVATIZATION

The methanol eluate was concentrated to near-dryness under a gentle stream of nitrogen gas. Prior to methylation, aliquots from selected samples were analyzed by gas chromatography/mass spectroscopy (GC/MS). The major portion of the concentrated eluate was derivatized using diazomethane for GC/MS analyses of methylated metabolites. In some cases, N-propyltolyltriazine was used for derivatization to distinguish between groups which had been excreted as methylated derivatives and those which had not been methylated in vivo.

"A portion of the nonextractable phase of urine from animals dosed at 160 mg/kg/day was derivatized using diazomethane after hydrolysis with 12 N HCl overnight (approximately 17 hours) at 100 °C. The derivatized sample was analyzed by GC/MS (pp. 3-5)."

Results:

Urinary pH was increased in response to dosing with chlorothalonil. Increases of approximately 1 pH unit were seen following replicate doses of 50 mg/kg and less, and 1.7 pH units following repeated dosing at the 160 mg/kg dose (table 1, p. 17).

The extractability (ethyl acetate) of radiolabel following the initial dose was greatest (84%) for low dose urine samples and decreased with increasing doses to 71 percent extraction at the high dose. Furthermore, at each dose level extractability decreased progressively with replicate dosing. For example, at the 1.5 mg/kg dose, extractability declined from 84 percent to 74 percent from the first to the fifth dosing. Likewise, at the 160 mg/kg dose, extractability declined from 71 percent to 49 percent (table 2, p. 18).

GC/MS analyses were successfully conducted on samples obtained on days 1 through 4 at the 50 and 160 mg/kg dose levels, and on the day 1 sample obtained from the 5 mg/kg regimen. Practical limitations of the procedure precluded such determinations for the 1.5 mg/kg dose group.

Dithiodichloroisophthalonitrile (dithiol) and trithiochloroisophthalonitrile (trithiol) (as methyl derivatives in both cases) were identified. It should be mentioned that since methylation of the extractables using diazomethane was performed prior to GC/MS analysis, the investigators attempted to determine which methylated or unmethylated derivatives, as the case may be, were actually excreted as metabolites. Accordingly, portions of extractable fractions were reacted with N-propyltolyltriazine as a means of introducing propyl rather than methyl substituents at unmethylated thiol sites on the various metabolites. GC/MS results enabled the conclusion to be drawn that the dithiol metabolite is actually present in the extractable fraction of urine as a mixture of the monomethyl and dimethyl derivatives and that its trithiol exists in urine as a mixture of the monomethyl, dimethyl, and trimethyl derivatives of the parent molecule. For chemical names and structures of chemical entities in question see pages 10 and 21 of the petitioner's report.

The total of the two metabolites (dithiol and trithiol) quantitated in the extractable fraction of urine on day 1 represented 20.9, 24.9, and 32.2 percent of the total radiolabel in urine at the 5, 50, and 160 mg/kg dose levels, respectively. The remaining 70 to 80 percent radiolabeled material in urine was not well characterized, however, with respect to the nonextractable urine components evidence permits speculation that likely radiolabeled metabolites include cysteinyltrichloroisophthalonitrile and cysteinyltrichlorocyanobenzoic (p. 10).

As to the extractable components, table 3 (p. 19) shows that on day 1 absolute amounts of dithiol and trithiol increased with increasing dose of chlorothalonil, however as a percentage of extractable radioactive materials, day 1 samples were constant for trithiol (i.e., independent of dose) at about 16 percent, but a dose-dependent increase for dithiol was evident, i.e., 5.2 percent (5 mg/kg), 9.0 percent (50 mg/kg), and 15.4 percent (160 mg/kg). Following replicate dosing at 50 and 160 mg/kg, there were marked declines in both absolute amounts of the two thiols and their percentages of extractable radiolabeled material, with dithiol exhibiting the more dramatic decline (table 3, p. 19).

The ratio, trithiol/dithiol, as determined at the various time points is presented in the following reproduction of table 4 (p. 20) from the submitted study.

THE RATIO OF TRI- AND DITHIOLS IN URINE

Dose mg/kg/day	Day	*Ratio ug Tri/ug Di
5	1	3.00
50	1	1.77
	2	2.84
	3	3.87
	4	3.91
160	1	1.09
	2	2.31
	3	3.56
	4	52

* ug = microgram equivalents.

As revealed in this table, at day 1 the ratio was inversely related to dose. When chlorothalonil was repeatedly administered to male rats at 50 and 160 mg/kg/day, the ratio increased with each dose administered (excepting day 4 of the 50 mg/kg dose group, which appears to have plateaued at day 3). After 4 days of administration, ratios at 50 and 160 mg/kg were 4 and 52, respectively.

Discussion:

Since there was a decreasing percentage of extractable radiolabeled material with increasing dose and with replicate dosing at all dose levels Table 2 (p.18) it is reasonable to conclude that shifts in distribution favoring polar metabolites occurs. This suggests, as the study authors note, that changes occur in the metabolism of chlorothalonil as dose level increases and upon multiple dose administration.

Data presented in Table 3 (p.19) reveal marked declines in urine levels of di- and trithiols with increasing daily dosing of chlorothalonil at both the 50 and 160 mg/kg/day dose levels. A more dramatic effect was observed at the higher dose, where between days 1 and 4 of replicate dosing the urinary dithiol level declined from 1542 to 9 ug. Since the decline in dithiol was much more precipitous than that of the trithiol, the ratio, trithiol/dithiol, rose to 52 as shown in the above table. A proportionate increase in urine trithiol to that of the decrease in dithiol was not seen, suggesting the induction or enhancement of a metabolic pathway

for the dithiol which would compete with that of the proposed dithiol ----> trithiol pathway, thus markedly enhancing the urinary trithiol/dithiol ratio.

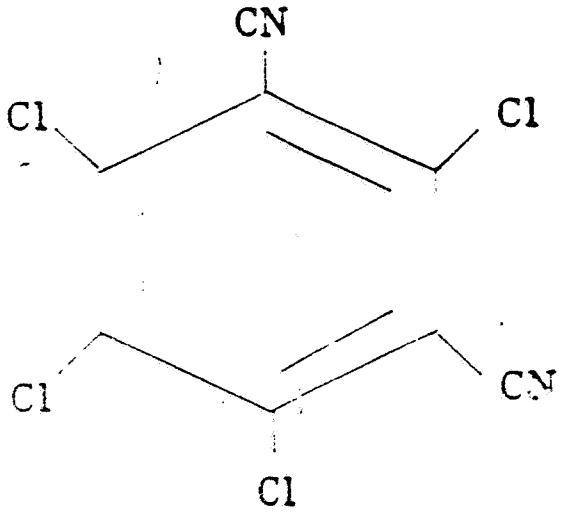
Insufficient data are available to specifically explain why the amount of dithiol excreted in the urine decreased more rapidly than the trithiol with repeated dosing at 50 and 160 mg/kg. The petitioner speculates that diversions of the dithiol or a preceeding metabolite from the glutathione pathway to a pathway producing highly polar metabolites could explain the phenomenon. It is reported that extractable fractions are undergoing further analysis to identify additional metabolites.

The dithiol and trithiol metabolites are speculated to have been derived via glutathione conjugation at two/three sites of the chlorothalonil molecule, respectively. This is supported, according to the petitioner, by another study as cited which demonstrated glutathione conjugation with chlorothalonil, in vitro and in vivo. Dithiol and trithiol metabolites accordingly would arise via enzymatic cleavage of glutathione conjugates followed by varying degrees of methylation.

Conclusions: See page 2.

Core Rating: Minimum.

ANIMAL METABOLISM OF CHLOROTHALONIL



^{14}C -UNIFORMLY DISTRIBUTED IN BENZENE RING

GENERAL METABOLISM

PARTIAL ABSORPTION

RAPID ABSORPTION

TISSUE DISTRIBUTION – KIDNEYS

DECREASED ELIMINATION RATE WITH DOSE

METABOLIC PATHWAY

ABSORPTION

ESTIMATED FROM ^{14}C IN:

BLOOD

BILE

URINE

CARCASSES

PHARMACOKINETIC DATA

– ABSORPTION VS. DOSE

PARAMETER	5mg/kg	50mg/kg	200mg/kg
DOSE RATIO	1	10	40
PEAK CONCN RATIO	1	8.5	10
AUC RATIO	1	9.9	16
PEAK TIME (hrs)	6	9	16

– NON–LINEAR KINETICS

– SATURATION

PARTICLE-SIZE DEPENDENCE

DOSE = 5mg/kg

	<5 um	>5 um
REL. BLOOD CONCN.	1.8	1
REL. BILE EXCRETION	3-4	1

SEX RELATIONSHIP

SEX	% ABSORBED AT 5 mg/kg	REL. BLOOD CONCENTRATIONS NON-PEAK 5,50,200 mg/kg	PEAK 5,50	PEAK 200 mg/kg
M	30-35%	1	1	1
F	30-35%	2	2	1

BLOOD CONCENTRATIONS (ng eq/ml)

DOSE mg/kg/d	NO. OF DOSES	HOURS POST-DOSE	CONCENTRATION (ng eq/ml)
160	1	24	11,100 ± 2400
160	5	2	14,300 ± 1600
160	5	6	10,300 ± 2500
160	5	9	16,100 ± 4600
		$\bar{x}$ ± SD	12,950 ± 2720
200	1	24	13,400 ± 7190

$\bar{x}$ ∓ SD = 13,040 ± 2364

MALE
KIDNEY CONCENTRATIONS
(UG EQ/G)

HOURS POST-DOSE	DOSE - MG/KG/D 5	50	200
	S	S	S
2	3.52 ±0.67	17.7 ±2.4	16.7 ±2.1
9	2.40 ±0.30	18.1 ±3.1	34.1 ±8.2
24	1.81 ±0.27	14.5 ±1.9	44.1 ±7.7
96	0.91 ±0.12	7.18 ±1.67	16.7 ±2.6
168	0.53 ±0.09	2.94 ±0.58	10.1 ±2.3

MALE
KIDNEY CONCENTRATIONS
(UG EQ/G)

HOURS POST-DOSE	DOSE - MG/KG/D 1.5 (5 Rep—ac)	5 R	50 R	160 R
2	3.12 ±0.43	8.03 ±1.22	31.5 ±3.1	105 ±34
9	2.13 ±0.39	5.38 ±0.40	30.0 ±3.4	71.9 ±12.1
24	1.35 ±0.21	4.00 ±0.77	25.6 ±2.6	52.3 ±4.9
96	0.72 ±0.02	1.99 ±0.23	12.2 ±1.5	34.2 ±4.9
168	0.44 ±0.02	1.30 ±0.23	7.11 ±1.10	26.5 ±3.4

DOSE RELATIONSHIP

PARAMETER	DOSE LEVEL (mg/kg/d) 1.5	5	50	160
DOSE RATIO	1	3.3	33.3 (1)	106.7 (3.2)
MAX KIDNEY CONCN (ug eq/g)	3.12	8.03	31.5	105
KIDNEY CONCN RATIO	1	2.6	10 (1)	34 (3.3)

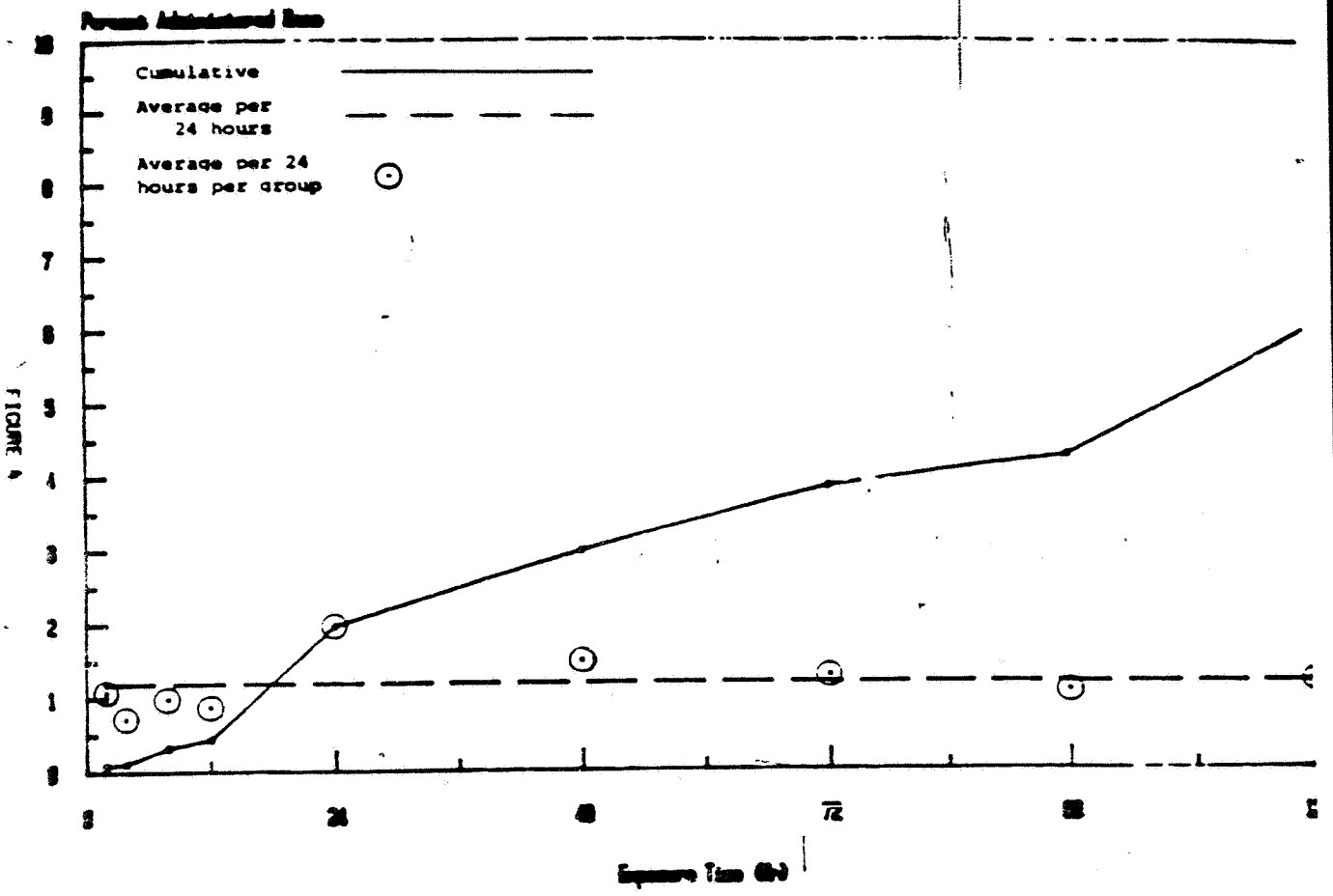
KIDNEY DEPLETION

PARAMETER	DOSE LEVEL (mg/kg/d) 1.5	5	50	160
k_1 (Hr^{-1}) x 10^2	3.69	2.98	0.96	3.00
k_2 (Hr^{-1}) x 10^2	0.78	0.78	0.89	0.47
% PEAK AT 7 DAYS	14	16	23	25

MEAN URINARY EXCRETION 0-7 DAYS POST-DOSE AS A PERCENT OF ADMINISTERED DOSE

	DOSE (mg/kg)		
	5	50	200
MALES	6.7%	5.7%	5.3%
FEMALES	11.5%	8.2%	5.4%
TIME FOR 90% COMPLETION M/F	24 HRS	48 HRS	72 HRS

Cumulative and Average Urinary Excretion



RCB Response:

The data upon which TOX based their conclusions were not field residue data. Rather, the TOX reviewer summarized fortification levels used in method validation for PCBN and chlorothalonil. These levels were obtained from the fortification/recovery tables (Tables 2 and 3) in the Analytical Methods section of the Residue Chemistry chapter.

To provide the TOX Branch with actual comparisons of PCBN and chlorothalonil residues in crops for which data are currently available, RCB has rereviewed the PCBN data summarized in the Residue Chemistry chapter of the FRSTR. Reported PCBN values were adjusted to a theoretical maximum based on batch analysis of the product used in the residue trial. For example, the maximum theoretical PCBN for broccoli was obtained by multiplying the actual reported value by 1.9. This conversion factor was based on analysis of the 4 lb/gal FlC (41.8%) batch used in the residue trial (the batch contained [redacted] PCBN but could legally have contained [redacted] In general, theoretical maximum PCBN residue levels constitute ≤ 4% of the chlorothalonil levels (see table below). However, in the cases of broccoli and bulb onions, the maximum level of PCBN relative to chlorothalonil is significantly higher (14 and 63%, respectively).

Crop	Maximum Theoretical PCBN Level (ppm)[a]	Chlorothalonil SDS 3701[b] (ppm)	% PCBN
Broccoli	0.27	1.38	14.4
Brussels sprouts	0.107	4.48	2.4
Bulb onions	0.038	0.06	63
Cabbage	0.082	5.02	1.6
Carrots	0.04	1.01	4
Cauliflower	<0.005[c]	2.23	[illegible]
Celery	<0.005	3.3	[illegible]
Cranberries	0.125	4.28	[illegible]
Cucumbers	0.13	4.32	[illegible]
Green onions	0.564	25.06	[illegible]
Melons	0.05	2.6	[illegible]
Soybeans	<0.005	0.029	[illegible]
Summer Squash	0.11	4.25	[illegible]
Winter Squash	0.068	2.[illegible]	[illegible]

[a]Maximum actual value adjusted to theoretical maximum based on batch analysis of product used in residue trial.

[b]SDS-3701 = 4-hydroxy-2,5,6-trichloroisophthalonitrile (metabolite currently included in tolerance definition)

[c]Nondetectable (<0.005) - Actual values were ≤ untreated control values.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

RCB defers to the TOX Branch regarding the need for PCBN field residue data for potatoes, succulent beans, tomatoes, dried plums, sweet corn forage, mint, papayas, passion fruit, peanuts, and grass grown for seed (crops for which adequate PCBN data are not currently available).

RDI:D.Edwards:7/89:(557-4353):W.Boodee:7/88:R.Schmitt:7/88
cc:PMSD/ISB:RF:SF:Reg.Standard File:Circu

REVIEWER

3/10/88

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

006672

JUN 16 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Lois Rossi, PM # 21
Fungicides, Herbicides Branch
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. 1 Toxicology Branch
Hazard Evaluation Division (TS-769C

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

FROM: D. Ritter, Toxicologist
Rev. Sec. 1 Toxicology Branch
Hazard Evaluation Division (TS-769C

SUBJECT: EPA # 50534-T - Chlorothalonil, submitting additional toxicity data.

Registrant: Fermenta Plant Protection, Menlo, CA.

Caswell #: 215B

Fermenta is submitting additional toxicity data in support of an Amended Registration. The data include an interim report of a tumorigenicity study in rats. The DER is appended.

"A Tumorigenicity Study of Technical Chlorothalonil in Rats: One Year Interim Report." Study # 1130-84-[illegible]

Chlorothalonil was fed in the diet of male and female [illegible] animals per sex per dose at levels of [illegible] mg kg BW day for one year. Ten animals per group were sacrificed and kidneys and stomachs were subjected to microscopic examination. Animals receiving [illegible] or more demonstrated renal epithelial hyperplasia, [illegible] hyperplasia and hyperkeratosis of the stomach [illegible]

065

renal tubular adenoma. Chlorothalonil also induced squamous epithelial hyperplaisa and hyperkeratosis of the forestomach in male and female rats receiving 15.0 and 175.0 mg/kg bw/day. The NOEL for renal effects is 2.0 mg/kg bw/day; for gastric effects the NOEL is 4.0 mg/kg bw/day.

2. Clarification of the In Vitro Chromosome Aberration Assay in Chinese Hamster Ovary Cells with Technical Chlorothalonil.Study # T4481.337. Toxicology Branch memo of 4/9/87, B. Dementi. Acc. # 405591-03. 1/4/88.

Dr. Chen's review of these data is appended. He found that they supported a classification of the study of "Acceptable".

Reviewer: D. Ritter, Toxicologist_ _Caswell #: 215B
Rev. Sec. # I/Toxicology Branch
Secondary Reviewer: R. Bruce Jaeger, Section Head
Rev. Sec. # I/Toxicology Branch

DATA EVALUATION RECORD

Study: Two Year Feeding Study in Rats: One year interim report.

MRID: 40559102.

Performing Laboratory: International Research & Development Corp.
Mattawan, MI.

Author(s): N. B. Wilson and J. C. Killeen.

Study ID Number: 1102-84-0103-TX-004.

Date of Study: 9/17/87.

Title: A Tumorigenicity Study of Technical Chlorothalonil in Rats.
A One Year Interim Report.

CORE Rating: Minimum Data. This is an interim report.

QA Statement: Satisfactory.

CONCULSIONS: Chlorothalonil induces microscopic alterations, consisting of epithelial hyperplaisa, clear cell hyperplaisa and karyomegaly in the kidneys of male rats receiving dietary Chlorothalonil at 4, 15 and 175 mg/kg bw day, and in female rats receiving 175 mg/kg bw day. Chlorothalonil also induces squamous epithelial hyperplasia and hyperkeratosis in the forestomachs of male and female rats receiving 15 and 175 mg/kg bw/day. The overall NOEL based on hyperplastic changes in the renal cortex is 2.0 mg kg bw/day.

Dark urine was reported in the high dose males and females. No explanation was offered for this finding.

One tubular adenoma was reported in a high dose male rat.

These findings are similar to those reported in earlier chronic studies using Chlorothalonil.

METHODS:

Purpose -

"This study was conducted to determine, if possible, the no-effect level for potentially preneoplastic and tumorigenic

effects in the kidney and forestomach in Fischer 344 rats following dietary adninistration of technical chlorothalonil."

Material Tested -

A standard batch of technical Chlorothalonil was used and was analyzed initially and at six, eight and twelve months. Blind No.: T-117-12.

Animals -

Fischer 344 rats were assigned to five groups of 65 male and 65 females each which were offered diets containing 0, 1.5, 3.0, 15 or 175 mg/kg bw/day of technical Chlorothalonil.

Diets -

The test material was mixed into standard laboratory diet at levels of 2.0 (1.5)*, 4.0 (3.0)*, 15 and 175 mg/kg bw/day. These preparations were assayed regularly throughout the study. The dietary preparations were made available to the animals ad libitum. Fresh diets were prepared every four days. Husbandry - Standard GLP. See Table I on compound ingestion.

Feed and water - Available ad libitum.

In-Life Measurements -

Animals were observed twice daily for mortality, morbundity and signs of toxicity.

Detailed physical examinations were done weekly.

Body weights were recorded from one week prior to initiation of diet, weekly through week 14, then biweekly thereafter.

Feed consumption was recorded similarly.

At 12 months blood samples were obtained from the orbital sinuses of 10 animals of each sex in each group and a hematological evaluation was done.

* Mixed at the higher level to account for possible dietary binding at low levels.

Blood parameters evaluated were:

Leukocytes
Red Cells
Hemoglobin
Hematocrit
MCV, MCH, MCHC
Platelets & Differentials

The same animals were then killed and subjected to a one year post-mortem examination.

Post Mortem Examinations -

All animals sacrificed in extremis were autopsied, as were all those that died during the one year period.

The brain, liver and kidneys were weighed. A full battery of tissues and organs were reserved for future histopathological examination. The kidneys, stomach and renal and mesenteric lymph nodes were prepared and examined histopathologically (W. M. Busey, DVM, PhD., Experimental Pathology Labboratories, Inc., of Herndon, VA).

RESULTS:

Morbidity and Signs of Toxicity -

One male in the 2, 4, and 15 mg/kg bw/day groups died on test. One 2 mg/kg bw/day female and two 15 mg kg bw/day females died on test. These are not considered to be compound-related deaths.

The authors reported that dark yellow urine was noted in the majority of 175 mg/kg bw/day males and females, beginning at week five and persisting through week fifty-two. The females in the 4, 15 and 175 mg/kg bw/day groups exhibited yellow anogenital staining in the latter half of the interim period. No similar effects were reported for the males in these groups or for the 2 mg/kg bw/day animals of either sex.

Body Weights -

Statistically significant deviations from control values were reported in the three lower-dose groups at various times; however, these deviations occured at random and did not occur in a dose-related fashion. Those of the males and female in the highest dose groups were significantly reduced when compared to those of the controls. The differences became larger as the study progressed.

Diets and Compound Consumption -

The amount of extractable Test Material in the lower two doses decreased with time over each four day storage period. Freezing the diets prevented loss of biologically available Test Material.

Actual compound consumption was satisfactory for the study.

Feed consumption relative to body weight in the highest dose males became increasingly greater during the study when compared to that of the controls. The differences amounted to about ten percent. At the end of the interim period the females on the highest dose likewise were consuming 10 % more feed.

Blood Analyses -

No alterations in hematological values were reported at any level tested.

Organ Weights -

Kidney - Absolute kidneys weights were significantly increased in the 175 mg/kg bw day males and females. Kidney weights relative to body weight and to brain weight also were significantly increased in these animals.

Liver - Absolute liver weights were significantly increased in the 175 mg/kg bw/day males and females. Liver weights relative to body weight and to brain weight also were significantly increased in these animals.

Gross Necropsy -

10 males in the high dose group exhibited granular kidneys. No other groups showed this effect. No other compound-related abnormalities were reported. See Table II.

Microscopic Examination -

A single tubular adenoma of the renal cortex was reported for one high dose male rat.

Interstitial fibrosis and regenerative epithelium occured in all groups and in both sexes. There was a trend toward greater severity as dose increased. See Table III.

Epithelial hyperplasia, clear cell hyperplasia and karyomega of the renal cortex were reported for all males receiving 4, or 175 mg/kg bw/day, and for females receiving 175 mg/kg bw/ (Table IV). The severity of these lesions increased with increasing dose (Table V).

Male and female rats receiving 15 and 175 mg/kg bw/day showe squamous epithelial hyperplasia and hyperkeratosis of the gastric mucosa. No lesions were reported for groups receivi or 4 mg/kg bw/day. This finding was associated with thicken mucosa (Table VI).

TABLE I

MEAN COMPOUND CONSUMPTION DURING THE FIRST YEAR OF THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Dose Group	Mean Compound Consumption, mg/kg/day					
	Nominal[a]		Analytical			
			Complete Availability[b]		Partial Availability[c]	
	Males	Females	Males	Females	Males	Females
Low	2.09	2.07	1.96	1.99	1.75	1.75
Low-Mid	4.18	4.15	4.03	3.98	3.65	3.63
High-Mid	15.7	15.7	15.2	15.1	15.2	15.2
High	181	181	180	180	183	181

[a] $\frac{\text{Food consumption (g/kg/day)}}{1000} \times \text{Nominal diet concentration (ppm)}$

[b] Assumes availability of chlorothalonil to the animals is unaffected by binding in the diet.

$$\frac{\text{Food consumption (g/kg/day)}}{1000} \times \text{Analytically determined diet concentration (ppm) on day of preparation (Day 0)}$$

[c] Assumes binding of chlorothalonil in the diet has some effect on its availability to the animals.

$$\frac{\text{Food consumption (g/kg/day)}}{1000} \times \frac{\text{Analytically determined Day 0 diet concentration (ppm)} + \text{Day 0 diet concentration adjusted for average binding (ppm)}}{2}$$

TABLE II

INCIDENCE[a] OF MACROSCOPIC AND MICROSCOPIC OBSERVATIONS INDICATIVE OF CHRONIC PROGRESSIVE NEPHROPATHY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Necropsy Observation	Histopathologic Observation	
		Granular Kidney	Regenerative Epithelium	Interstitial Fibrosis
Males/				
Control	0	0/10	9/10	6/10
Low	1.75	0/11	5/11	6/11
Low-Mid	3.63	0/11	9/11	7/11
High-Mid	15.2	0/11	10/11	6/11
High	183	7/10	10/10	10/10
Females/				
Control	0	0/10	5/10	1/10
Low	1.75	0/11	6/11	0/11
Low-Mid	3.63	0/10	6/10	1/10
High-Mid	15.2	0/12	8/12	1/12
High	181	0/10	9/10	2/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

TABLE III

SEVERITY OF INTERSTITIAL FIBROSIS AND REGENERATIVE EPITHELIUM IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Finding: Severity	Dose Group									
	Control		Low		Low-mid		High-mid		High	
	M	F	M	F	M	F	M	F	M	F
Interstitial Fibrosis:										
minimal	5	0	6	0	5	0	3	0	1	2
mild	1	1	0	0	1	1	3	0	3	0
moderate	0	0	0	0	1	0	0	0	6	0
marked	0	0	0	0	0	0	0	0	0	0
severe	0	0	0	0	0	0	0	0	0	0
Regenerative Epithelium:										
minimal	5	4	2	6	6	5	2	8	2	3
mild	3	1	3	0	2	0	6	0	1	6
moderate	1	0	0	0	1	1	2	0	5	0
marked	0	0	0	0	0	0	0	0	1	0
severe	0	0	0	0	0	0	0	0	0	0

TABLE IV

INCIDENCE[a] OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Histopathologic Finding		
		Epithelial Hyperplasia	Clear Cell Hyperplasia	Karyomegaly
Males/				
Control	0	0/10	0/10	2/10
Low	1.75	0/11	0/11	5/11
Low-Mid	3.65	8/11	0/11	6/11
High-Mid	15.2	8/11	2/11	7/11
High	183	10/10	10/10	10/10
Females/				
Control	0	0/10	0/10	3/10
Low	1.75	0/11	0/11	3/11
Low-Mid	3.63	0/10	0/10	2/10
High-Mid	15.2	0/12	0/12	3/12
High	181	7/10	4/10	9/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

TABLE V

SEVERITY OF SEVERAL HISTOPATHOLOGIC FINDINGS IN THE KIDNEY AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Finding: Severity	Dose Group									
	Control		Low		Low-mid		High-mid		High	
	M	F	M	F	M	F	M	F	M	F
Epithelial Hyperplasia:										
minimal	0	0	0	0	5	0	5	0	1	2
mild	0	0	0	0	3	0	3	0	1	2
moderate	0	0	0	0	0	0	0	0	3	3
marked	0	0	0	0	0	0	0	0	5	0
severe	0	0	0	0	0	0	0	0	0	0
Clear Cell Hyperplasia:										
minimal	0	0	0	0	0	0	2	0	1	2
mild	0	0	0	0	0	0	0	0	2	2
moderate	0	0	0	0	0	0	0	0	4	0
marked	0	0	0	0	0	0	0	0	3	0
severe	0	0	0	0	0	0	0	0	0	0
Karyomegaly:										
minimal	2	3	5	3	4	2	3	3	2	2
mild	0	0	0	0	1	0	3	0	5	6
moderate	0	0	0	0	1	0	1	0	3	[illegible]
marked	0	0	0	0	0	0	0	0	0	[illegible]
severe	0	0	0	0	0	0	0	0	0	[illegible]

TABLE VI

INCIDENCE[a] OF SELECTED MACROSCOPIC AND MICROSCOPIC FINDINGS IN THE FORESTOMACH AT ONE YEAR IN THE TUMORIGENICITY STUDY IN RATS WITH TECHNICAL CHLOROTHALONIL

Sex/ Dose Group	Dose Level, mg/kg/day	Necropsy Finding	Histopathologic Finding	
		Thickened Mucosa	Squamous Hyperplasia	Hyperkeratosis
Males/				
Control	0	0/10	0/10	0/10
Low	1.75	0/11	0/11	0/11
Low-Mid	3.65	0/11	0/11	0/11
High-Mid	15.2	2/11	6/11	4/11
High	183	10/10	10/10	10/10
Females/				
Control	0	0/10	0/10	0/10
Low	1.75	0/11	0/11	0/11
Low-Mid	3.63	0/10	0/10	0/10
High-Mid	15.2	1/12	7/12	10/12
High	181	7/10	10/10	10/10

[a]Number of affected animals/Number of animals at the one year interim necropsy and which died during the first year of the study

Review of Registrant's Response to the Previous TB Review Comments Concerning the In-Vitro Chromosomal Aberration Assay in Chinese Hamster Ovary Cells with Technical Chlorothalonil, Study No. T4481.337 (Toxicology Branch Memo 4/9/87 B. Dementi) Accession No. 405591-03
January 4, 1988

Registrant's Response:

" The purpose of this report amendment is to include in the contract laboratory report in Appendix B a report amendment clarifying the selection of harvest times for the chromosomal aberration study from the preliminary toxicity test.

With metabolic activation at 3 ug/ml, the highest dose at which dividing cells were observed in the preliminary toxicity test, the percent of dividing cells in M_1, M_2 and M_3 was 20%, 79% and 1% respectively. In the solvent control the distribution was 5%, 94% and 1% respectively. The differences in percent of cells in first and second metaphase between the cells exposed to chlorothalonil and the solvent control were within experimental variation observed at the laboratory, and not due to cell cycle delay. Therefore, for the chromosomal aberration assay with metabolic activation the cells were harvested at the standard 10 hours. "

Reviewer's Comments:

The provided report amendment for clarifying the selection of harvest times for the study with S9 activation is considered to be justified.

Recommendation:

The test compound, chlorothalonil (T-117-12), was not considered to be a clastogenic agent in the S9 activated study at the concentrations tested (0.6 through 6 ug/ml). However, T-117-12 was considered positive in the nonactivated test system only. The study is upgraded to acceptable.

John H.S. Chen 6/7/88

Reviewed by John H.S. Chen
Review Section I
Toxicology Branch

7/23/87

DEC 13

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Reto Engler, Ph.D., Chief
Mission support Staff
Toxicology Branch/HED TS-769C

THRU: R. Bruce Jaeger, Section Head
REv. Sec. # 1/Toxicology Branch/HED TS-769C

FROM: D. Ritter, Toxicologist D R 7-23-87
Rev. Sec. # 1/Toxicology Branch/HED TS-769C

Subject:

Applicator Risk Assessment for worker exposure to Chlorothalonil.

Caswell #: 215B.

This new Risk Assessment is for workers exposed to the B2 oncogen, Chlorothalonil, in the field. It should be appended to the final Peer Review document for Chlorothalonil that is due 7/30/87. The new assessment is based on a recently re-calculated potency estimate, $Q_1^* = 1.1 \times 10^{-2}$ (mg/kg bw/day)$^{-1}$, derived from a recent rat two year feeding study. The new value was calculated by Bernice Fisher of the Statistical Support Team in her review of 7/20/87.

Exposure estimates were obtained from the S. Noren memo of 12/17/84. Lifetime Average Daily Doses (LADD) were calculated from these by H. Lacayo in his original Risk Assessment memo of 5/17/85. These estimates are being used because no new use patterns have been approved since 1984 for CTN products.

Two Risk estimates are given; one is based on an assumption that 100% of the exposure level is dermally absorbed, and one is based on a dermal absorption rate of 6.3 %. This value was determined experimentally in a study evaluated on 2/20/86 by myself.

370

WORKER RISK ASSUMING 100% AND 6.3% DERMAL ABSORPTION

Ground Application	LADD[1]	RISK[2]	RISK[3]
Sprayer/Mixer	0.0415	4.6×10^{-4}	6.3×10^{-5}
Aerial Application			
Mixer	0.029	3.2×10^{-4}	4.4×10^{-5}
Flagman	0.011	1.2×10^{-4}	1.6×10^{-5}
Pilot	0.005	5.5×10^{-5}	7.5×10^{-6}

---

[1] LADD values from H. Lacayo Risk Assessment of 5/12/85.

[2] Calculation assumes 100% dermal absorption.

[3] Calculation assumes 6.3% dermal absorption.

C 7/23/87

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Esther Saito
SIS/HED TS769C

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

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

Subject: Chlorothalonil Dermal Absorption values.

In response to your request for dermal absorption data to complete your Risk Assessment for Applicator Exposure to Chlorothalonil, we are providing the following:

In a dermal absorption study in male Sprague-Dawley rats(MRID DLR007), 14-C Chlorothalonil was applied dermally in acetone and the rate of absorption in the blood was measured at 2, 4, 8, 12, 24, 48, 72, 96 and 120 hours. The rate of dermal absorption at 2 and 4 hours was about the same (15.1 and 164 ugm-equivalents per day, respectively). The average daily absorption rate stabilized at 24 hours at 73.2 +/- 15.3 ugm 14-C Chlorothalonil per day. This represents rate of dermal absorption of 6.3 % of the administered dose per 24 hours.

38

CC, 713

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C 20460

MEMORANDUM: JUL 21 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Don Stubbs
RSERB
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaulation Division TS-769C

THRU: T. M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaulation Division TS-769C

FROM: D. Ritter, Toxicologist
Rev. Sec. # 1/Toxicology Branch
Hazard Evaulation Division TS-769C

DLR 7-20-87

7/21/87

Subject:

87-CA-03: Chlorothalonil Section 18 on Mushrooms in California.

In our most recent review of this request (D. Ritter, 6/17/87 we defered to EAB as to worker exposure under the proposed exemption. This was necessary because CTN is a B2 oncogen and a Risk Analysis was needed. EAB has calculated worker exposures based on dermal monitoring data (review of K. Warkentien, 7/13/87). She has calculated that dermal exposure to a person mixing/loading and drenching in a greenhouse (an environment similar to that for mushroom culture) would be 50 ug/kg bw/day based on worst case exposure.

The Toxicology Branch has completed its statistical evaluation of a recently completed rat two year feeding study and has determined that the potency estimate, Q_1^* for this study is 1.1×10^{-2} mg/kg bw/day (Bernice Fisher, 7/14/87).

If one assumes that dermal penetration is 100% in man, the lifetime oncogenic risk to applicators from dermal exposure in mushroom culture is 3.3×10^{-5}.

If one assumes that dermal penetration in man is 6.3 % of the total dermal dose (based on our review of a rat dermal absorption study, D. Ritter, 2/20/86), the lifetime oncogenic risk to applicators from dermal exposure in mushroom culture is 2.4×10^{-6}.

382

Since no detectable exposure by inhalation is anticipated, we are not calculating an oncogenic risk from this route of exposure.

Risk Assessment[1]

$$\text{Risk} = Q_1^* \times \text{LADD (Lifetime Average Daily Dose)}^{[2]}$$

$$= Q_1^* \times (50 \text{ ug/kg/day}) \times \frac{(5 \text{ days} \times 10 \text{ weeks})}{365} \times 35/70$$

$$= 1.1 \times 10^{-2} \text{ mg/kg bw/day} \times 3.42 \text{ ug/kg bw/day}$$

$$\text{Risk} = 3.8 \times 10^{-5} \text{ (assuming 100\% dermal absorption).}$$

$$\text{Risk} = 1.1 \times 10^{-2} \text{ mg/kg bw/day} \times 2.16 \times 10^{-1} \text{ ug/kg bw/day}$$

$$= 2.4 \times 10^{-6} \text{ (assuming 6.3\% dermal absorption).}$$

---

1 Equations used in the H. Lacayo Risk Assessment of 5/17/85 (copy attached).

2 LADD = (Dose for one working day) x (No. days worked with chemical) /365 X (35 working years/70 year lifetime) assuming 100% dermal absorption.

= (one day exposure) x $\frac{\text{(days exposed per year)}}{365}$ x 35/70

II. FORMULAS

A. LADD Formula

The Lifetime Average Daily Dose (mg/kg/day is approximated by:

LADD = (Dose acquired in one working day in mg/kg/day)

x (No. of working days per year with the chemical)/365

x (35 years of working)/(70 years lifetime)

$$= (\text{One day exposure}) \times \frac{\text{days exposed/yr}}{365} \times \frac{35}{70}$$

B. Conversion of ppm to mg/kg/day

1 ppm in mouse diet = .150 mg/kg/day

Quick Conversion (for ppm only)

$$1 \text{ ppm in diet for animal} = \frac{\text{(Wt of diet in grams)}}{\text{(Wt of animal in grams)}}$$

= mg/kg/day for animal

C. Interspecies Conversion Factor

Let SA = Surface Area

W_h = body weight of humahn
W_a = body weight of animal
d_h = dose for human (mg/kg/day)
d_a = dose for animal (mg/kg/day)

If we assume the surface ares is proportional to $W^{2/3}$ and that equivalent doses (in mg/day) are proporational to surface areas, then $d_h = d_a \times (W_a/W_h)^{1/3}$.

For example extrapolation of mouse to an "equivalent" human dose can be done as follows:

1. Convert mouse dose which is usually in ppm to mg/kg/day.

   .15 x (mouse dose in ppm) = mouse dose in mg/kg/day.

2. Therefore,

   Human Equiv. Dose = (mouse dose in mg/kg/day)x(25/65000)$^{1/3}$

6/21/87

SECTION HEAD

00713

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

MEMORANDUM: JUL 21 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Don Stubbs
RSERB
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaulation Division TS-769C

THRU: T. M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaulation Division TS-769C

FROM: D. Ritter, Toxicologist
Rev. Sec. # 1/Toxicology Branch
Hazard Evaulation Division TS-769C

DLR 7-20-87
7/21/87

Subject:

87-CA-03: Chlorothalonil Section 18 on Mushrooms in California.

In our most recent review of this request (D. Ritter, 6/17/87) we defered to EAB as to worker exposure under the proposed exemption. This was necessary because CTN is a B2 oncogen and a Risk Analysis was needed. EAB has calculated worker exposures based on dermal monitoring data (review of K. Warkentien, 7/13/87). She has calculated that dermal exposure to a person mixing/loading and drenching in a greenhouse (an environment similar to that for mushroom culture) would be 50 ug/kg bw/day based on worst case exposure.

The Toxicology Branch has completed its statistical evaluation of a recently completed rat two year feeding study and has determined that the potency estimate, Q_1^* for this study is 1.1×10^{-2} mg/kg bw/day (Bernice Fisher, 7/14/87).

If one assumes that dermal penetration is 100% in man, the lifetime oncogenic risk to applicators from dermal exposure in mushroom culture is 3.8×10^{-5}.

If one assumes that dermal penetration in man is 6.3 % of the total dermal dose (based on our review of a rat dermal absorption study, D. Ritter, 2/20/86), the lifetime oncogenic risk to applicators from dermal exposure in mushroom culture is 2.4×10^{-6}.

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

JUL 8 1988

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Chlorothalonil Final Registration Standard and Tolerance Reassessment (FRSTR): Response to TOX Branch Memo Regarding Residues of the Chlorothalonil Manufacturing Impurity, pentachlorobenzonitrile (PCBN), in food and feed crops

FROM: Debra F. Edwards, Ph.D.
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

Debra Edwards

THROUGH: Charles L. Trichilo, Ph.D., Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769C)

TO: Esther Saito
Science Integration and Policy Staff
Hazard Evaluation Division (TS-769C)

and

William Burnam, Deputy Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Introduction:

In the Residue Chemistry chapter of the Chlorothalonil FRSTR (3/11/88), RCB requested data depicting the residues of PCBN, a manufacturing impurity of chlorothalonil, in several raw agricultural commodities (RACs) and processed products following treatment with registered chlorothalonil end-use products. Although acceptable PCBN data are available for several crops (see table on following page), additional data were required for several major food and feed items, including potatoes, succulent beans, tomatoes, dried plums, sweet corn forage, mint, papayas, passion fruit, peanuts and grass grown for seed. Subsequently, the TOX Branch issued a memo (D. Ritter, 5/2/88), based on their review of the currently available PCBN data in the Residue Chemistry chapter, which stated that residues of PCBN in raw agricultural commodities are in approximately the same proportion relative to residues of chlorothalonil as in technical chlorothalonil products. Thus, the TOX Branch inferred that "the toxicological profile of the technical product reflects the toxicity of PCBN as an impurity." They concluded, "our concern for residues of potentially toxic PCBN in racs is alleviated."

Since no detectable exposure by inhalation is anticipated, we are not calculating an oncogenic risk from this route of exposure.

Risk Assessment[1]

$$\text{Risk} = Q_1^* \times \text{LADD (Lifetime Average Daily Dose)}^{[2]}$$

$$= Q_1^* \times (50 \text{ ug/kg/day}) \times \frac{(5 \text{ days} \times 10 \text{ weeks})}{365} \times 35/70$$

$$= 1.1 \times 10^{-2} \text{ mg/kg bw/day} \times 3.42 \text{ ug/kg bw/day}$$

$$\text{Risk} = 3.8 \times 10^{-5} \text{ (assuming 100\% dermal absorption).}$$

$$\text{Risk} = 1.1 \times 10^{-2} \text{ mg/kg bw/day} \times 2.16 \times 10^{-1} \text{ ug/kg bw/day}$$

$$= 2.4 \times 10^{-6} \text{ (assuming 6.3\% dermal absorption).}$$

---

1 Equations used in the H. Lacayo Risk Assessment of 5/17/85 (copy attached).

2 LADD = (Dose for one working day) x (No. days worked with chemical) /365 X (35 working years/70 year lifetime) assuming 100% dermal absorption.

$$= \text{(one day exposure)} \times \frac{\text{(days exposed per year)}}{365} \times 35/70$$

II. FORMULAS

A. LADD Formula

The Lifetime Average Daily Dose (mg/kg/day is approximated by:

LADD = (Dose acquired in one working day in mg/kg/day)

x (No. of working days per year with the chemical)/365

x (35 years of working)/(70 years lifetime)

$$= (\text{One day exposure}) \times \frac{\text{days exposed/yr}}{365} \times \frac{35}{70}$$

B. Conversion of ppm to mg/kg/day

1 ppm in mouse diet = .150 mg/kg/day

Quick Conversion (for ppm only)

$$1 \text{ ppm in diet for animal} = \frac{\text{(Wt of diet in grams)}}{\text{(Wt of animal in grams)}}$$

= mg/kg/day for animal

C. Interspecies Conversion Factor

Let SA = Surface Area

W_h = body weight of humahn
W_a = body weight of animal
d_h = dose for human (mg/kg/day)
d_a = dose for animal (mg/kg/day)

If we assume the surface ares is proportional to $W^{2/3}$ and that equivalent doses (in mg/day) are proporational to surface areas, then $d_h = d_a \times (W_a/W_h)^{1/3}$.

For example extrapolation of mouse to an "equivalent" human dose can be done as follows:

1. Convert mouse dose which is usually in ppm to mg/kg/day.

   .15 x (mouse dose in ppm) = mouse dose in mg/kg/day.

2. Therefore,

   Human Equiv. Dose = (mouse dose in mg/kg/day)$\times(25/65000)^{1/3}$

5/17/85

007718

~~CASWELL FILE~~

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

MAY 17 1985

004455

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Risk Assessment for Chlorothalonil Based on Diamond Shamrock's Two Year Chronic Mouse Feeding Study. Accession No. 071541.

Caswell No. 215B

FROM: Herbert Lacayo, Statistician Herbert Lacayo 5/16/85
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Dianne Beavers, Product Manager Team #21
Herbicide Fungicide Branch
Registration Division (TS-767)

THRU: Bertram Litt, Leader
Statistics Team, Mission Support Staff
Toxicology Branch/HED (TS-769)

THRU: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769) Reto Engler 5/17/85

Summary:

The study data analyzed below indicate that chlorothalonil (CTN) is a renal carcinogen in male CD-1 mice. The weight of evidence determination with respect to human carcinogenicity will be made by the Toxicology Branch Cancer Review Committee.

Chlorothalonil has a potency factor Q_1^* of 2.4×10^{-2} for exposure expressed in mg/kg body weight/day.

Background:

The Registrant submitted their own risk assessment. Sufficient methodological detail was not given in their submission to determine precisely why the Diamond Shamrock results were two orders of magnitude lower than that obtained by Crump's multi-stage model (Ref. 1), where this latter model was implemented is accordance to procedures recommended by the EPA draft guidelines.

Study Description:

The National Cancer Institute Study (NCI-CG-TR-41, 1978) contains evidence that CTN induces renal neoplasm in Osborne-Mendel male and female rats. This prompted Diamond Shamrock Corporation to perform a second study in mice ("a Chronic Dietary Study in Mice with Technical Chlorothalonil," dated April, 1983) to test the null hypothesis that chlorothalonil does not cause kidney tumors. Their two year feeding study used 97.7% CTN, CD-1 mice and was carried out by Bio/Dynamics.

Test mice were assigned randomly to four groups of 60 males and 60 females per treatment. The treatment groups consisted of control, low, medium, and high dose respectively as shown below.

TABLE 1

Experimental Design for the Chlorothalonil Feeding Study

Group	Dose (ppm)	Number of Males	Number of Females
I	0	60	60
II	750	60	60
III	1500	60	60
IV	3000	60	60

The study was initiated February, 1980 and terminated after 24 months. All surviving mice were sacrificed at the end of the study period. Animals dying or sacrificed during the study or at termination were necropsied.

Qualatative Analysis:

The Registrant and D. Ritter, EPA Toxicologist, note average survival in all groups except high dose males; and "food consumption and weight gain were comparable among groups." They both summarize the results by noting that there is nothing in the study which would either cause the tumor data to be excluded or cause difficulties in its interpertation.

Statistical review indicates no discernable strong dose related trends in the mortality of the test animals. However, as noted by the Registrant, mortality is significantly higher for high dose males when compared to controls ($p = .07$ by Fischer's Exact test). Second, female mortality by 18 months was significantly higher than male mortality for corresponding study groups ($p < .01$ by Fischer's Exact test). These mortality data are summarized below in Table 2.

389

TABLE 2

Cummalative Mortality At Six Month Intervals

	MALES				FEMALES			
DOSE (ppm)	6	12	18	24	6	12	18	24
0	1	3	8	29	4	8	20	42
750	0	2	10	35	2	3	18	38
1500	5	7	8	26	3	6	17	37
3000	2	10	13	38	3	9	20	41

Body weights for both male and female for all treatment groups means were comparable to controls for both sexes. Although significant differences were not noted within either sex, the female mice appeared to exhibit greater variability for both within and between group variances.

The tumors of greatest interest were renal tumors in male mice. The data are summarized in Table 3.

TABLE 3

Dose (ppm)	0	750	1500	3000
Response	0/57	6/59	4/59	4/56

Because the tumor rate rises then flattens out by 1500 ppm, it is clear that the departure from linearity explains the lack of a statistically significant dose-response trend ($p = .14$ by the Peto or Armitage-Cochran tests). However, when historical data are utilized (Ref. 2,3) it may be shown that the effect is dose related. This is done by reasoning similar to that given in Ref. 2. Using a background tumor rate of $p = .002$ (estimated from data in Ref. 3), binomial distribution theory implies that the probability of having 14 or more male mice with renal tumors in a group of 231 is less than .0001. Stated more formally, the dose effect of chlorothalonil is statistically significant at the $p = .0001$ level, compared to the referenced historical controls under the binomial distribution assumption.

Quantitative Risk Assessment:

In addition to the renal tumors noted above, all treatment groups (in both sexes) exhibited gastric carcinomas. These are summarized below.

TABLE 4

Gastric
(Number of Tumors/Number of Animals at Risk)

	0 ppm	750 ppm	1500 ppm	3000 ppm
Female				
Squamous cell Carcinoma	0/57	2/60	6/58	5/58
Glandular	0/57	1/60	1/58	2/56
Total	0/57	3/60	7/58	7/58
Male				
Squamous cell Carcinoma	0/55	2/59	5/59	1/51
Glandular	0/55	1/59	2/59	0/51
Total	0/55	3/59	7/59	1/102

Squamous cell and Glandular carcinomas are not normally additive. However, in this case Dr. L. Kasza, Staff Pathologist suggests that there may be evidence of multiple tissue tumors that may be due to the same causative agent or mechanism.

For risk assessment purposes we will use the rare renal tumors rather than gastric tumors because that effect is detected at a lower dose. The problem of the non monotonicity of the dose response with the renal tumors can be dealt with by eliminating the 1500 and 3000 ppm dose groups as recommended by the Crump multi-stage procedure and the Mantel/Tukey paper (Ref. 6). This approach is consistent with EPA policy (see Ref. 4) that tends to select the data groups giving the highest potency (Q_1^*).

Crump's multi-stage procedure was applied to the following renal-tumor-data set where human equivalent dose is expressed in mg/kg/day.

TABLE 5

Renal Tumors

Human Equivalent Dose (mg/kg/day)	0	8.2
Response	0/57	6/59

The human equivalent dose (in the absence of experimental data) was calculated by standard methods (see Appendix for formulas).

The results of the multi-stage modeling are given below.

MLE of Q_1	Est of Q_1^*
1.31×10^{-2}	2.4×10^{-2}

Note that the Chi Square value is not shown, as it is not relevant because there are only two dose groups to fit. Note that the MLE (maximum likelihood estimate) of Q and Q_1^* are close. Hence, there is a close correspondence between the point estimate of the slope based on the data, and the 95% upper bound on this slope.

Diamond Shamrock carried out their own independent risk assessment producing results which differ from ours by about two orders of magnitude. This discrepancy might be reconciled as follows:

1. If the Registrant used all four groups without surface area adjustment of the dose and if they used the maximum likelihood estimate for potency (instead of $Q_1^* = 2.4 \times 10^{-2}$), their estimate would be 2.8×10^{-3}.

2. If the Registrant also performed a surface-area correction of say $(6000/40)^{1/3} = 11.4$, they would find a potency, Q_1^*, of about 2.45×10^{-4}.

3. By working backwards from the Registrant's risk data we have found that their potency was about 2.28×10^{-4} to 2.46×10^{-4}. This includes the 2.45×10^{-4} value calculated above. That possibly clarifies the two orders of magnitude differences between the results.

For completeness, we list two other possible sources of error:

1. The Registrant appears to count all animals on test while Toxicology Branch reviewers count only non-autolyzed mice.

2. The Registrant appears to over estimate the "Annualized Daily Exposure" by not taking into consideration that a worker will generally be exposed for only 1/2 his(her) life time.

Characterization of Risk:

The risk for the TMRC and some of the published tolerances (see Appendix for complete list) are given below where the risk are based on a $Q_1^* = 2.4 \times 10^{-2}$.

TABLE 6

	Exposure (mg/kg/day)	Risk
Celery	.001073	10^{-5}
Cucumber	.000907	10^{-5}
Melons	.002504	10^{-5} to 10^{-4}
Beans (snap)	.001226	10^{-5}
Tomatoes	.00359	10^{-4}
Cabbage	.0009198	10^{-5}
TMRC	.011905	10^{-4}

Worker risks were obtained from S.E. Noren's memo to R. Engler dated December 17, 1984 (Ref. 5), the basic data and risks are given below.

TABLE 7

Worker Risks Based on $Q_1^* = 2.4 \times 10^{-2}$ and 100% Dermal Penetration

Ground Application	LADD[a]	Risk[b]
Sprayer Mixer	.0415	10^{-3}
Aerial Application		
Mixer	.029	10^{-4} to 10^{-3}
Flagman	.011	10^{-4}
Pilot	.005	10^{-4}

[a] LADD = Lifetime Average Daily Dose (see Appendix for detail).

[b] Risk = $Q_1^* \times$ LADD

APPENDIX

I. Reference

II. Formulas

III. Published Tolerances

I. REFERENCE

1. Crump, K. S. (1982) An improved procedure for low-dose carcinogenic risk assessment from animal data. Journal of Environmental Pathology and Toxicology Vol. 5, No. 2, 675-684.

2. Memorandum, H. Lacayo to R. Engler. Subject: Use of Historical Data..... dated Feb. 29, 1985.

3. Letter. R. P. Burton of Biotech to H. M. Jacoby of EPA dated Dec. 19, 1983.

4. Water Quality Criteria Documents, Federal Register, Vol. 45. No. 231, Friday, Nov. 28, 1980.

5. Memorandum. S. E. Noren to R. Engler, Subject: Applicator Exposure for Chlorothalonal.

6. N. Mantel, N. R. Bohidar, C. C. Brown, J. L. Ciminera, and J. W. Tukey. An improved Mantel-Bryan procedure for "safety" testing of carcinogens. Cancer Research 345, 865-872 (1975).

II. FORMULAS

A. LADD Formula

The Lifetime Average Daily Dose (mg/kg/day is approximated by:

LADD = (Dose acquired in one working day in mg/kg/day)

x (No. of working days per year with the chemical)/365

x (35 years of working)/(70 years lifetime)

$$= (\text{One day exposure}) \times \frac{\text{days exposed/yr}}{365} \times \frac{35}{70}$$

B. Conversion of ppm to mg/kg/day

1 ppm in mouse diet = .150 mg/kg/day

Quick Conversion (for ppm only)

$$1 \text{ ppm in diet for animal} = \frac{\text{(Wt of diet in grams)}}{\text{(Wt of animal in grams)}}$$

= mg/kg/day for animal

C. Interspecies Conversion Factor

Let SA = Surface Area

W_h = body weight of humahn
W_a = body weight of animal
d_h = dose for human (mg/kg/day)
d_a = dose for animal (mg/kg/day)

If we assume the surface ares is proportional to $W^{2/3}$ and that equivalent doses (in mg/day) are proporational to surface areas, then $d_h = d_a \times (W_a/W_h)^{1/3}$.

For example extrapolation of mouse to an "equivalent" human dose can be done as follows:

1. Convert mouse dose which is usually in ppm to mg/kg/day.

.15 x (mouse dose in ppm) = mouse dose in mg/kg/day.

396

2. Therefore,

Human Equiv. Dose = (mouse dose in mg/kg/day)$\times(25/65000)^{1/3}$

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil - Rat Study, Qualitative and Quantitative Risk Assessment — caswell no. [illegible]

FROM: Bernice Fisher, Biostatistician
Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C) Bernice Fisher 7/20/87

TO: David Ritter, Toxicologist
Section I, Toxicology Branch
Health and Evaluation Division (TS-769C)

THRU: for Richard Levy, M.P.H., Leader-Biostatistics Team e Jacobs 7/20/
Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C)

and

for Reto Engler, Ph.D e. Kinde
Chief, Scientific Mission Support Staff
Toxicology Branch
Health and Evaluation Division (TS-769C)

SUMMARY

The potency estimate, Q_1^* of Chlorothalonil is 1.1×10^{-2} $(mg/kg/day)^{-1}$ in human equivalents $[B_2]$. This estimate is based upon female rat renal tumors (carcinomas and adenomas).

In female rats there was a significant survival disparity in the pairwise comparison of controls with the mid dose group.

In males rats, there was a significant increase in mortality with dose increments of the chemical, primarily due to the significant increase of deaths in the high dose group as compared with controls.

Background

The May 28, 1987 Peer Review Committee for Chlorcthalonil decided that a qualitative and quantitative Risk Assessment was needed and should be based upon the renal tumor formations in rats of the SDS Biotect study of Fisher 344 strain, dosed with 0, 40, 80 and 175 mg/kg of the chemical.

Qualitative Review

Survival analysis was prepared by the use of the D.G. Thomas, H. Breslow and J.J. Gart computer program. The results of the analysis indicated that mortality did not significantly increase with increasing doses of Chlorothalonil in female rats. However, in the pairwise comparison of conrols with the mid dose (80 mg/kg) group, there was a significant ($p = .02$) difference.

In male rats, survival was significantly ($p<.02$) decreased with dose increments of Chlorothalonil. In addition the pair wise comparison of control with the highest dose (175 mg/kg) was also statistically significant ($p=.03$). See Table 1. for details.

In spite of the fact that survival was a problem in the study, the renal tumor formations only started to appear at the beginning of the 79th week of the study and most of the tumors were found in the final kill of the study in both sexes. In addition deaths on the study began about one year after it started.

Because of the late appearance of both deaths and also renal tumors, the use of the Cochran-Armitage Trend test and Fisher's Exact pairwise comparisons with controls were deemed most appropriate+ for the qualitative evaluation of the data.

The Cochran-Armitage Trend test on renal carcinomas, renal adenomas, and combined renal carcinomas and adenomas for both sexes, were all highly significant ($p<.02$). Also, all of the aforementioned groups for both sexes showed consistently significant diferences in tumor rates in the pairwise comparisons (Fisher Exact test) of controls with the highest dose (175 mg/kg) group. See Table II. for details.

+ There is no appropriate way to adjust for the survival disparties since the Peto Prevalence test would be collapsed onto too few time intervals.

Dose- Response Review

On the basis of the qualitative evaluation of renal tumors in rats, the potency estimate, Q_1^* of Chlorothalonil was based upon the proportions in females, which were the most sensitive to the chemical. This estimate was obtained from the Multi-Stage (K. Crump's computer program) Model in terms of rat mg/kg/day doses and then converted to human equivalents by the interspecies surface area adjustments as recommended by EPA Cancer Guidelines. See Table IV. for details.

Table I. Chlorothalonil - Rat Study, Mortality Rates+ and Life Table Analysis Resulta

A. Males

Dose	Weeks				
mg/kg	0-52	53-78	79-104	105-115[a]	Total
0	0/66	3/66	10/63	15/53	28/66 (42)*
40	1/61	1/66	10/60	16/50	27/61 (44)
80	1/60	1/58	14/57	9/43	26/60 (43)
175	0/60	1/60	16/59	21/43	38/60 (63)*

B. Females

Dose	Weeks				
mg/kg	0-52	53-78	79-104	105-128[b]	Total
0	0/60	1/60	10/59	18/49	29/60 (48)
40	0/60	0/60	11/60	28/49	39/60 (65)
80	1/61	3/60	6/57	33/51	43/61 (70)*
175	0/59	1/59	11/58	22/47	34/59 (58)

+ Number of animals died/ Number of live animals at beginning of interval

( ) percent

a final sacrifice at 115 weeks.

b final sacrifice at 128 weeks.

Note: The above time intervals were selected for display only. Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level.

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

Table II - Chlorothalonil - Rat Study, Renal Tumor Rates Cochran-Armitage Trend test and Fisher Exact test Results

A. Males

Dose mg/kg Renal Tumor Rates[1]	0	40	80	175
Carcinomas	1/66(2)*	3/61(5)	1/60(2)	6/60(10)*
Adenomas	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(32)**

B. Females

Dose mg/kg Renal Tumor Rates[1]	0	40	80	175
Carcinomas	0/60(0)**	1/60(2)	3/61(5)	12/59(20)**
Adenomas	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
( ) per cent

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

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

Table III. Chlorothalonil - Rat Study, Stomach Tumor Rates+ (Gastric Squamous Mucosa - Papilloma and Carcinoma) Cochran-Armitage Trend test and Fisher Exact test Results

A. Males

Tumor	Dose - mg/kg 0	40	80	175
Stomach Gastric Squamous Mucosa				
Carcinoma	1/66/(2)	0/60(0)	0/60(0)	1/60(2)

B. Females

Tumor				
Stomach Gastric Squamous Mucosa				
Carcinoma	0/60	0/60	1/61	1/59
Papilloma	0/60	1/60	2/61	2/59
Both	0/60(0)*	1/60(2)	3/61(5)	3/59(5)

+ Number of tumor bearing animals/Number of animals examined

( ) Percent

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

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

Table IV. Chlorothalonil - Rat Study - Potency Estimate, Q_1^* $(mg/kg/day)^{-1}$

	Rat	Human Equivalents
Female	2.0×10^{-3}	1.1×10^{-2}
Male	2.3×10^{-3}	1.2×10^{-2}

References

Armitage, P. (1955) Tests for Linear Trends in Proportious, Biometrics 11, 375-386.

Cochran, W.G. (1954) Some Methods for Strengthening the Common X^2 test, Biometrics 10, 417-451.

Cox, D.r. (1972) Regression Models and Life Tables (with discussion) J. Roy. Stat. Soc. Ser. B. 34, 187-220.

Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogenity Analysis of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381.

Blood Concentrations Following Dermal Exposure

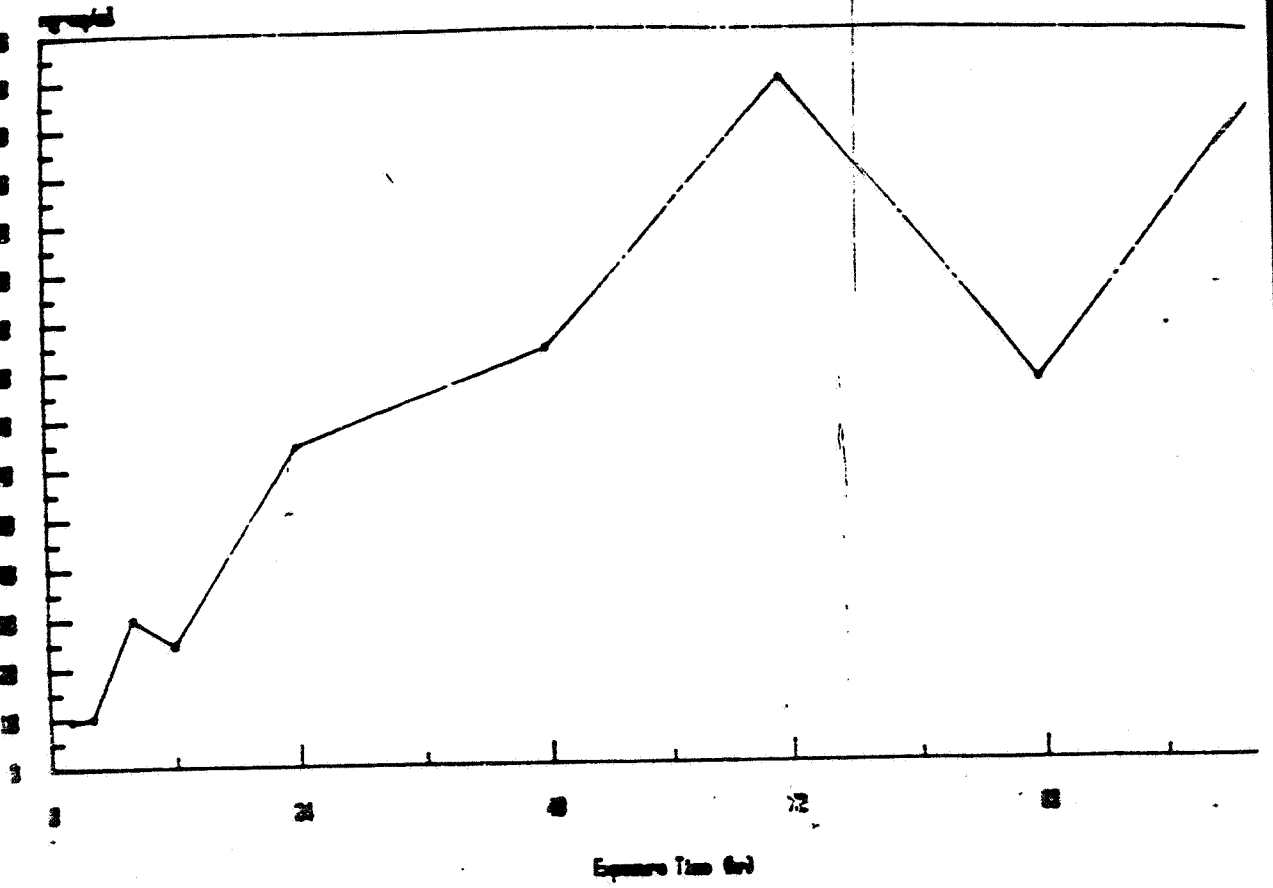


FIGURE 2

00,713

DATA EVALUATION REPORT

STUDY: Three Generation Rat Reproducion Study

LABORATORY: Hazleton Laboratories, Falls Church, VA

STUDY NUMBER & DATE: # 200-150 2/2/67 O. E. Paynter, Ph.D.

ACCESSION NUMBER:

MRID: 00038913

MATERIAL TESTED: A "Blend" of materials resembling technical Chlorothalonil (93.6% pure)*.

ANIMALS: 10 males and 20 females per group.

METHODS:

Diets containing 0, 0.15, 1.5 and 3.0/2.0 % (3.0 for only 14 days, then 2.0%) of test material were offered throughout the study.

RESULTS:

See the review of E. Long, PP # 7F-0743, 1/31/69.

Growth depression at all levels. Gross renal discoloration and pitted kidney sufraces. Gastric acanthosis and hyperkeratosis. Negative for teratogenic effects.

CONCLUSIONS:

NOEL for reproductive effects is less than 0.15 % based on reduced growth and renal and gastric effects.

Not a rat teratogen.

CORE RATING:

Supplemental. A NOEL not demonstrated. Not repairable.

[redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

reduced in the 10/15 and 20/30 mg/kg males and females throughout the study, even after reduction of doses. Food consumption was unremarkable except for decreases in 10/15 and 20/30 mg/kg females and 20/30 mg/kg males, consistent with decreased body weights and increased mortality during the first 30 weeks. There were similar decreases in total serum protein, albumin, globulin, and cholesterol in 20/30 mg/kg males and females and 10/15 mg/kg females after 6 months. These returned to control levels for the remainder of the study, after doses were reduced to 20 and 10 mg/kg, respectively.

There were significant hemopoietic effects in the 10/15 and 20/30 mg/kg animals, particularly females, during the first 6 months. Evidence of microcytic anemia was provided by reduced RBC counts, hematocrit, hemoglobin, MCV, and MCH with accompanying increases in MCHC, reticulocytes and metarubricytes. Segmented neutrophiles were increased with corresponding decrease in percentage of lymphocytes. Specially stained bone marrow presented evidence of hypocellularity. Wallory's stain of liver tissue revealed an increased iron content (hemosiderin). After 18 and 24 months exposure the 10/15 mg/kg group females continued to present evidence of anemia (decreased Hct, Hgb, MCV, MCH and increased MCHC) with a positive bone marrow response (increased cellularity with a shift to increasing number of immature erythyroid cell types and increase number animals with a low M/E ratio). Prussian Blue staining demonstrated the presence of hemosiderin in the 10 mg/kg males and females, not considered significant at 3 mg/kg. After 24 months exposure there were decreased serum potassium levels in all dosed females. Urinalyses and examination for fecal occult blood were unremarkable, except for increased urine volume at 6 months in the high dose group animals.

Ophthalmological examination at 6 months revealed increased pale ocular structures and spontaneous hemorrhage in high dose male and female animals. At 24 months there were increased numbers of dilated pupils (not responding to light) and increased bilateral cataract disease in high dose males.

Comparison of selected organ weights demonstrated decreased absolute organ weights for kidney, heart and brain in high dose males with no significant relative organ-to-body weight changes. High dose females had decreased absolute kidney and heart weights with no relative weight changes except for spleen and brain. Microscopic examination failed to confirm any compound related effects on these organs. There were no significant compound related non-neoplastic organ changes except for hemosiderin in the liver of high dose females and hemorrhage in CNS tissues, hypocellular bone marrow and post-mortem congestion of lymph in high dose male and female rats.

Examination of tissues/organs for neoplastic changes did not indicate any compound related effects at any level tested.

Data presented in this study demonstrate that the metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile, is without adverse effects in male and female rats at levels up to and including 3 mg/kg/day for 2 years (McGee et al., 1991).

CORE Rating: Guideline.

Results of mutagenicity assays of [illegible]

Test Organism	Test Substance (% purity)	Results	Reference
GENE MUTATION STUDIES			
Bacteria			
S. typhimurium	97.8	No mutagenic activity was reported in TA98, TA100, TA1535, TA1537 and TA1538 with or without metabolic activation.	Ganzer 1977a
	99.6	Negative response in G46, C3007, TA1538, TA1531, C3076, TA1700, D3052 and TA1727 according to host mediated assay.	Legator 1977a
	99.7	No mutagenic activity was reported in TA98, TA100, TA1535, TA1537 and TA1538 without metabolic activation.	Shirasu et al 1977
E. coli	99.3	No mutagenic activity was reported in WP2 hcr^+ and WP2 hcr^- with or without metabolic activation.	Shirasu et al 1977
Cultured Mammalian Cells			
Chinese hamster cells (V79) and Mouse Fibroblast Cells (Balb 3T3)	97.8	No mutagenic effect was reported in these two mammalian cell lines with or without metabolic activation.	Ganzer 1977b
CHROMOSOME MUTATION			
Cytogenetics - In Vivo Rat, Mouse, Chinese hamster	98.2	No induced chromosomal aberrations were reported in bone marrow cells of rat, mouse and Chinese hamster.	Dieu et al 1981b
Micronucleus - In Vivo Rat, Mouse, Chinese hamster	98.2	Negative responses were reported in the polychromatic erythrocytes of treated animals.	Legator 1977a & Dieu et al 1981b
Dominant Lethal - Mice		No mutagenic activity to induce dominant lethals in male mice were reported.	Legator 1977a
DNA DAMAGE AND REPAIR - Bacteria			
S. typhimurium	97.8	Positive response in demonstrating significantly preferential cell killing between the TA1535 and TA1978 with or without metabolic activation.	Ganzer 1977b
B. subtilis	99.3	Negative response. No marked difference in the inhibition zones of the strain H17 and M45 as reported.	Shirasu et al 1977

Results of Mutagenicity Assays of 4-hydroxy-2,5,6-trichlorcisophthalonitrile

Test Organism	Test Substance (% purity)	Results	Reference
GENE MUTATION STUDIES			
Bacteria			
Salmonella typhimurium	99.0	No mutagenic activity was reported in TA98,TA100,TA1535,TA1536 and TA1538 with or without metabolic activation.	Banzer 1977d
S. typhimurium	99.0	Negative response in G46, TA1530, C270,TA1531,C3076,TA1700,D3052 and TA1724 according to host mediated assay.	Legator 1974b
Cultured Mammalian Cells			
Chinese hamster Cells (V79) and Mouse Fibroblast Cells (Balb/3T3)	99.0	No mutagenic activity was reported in TA98,TA100,TA1535,TA1537 and TA1538 without metabolic activation.	Banzer 1977e
CHROMOSOME EFFECT			
Micronucleus - In Vivo Mouse	99.0	Negative response was reported in the polychromatic erythrocytes of treated mice.	Legator 1974b
Dominant Lethal - Rodent			
Rat	99.0	No induced dominant lethals were reported in treated rats.	Hastings et al 1975
Dominant Lethal - Rodent			
Mouse	99.0	No induced dominant lethals were reported in treated mice.	Legator 1974b, 1975
DNA DAMAGE AND REPAIR - Bacteria			
S. typhimurium	99.0	Negative response. No marked difference in the inhibition zones of the strains TA1538 and TA1978 were reported.	Banzer 1977f
CELL TRANSFORMATION - IN VITRO			
Fischer Rat Embryo Cell Lines (F1706 and H4536)	99.0	Negative responses were reported in both treated cell lines.	Price 1978b

FAO
PLANT
PRODUCTION
AND PROTECTION
PAPER

72/2

Pesticide residues in food - 1985

Report sponsored jointly by FAO and WHO with the support of the International Programme on Chemical Safety (IPCS)

Joint meeting of the
FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Expert Group on Pesticide Residues
Geneva, 23 September - 2 October 1985

Table 7. **Results of mutagenicity assays of chlorothalonil**

Test system	Test substance	Dose level/ concentration	Results	Reference
Mouse bone marrow cytogenetics assay - in vivo	Chlorothalonil	250, 1250, & 2500 mg/kg, orally	Negative[a]	Mitzens et al., 1985a
Rat bone marrow cytogenetics assay - in vivo	Chlorothalonil	500, 2500, & 5000 mg/kg, orally	Negative[b]	Mitzens et al., 1985b
Chinese hamster bone marrow cytogenetics assay - in vivo	Chlorothalonil	500, 2500, & 5000 mg/kg given as single treatment; 50, 125, & 250 mg/kg given as 5 daily treatments orally	Weak clastogen positive in treated groups: 5 x 50 & 5 x 250 mg/kg. No dose-response relationship.	Mitzens et al., 1985c

a Positive control (MMS) gave expected positive response at 65 mg/kg.
b Positive control (MMS) gave expected positive response at 130 mg/kg.

Table 8. **Results of mutagenicity assays of technical chlorothalonil, manufacturing impurities, and possible metabolites of technical chlorothalonil**

Test system (Ames test)	Test substance	Dose level/ concentration	Results	Reference
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	Chlorothalonil	Non-activation 0.16, 0.8, 4.0, 8.0, & 16.0 µg/plate. Activation 0.5, 2.5, 12.5, 25, & 50 µg/plate	Negative	Jones et al., 1984
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,5,6-Trichloro-3-cyanobenzamide	20, 100, 500, 1000, & 2000 µg/plate (for both activation & non-activation	Negative	Jones et al., 1985j

(Table 8 continued)

Test system (Ames test)	Test substance	Dose level/ concentration	Results	Reference
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,4,5,6-Tetrachloro-3-cyanobenzamide	Non-activation 6, 30, 150, 300, & 600 µg/plate. Activation 10, 50, 250, 500, & 1000 µg/plate	Negative	Jones et al., 1985k
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,5,6-Trichloro-4-hydroxy-3-cyanobenzamide	Non-activation 20, 100, 400, 800, & 2000 µg/plate. Activation 40, 400, 1000, 3000, & 6000 µg/plate	Negative	Jones et al. 1985l
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,3,5,6-Tetrachlorobenzonitrile	20, 100, 500, 1000, & 2000 µg/plate (for both activation & non-activation)	Negative	Jones et al. 1985d
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,4,5,6-Tetrachlorobenzamide	100, 500, 2500, 5000 & 10,000 µg/plate (for both activation & non-activation)	Negative	Jones et al. 1985e
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,4,5-Trichloro-3-cyanobenzamide	20, 100, 500, 1000, & 2000 µg/plate (for both activation & non-activation)	Negative	Jones et al. 1985f
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,5,6-Trichloro-4-thioisophthalonitrile	Non-activation 250, 400, 630, 1000, 1600, & 2500 µg/plate. Activation 400, 630, 1000, 1600, 2000, 2500, 3000, 4000, & 5000 µg/plate	Negative	Jones et al. 1985g

(Table 8 continued)

Test system (Ames test)	Test substance	Dose level/ concentration	Results	Reference
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,5,6-Trichloro-3-carboxybenzamide	100, 500, 2500, 5000, & 10,000 µg/plate (for both activation & non-activation)	Negative	Jones et al., 1985h
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,4,5-Trichloroisophthalonitrile	0.5, 2.5, 10, 35, & 70 µg/plate (for both activation & non-activation)	Negative	Jones et al., 1985i
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	2,3,5,6-Tetrachloroterphthalonitrile	4, 20, 100, 200, & 400 µg/plate (for both activation & non-activation)	Negative	Jones et al., 1985a
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	Isophthalonitrile	40, 200, 1000, 2000, & 4000 µg/plate (for both activation & non-activation)	Negative	Jones et al., 1985b
S. typhimurium TA98, TA100, TA1535, TA1537, & TA1538 W/S9 and W/O S9*	Pentachlorobenzonitrile	10, 50, 250, 500, & 1000 µg/plate (for both activation & non-activation)	Negative	Jones et al. 1985c

* The S9 fraction was prepared from rat kidney homogenate

the proximal convoluted tubule in males at all levels (e.g ≥ 40 mg/kg) and in females at ≥ 175 mg/kg b.w. Cytoplasmic inclusion bodies or "hyaline droplets" were also identified using neutral red stain in both sexes at all doses. The angular material (cytoplasmic inclusions), which represent an analogous finding to the E.M. evaluation, was seen only in males (Trump et al., 1985; Busey 1985a).

COMMENTS

Chlorothalonil was evaluated by the Joint Meetings in 197-, 197-, 1979, 1981 and 1983 and additional metabolism data and a rat carcinogenicity study were requested. Chlorothalonil has also been evaluated by IARC (1-83) and

00773

DP2 3 4-21-84

DATA EVALUATION REPORT

STUDY: Rat Two Year Dietary Exposure[1]

LABORATORY: International Research and Development Corporation, Mattawn, MI.

STUDY NUMBER & DATE: DTX-80-0016

ACCESSION NUMBER: 071527

MRID:

MATERIAL TESTED: DS 3701 (100%)

ANIMALS: Charles River CD Rats

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

"Groups of Sprague-Dawley CD rats (75 males and 75 females/group) were administered 4-hydroxy-2,5,6-trichloroisophthalonitrile in the diet at dosage levels of 0, 0.5 and 3 mg/kg/day for 104 weeks. Original dosage levels of 15 and 30 mg/kg/day were reduced at week 30 to 10 and 20 mg/kg/day, respectively, because of poor survival and anemia. Animals were observed daily for mortality and gross signs of toxicity/general appearance. Individual body weights and food consumption were measured regularly during the study. Clinical laboratory studies were performed periodically throughout the study on 10 rats/sex/group at six month intervals. Ophthalmological examinations and urinalyses were performed routinely, and feces were collected and examined to evaluate the observed anemia. Interim sacrifices were performed after 1 year on 10 rats/sex in all groups except for the high dose animals which were all necropsied. Terminal necropsies were performed on all surviving animals after 2 years, selected organs weighed, and complete histopathological examinations conducted.

RESULTS:

Pale skin and eyes were evident for the first 30 weeks in high dose males and females with similar but less marked findings in the 15 mg/kg group. Mortality was significantly increased in the 30 mg/kg group males and females, and in the 15 mg/kg group females. The high dose group was sacrificed at 12 months after the dose level had been reduced to 20 mg/kg at week 30. Decreasing the 15 mg/kg/day dose level at week 30 to 10 mg/kg similarly improved the survivability, which was comparable to controls for the remainder of the study. Body weight was

---

1 From: Jaeger, R.B., et al. WHO/FAO Report, 1983, Geneva.

reduced in the 10/15 and 20/30 mg/kg males and females throughout the study, even after reduction of doses. Food consumption was unremarkable except for decreases in 10/15 and 20/30 mg/kg females and 20/30 mg/kg males, consistent with decreased body weights and increased mortality during the first 30 weeks. There were similar decreases in total serum protein, albumin, globulin, and cholesterol in 20/30 mg/kg males and females and 10/15 mg/kg females after 6 months. These returned to control levels for the remainder of the study, after doses were reduced to 20 and 10 mg/kg, respectively.

There were significant hemopoietic effects in the 10/15 and 20/30 mg/kg animals, particularly females, during the first 6 months. Evidence of microcytic anemia was provided by reduced RBC counts, hematocrit, hemoglobin, MCV, and MCH with accompanying increases in MCHC, reticulocytes and metarubricytes. Segmented neutrophiles were increased with corresponding decrease in percentage of lymphocytes. Specially stained bone marrow presented evidence of hypocellularity. Mallory's stain of liver tissue revealed an increased iron content (hemosiderin). After 18 and 24 months exposure the 10/15 mg/kg group females continued to present evidence of anemia (decreased Hct, Hgb, MCV, MCH and increased MCHC) with a positive bone marrow response (increased cellularity with a shift to increasing number of immature erythyroid cell types and increase number animals with a 1:1 M/E ratio). Prussian Blue staining demonstrated the presence of hemosiderin in the 10 mg/kg males and females, not considered significant at 3 mg/kg. After 24 months exposure there were decreased serum potassium levels in all dosed females. Urinalyses and examination for fecal occult blood were unremarkable, except for increased urine volume at 6 months in the high dose group animals.

Ophthalmological examination at 6 months revealed increased pale ocular structures and spontaneous hemorrhage in high dose male and female animals. At 24 months there were increased numbers of dilated pupils (not responding to light) and increased bilateral cataract disease in high dose males.

Comparison of selected organ weights demonstrated decreased absolute organ weights for kidney, heart and brain in high dose males with no significant relative organ-to-body weight changes. High dose females had decreased absolute kidney and heart weights with no relative weight changes except for spleen and brain. Microscopic examination failed to confirm any compound related effects on these organs. There were no significant compound related non-neoplastic organ changes except for hemosiderin in the liver of high dose females and hemorrhage in CNS tissues, hypocellular bone marrow and post-mortem congestion of lymph in high dose male and female rats.

Examination of tissues/organs for neoplastic changes did not indicate any compound related effects at any level tested.

Data presented in this study demonstrate that the metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile, is without adverse effects on male and female rats at levels up to and including 3 mg/kg/day for 2 years (McGee et al., 1983).

CORE Rating: Guideline.

DATE: January 25, 1978

SUBJECT: Review by Eleanor L. Long, M.D., Pathologist, U.S. Environmental Protection Agency (TOX, RD, CPP), of 78-Week Feeding Study in Rats on DAC-3701 (4-Hydroxy Metabolite of Chlorothalonil [Daconil])

Note. This study was previously reviewed by Mr. Robert Ceberly of TOX. It was done by Bio/Tox Research Laboratories, Inc., and the pathologist was John F. Ferrell, D.V.M., of Experimental Pathology Laboratories, Inc.

TABLE I. MISCELLANEOUS DATA FROM 78-WEEK RAT FEEDING STUDY ON DAC-3701

PPM Fed	Group	Rats Started		78-Week Survival				Mean Terminal Weight in Grams		Microscopic Examination	
				Males		Females					
		M	F	No.	%	No.	%	Males	Females	M	F
0	I	30	30	26	87	23	77	618	371	15	17
10	II	30	30	23	77	25	83	554	346	12	22
50	III	30	30	26	87	24	80	620	358	14	22
100	IV	30	26	20	67	24	92	610	359	12	23
200	V	27	17	18	67	14	82	532	319	14	15

Methods.

A 78-week feeding study using Spartan strain, Sprague-Dawley-derived rats (from Spartan Research Animals, Haslett, Michigan) was done on DAC-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile, the 4-hydroxy metabolite or major metabolite of chlorothalonil). This study was required as previous metabolism studies had shown that this metabolite may be found in milk in significant amounts when the parent compound is ingested by the cow due to soil degradation resulting in deposition on plants but that neither the rat nor the dog had been adequately exposed to it in the numerous subacute and chronic toxicity tests already run on chlorothalonil, as in both species almost all the parent compound fed is excreted unchanged in the feces. Animals used were the F1 generation of a 3-generation reproduction study (Bio/Tox 21-251A) at the same dosage levels, 0, 10, 50, 100, and 200 ppm, the groups corresponding to parental groups. As Table I shows, 30 rats of each sex per dosage level were started on the study, except for 27 males on 200 ppm, and 26 and 17 females on 100 and 200 ppm, respectively, the lower numbers being attributable to the reduced numbers of offspring available at these levels. The study was terminated after 78 weeks of treatment when the average rat age was 84.7 weeks. Observations and laboratory tests during life included body weight, survival, food consumption, any clinical abnormalities, hematology (hematocrit, hemoglobin, erythrocyte count, and total and differential leukocyte count), blood chemistry (sodium, potassium, chloride, glucose, BUN, bilirubin, alkaline phosphatase, SGPT, and SGOT), and urinalysis. All rats were autopsied and liver, kidneys, testes with epididymides, heart, spleen, thyroid, and adrenals were weighed. Microscopic examination was performed on (1) pituitary, thyroid, lung, heart, liver, spleen, kidneys, adrenals, stomach, small and large intestine, pancreas, mesenteric lymph node, bladder, testes, ovaries, sternum or femur), and any unusual lesion from at least 10/sex/group among survivors, (2) the organs just listed in all rats with definite or suspected neoplasms, and (3) selected lesions from non-survivors.

Effects.

Effects of treatment were (1) decreased survival in males at 100 and 200 ppm (20/30 or 67% and 18/27 or 67% at 100 and 200 ppm, respectively, versus 26/30 or 87% for the controls), and (2) roughening of the fur and irritability at the highest dose. While other sporadic and inconsistent changes, including corneal ulcers, occurred in all 5 groups, control as well as treated, there were no other changes which were clearly related to the administration of DAC-3701. Terminal body weights in both sexes and testicular weights in the males were lower at the high dose than in the controls. However, this was attributed by the company to the fact that the high dose animals were smaller and weighed less at the beginning of the study, a reasonable view, especially since the rate of growth in both sexes was actually greater at this level than in the controls. Though the parent compound produced serious changes in the kidneys at high and intermediate levels of dosage, there was no indication in this study of any effect of the 4-hydroxy metabolite on this organ, as there was no more renal disease (apparently chronic nephritis [called by some "chronic nephrosis"], the most common renal disease in old rats, from the descriptions) in treated than in control animals. Two tables accompanying this review show the incidence of all the different types of tumors which were reported; neither shows any evidence of a neoplastic effect. Moreover, no renal tumors were reported in any animal.

Evaluation of Study.

This study was terminated after only 78 weeks of treatment, though the total time of exposure, according to some of the data, may be considered to have been actually 87 weeks, if the in utero and pre-weaning periods are included. Unfortunately, there is some confusion concerning the true time of exposure. I quote a note in Mr. Coberly's review of 1-12-77 which would indicate that the total exposure period, counting the in utero weeks, is in fact 88 to 90 weeks. "On 12-2-76 Dr. Budny (of Diamond Shamrock) informed me by letter of the time table. According to his dates the rats appear to have been exposed to the chemical for from 85 to 87 weeks. This does not include the in utero exposure period of 21 days." While no evidence of oncogenesis was found, none of these periods is long enough to completely rule out an oncogenic effect. Also, microscopic examination was inadequate to eliminate the possibility of such an effect, as less than half the rats in 4 of the 5 groups were so examined. On the other hand, the study can probably be considered long enough and otherwise adequate to satisfy the requirements for chronic toxicity, though here again more complete microscopic study would have been desirable. For chronic toxicity the no-effect level is 50 ppm.

Eleanor L. Lee

TABLE II

78 WEEK FEEDING STUDY [redacted] RATS (SPARTAN, SPRAGUE-DAWLEY DERIVED) ON DAC-3711: NEOPLASMS AND PRENEOPLASTIC LESIONS

Histopathologic diagnoses are by John F. Gerrell, D.V.M., Experimental Pathology Laboratories, Inc.

Group IA. Male Controls.

Rat Number	Wks. on Test	Organ	Histopathologic Diagnosis
1-1#1	78	Adrenal	Pheochromocytoma
1-1#2	78	Thyroid	Parafollicular hyperplasia
1-1#5	78	Adrenal	Cortical hyperplasia
2-1#1	78	Pituitary	Chromophobe hyperplasia
2-1#2	78	Adrenal Thyroid	Cortical hyperplasia Parafollicular adenoma
2-1#4	78	Lymph node Adrenal	Angioma Cortical hyperplasia
2-1#5	78	Thyroid Adrenal	Parafollicular hyperplasia Cortical hyperplasia
2-2#4	78	Adrenal	Cortical hyperplasia
2-2#5	78	Heart, adrenal	Sarcoma
3-1#3	78	Pituitary	Chromophobe hyperplasia
3-2#4	78	Pituitary Lymph node	Chromophobe hyperplasia Angioma
3-1#1	Under 78	Hind leg	Undifferentiated sarcoma with metastases to lung
		Liver	Bile duct adenoma
3-2#5	Under 78	Tissue mass Adrenal	Fibroma Pheochromocytoma

Group IIA. Males on 10 PPM.

6-1#1	78	Pituitary Thyroid	Chromophobe adenoma Parafollicular hyperplasia
6-2#2	78	Thyroid Tissue mass	Parafollicular hyperplasia Fibroma
6-2#4	78	Adrenal	Cortical hyperplasia
7-1#5	78	Adrenal	Cortical hyperplasia
7-2#3	78	Thyroid Adrenal	Parafollicular hyperplasia Cortical hyperplasia
7-2#4	78	Thyroid	Parafollicular hyperplasia
8-1#4	78	Skin	Squamous cell carcinoma
7-2#5	Under 78	Mammary gland	Fibroadenoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group IIIA. Males on 50 PPM.

Rat Number	Wks. on Test	Organ	Histopathologic Diagnosis
11-1#2	78	Adrenal Tissue mass	Pheochromocytoma Neurofibroma
11-2#1	78	Pituitary	Chromophobe adenoma
11-2#4	78	Pituitary Thyroid Tissue mass	Chromophobe adenoma Parafollicular hyperplasia Carcinoma
12-1#5	78	Thyroid	Parafollicular hyperplasia
12-2#3	78	Thyroid	Parafollicular carcinoma
12-2#4	78	Thyroid Skin Tissue mass	Parafollicular hyperplasia Squamous cell carcinoma Lymphangioma
13-1#2	78	Thyroid Tissue mass	Parafollicular hyperplasia Fibroma
13-1#4	78	Pituitary Skin	Chromophobe hyperplasia Sebaceous cystadenoma
13-1#5	78	Thyroid	Parafollicular hyperplasia

Group IVA. Males on 100 PPM.

16-1#2	78	Tissue mass	Fibroma
17-2#4	78	Tissue mass	Sarcoma
18-1#3	78	Adrenal	Cortical hyperplasia
16-1#4	Below 78	Pancreas	Islet cell adenoma
17-1#4	Below 78	Tissue mass	Sarcoma
17-1#5	Below 78	Tissue mass	Fibroma
18-2#1	Below 78	Tissue mass	Schwannoma

Group VA. Males on 200 PPM.

21-1#2	78	Tissue mass Thyroid Pituitary Adrenal	Fibroma Parafollicular hyperplasia Chromophobe hyperplasia Cortical hyperplasia
21-1#4	78	Thyroid Adrenal	Parafollicular adenoma Pheochromocytoma
21-2#4	78	Thyroid	Parafollicular carcinoma
22-2#1	78	Thyroid	Parafollicular hyperplasia
22-2#3	78	Pituitary Thyroid	Chromophobe adenoma Parafollicular adenoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group VA. Males on 200 PPM. (continued)

Rat Number	Wks on Test	Organ	Histopathologic Diagnosis
22-2#4	78	Pituitary Thyroid	Chromophobe adenoma Parafollicular hyperplasia
23-1#1	78	Adrenal	Cortical hyperplasia
23-2#4	78	Pituitary	Chromophobe adenoma
21-1#1	Below 78	Pituitary	Chromophobe adenoma
21-2#1	Below 78	Tissue mass	Sarcoma
22-1#1	Below 78	Mammary gland	Fibroadenoma
23-2#5	Below 78	Parathyroid	Adenoma

Group IB. Female Controls.

4-1#3	78	Mammary gland	Fibroadenoma
4-1#4	78	Pituitary	Chromophobe adenoma
4-1#5	78	Mammary gland	Adenoma
4-2#1	78	Thyroid Mammary gland	Parafollicular adenoma Fibroadenoma
4-2#2	78	Pituitary Thyroid	Chromophobe adenoma Parafollicular carcinoma
4-2#5	78	Mammary gland	Fibroadenoma
4-2#8	78	Thyroid Pituitary Adrenal Mammary gland	Parafollicular hyperplasia, [illegible] Chromophobe adenoma Cortical hyperplasia, minimal Fibroadenoma
5-1#2	78	Mammary gland Thyroid	Fibroadenoma Parafollicular hyperplasia
5-1#6	78	Mammary gland	Fibroadenoma
5-2#1	78	Adrenal Thyroid	Cortical hyperplasia Parafollicular adenoma
5-2#2	78	Mammary gland Thyroid	Adenoma Parafollicular hyperplasia, [illegible]
5-2#3	78	Adrenal	Cortical hyperplasia
5-2#4	78	Mammary gland Adrenal	Adenoma Cortical hyperplasia
5-2#7	78	Mammary gland Pituitary	Fibroadenoma Chromophobe hyperplasia
4-2#3	Below 78	Mammary gland	Fibroadenoma
4-2#6	Below 78	Mammary gland	Fibroadenoma
4-2#7	Below 78	Mammary gland	Fibroadenoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group IIB. Females on 10 PPM.

Rat Number	Weeks on Test	Organ	Histopathologic Diagnosis
9-1#3	78	Mammary gland	Adenoma
9-1#4	78	Mammary gland	Fibroadenoma
9-1#5	78	Mammary gland Pituitary	Fibroadenoma Chromophobe adenoma
9-1#6	78	Thyroid Pituitary	Parafollicular adenoma Chromophobe carcinoma
9-1#7	78	Adrenal Pituitary Large intestine Mammary gland	Cortical hyperplasia Chromophobe adenoma Lymphosarcoma Fibroadenoma
9-2#1	78	Mammary gland Mammary gland	Adenoma Fibroadenoma
9-2#2	78	Thyroid	Parafollicular adenoma
9-2#4	78	Mammary gland Thyroid	Adenoma Parafollicular carcinoma
9-2#5	78	Thyroid Pituitary	Parafollicular adenoma Chromophobe adenoma
9-2#7	78	Mammary gland	Fibroadenoma
9-2#8	78	Mammary gland Pituitary	Fibroadenoma Chromophobe adenoma
10-1#2	78	Mammary gland Thyroid	Fibroadenoma Parafollicular adenoma
10-1#3	78	Mammary gland	Adenocarcinoma
10-1#7	78	Thyroid	Parafollicular adenoma
10-2#1	78	Pituitary Mammary gland Mammary gland	Chromophobe adenoma Fibroadenoma Adenoma
10-2#2	78	Mammary gland	Fibroadenoma
10-2#3	78	Mammary gland	Fibroadenoma
10-2#4	78	Mammary gland Adrenal	Adenoma Cortical hyperplasia
10-2#6	78	Adrenal	Cortical hyperplasia
10-2#7	78	Pituitary	Chromophobe adenoma
9-1#2	Below 78	Mammary gland	Fibroadenoma
10-1#4	Below 78	Mammary gland	Fibroadenoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group IIIB. Females on 50 PPM.

Rat Number	Weeks on Test	Organ	Histopathologic Diagnosis
14-1#2	78	Thyroid Pituitary Adrenal Mammary gland	Parafollicular hyperplasia Chromophobe hyperplasia Cortical hyperplasia Fibroadenoma
14-1#3	78	Mammary gland	Fibroadenoma
14-1#7	78	Pituitary	Chromophobe adenoma
14-2#2	78	Thyroid Tissue mass	Parafollicular adenoma No micro.(hence not in tumor tabl
14-2#3	78	Mammary gland Pituitary	Adenocarcinoma Chromophobe adenoma
14-2#_	78	Mammary gland Thyroid	Fibroadenoma Parafollicular adenoma
14-2#5	78	Pituitary	Chromophobe adenoma
14-2#7	78	Pituitary	Chromophobe adenoma
15-1#_	78	Mammary gland Thyroid	Fibroadenoma Parafollicular adenoma
15-1#5	78	Mammary gland Mammary gland Liver	Fibroadenoma Adenoma Nodular hyperplasia
15-1#7	78	Mammary gland	Adenoma
15-2#2	78	Mammary gland Liver	Fibroadenoma Nodular hyperplasia
15-2#3	78	Liver Thyroid Mammary gland	Nodular hyperplasia Parafollicular hyperplasia, mini Fibroadenoma
15-2#5	78	Mammary gland Pituitary	Fibroadenoma Chromophobe adenoma
14-1#_	Below 78	Cerv.mass, probably mammary	Adenocarcinoma
15-1#1	Below 78	Mammary gland	Fibroadenoma
15-1#1	Below 78	Pituitary	Chromophobe adenoma
15-2#1	Below 78	Mammary gland	Fibroadenoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group IVB. Females on 100 PPM.

Rat Number	Weeks on Test	Organ	Histopathologic Diagnosis
19-1#1	78	Mammary gland	Fibroadenoma
19-1#2	78	Thyroid Ovary	Parafollicular hyperplasia Cystadenoma
19-1#3	78	Mammary gland	Fibroadenoma
19-1#4	78	Mammary gland Mammary gland	Adenoma Fibroadenoma
19-1#5	78	Mammary gland	Adenoma
19-2#1	78	Tissue mass	Lymphosarcoma
19-2#3	78	Pituitary	Chromophobe adenoma
19-2#4	78	Pituitary	Chromophobe hyperplasia
19-2#5	78	Pituitary Mammary gland	Chromophobe hyperplasia Fibroadenoma
19-2#6	78	Mammary gland	Fibroadenoma
19-2#7	78	Adrenal Mammary gland	Cortical hyperplasia Fibroadenoma
20-1#2	78	Mammary gland Mammary gland Thyroid Liver	Adenoma Fibroadenoma Parafollicular adenoma Nodular hyperplasia
20-1#4	78	Thyroid Mammary gland	Parafollicular hyperplasia Adenoma
20-1#6	78	Thyroid Mammary gland	Parafollicular carcinoma Fibroadenoma
20-2#2	78	Mammary gland Mammary gland Thyroid	Adenoma Fibroadenoma Parafollicular hyperplasia, minimal
20-2#5	78	Ovary	Cystadenoma
20-2#6	78	Pituitary	Chromophobe hyperplasia
19-2#2	Below 78	Uterus	Endometrial stromal sarcoma

RATS WITH NEOPLASMS AND PRENEOPLASTIC LESIONS IN 78 WEEK FEEDING STUDY ON DAC-3701 (continued)

Group VB. Females on 200 PPM.

Rat Number	Weeks on Test	Organ	Histopathologic Diagnosis
24-1#1	78	Mammary gland Adrenal	Fibroadenoma Cortical hyperplasia
24-1#2	78	Mammary gland	Fibroadenoma
24-1#3	78	Pituitary	Chromophobe adenoma
24-1#4	78	Mammary gland	Fibroadenoma
24-1#5	78	Pituitary Thyroid	Chromophobe adenoma Parafollicular adenoma
24-1#6	78	Thyroid	Parafollicular carcinoma
24-1#7	78	Pituitary	Chromophobe hyperplasia
24-1#8	78	Mammary gland Thyroid	Fibroadenoma Parafollicular adenoma
24-2#1	78	Pituitary	Chromophobe adenoma
24-2#2	78	Pituitary	Chromophobe adenoma
24-2#5	78	Pituitary	Chromophobe hyperplasia
24-2#6	78	Thyroid Pituitary Adrenal Mammary gland	Parafollicular carcinoma Chromophobe hyperplasia Cortical hyperplasia Fibroadenoma
24-2#7	78	Thyroid Pituitary Adrenal Mammary gland	Parafollicular adenoma Chromophobe adenoma Cortical hyperplasia Fibroadenoma
24-2#3	Below 78	Mammary gland	Adenoma

TABLE III

TUMORS AND PRENEOPLASTIC HYPERPLASIAS IN 78 WEEK RAT FEEDING STUDY ON DAC-3701

Organ and Tumor (or Hyperplasia)	Animals With Tumor Or Hyperplasia — Dosage in PPM: Males 0	10	50	100	200	Females 0	10	50	100	200
Mammary gland, fibroadenoma	0	1	0	0	1	10	12	10	9	6
Mammary gland, adenoma	0	0	0	0	0	3	5	2	5	1
Mammary gland, adenocarcinoma	0	0	0	0	0	0	1	2	0	0
Pituitary, chromophobe hyperplasia	3	0	1	0	1	1	0	1	3	1
Pituitary, chromophobe adenoma	0	1	2	0	4	3	6	6	1	5
Pituitary, chromophobe carcinoma	0	0	0	0	0	0	1	0	0	0
Thyroid, parafollicular hyperplasia	2	4	5	0	3	3	0	2	3	0
Thyroid, parafollicular adenoma	1	0	0	0	2	2	5	3	1	2
Thyroid, parafollicular carcinoma	0	0	1	0	1	1	1	0	1	3
Adrenal, cortical hyperplasia	5	3	0	1	2	4	3	1	1	0
Adrenal, pheochromocytoma	2	0	1	0	1	0	0	0	0	0
Parathyroid, adenoma	0	0	0	0	1	0	0	0	0	0
Pancreas, islet cell adenoma	0	0	0	1	0	0	0	0	0	0
Liver, nodular hyperplasia	0	0	0	0	0	0	0	3	1	0
Liver, bile duct adenoma	1	0	0	0	0	0	0	0	0	0
Ovary, cystadenoma	–	–	–	–	–	0	0	0	2	0
Uterus, endometrial stromal sarcoma	–	–	–	–	–	0	0	0	1	0
Large intestine, lymphosarcoma	0	0	0	0	0	0	1	0	0	0
Tissue mass (?lymph node), lymphosarcoma	0	0	0	0	0	0	0	0	1	0
Lymph node, angioma	2	0	1	0	0	0	0	0	0	0
Skin, sebaceous cystadenoma	0	0	1	0	0	0	0	0	0	0
Skin, squamous cell carcinoma	0	1	1	0	0	0	0	0	0	0
Tissue mass (?subcutaneum), fibroma	1	1	1	2	1	0	0	0	0	0
Tissue mass (?subcutaneum), neurofibroma	0	0	1	0	0	0	0	0	0	0
Tissue mass (?subcutaneum), Schwannoma	0	0	0	1	0	0	0	0	0	0
Tissue mass (?subcutaneum), sarcoma	1	0	0	2	1	0	0	0	0	0
Heart and adrenal, sarcoma	1	0	0	0	1	0	0	0	0	0
Tissue mass, carcinoma	0	0	1	0	0	0	0	0	0	0
Number of rats started on study	30	30	30	30	30	30	30	30	30	30
Total rats with tumor	7	4	7	6	10	16	22	18	16	12
Rats with malignant tumor	2	1	3	2	2	1	4	2	3	2
Rats with multiple tumors	2	0	3	0	2	3	9	5	1	1
Rats with pituitary chrom.hyperpl. or tumor	3	1	3	0	5	4	7	7	4	5
Rats with thyroid parafol. hyperpl.or tumor	3	4	6	0	5	5	6	5	4	3
Rats examined microscopically	15	12	14	12	14	17	22	22	20	14
Rats with pituitary micros. examined	12	11	13	9	12	17	21	18	14	11
Rats with thyroid micros. examined	13	12	14	11	14	17	20	20	16	11
Rats surviving 78 weeks	26	23	26	20	18	23	25	24	21	14

DATA EVALUATION REPORT

STUDY: Chronic Mouse Dietary Study

LABORATORY: T.R. Evans Research Center, Painesville, OH

STUDY NUMBER & DATE: 098-5TX-78-0024-001 2/17/82

ACCESSION NUMBER: 071531

MRID:

MATERIAL TESTED: DS-3701 (4-hydroxy - lite) 99.6% pure.

ANIMALS: CD-1 mice

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

ROUTE OF ADMINSTRATION: Dietary. Prepared fresh weekly. Analyzed for DS-3701.

LEVELS OFFERED: 0, 375, 750 and 1500 ppm.

SCHEME OF ADMINSTRATION:

Group	Dose (ppm)	No. Males	No. Females
I	0	60	60
II	375	60	60
III	750	60	60
IV	1500	60	60

OBSERVATIONS:

- 2X daily for mortality and signs of toxicity. Detailed physical examination weekly.
- Food consumption and body weights obtained weekly through week 14; then biweekly through week 26. Monthly thereafter.
- Diets were prepared fresh weekly. Samples were then obtained for subsequent analysis for test material.

BIOLIGICAL MEASUREMENTS:

Hematology at 12 and 18 months and at termination:

Hemoglobin
Hematocrit
Total RBCs and WBCs
Differentials

Added at termination: Bone marrow differentials and reticulocytes.

POST MORTEM EXAMINATION:

- All animals dead or moribund during the study were necropsied. All surviving males were terminated at 24 months. All surviving females in the low and middle doses and 10 control females were necropsied at 20 months. Surviving females in the high dose and all remaining control females were necropsied at 22 months.

- The following organs and tissues were preserved, prepared and examined histologically:

Brain *	Thyroid Gl.	Parathyroid Gl.
Trachea	Esophagus	Lungs
Heart *	Pancreas	Salivary Gl.
Stomach	Duodenum	Ileum
Colon	Liver *	Gallbladder
Spleen *	Kidney *	Gonads *
Epididymus	Prostate	Seminal Vesicle
Uterus	Vagina	Eye
Pituitary	Ovary	Lymph nodess
Spinal Cord	Adrenal Gl.	Ureter
Urethra	Aorta	Muscle
Thymus	Peripheral Nerve	Skin
Mammary Gl.	Bone & Marrow	

* organ weights obtained

NOTE:

We combined all reported neoplasms, both malignant and benign, to construct incidence tables for the lungs, the liver, the kidneys and miscellaneous organs and tissues. Every animal bearing one or more neoplasms was counted as one "hit"; thus, an animal with more than one neoplasm counted only once. Table I shows the distribution of neoplastic response in this assay.

RESULTS:

OBSERVATIONS:

- Body weights were significantly lower overall for the high dose males and females.
- Food consumption in the low and middles dose groups was about the same as that for the controls. Food consumption in the high dose male and female groups was increased significantly.
- Actual Dietary Assay

Group	Nominal Concentration (ppm)	Mean (ppm for all analyses)
I	0	0
II	375	384
III	750	780
IV	1500	1552

BIOLOGICAL MEASUREMENTS:

Hematology

Hematological evaluation showed reduced RBCs at twelve months in low and high dose males and in all female treatment groups. At eighteen months RBCs were significantly reduced in the middle dose males and in the middle and high dose females. WBCs were significantly increased in the high dose terminal (22 months) females.

Hemoglobin and hematocrit were not remarkable for toxic effect of DS-3701.

There was a reduction in RBCs at termination (24 months) in the high dose males. WBCs were significantly increased in the middle dose terminal males.

Bone and bone marrow values were not conclusively affected, nor were the total and differential leukocyte counts at the 12 and 18 month intervals or at termination.

POST MORTEM EXAMINATION:

Body Weights and Organ Weights

Liver-to-body weight ratios were significantly decreased in the 750 ppm females sacrificed at 20 months and were significantly increased in the 1500 ppm females scarificed at 22 months. Liver-to-body weight ratios were significantly increased in all treated group males sacrificed at 24 months.

Spleen-to-body weight ratios were significantly increased in the 750 ppm females sacrificed at 20 months.

Brain-to-body weight ratios were slightly, but significantly, increased in the 1500 ppm females sacrificed at 22 months.

DISCUSSION:

Hematology

Overall, apart from the finding that there were increased RBC values in treated female mice at all dose levels at the 12 month test period, we consider that variations noted in the hematological parameters studies were within normal limits. For the twelve month female RBCs, this was confirmed as a treatment-related effect in the 18 month female which showed significant reductions in the middle and high dose treatment groups. Therefore, the observed NOEL for this parameter is less than 375 ppm in the diet.

Organ and Body Weights

We consider the finding of reduced liver-to-body weight ratios in all treatment group males to be compound related; therefore, the NOEL for this parameter is less than 375 ppm in the diet.

Histopathology

Table I summarizes Toxicology Branch's evaluation of the neoplasms reported in the individual animal necropsy reports. The great majority of neoplasms were confined to the lungs, liver and kidney and were composed of benign (adenoma and hepatoma) and carcinogenic (lymphosarcoma; hepatocellular carcinomas, etc.) tumors. All benign and malignant neoplasms were considered to be tumors within the definition of the Science Advisory Panel (SAP): "...With regard to lung tumors in CD-1 mice, the Panel agrees that the data for adenomas and carcinomas should be combined..." (Gray, 1983).

With the exception of the low dose males, dietary challenge with DS-3701 in CD-1 mice resulted in decreasing overall tumor incidence with increasing dose. The difference in tumor incidence between the control males and the low dose males is not considered to be significant when taking into acount the lower tumor incidences in the middle and high dose groups.

Examination of the tumor response of individual animals failed to reveal a dose-related effect of DS-3701 on specific carcinogenic lesions. In males the incidence of benign vs. malignant lesions was roughly 1:1 in control and treatment group males in the lungs and liver. In the females the benign lesions occured more frequently in those dose groups where malignant lesions were noted.

There was no dose-related occurrence of animals bearing more than two specific lesions in either males or females at any dose tested.

CONCLUSIONS:

The systemic NOEL for this study is less than 375 ppm based on reduced liver-to-body weight ratios in males.

Overall, we conclude that DS-3701 does not induce tumors in CD-1 male or female mice when offered in the diet for the lifetime of the animal at levels up to 1500 ppm.

CORE RATING:

1. For systemic effect: Supplemental - no NOEL demonstrated.

2. For tumorigenic response: Guideline.

TABLE 1

Combined Neoplasms per Sex per Dose Level

FEMALES

Site of Neoplasm	0 ppm	Incidence	375 ppm	Incidence	750 ppm	Incidence	1500 ppm	Incidence
Liver	2(44)*	4.5 %	2(57)	3.5 %	0(48)	0.0 %	0(56)	0.0 %
Lungs	9(59)	15.3 %	7(58)	12.1 %	2(57)	3.5 %	2(59)	3.4 %
Kidney	1(44)	2.3 %	0(59)	0.0 %	0(52)	0.0 %	0(58)	0.0 %
Other	1(43)	2.3 %	2(59)	3.3 %	5(57)	8.8 %	1(58)	1.7 %
Total Lesions	13(59**	22.0 %	11(59)	18.6 %	7(57)	13.3 %	3(59)	5.1 %

MALES

Liver	19(58)	32.8 %	15(57)	26.3 %	10(52)	19.2 %	4(54)	7.4 %
Lung	4(58)	6.9 %	11(58)	19.0 %	6(57)	10.5 %	9(57)	15.9 %
Kidney	1(58)	1.7 %	1(57)	1.8 %	2(54)	3.7 %	0(57)	0.0 %
Other	1(58)	1.7 %	1(57)	1.8 %	3(57)	5.3 %	2(57)	3.5 %
Total Lesions	25(58)	43.1 %	28(58)	48.3 %	21(57)	36.8 %	15(57)	26.3 %

* Figures in parentheses represent that number of organs actually examined and reported on the individual necropsy reports.

** Figures in parentheses in Total Lesions line are the maximum number of animals examined.

Miscellaneous: 10 documents, 5/17/85 - 11/03/88.

11/3/88

CC7713

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

3 NOV 1988

OFFICE OF
PESTICIDES AND TOXIC SUBST

MEMORANDUM

SUBJECT: PP# 5F3183. Chlorothalonil on Cherries. Evaluation of Amendment dated June 22, 1988. MRID Nos. 406848-00 thru 02. DEB No. 4193.

FROM: Stephanie H. Willett, Chemist
Tolerance Petition Section 2
Dietary Exposure Branch
Health Effects Division (TS-769C)

TO: Lois Rossi, PM 21
Registration Division (TS-769C)
and
Toxicology Branch, Herbicide-Fungicide Support
Health Effects Division (TS-769C)

THRU: Charles L. Trichilo, Ph.D., Chief
Dietary Exposure Branch
Health Effects Division (TS-769C)

Background

Fermenta Plant Protection Company, formerly SDS Biotech Corporation and Diamond Shamrock Corporation, proposed to increase the 0.5 ppm tolerance for the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) and its metabolite 4-hydroxychlorothalonil (4-hydroxy-2,5,6-trichloroisophthalonitrile) in or on cherries (sweet and tart) to 3.0 ppm in the subject petition. The petition was placed in reject status pending resolution of several deficiencies cited in the initial review by DEB (see memo of M.P. Firestone dated March 7, 1985).

Chlorothalonil tolerances are established on several RACs in 40 CFR 180.275 at levels ranging from 0.05 to 15 ppm.

The Residue Chemistry Chapter of the Chlorothalonil FRSTR was issued on March 11, 1988.

Summary of Deficiencies That Still Need Resolution

1. A permanent tolerance established on only washed tart cherries is not acceptable to DEB. A tolerance must be high enough to cover residues found in both washed and dry tart cherries. This needs to be reflected in a revised Section F.

2. The proposed use and label directions are not clear. A revised Section B needs to be submitted.

3. A ^{14}C-Chlorothalonil foliar metabolism study is needed on cherries or some other related tree fruit.

4. Residue data are needed on unwashed tart cherries (see DEB's Comments/Conclusions, re: Deficiencies 5a-d that follow in this review).

5. Sample storage information and stability data must be submitted.

Recommendations

DEB recommends against the establishment of a 3.0 ppm tolerance on tart cherries for reasons cited in conclusions 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. Additional metabolism and residue data are needed, as well as revisions to Section B, and possibly Section F.

Conclusions Resulting from the Review of the Present Submission

1. The revised Section F proposing separate permanent tolerances on sweet and sour cherries is acceptable. However, the proposed tolerance level on tart cherries may have to be changed, depending upon the indications of the additional residue data requested.

2. Residue data should be obtained from unwashed cherries so that an established permanent tolerance would cover a worst case situation. Some data from washed cherries may be appropriate for comparison purposes.

3. The proposed use and label directions are not clear. The total number of post shuck split applications that will be allowed should be specified, and a seasonal maximum application rate expressed in lb ai/A/yr should appear on the label. Residue data are needed from trials where the pesticide is applied by both ground and aerial equipment in order to support the proposed use.

4. The petitioner will need to revise Section B/label to include a restriction against grazing treated orchards/groves and cutting cover crops for feed.

5. The petitioner should conduct a metabolism study where ^{14}C-Chlorothalonil is foliar applied to cherries, or some related tree crop. The rate and frequency of application should be sufficiently high to permit adequate identification of residues.

6. Additional analytical methodology may be needed if sufficient levels of other metabolites are found to form in cherries.

7. The petitioner will need to submit additional residue data from unwashed cherries treated at the maximum application rate with at least the maximum number of post shuck split applications specified in the Section B/label.

8. Residue data are typically required for cherries grown in the major cherry growing regions in the US. These are California, Oregon or Washington, Michigan, Utah or Montana or Idaho, and New York or Philadelphia, according to Agricultural Statistics. The petitioner will need to submit additional residue data from field trials conducted in these areas.

9. Storage conditions and intervals should be submitted for all samples. Presently storage data support stability of chlorothalonil and its 4-hydroxy metabolite for 6 months. Additional storage stability data depicting the percent decline in residues of chlorothalonil and its 4-hydroxy metabolite, HCB and PCBN under the storage conditions used and for the same storage period as the field trial samples must be submitted.

10. All questions concerning the nature of the residue in plants have not been adequately addressed. Additional residue data, including storage stability data, on other components may be needed depending on the final outcome of the requested foliar metabolism studies.

11. The proposed US tolerance level and expression are incompatible with Codex.

Present Considerations

This submission is a response to the dietary exposure deficiencies previously cited. The submission consists of revisions to Sections B and F, a plant metabolism study and additional residue data.

The deficiencies will be restated below, followed by the petitioner's response and DEB's comments and conclusions.

Deficiency 1a

The petitioner will need to submit a revised Section F in which a permanent tolerance is proposed.

Petitioner's Response to Deficiency 1a

The petitioner now proposes to retain the tolerance for sweet cherries at 0.5 ppm as presently established in 40 CFR 180.275, and raise the tolerance for tart (sour) cherries to 3.0 ppm in order to support the proposed amended use pattern.

DEB's Comments/Conclusions, re: Deficiency 1a

A tolerance was previously established for chlorothalonil and its primary metabolite on sweet and sour cherries at 0.5 ppm (see PP# 2F2602). This tolerance was based on residues remaining on the fruit after early season applications of chlorothalonil, where the seasonal maximum application was 8.4 lb ai/A and the PHI is about 55 days.

The revised Section F proposing separate permanent tolerances on sweet and sour cherries is acceptable.

The adequacy of the proposed tolerance level on sour cherries is discussed under deficiencies 3a and 5.

Deficiency 1b

The petitioner should be informed that RCB considers the RAC to be unwashed cherries as they are picked from the tree since cherries can be harvested dry.

Petitioner's Response to Deficiency 1b

The petitioner contends that cherry agricultural practices and processing companies consider the harvesting of tart cherries (e.g. Montmorency) into cold water vats as essential, and only those cherries harvested in this manner are acceptable for removal from the farm. Therefore the petitioner wishes to define tart cherries as washed, which is how they leave the farm gate following normal agricultural harvesting practices (see also notes from meeting with Fermenta, 8/30/88, D. Edwards). The amended use is not intended for sweet cherries.

DEB's Comments/Conclusions, re: Deficiency 1b

DEB reiterates the previous conclusion concerning the present definition of cherries. DEB has no information wherein all (100%) tart cherries will leave the various farms in water vats. For example, if a grower's cherry crop has been damaged by windstorm or some other act of nature, would the grower risk the income from his crop by placing these cherries into water vats where there could be a loss of natural juices, etc.? Chlorothalonil is a systemic fungicide, and washing may have little effect on the residue levels, depending on the length of the treatment period, application intervals, amount of pesticide applied, the PHI and other factors.

Residue data should be obtained from unwashed cherries. Some data from washed cherries may be appropriate for comparison purposes. Some safety evaluations could be based on the lower residue levels. The tolerance, however, will still be set based on residues in/on unwashed cherries (see Section 171-4(c)(2)(iv)(b) of Assessment Guidelines, Subdivision O for guidance). This will cover residues resulting from a worst case situation.

Deficiency 2a

The petitioner will need to submit a revised Section B/label in which a restriction is included which will limit the number of post-shuck-split applications allowed. The petitioner should be informed that the proposed use must be supported by the submitted residue data.

Petitioner's Response to Deficiency 2a

The established use on cherries allows for application of BRAVO 500 at popcorn stage (pink, red or early white bud), a second application at full bloom and a third application at petal fall, all at a rate of 4 1/2 to 8 pints/A (2.3 to 4.2 lb ai/A) to control blossom blight and brown rot. To control cherry leaf spot, a fourth application is allowed at shuck split and post-harvest applications are allowed within 7 days after fruit is removed and also at 10 to 14 days later, all at reduced rates of 4 1/2 to 6 pints/A. Pre-bloom applications for control of leaf curl are also allowed.

The petitioner proposes to amend the present product label to allow additional applications of Bravo 500 (4.17 lb ai/gal), Bravo 720 (6 lb ai/gal) and Bravo 90DG (90% water dispersable granules) only to tart cherries mechanically harvested into water. In addition to the bloom and shuck split applications already specified, applications are to be made after shuck split at 10 to 14 day intervals until 7 days prior to harvest. The spray volume specified for tart cherries is 20 to 300 gallons.

Both ground and aerial applications are permitted. There is a restriction against allowing livestock to graze in treated areas.

DEB's Comments/Conclusions, re: Deficiency 2a

The proposed use and label directions are not clear. The total number of post-shuck-split applications that will be allowed should be specified, and a seasonal maximum application rate expressed in lb ai/A/yr should appear on the label. Residue data are needed from trials where the pesticide is applied by both ground and aerial equipment in order to support the proposed use.

This deficiency is not resolved. The petitioner will need to modify the Section B/label.

Deficiency 2b

RCB does not consider split PHI's (i.e., 7 days for cherries harvested into water and 30 days for cherries not harvested into water) acceptable (see also Conclusion 1b above). Furthermore, water can not remove any systemic residues. The petitioner will need to propose only a single PHI in a revised Section B/label.

Petitioner's Response to Deficiency 2b

The minimum PHI specified on the label is 7 days. The treatment period and application rates will differ for sweet and tart cherries.

DEB's Comments/Conclusions, re:Deficiency 2b

This deficiency is resolved. However, other revisions to Section B/label will be needed (see also deficiencies 2a and 2c).

Deficiency 2c

The revised Section B/label should contain a restriction against grazing treated orchards/groves and cutting cover crops for feed.

Petitioner's Response to Deficiency 2c

None

DEB's Comments/Conclusions, re: Deficiency 2c

The petitioner will need to revise Section B/label to include a restriction against grazing treated orchards/groves and cutting cover crops for feed.

This deficiency has not been resolved.

Deficiency 3a

In RCB's review of PP# 4F3025, the petitioner was advised of the need for a ring-labeled ^{14}C-chlorothalonil foliar-applied apple metabolism study (see M. Kovacs memo of 5/30/84). RCB reiterates the need for such a plant metabolism study in support of the proposed post-shuck split chlorothalonil use on cherries. Thus, the nature of the residue in plants is not adequately understood.

Note: Earlier plant metabolism studies primarily reflect soil applications.

Petitioner's Response to Deficiency 3a

The petitioner has submitted a study on the metabolism of ^{14}C-chlorothalonil on tomatoes (MRID# 406848-01).

In this study, four tomato plants received three weekly applications of ^{14}C-chlorothalonil of approximately 7.35 mg chlorothalonil/plant/application, equivalent to 4.0 pints of Bravo 500 per acre. The radiochemical purity was approximately 98.1%. Tomato fruit and vines were harvested after the third and final application. Vine samples were chopped and ground and stored frozen. Tomato fruit samples were immediately subjected to dichloromethane surface strip and macerated or frozen whole. Tomato fruit was blended with acidified acetone. The radioactive content of the post extraction solids (PES) was determined by combustion. Tomato foliage (vine) samples were analyzed similarly. Radioactivity remaining in the extracted solids was also determined by combustion. After removal of the acetone the aqueous sample was extracted with diethyl ether. The radioactive content of the combined organic phase and the aqueous phase was determined by LSC. A summary of the analyses follows in Table I.

TABLE I. TOTAL ^{14}C-RESIDUES FOUND ON TOMATO FRUIT AND TOMATO VINE SAMPLES AFTER THREE WEEKLY APPLICATIONS OF ^{14}C-CHLOROTHALONIL

Sample Type	Days After Last Application		Total ^{14}C-Residue ppm
Fruit	1	Rep 1	3.3
Fruit		Rep 2	2.9
Fruit	7	Rep 1	0.8
Fruit		Rep 2	0.5
Fruit	14	Rep 1	0.8
Fruit		Rep 2	0.4

Sample Type	Days After Last Application	Total ^{14}C-Residue[1] ppm
Vine	1 Rep 1	20.6
Vine	Rep 2	20.5
Vine	7 Rep 1	12.6
Vine	Rep 2	12.8
Vine	14 Rep 1	13.9
Vine	Rep 2	14.1

[1]Chlorothalonil equivalents
(Fruit had been surface rinsed with dichloromethane)

The distribution through the extraction procedure of the terminal residues in tomato fruit and vine samples is presented in Table II.

TABLE II. PERCENT DISTRIBUTION OF ^{14}C-RESIDUE IN TOMATO FRUIT AND VINE SAMPLES THROUGH THE EXTRACTION PROCEDURE[1]

Sample Type	Days After Last App.	Organic Rinse	Organic Extract	PNE	PES
Fruit	1 Rep 1	71.4	4.0	22.2	2.5
	Rep 2	78.6	4.4	15.5	1.5
	Avg	75.0	4.2	18.9	2.0
	7 Rep 1	52.5	5.0	39.6	2.8
	Rep 2	58.7	1.9	24.1	15.3
	Avg	55.6	3.5	31.9	9.1
	14 Rep 1	59.9	2.6	32.3	5.2
	Rep 2	62.4	3.9	30.6	3.1
	Avg	61.2	3.3	31.5	4.2
Vines	1 Rep 1		80.7	13.7	5.6
	Rep 2		79.2	12.8	8.0
	Avg		80.0	13.3	6.8
	7 Rep 1		67.1	19.0	13.9
	Rep 2		66.4	19.2	14.5
	Avg		66.8	19.1	13.6
	14 Rep 1		56.8	29.7	13.6
	Rep 2		54.2	29.6	16.1
	Avg		55.5	29.7	14.9

1 - % of total ^{14}C-activity recovered
PNE-Polar Non Extractable (water soluble)
PES-Post Extraction Solids

According to the petitioner, the data indicate that the percentage of water soluble ^{14}C PNE in tomato fruit increased as the amount of ^{14}C extractable material (dichloromethane and diethyl ether) decreased with time between days 1 and 7, and changed little after day 7. However, in terms of ppm, the amount of residue in the PNE declined, on average, from 0.481 to 0.219 to 0.190 ppm during this interval, in parallel with total residues. In tomato vines, the percentage and amount of radiolabel in the water soluble non-extracted fraction (PNE) increased with time, and in vines this continued throughout the entire 14 days (from 2.723 ppm at day 1 to 4.151 ppm at day 14). Additional testing indicated that the majority of the tomato fruit PNE residue is contained in the pulp of the tomato fruit, while the majority of the tomato fruit PES residue is contained in the tomato fruit skin. Also, it appears that the PNE is most likely translocated into the fruit from foliage and vine or root uptake of ^{14}C-Chlorothalonil related metabolite residues, and /or results from direct penetration of the fruit surface by chlorothalonil. While the skin may play a role in preventing or facilitating transport of chlorothalonil into the body of the tomato fruit, it does not appear to be essential to the process of PNE generation.

Most of the residue extracted in the organic phases (59-79% in tomato fruit, 56-80% in vines) was identified by HPLC as the parent (91 to 95% in fruit, 74 to 93% in vines) and 4-hydroxychlorothalonil (2 to 6% in fruit, 3 to 14% in vines). The total amount of unidentified residues in these fruit samples ranged from 3.4 to 4.8%. The amount present as parent declined from 1.96 ppm at day 1 to 0.35 ppm at day 7 after the last application, and remained virtually unchanged after day 7. A similar pattern was displayed for 4-hydroxychlorothalonil (SDS-3701), which decline from about 0.04 to 0.02 ppm through the same time interval. The amount of parent present in vines also declined over the post harvest interval. The maximum level of SDS-3701 found in tomato vines was 13.8% of the level of chlorothalonil residue, or about 1.07 ppm at 14 days post application.

The amount of ^{14}C-residues contained in the polar nonextractable (PNE) fraction of the tomato fruit approached 32%, or about 0.1 ppm equivalent, of the total residue at both 7 and 14 days. Various analytical methods were used in an effort to identify the components. GPC showed that the PNE fraction contained at least three component accounting for 15.8, 35.1 and 9.0% (total about 60%) of the PNE residue, equivalent to 0.03, 0.067 and 0.017 ppm chlorothalonil equivalents. GPC behavior of the first

two fractions suggested that they were conjugated species (disaccharide and monosaccharide respectively), and that all three likely contained at least one phenolic -OH group, or similar acidic moiety. Enzyme catalyzed hydrolysis suggested that one-fourth of the tomato fruit PNE may be conjugated with glucose or a closely related sugar. Base catalyzed solvolysis indicated that at least one-half of the PNE component contained two intact cyano groups, and that this portion is capable of base reaction leading to the conjugate free species, 5-chloro-2,4,6-trimethoxyisophthalonitrile (SDS-3316), after methylation. Acid catalyzed n-butanol solvolysis indicated that nearly all of the tomato fruit PNE could be butylated, leading to an uncertain number of products, probably more than 3. The extract of acid butanol solvolysis of the PNE could not be cleaned up enough to provide adequate GC/MS data for further analytical evaluation.

The amount of ^{14}C-residues contained in the post extraction solids (PES) fraction of the tomato fruit was less than 5% (<0.025 ppm) of the total residue at 14 days after the last application and identification was not pursued.

DEB's Comments/Conclusions, re:Deficiency 3a

The metabolism data on tomatoes suggest that the residues are mostly comprised of chlorothalonil and the 4-hydroxy metabolite. The residues appear to be mostly surface in nature. Some translocation from foliage (vine), root uptake and possibly skin of ^{14}C-Chlorothalonil related metabolite residues does appear to occur, although no other metabolites were specifically identified.

The metabolism study data on tomatoes are insufficient to support the proposed use on cherries. To date DEB files show that ^{14}C-Chlorothalonil has been foliar applied only to lettuce. Those data and these data on tomatoes are not readily translatable to cherries, which is a tree crop. Additionally, the treatment levels, application rates and use patterns used in the tomato metabolism study are quite different from those to be used on cherries.

The petitioner should conduct a metabolism study where ^{14}C-Chlorothalonil is foliar applied to cherries, or some related crop (stone fruit or apples would be acceptable). The petitioner was informed about the need for a ^{14}C-Chlorothalonil metabolism study on a tree crop in DEB's 5/30/84 review of PP# 4F 2025 see also Chlorothalonil Registration Standard, 11/4/83, Residue Chemistry Chapter; Nature of the Residue in Plants, p. 2 under Conclusions). The rate and frequency of application should be sufficiently high to permit adequate identification of residues.

This deficiency has not been resolved. Additional metabolism data are needed.

Deficiency 4.

RCB can not conclude at this time that adequate analytical methodology is available to enforce tne proposed tolerance on cherries until the nature of the residue in plants has been adequately resolved (see Conclusion 3a).

Petitioner's Response to Deficiency 4

None

DEB's Comments/Conclusions, re:Deficiency 4

DEB reiterates the previous conclusion. Additional analytical methodology may be needed if sufficient levels of other metabolites are found to form in cherries. Additional plant metabolism data are needed.

This deficiency remains unresolved.

Deficiencies 5a-d

RCB considers the residue data inadequate to support the proposed 3 ppm chlorothalonil tolerance on cherries.

Since RCB considers the RAC to include dry harvested cherries (see Conclusion 1b), residue data generated on washed tart cherries are not considered adequate to support any proposed tolerance. Therefore, the petitioner will need to submit additional residue data generated on cherries harvested dry (sweet and tart) and reflective of the proposed use (i.e., maximum number of post-shuck-split treatments, maximum application rate, etc.). These residue data must be geographically representative of the major cherry growing regions of the country. Thus the petitioner will need to generate additional residue data on field-treated cherries grown in the states of CA, OR or WA, MI, and NY or PA (note: if these treated samples are stored more than 6 months prior to analysis, additional storage stability data will be required).

Pending RCB's final conclusion concerning the nature of the residue in plants (see Conclusion 3a), the petitioner may need to submit residue data on components of the terminal residue other than chlorothalonil, 4-hydroxychlorothalonil, HCB and PCBN.

Petitioner's Response to Deficiencies 5a-d

The petitioner has submitted a study entitled "Residues of Tetrachloroisophthalonitrile, SDS-3701, SDS-46851, HCB and PCBN on Mechanically Harvested Tart Cherries" (MRID# 406848-02). HCB and PCBN are impurities in the technical grade active ingredient.

Field trials were conducted in New York and Michigan. The petitioner claims that 90% of tart cherries are grown in Michigan. A spray solution of Bravo 500 was applied at a rate of 6 pts/A using commercial air carrier sprayers. The number of applications ranged from 4 to 10, with PHI's ranging from 7 to 50 days. The number of applications after shuck split ranged from 0 for samples with a PHI of 50 days, to 6 for samples with a 7 day PHI. The treated cherries as well as the control samples were mechanically harvested, which involved shaking the trees and allowing the cherries to fall into a large container of cool water according to normal commercial practices for harvesting this crop.

Residues of chlorothalonil, SDS-3701, SDS-46851, HCB and PCBN were extracted from the cherry samples and selectively partitioned into an organic solvent. The residues of chlorothalonil, HCB and PCBN were separated by column chromatography prior to subsequent quantitation by electron capture gas chromatography. The residue of SDS-3701 was derivatized to its methyl ether prior to quantitation. The residue of SDS-46851 was derivatized to its methyl ester for quantitation. The residues of derivatized SDS-3701 and SDS-46851 were cleaned up by column chromatography prior to quantitation. When untreated tart cherries were fortified with chlorothalonil, the following recovery results were obtained:

TABLE III. RECOVERIES OF CHLOROTHALONIL AND RELATED COMPONENTS FROM SPIKED CHERRY SAMPLES

Component Added	Fortification Range (ppm)	Recovery Range (%)
Chlorothalonil	0.03-4.91	80-90
SDS-3701	0.03-0.491	68-90
SDS-46851	0.03-0.495	82-117
HCB	0.01-0.049	67-90
PCBN	0.015-0.098	73-87

The limits of determination are approximately 0.01 ppm for parent, 0.01 ppm for SDS-3701, 0.03 ppm for SDS-46851, 0.003 ppm for HCB and 0.005 ppm for PCBN.

Maximum chlorothalonil residues were found to be 1.35 ppm after 8 applications (4 after shuck split) and a 7 day PHI, 0.63 ppm after 10 applications (6 after shuck split) and a 7 day PHI, and 0.08 ppm after 4 applications (0 after shuck split) and a 50 day PHI. PCBN levels did not exceed 0.015 ppm and occurred in the same ratio to chlorothalonil as found in the technical material

used to manufacture the Bravo 500 applied in the studies. Levels of HCB did not exceed 0.006 ppm. A mean residue of 0.02 ppm SDS-3701 was observed in the cherries treated with 8 applications. No detectable residues of SDS-3701 where found in cherries treated with 10 applications. No SDS-46851 residues were detected in any samples.

DEB's Comments/Conclusions, re:Deficiencies 5a-d

DEB considers the residue data inadequate to support the proposed 3 ppm chlorothalonil tolerance on tart cherries.

Since the RAC is considered to include dry harvested cherries as well as wet harvested cherries, some residue data are needed on unwashed cherries. The petitioner will need to submit additional residue data from unwashed cherries treated at the maximum application rate with the maximum number of post shuck split applications specified in the Section B/label. In this case, the effects of washing are not considered in the establishment of tolerances.

Residue data are typically required for cherries grown in the major cherry growing regions in the US. These are California, Oregon or Washington, Michigan, Utah or Montana or Idaho, and New York or Philadelphia, according to Agricultural Statistics. (Note: Residue data on cherries submitted in support of PP# 2F2602 were from field trials conducted in Oregon and New York). The petitioner will need to submit additional residue data from field trials conducted in these areas. Application should be made with both ground and aerial equipment if both application techniques are to be used.

Storage conditions and intervals should be submitted for all samples. Presently storage data support stability of chlorothalonil and its 4-hydroxy metabolite in frozen storage for 6 months. The residue data submitted here indicate that the field trials were conducted in 1984 which further questions the usefulness of these data. Additional storage stability data depicting the percent decline in residues of chlorothalonil, 4-hydroxychlorothalonil, HCB and PCBN under the storage conditions used and for the same storage period must be submitted. The analytical methodology used to assess residue levels appears to be adequate.

All questions concerning the nature of the residue in plants have not been adequately addressed. Additional residue data including storage stability data on other components may be needed depending on the final outcome of the requested metabolism studies.

Deficiencies 5 a through d have not been resolved.

Other Considerations

An updated International Residue Limit Status sheet is attached to this review. There are no Canadian or Mexican tolerances established for chlorothalonil on cherries. Codex has established a 10 ppm limit (parent compound only) for chlorothalonil on cherries. Thus, there is incompatibility in the tolerance levels and the tolerance expression.

Attachment: International Residue Limit Status Sheet

cc: SHWillett, PP# 5F3183, E. Eldredge (ISB/PMSD), Circ., RF
TS769C: DEB:CM#2:RM810:X1669:SHWillett:shw-10/27/88
RDI: JHOnley, 11/1/88; RDSchmitt, 11/1/88

00771

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL ~~Chlorothalonil~~

CODEX NO. 081

CODEX STATUS:

☐ No Codex Proposal
Step 6 or above

Residue(if Step 8): ~~Chlorothalonil~~

Crop(s)	Limit (mg/kg)
Cherries	10

PROPOSED U.S. TOLERANCES:

Petition No. 5F3183

RCB Reviewer S.H. Willett

Residue: 2,4,5,6-tetrachloroisophthalonitrile and its metabolite *

Crop(s)	Lim (mg
cherries (tart)	3
cherries (sweet)	3

CANADIAN LIMITS:

☑ No Canadian limit (for cherries)

Residue:

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

☑ No Mexican limit (for cherries)

Residue:

Crop(s)	Lim (mg/

NOTES:

* 4-hydroxy-2,5,6-trichloroisophthalonitrile

10/19/88

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

OCT 19 1988

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

TO: Lois Rossi, PM # 21
Herbicide/Fungicide Branch
Registration Division TS-767C

THRU: Quang Bui, Ph.D., Section Head
Rev. Sec. I/HFASB
Health Effects Division TS-769C
10/7/88

THRU: William L. Burnam, Chief
HFASB
Health Effects Division TS-769C

FROM: D. Ritter, Toxicologist
Rev. Sec. I/HFASB
Health Effects Division TS-769C
10-6-88

Subject: Chlorothalonil Registration Standard Data Call In; request to evaluate Acute Inhalation Study in the Rat. Chlorothalonil Product GX-198.

Registrant: Griffin Corporation, Valdosta, GA.

Caswell #: 215B.

TOX Project #: 8-1069.

Griffin Corporation is requesting our review of a Rat Acute Inhalation Toxicity study to support continued registration and re-registration of products containing Chlorothalonil. The study is identified as:

Acute Inhalation Toxicity, Rat, Study # 3159.37, dated 6/8/88.
Performing Laboratory: Springborn Life Sciences, Inc., Spencerville, OH.
Author: K. G. Michelwicz, Ph.D.

The DER is attached. The study was rated CORE Guideline with a TOX Category of II. The Registrant should provide a Confidential Statement of Formulation for this product.

Primary Reviewer: D. Ritter, Toxicologist DR 10-6-88 Caswell #: 215B
Rev. Sec. # I, HFASB
Secondary Reviewer: Quang Bui, Ph.D. Quang Bui
Section Head, Rev. Sec. # I, HFASB

DATA EVALUATION RECORD

STUDY: Acute Inhalation Toxicity, Rat EPA Guideline # 81-3.

LABORATORY: Springborn Life Sciences, Inc., Spencerville, OH.

STUDY NUMBER & DATE: 3159.37 6/8/88.

ACCESSION NUMBER: 40729701

MRID: 40729701

MATERIAL TESTED: Chlorothalonil Product GX-198, Batch # 88012731, AN #80041[1]
Gray Liquid. Diluted to 70% (w/v) with distilled water.

ANIMALS: Young adult Sprague-Dawley rats obtained from Charles River Laboratories, Inc., Portage, MI. Initial weight range 212 - 307 Gm.

TITLE OF REPORT: Acute Nose Only Inhalation Toxicity of Chlorothalonil, Product GX-198 in Rats.

AUTHOR OF REPORT: Kevin G. Michelwicz, Ph.D.

CONCLUSIONS:

All levels of exposure to Test Material resulted in high mortality and a precise LC_{50} cannot be determined, but it is substantially less than 0.3 mg/L. The lowest LC_{50} for a TOX Category II rating is 2.0 mg/L. The results of this study therefore support a TOX Category of II for this product.

CORE RATING: Guideline data.

TOXICITY CATEGORY: II

METHODS:

A. STUDY DESIGN

1. Animal Assignment -

Five males and five females each were assigned to one of four groups to receive a nose-only liquid aerosol containing the Test Material or distilled water for a single exposure of 240 minutes duration.

2. Husbandry - Standard GLP.

3. Feed and Water - Available ad libitum except during compound administration.

---

1 NOTE: CSF not provided; this may be a 4 lb/gallon formulation.

4. Compound Administration -

Animals were immobilized in plastic tubes with only the nose exposed to the breathing zone of the apparatus, which was a 270 Liter Rochester-type inhalation chamber.

A high pressure air source piped through an atomizing nozzle was used to generate the test atmosphere. A peristaltic pump delivered the diluted test material to the nozzle. Atmosphere flow rate was monitored continuously using Dwyer meters.

Temperature, relative humidity, % O_2 content and air flow rate were recorded at ca 30 minute intervals during the exposure period.

Nominal Aerial Concentration of Test Material in mg/L was determined by dividing the amount of Test Material used in mg by the total amount of air used in Liters, and adjusting for the 70% concentration of product in the Test Material.

Gravimetric Concentration was derived by drawing a sample of test atmosphere through a pre-weighed filter and recording its weight differential in mg, then dividing this value by the amount of test atmosphere used in Liters, and adjusting for the 70% concentration of product in the Test Material.

Particle Size was determined hourly by drawing test atmosphere through an Anderson 2000 impactor, weighing the different pre-weighed filters and dividing by the volume of air used. The Mass Median Aerodynamic Diameter and geometric Standard Deviation were also determined from these data.

5. Observations -

For mortality, twice daily. For signs of overt toxicity, two or three times during the day following exposure, then thereafter for 14 days.

6. Termination -

All animals were necropsied at death or were killed on day 14 and subjected to gross necropsy. Special attention was paid to the respiratory system.

B. RESULTS:

1. Atmospheric parameters -

TABLE I

Test Material Exposure Levels

Exp. #	Treatment	Ave. Grav. Conc. (mg/L)	Ave. Nominal Conc. (mg/l)	Mass Median Aerodynam. Diam.(u)
1	Dist. HCH	5.2	254	7.4
2	CTN	3.4	193	4.4
3	CTN	1.8	68	4.9
4	CTN	0.3	18	3.6

2. Mortality -

The mortality associated with inhalation exposure to Chlorothalonil is shown in Table II.

TABLE II

MORTALITY

Dose mg/L	Males[1]	Females	Combined
0	0/5	0/5	0/0
3.4	4/5	4/5	8/10
1.8	5/5	4/5	9/10
0.3	4/5	5/5	9/10

1 Number dead/number exposed

3. Cageside Observations -

Control and test animals were soiled with urine and feces and was thought to be due to the restraint system used.

Signs of toxicity in all test group animals included hypoactivity, respiratory distress, dehydration, tremors, prostration and possible hypothermia.

4. Body Weights -

Control animals exhibited weight gain during the observation phase of the study. All moribund animals suffered weight loss, while the three surviving rats showed decreased weights during days 1 through 8 but then partially recovered this loss.

5. Gross Necropsy Findings -

Treatment-related abnormalities were reported for all animals that died during the study. These included mottled, red and tan lungs, white rubbery material was found in the tracheas; there was congestion in the cerebral vessels and yellow gelatinous material in the intestines.

CONCLUSIONS:

All levels of exposure to Test Material resulted in high mortality and a precise LC_{50} cannot be determined, but it is substantially less less than 0.3 mg/L. The lowest LC_{50} for a TOX Category II rating is 0.2 mg/L. The results of this study therefore support a TOX Category of I for this product.

CORE RATING: Guideline data.

TOXICITY CATEGORY: I.

8/19/88

REVIEWER

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

AUG 19 1988

006828

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Lois Rossi, PM # 21
Herbicides/Fungicides Branch
Registration Division TS-767C

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

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

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

Subject: EPA # 210 - Chlorothalonil Data Call In. Submission of additional toxicity data.

Caswell #: 215B

Sponsor: Griffin Corporation, Valdosta, GA.

TOX Proj. #: 8-0539

Griffin Corporation is submitting four acute toxicity studies on Flowable Chlorothalonil. These have been reviewed and the DERs are attached. Except for the missing Confidential Statement of Formulation (CSF) of this product, three of the four studies are acceptable and support the continued Registration of products containing Chlorothalonil. The Primary Dermal Irritation study, likewise rated CORE Invalid, can be upgraded to CORE Supplemental upon submission of the CSF, but cannot be further repaired.
Studies reviewed are:

1. Acute Oral Toxicity, Rat, Study # 3159.5.1
LD_{50} > 5.0 Gm/kg body weight. TOX Category III.
CORE - Invalid. The CSF must be provided.

2. Acute Dermal Toxicity, Rabbit, Study # 3159.5.2.
LD_{50} > 2.0 Gm/kg body weight. TOX Category III.
CORE - Invalid. The CSF must be provided.

3. Primary Eye Irritiation, Rabbit, Study # 3159.5.3
Moderate eye irritant. PII = 13.8/110. TOX Category II.
CORE - Invalid. The CSF must be provided.

4. Primary Dermal Irritiation, Rabbit, Study # 3159.5.4
Mild dermal irritant. PII = 1.22/8.0 TOX Category - Unknown.
CORE - Invalid. Repairable to Supplemental with submission of CSF.

Reviewed by: D. Ritter, Toxicologist DVR 8-18-88 Guideline #: 81-1
Section I , Tox. Branch (TS-769C)
Secondary reviewer: R. Bruce Jaeger, Section Head
Section I , Tox. Branch (TS-769C) 8/18/88

CC 7 3
006828

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity, Rat TOX. CHEM. NO. 215B

ACCESSION NUMBER: NA MRID NO.: 40509901

TEST MATERIAL: Flowable Chlorothalonil

SYNONYMS: NA

STUDY NUMBER(S): 3159.5.1

SPONSOR: Griffin Corporation, Valdosta, GA.

TESTING FACILITY: Springborn Institute for Bioresearch, Inc.
Spencerville, OH.

TITLE OF REPORT: "Acute Oral Toxicity Study of Flowable Chlorothalonil in Rats"

AUTHOR(S): Joseph P. Siglin, BA.

REPORT ISSUED: 3/25/87

CONCLUSIONS: The Acute Oral LD_{50} is > 5.0 gm/kg. TOC Category III.

Classification: CORE - Invalid. May upgraded to Guideline by submitting thepConfidential Statement of Formulation for this product,

Special Review Criteria (40 CFR 154.7) None exceeded.

A. MATERIALS

1. Test compound: As cited above.
Description: Cream colored liquid
Batch #: G-Chlor-2 Purity: Not given.

2. Test animals: Species: Rat. Strain: Harlan, Sprague Dawley

Age: Young Adult. Weight: 180 - 300 gm.

Source: Harlan.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned 5 M & 5 F to a single test group receiving 5.0 Gm/kg body weight.

Husbandry - Standard GLP.

353

2. Compound Administration:

   Test Material was administered undiluted once by gavage on Day 1.

3. Quality assurance procedures were satisfactory.

4. Animals were observed for toxic effects three times on day one, then once daily thereafter for 15 days.

5. Animals received feed and water ad libitum.

6. Animals were weighed on days 1, 8 and 15 or at death.

7. Animals were inspected twice daily for mortality.

8. All animals surviving to termination and all animals dying or moribund during the study were subjected to gross necropsy.

RESULTS:

Mortality -

Two males and one female died by day 2 and one female died on day 4.

	DAYS		
	1	5	15
Males	5/5*	3/5	3/5
Females	4/5	3/5	3/5
	9/5	6/10	6/10

LD_{50} > 5.0 gm/kg body weight.

Signs of Toxicity -

Clinical signs of toxicity occured in all animals and included dark material around the mouth, decreased activity, soft stools, diarrhea, and fecal and urinary staining. Those animals which died had 8 - 11% weight loss; survivors gained weight during the in-life phase.

* Rats alive/rats dosed

Gross Pathology -

Colored fluid and gelatinous material was reported in the GI tracts of the animals which died during the study. No gross effects were reported for the surviving rats.

CONCLUSIONS:

The Acute Oral LD_{50} in this study is greater than 5.0 Gm/kg body weight.

Note: The composition of the Test Material must be provided.

Reviewed by: D. Ritter, Toxicologist
Section I , Tox. Branch (TS-769C)
Secondary reviewer: R. Bruce Jaeger, Section Head
Section I , Tox. Branch (TS-769C)

Guideline # 81-2

00171?
006828

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity, Rabbit TOX. CHEM. NO. 215B

ACCESSION NUMBER: NA MRID NO.: 40509902

TEST MATERIAL: Flowable Chlorothalonil

SYNONYMS: NA

STUDY NUMBER(S): 3159.5.2

SPONSOR: Griffin Corporation, Valdosta, GA

TESTING FACILITY: Springborn Life Sciences, Inc., Spencerville, OH.

TITLE OF REPORT: "Acute Dermal Toxicity Study of Flowable Chlorothalonil in Rabbits Limit Test"

AUTHOR(S): Joseph C. Siglin, BA

REPORT ISSUED: 2/5/88.

CONCLUSIONS: LD_{50} > 2.0 gm/kg body weight TOX Category III.

Classification: CORE - Invalid. May be upgraded to Guideline by submitting the Confidential Statement of Formulation of the product.

Special Review Criteria (40 CFR 154.7) None exceeded.

A. MATERIALS:

1. Test compound: Cream-colored liquid, Lot # G-Chlor-2. Composition and purity not given.

2. Test Animals: 5 male & 5 female young adult New Zealand Albino Rabbits.

Husbandry - Standard GLP.

Feed & Water - Available ad libitum.

B. STUDY DESIGN:

1. Animal assignment -

Animals were assigned 5 M & 5 F to the single test group.

Administration of Test Material -

On day 0 the animals were clipped free of hair over the dorsal trunk, exposing an area of ca. 12 x 20 cm, about 10% of total body surface area. The following day, Day 1, the Test Material was applied at a rate of 2.0 gm/kg bw. The site was then occluded with gauze dressings and a body sock. The animals were then restrained using a leather harness.

24 hours later (day 2) the harness and dressings were removed and the application sites were cleansed with distilled water.

The animals were observed for toxic effect three times daily on day 2, then once daily thereafter for 15 days. Animals were checked twice daily for mortality.

Body weights were obtained on days 1, 8 and 15.

Gross necropsy was performed on all survivors at the end of 15 days.

3. RESULTS

Mortality -

No animals died during the study.

Signs of toxicity -

None reported.

Body weights -

All animals gained weight satisfactorily.

Gross necropsy -

No gross evidence of toxic effect was reported.

4. CONCLUSIONS:

The Dermal LD_{50} is > 2.0 gm/kg body weight.

The composition of the Test Material must be provided.

Reviewed by: D. Ritter, Toxicologist DLR 8-18-88
Section I , Tox. Branch (TS-769C)
Secondary reviewer: R. Bruce Jaeger, Section Head
Section I , Tox. Branch (TS-769C) RBJ 8/18/88

Guideline #: 81-4

0068

DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irr., Rabbit TOX. CHEM. NO. 2158

ACCESSION NUMBER: NA MRID NO.: 40509903

TEST MATERIAL: Flowable Chlorothalonil

SYNONYMS: NA

STUDY NUMBER(S): 3159.5.3

SPONSOR: Griffin Corporation, Valdosta, GA.

TESTING FACILITY: Springborn Institute for Bioresearch, Inc., Spencerville, OH.

TITLE OF REPORT: "Primary Eye Irritation Study of Flowable Chlorothalonil in Rabbits".

AUTHOR(S): Joseph C. Siglin, BA

REPORT ISSUED: 4/2/87,

CONCLUSIONS: Flowable Chlorothalonil induces moderate eye irritation in rabbits, PII of 13.8/110. This is a Moderate Irritant.

Classification: CORE - Invalid. May be upgraded to Guideline by submission of the Confidential Statement of Formulation for this product.

TOX Category II. Corneal opacity reversible in 7 days.

Special Review Criteria

(40 CFR 154.7) None exceeded.

A. MATERIALS:

1. Test compound: Cream-colored liquid, Lot # G-Chlor-2. Composition and purity not given.

2. Test Animals: 9 young adult New Zealand Albino Rabbits.

Husbandry - Standard GLP.

Feed & Water - Available ad libitum.

B. STUDY DESIGN:

1. Animal assignment -

All animals were assigned to the single test group. Animals were acclimated for at least five days prior to initiation of the experiment.

2. Husbandry - Standard GLP.

3. Feed & water - Available ad libitum

4. Compound Administration -

Animals were weighed prior to exposure. Both eyes were examine UV and Na fluorescein prior to exposure. 0.1 ml of Test Materi instilled into the conjunctival sac of the right eye. The eyeli held closed for ca. one second. The left eye served as the untr control. Three rabbits had their eyes washed with physiological for 30 seconds following exposure.

Eyes were evaluated for compound-related effects according to Dr at 1, 4, 48 and 72 hours after dosing. The non-washed group was on days 4 and 7 as well.

All animals were killed and discarded after the conclusion of th

C. RESULTS:

1. Body Weights -

No compound-related effect was reported since no post-treatment was made.

2. Ocular Effects -

Unwashed eyes showed mild to moderate corneal opacities, and ir conjunctival irritation, consisting of redness and swelling, wit maximum combined score of 13.8/110 at 24 hours, decreasing to 1. days. Conjunctival irritation was noted in 6/6 rabbits; iris ef 1/6 rabbits, and corneal effects in 2/6 rabbits.

The washed eyes showed a slight irritation score of 4.0 111 at 1 had cleared by 48 hours.

4. CONCLUSIONS:

Flowable Chlorothalonil induces moderate eye irritation in rabbits, PI 13.8/110. Corneal opacity reversible in 7 days. This is a Moderate Ir

Note: The CSF Flowable Chlorothalonil must be provided.

Reviewed by: D. Ritter, Toxicologist DKR 5-18-88 Guideline #: 8
Section I , Tox. Branch (TS-769C)
Secondary reviewer: R. Bruce Jaeger, Section Head
Section I , Tox. Branch (TS-769C) RBJ 8/18/88

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irr., Rabbit TOX. CHEM. NO. 215B

ACCESSION NUMBER: MRID NO.: 40509904

TEST MATERIAL: Flowable Chlorothalonil

SYNONYMS: NA

STUDY NUMBER(S): 3159.5.4

SPONSOR: Griffin Corporation, Valdosta, GA.

TESTING FACILITY: Springborn Institute for Bioresearch, Inc., Spencerville,

TITLE OF REPORT: "Primary Dermal Irritation study of Flowable Chlorothalon: in Rabbits".

AUTHOR(S): Joseph C. Siglin, BA.

REPORT ISSUED: 3/29/87.

CONCLUSIONS: PII = 1.22/8.0.

Classification: CORE - Invalid. Reversibility of effects in accordance with 81-5 not demonstrated. Repairable to Supple with submission of CSF.

TOX Category - Unknown.

Special Review Criteria (40 CFR 154.7) None exceeded.

A. MATERIALS:

1. Test compound: Cream-colored liquid, Lot # G-Chlor-2. Composition s not given.

2. Test Animals: 6 young adult New Zealand Albino Rabbits.

Husbandry - Standard GLP.

Feed & Water - Available ad libitum.

B. STUDY DESIGN:

1. Animal assignment -

6 animals were assigned to the single test group.

CD682

[redacted]

2. Compound Administration:

One day prior to exposure the fur was clipped from the dorsal trunk area. The next day, the animals were weighed and an area approximately 1 inch squa received 0.5 ml Test Material. The area was covered with gauze and secured with a body stocking and taped.

Four hours later the dressings were removed and the test site was cleansed w distilled water. The test site was examined at 30 and 60 minutes and at 24 48 and 72 hours following removal of the dressings. The dermal response was graded according to the method of Draize. The animals were then killed and discarded.

C. RESULTS:

1. Body Weights -

No compound-related effects were reported since no additional weighings were

2. Dermal Effects -

The Test Material was slightly irritating, inducing well defined dermal erythema and edema with some eschar formation. Irritation was still presen at termination at 4 days. The PII was 1.22/8.0.

D. CONCLUSIONS:

The irritancy of Flowable Chlorothalonil is not fully defined. The reversibili of irritation was not demonstrated since 6/6 rabbits had erythema and eschar formation that persisted through 4 days when the study was terminated. This deficiency cannot be repaired.

Draize Score = 1.22/8.0.

Note: The composition of the Test Material must be provided.

7/5/85

CONT 3

JUL 8 1988

RCB SCIENCE INTEGRATION/DEFERRAL

To: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch

From: Charles L. Trichilo, Ph.D.
Chief, Residue Chemistry Branch

Subject: Request for Toxicology Input on PCBN
(Manufacturing Impurity in Chlorothalonil)

RCB Number: _____

Action: FRSTR

Chemical Name: Chlorothalonil

Purpose: Determination of Need for Additional Residue Data for PCBN

Due Date: _____

Background Data: Before RCB can complete it's work, input from TOX is needed. Refer to the attached memo for a discussion of the background information pertaining to this deferral.

Technical Contact: Debra Edwards -RCB (557-4353)

Deferral: Based on the data summarized in the attached memo, should RCB require field residue data for pentachlorobenzonitrile (PCBN) in or on additional crops, in order to obtain a complete PCBN residue data base?

cc: Lois Rossi (PM 21)
Amy Rispin (SIMS)
W. Boodee (RCB)
RCB Subject File
RCB Registration Standard File