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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Thiodicarb

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 5/14/96
Review Section II
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Health Effects Division (7509C)

and

Esther Rinde, Ph.D. E. Rinde
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TO: Dennis Edwards
Product Manager #19
Insecticide-Rodenticide Branch
Registration Division (7505C)
and
Jay Elllenberger
Special Review and Reregistration Division (7508W)

THROUGH: Stephanie R. Irene Ph.D. Stephanie R. Irene 6/6/96
Acting Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on November 29, 1995 to discuss and evaluate the weight-of-the-evidence on Thiodicarb with particular reference to its carcinogenic potential. The CPRC concluded that Thiodicarb should be classified as Group B2 - probable human carcinogen, based on statistically significant increases in hepatocellular adenomas, carcinomas and combined adenoma/carcinoma in both sexes of the CD-1 mouse and statistically significant increases in testicular interstitial cell tumors in male Sprague-Dawley rats. The CPRC recommended that for the purpose of risk characterization, a Margin of Exposure (MOE) methodology be used for the estimation of human risk, based on the hepatocellular combined adenoma/carcinoma in male mice.

SUMMARY

Administration of Thiodicarb in the diet to CD-1 mice resulted in increased incidences of hepatocellular tumors in both sexes. In both male and female mice, there were statistically significant increases in hepatocellular adenomas, carcinomas and combined adenomas/carcinomas at the highest dose (1000 mg/kg/day); there were also statistically significant positive dose-related trends for adenomas and carcinomas, alone and combined. The tumor incidences were unusually high (combined adenoma/carcinoma at the highest dose 76% vs 18% in controls for males; 62% vs 2% in controls for females.)

The incidence of adenomas and carcinomas at the highest dose exceeded that of historical controls in both sexes; in addition, in male mice, the incidence of adenomas at the mid-dose (70 mg/kg/day) exceeded that of historical controls.

The majority of the CPRC considered that the highest dose may have been excessive, based on effects on the hematopoietic system and other signs of toxicity. In male mice at the highest dose there were decrements in body weight gain >>15%; there was no effect on mortality, however. In female mice, body weight gains were comparable to that of controls, but there was increased mortality.

Administration of Thiodicarb to Sprague-Dawley rats was associated with a statistically significant increase in testicular interstitial cell tumors in males at the highest dose (900 ppm or 60 mg/kg/day) with a statistically significant positive trend. There were no apparent compound-related increases in tumors in female rats.

The incidence of testicular interstitial tumors in male rats exceeded that of historical controls.

The dosing in the rat study was considered to be adequate in both sexes, based on body weight gain depressions (10-14% at 52 weeks) and comparable survival (female rats actually had enhanced survival).

Administration of Thiodicarb to Fischer 344 rats at doses up to 10 mg/kg/day (200 ppm) did not result in any apparent increases in tumors of either sex. The highest dose (200 ppm) in this study was inadequate for assessing the carcinogenic potential of Thiodicarb.

Thiodicarb was tested in a variety of mutagenicity assays and was negative in all but the mouse lymphoma assay, in which there was only a weak to equivocal response and for mitotic gene conversion in Saccharomyces cerevisiae. Overall there is low concern for the mutagenicity of Thiodicarb.

Thiodicarb is structurally related to Methomyl. Both Methomyl and Thiodicarb have a common metabolite (Acetamide) that induced liver tumors in rodents. Acetamide was classified as a Group C (without a Q1*). Methomyl-oxime, another metabolite of Thiodicarb is an analog of acetone-oxime, which is also carcinogenic to the rodent liver.

The classification of Group B2 was based on the increases in liver tumors in both sexes of the CD-1 mouse, statistically significant by both pair-wise and trend analysis and statistically significant increases in testicular interstitial tumors in the male Sprague-Dawley rat. Data from related structural analogs provided additional support, particularly for the liver tumors.

The CPRC noted that while the highest dose in mice may have been excessive, the overall dose selection was improper with the highest dose more than 10 fold that of the mid-dose. The CPRC also noticed that there was a suggestive tumor response in the male mouse liver even at the mid-dose. The CPRC agreed that the liver tumors were compound related.

A. Individuals in Attendance at the meetings:

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

William Burnam

Karl Baetcke

Yiannakis Ioannou

Kerry Dearfield

Elizabeth Doyle

Hugh Pettigrew

Esther Rinde

Richard Hill

Yin Tak Woo

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

Linda Taylor[1]

Clark Swentzel

Lori Brunsman

Lucas Brennecke[2]
(PAI/ORNL)

3. Other Attendees:

Bernice Fisher (HED)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

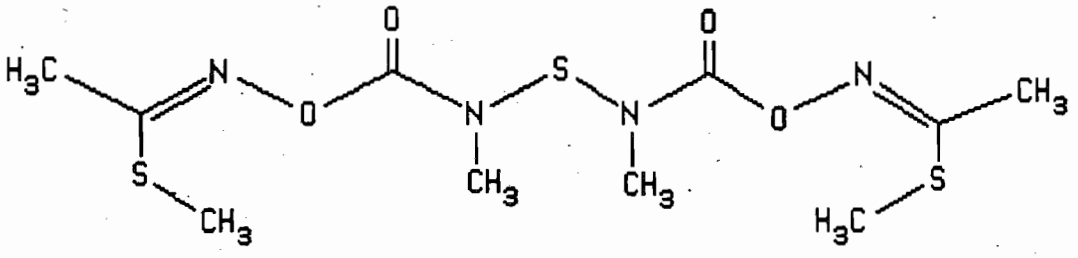
[2]Signature indicates concurrence with pathology report.

B. Material Reviewed

The material available for review consisted of DER's, one-liners, data from the literature and other data summaries prepared and/or supplied by Dr. Linda Taylor, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Thiodicarb [dimethyl N,N'-[thiobis[(methyliminocarbonyl)oxy]bis] ethanimidothioate; 3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetraazapentadeca-3,12-diene-6,10-dione] is a carbamate insecticide for control of insects in cotton, soybeans, corn, leafy vegetables [7/15/94], broccoli, cabbage, and cauliflower [8/15/94], peanuts, ornamentals, non-crop areas, seed treatment, tomatoes, peppers??. The trade name is Larvin. The CAS Registry Number [CAS NO.] is 59669-26-0. The PC Code is 114501. The Tox. Chemical No. is 900AA.



Structure of Thiodicarb

D. Evaluation of Carcinogenicity Evidence

1. Carcinogenicity Study in Mice

Reference: THIODICARB 97 Week Dietary Carcinogenicity Study in Mice with 52 Week Interim Kill (Results after 97 Weeks) [Study # IRI Project No. 439056; Report # 7749, dated September 8, 1993; MRID # 43000501; Document No. 011030]. **Addendum**: MRID # 43619301 [Supplemental Subchronic Data and Background Tumor Incidences]. **Position Paper**: Discussion on the Potential Oncogenicity of Thiodicarb in the Mouse, JP Rieth, dated September 6, 1994.

a. Experimental Design

Groups of Charles River CD®-1 mice of both sexes [50 mice/sex/dose (carcinogenicity); 15 mice/sex/group (interim group)] were administered Thiodicarb [96% pure] via the diet at dose levels of 0, 5, 70, or 1000 mg/kg/day for 97 weeks. There was an interim sacrifice of 10 mice/sex/group at week 52. Those in the interim group not sacrificed at 52 weeks were continued on the diets until termination and included with the carcinogenicity mice.

b. Discussion of Tumor Data

MALES - Male mice had significant increasing trends and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the control for hepatocellular adenomas, carcinomas, and adenomas and/or carcinomas [Table 1].

FEMALES - Female mice had significant increasing trend and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the control for hepatocellular adenomas, carcinomas, and adenomas and/or carcinomas [Table 2].

TABLE 1. THIODICARB - CD-1 Mouse Study

Male Hepatocellular Tumor Rates[+] and Exact Trend Test
Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	5	70	1000
Adenomas	7/44	7/46	13/49	32[a]/49
(%)	(16)	(15)	(27)	(65)
p =	0.000**	0.579[n]	0.161	0.000**
Carcinomas	2/44	0/46	3/49	14[b]/49
(%)	(5)	(0)	(6)	(29)
p =	0.000**	0.236[n]	0.551	0.002**
Combined	8[c]/44	7/46	14[d]/49	37[e]/49
(%)	(18)	(15)	(29)	(76)
p =	0.000**	0.462[n]	0.176	0.000**

[+]Number of tumor bearing mice/Number of mice examined, excluding those that died before week 52; also excludes week 53 interim sacrifice mice.
[a]First adenoma observed at week 53, dose 0 mg/kg/day, in an interim sacrifice mouse. Second adenoma observed at week 63, dose 1000 mg/kg/day, in a mouse that died on test.
[b]First carcinoma observed at week 52, dose 1000 mg/kg/day.
[n]Negative change from control.
[c]One mouse in the 0 mg/kg/day dose group had both an adenoma and a carcinoma.
[d]Two mice in the 70 mg/kg/day dose group had both an adenoma and a carcinoma.
[e]Nine mice in the 1000 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

TABLE 2. THIODICARB - CD-1 Mouse Study

Female Hepatocellular Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	5	70	1000
Adenomas	1/48	1/47	2/45	24[a]/45
(%)	(2)	(2)	(4)	(53)
p =	0.000**	0.584	0.310	0.000**
Carcinomas	0/40	0/43	1/40	8/34
(%)	(0)	(0)	(2)	(24)
p =	0.000**	-	0.177	0.001**
Combined	1/48	1/47	3/45	28[c]/49
(%)	(2)	(2)	(7)	(62)
p =	0.000**	0.584	0.185	0.000**

[+]Number of tumor bearing mice/Number of mice examined, excluding those that died or were sacrificed before observation of the first tumor not in an interim sacrifice mouse.

[a]First adenoma observed at week 53, dose 1000 mg/kg/day, in an interim sacrifice mouse. Second adenoma observed at week 55, dose 1000 mg/kg/day, in a mouse that died on test.

[b]First carcinoma observed at week 71, dose 70 mg/kg/day.

[c]Four mice in the 1000 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

MALES - When compared to historical controls of the testing facility, both the incidence of adenomas and carcinoma at 1000 mg/kg/day are greater than the historical control incidence, and the incidence of adenomas at 70 mg/kg/day exceeds the historical control [Table 3].

Table 3. Iveresk Research International♥ Historical Control Data [1990-1993] 92-104-Weeks Duration

Study #♣	Hepatocellular Tumor Incidence Rate - MALES	
	Adenoma	Carcinoma
618	8/50 (16)♦	8/50 (16)
670	2/49 (4)	0/49 (0)
617	4/50 (8)	0/50 (0)
293	9/60 (18)	7/70 (14)
694	0/50 (0)	0/50 (0)
483	9/50 (18)	5/50 (10)
056♪	6/50 (12)	2/50 (4)
001♫	5/50 (10)	5/50 (10)

♥ testing facility; ♦ (%); ♣ studies of 104-weeks duration unless noted otherwise; ♪ 97-week study; ♫ 92-week study

FEMALES: When compared to historical controls of the testing facility, the incidence of both hepatocellular adenomas and carcinomas in the high-dose **females** exceeds the historical control [Table 4].

Inveresk Research International♥ Historical Control Data (92-104 Weeks), 1990-1993

Study #	Table 4. Hepatocellular Tumor Incidence - FEMALES	
	Benign	Malignant
618	1/50 (2)♦	1/50 (2)
670	0/49 (0)	0/49 (0)
617	0/50 (0)	0/50 (0)
293	0/50 (0)	2/50 (4)
694	0/50 (0)	0/50 (0)
483	3/49 (6)	1/49 (2)
056♪	1/50 (2)	0/50 (0)
001♫	1/50 (2)	0/50 (0)

♥ testing facility; ♦ (%); ♪ 97 weeks duration; ♫ 92 weeks duration

c. Non-Neoplastic Lesions

MALES: The incidence of several non-neoplastic liver lesions was significantly increased at the high dose compared to the concurrent control [Table 5].

Tissue/Lesion/ Dose (mg/kg/day) MALES	Table 5. Microscopic Findings - Non-Neoplastic			
	0	5	70	1000
Liver N=	50	50	50	50
hepatocyte hypertrophy	2	6	6	20***
bile duct hyperplasia	2	1	1	12**
pigmented macrophages	0	0	1	48***
cellular change	5	1	10	21***
hepatocyte pleomorphism	5	10	13	29***
single cell necrosis	1	6	8*	7
focus(i) of hemopoiesis	4	2	2	15**
lobular hepatitis	0	0	0	6*

* p<0.05; ** p<0.01; *** p<0.001

FEMALES: The incidence of several non-neoplastic liver lesions in the high-dose females was greater than the concurrent control group [Table 6].

Tissue/Lesion/ Dose (mg/kg/day) FEMALES	Table 6. Microscopic Findings - Non-Neoplastic			
	0	5	70	1000
Liver N=	50	50	50	50
hepatocyte hypertrophy	1	0	2	13***
bile duct hyperplasia	0	1	0	13**
pigmented macrophages	4	3	1	46***
cellular change	2	3	1	21***
hepatocyte pleomorphism	2	3	2	30***
single cell necrosis	5	4	3	13
focus(i) of hemopoiesis	3	3	4	7
lobular hepatitis	0	1	0	10**

* p<0.05; ** p<0.01; *** p<0.001

d. Adequacy of the Dosing for Assessment of Carcinogenicity

The statistical evaluation of mortality indicated no significant incremental change with increasing doses of Thiodicarb in male mice, but the evaluation in female mice indicated a significant increasing trend for mortality with increasing doses of Thiodicarb. The highest dose tested is the limit dose, which is adequately high. Signs of toxicity at this dose level include (1) decreased body weight in males throughout most of the study [86% of control at week 32;, 78% of control at week 96], (2) decreased body-weight gains in males during the first year [54% of control] and overall [33% of control], (3) decreases in hemoglobin, hematocrit, and erythrocytes in both sexes, (4) increases in alanine aminotransferase and total bilirubin in both sexes, (5) increased liver and spleen weights in both sexes, (6) increased kidney weight in females, (7) increased incidences of liver and spleen lesions at both the interim and terminal sacrifices in both sexes, and (8) increased incidence of

kidney lesions in both sexes at termination. The Registrant argues that the MTD was exceeded and, since liver tumors occurred only at this dose level, they should not be considered treatment-related. **NOTE:** There were adequate numbers of mice available at study termination in both sexes with which to assess the carcinogenicity potential of Thiodicarb. **The NOEL was set at 70 mg/kg, the LOEL at 1000 mg/kg, based on increased mortality in females, decreased body-weight gain in males, increased bilirubin [total], increased alanine aminotransferase in females, and increased kidney, liver, and spleen lesions in both sexes.**

The majority of the CPRC considered that the highest dose may have been excessive, based on effects on the hematopoietic system and other signs of toxicity. In male mice at the highest dose there were decrements in body weight gain >>15%; there was no effect on mortality, however. In female mice, body weight gains were comparable to that of controls, but there was increased mortality. The CPRC noted that while the highest dose in mice may have been excessive, the overall dose selection was improper with the highest dose more than 10 fold that of the mid-dose. The CPRC also noticed that there was a suggestive tumor response in the male mouse liver at the mid-dose. Also, the tumor incidences were unusually high (hepatocellular combined adenoma/carcinoma at the highest dose: 76% vs 18% in controls for males; 62% vs 2% in controls for females.)

There is a 1980 Carnegie-Mellon Institute of Research study [initiated on 11/1/76] on Thiodicarb in which Charles River CH:COBS CD-L (ICR)BR [80/sex/group] mice were administered Thiodicarb at dose levels of 1, 3, and 10 mg/kg/day [2 control groups] in the diet for 24 months [interim sacrifice of 20/sex/group at 80 weeks; terminal sacrifice at 105 weeks]. Survival was not affected in males, but the original reviewer stated that the high-dose females displayed an increase in mortality over the last 6 months of the study. Based on Table 2-4 of the original report, page 20 [14], the increase appears to have occurred during months 17-19 and one of the control groups has a low mortality rate compared to the other control [see handout]. TB II notes that during months 17 and 18 twenty-three high-dose male mice died compared to 6 and 13 in the two control groups. Body weight changes and food consumption were comparable among the groups of both sexes throughout the study. No significant differences were reported in the frequency of non-neoplastic or neoplastic lesions in either sex. The incidence of liver tumors is shown in Table 7 [from Registrant submission of 9/21/94 (Position Paper)]. The NOEL was set at 3 mg/kg/day, the LOEL at 10 mg/kg/day, based on mortality in females.

Table 7. Liver Tumors [# with/# examined (%)]					
Dose [mg/kg/day]/Lesion/Sex	0	0	1	3	10
hepatocellular carcinoma MALES FEMALES	25/76(33)♦ 3/77	37/80(46) 1/80	29/78(37) 3/78	32/78(41) 1/76	26/80(33) 3/75
hepatocellular adenoma MALES FEMALES	19/76(25) 5/77	18/80(23) 2/80	22/78(28) 2/78	24/78(31) 2/76	24/80(30) 1/75

♦ # with tumor/# examined (%); those with both adenoma and carcinoma are listed only as carcinoma.

In support of their contention that the mid dose utilized in the 1993 mouse carcinogenicity study [70 mg/kg/day] is an adequately high dose level for assessing the carcinogenic potential of Thiodicarb, the Registrant submitted an acute oral [LD_{50}] study in mice that indicates an LD_{50} of ≈ 70 mg/kg [♂♂ 73 mg/kg (55-98)/♀♀ 79 mg/kg (62-101)] in mice. It is to be noted that the strain of mouse [OF1] used in this latter study differs from that used in the carcinogenicity study. The Registrant argues that feeding an LD_{50} dose each day over the entire lifespan should be adequately high. Additionally, the Registrant submitted a 4-week dietary study in mice {30 ppm [♂♂ 6.2/♀♀ 8.3 mg/kg], 1750 ppm [♂♂ 346/♀♀ 491 mg/kg], 3500 ppm [♂♂ 734/♀♀ 954 mg/kg], and 7000 ppm [♂♂ 1538/♀♀ 2030 mg/kg]} to support their contention. The findings include (1) decreased body weight at the 7000 ppm dose level [♂♂ 80%/♀♀ 88% of control], (2) a negative body-weight gain [-1.6 grams] overall in high-dose males, (3) a decreased body-weight gain overall in the high-dose females [47% of control], (4) a decreased overall body-weight gain in males at the 3500 ppm dose level [76% of control], and (5) slightly decreased overall food consumption in both sexes at 7000 ppm, which might be due to a palatability problem, (6) a dose-related increase in liver weight in females, a dose-related increase in spleen weight in males, and decreased ovarian weight at the high dose. There were no treatment-related effects observed on cholinesterase activity in plasma, RBC, or brain in either sex. **The NOEL is 30 ppm (♂♂ 6.2/♀♀ 8.3 mg/kg), the LOEL at 1750 ppm (♂♂ 346/♀♀ 491 mg/kg), based on increased liver weight in females and increased spleen weight in both sexes.** The Registrant concludes that the 1000 mg/kg/day dose level exceeds the MTD.

Based on the data available, it appears that the mid- dose tested in the 1993 mouse carcinogenicity study was not adequate to assess the carcinogenic potential of Thiodicarb in male and female CD®-1 mice.

2. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Reference: 104-Week Dietary Carcinogenicity Study in Rats [IRI Project # 450441; Report # 11026, dated 7/5/94; MRID # 43308201; Document No. 011359 (Interim sacrifice report; MRID # 43405001; Report # 7881, dated 1/5/94; Document No. 011378)].

a. Experimental Design

Sprague-Dawley rats [50/sex/group for 105 weeks; 15/sex/group for interim 52-week sacrifice] were fed Thiodicarb at dose levels of 0, 60 ppm [♂♂ 3.3/♀♀ 4.5 mg/kg/day], 200 ppm [♂♂ 12/♀♀ 15 mg/kg/day], and 900 ppm [♂♂ 60/♀♀ 80 mg/kg/day].

b. Discussion of Tumor Data

FEMALES: There were no compound-related tumors observed in female rats. **MALES:** Male rats had a significant increasing trend and a significant difference in the pair-wise comparison of the 900 ppm dose group with the controls for testicular interstitial cell tumors [Table 8].

Table 8. Thiodicarb - Sprague-Dawley Rat Study

Male Testes Interstitial Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	60	200	900
Tumor	5/49	3/48	3/48	12[a]/47
(%)	(10)	(6)	(6)	(26)
p =	0.002**	0.369[n]	0.369[n]	0.044*

[+]Number of tumor bearing rats/Number of rats examined, excluding those that died or were sacrificed before week 54.
[a]First adenoma observed at week 75, dose 900 ppm.
[n]Negative change from control.
Note: Interim sacrifice rats are not included in this analysis. There were no testes interstitial cell tumors observed in any interim sacrifice rats.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

When compared to historical controls of the testing facility, the incidence at the high-dose level exceeds the historical control incidence [Table 9].

Table 9. Historical Control Incidence of Testicular Interstitial Cell Tumors and Focal Hyperplasia in Sprague-Dawley Rats

Study #/ # Examined	Incidence		
	Benign	Malignant	Focal Hyperplasia
593/50	1	0	1
609/50	0	0	1
403/48	4	0	5
504/50	3	0	5
090/49	3	0	2
140/50	5	0	6
728/50	5	0	11
059/50	4	0	0
411/50	2	0	2
944/49	2	0	3

c. Non-Neoplastic Lesions

There were significantly fewer testes at the high-dose level with no abnormality. The incidence of testicular tubular atrophy was slightly increased at the high dose compared to the control [Table 10]. At the interim sacrifice [MRID #43405001; Document No. 011378], 2 males of 15 sacrificed displayed tubular atrophy of the testes [grades ± and +].

Table 10. Non-neoplastic Lesions in the Testes

Lesion/Dose	0 ppm	60 ppm	200 ppm	900 ppm
Tubular Atrophy				
# males examined	50	50	50	49
TOTAL N=	19 [3] ♦	16 [7]	22 [11]	27 [10]
grade ±	7	5	12	10
grade +	4	6	0	6
grade ++	6	3	6	8
grade +++	2	2	4	3
Focal Interstitial Cell Hyperplasia				
# males examined	50	50	50	49
TOTAL	2	4 [1]	7 [2]	6 [2]
grade ±	1	2	2	2
grade +	1	2	5	3
grade ++	0	0	0	1
# Testes with No Abnormality	28	30	23	17*

♦ [# bilateral]; * p <0.05

d. Adequacy of Dosing for Assessment of Carcinogenicity

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Thiodicarb in male rats. Female rats indicated a significant **decreasing** trend for mortality with increasing doses of Thiodicarb. In the 52-week interim report of this study [Document No. 011378], decreased body weight was observed in both sexes at the **900** ppm dose level [♂♂ 86%/♀♀ 90% of control at week 13; ♂♂ 80%/♀♀ 86% of control at week 38] compared to the controls. Body-weight gains were decreased also at this dose level in both sexes, with males displaying a gain that was 79% of the control value and females with a gain that was 82% of the control value for the 0-13 week interval [no statistics were performed on body-weight gain data]. Both sexes at the 900 ppm dose level displayed decreased food consumption during week 1, and the 900 ppm males also ate less during week 9. At all other times, food consumption was comparable among the groups. The dose levels appear adequate to assess carcinogenicity.

There is a 1977-79 chronic toxicity/carcinogenicity study on Thiodicarb in which Fischer 344 rats [120/sex/group] were administered Thiodicarb at dose levels of 0, 0, 0.5, 1.0, 3.0, and 10.0 mg/kg/day in the diet for 24 months [interim sacrifices at 6 (10/sex/group), 12 (10/sex/group), and 19 (20/sex/group) months]. Survival overall was lowest in the control groups, and food consumption was variable throughout the study. Between days 503 and 531 of dosing, males in all groups displayed a significant weight loss, which was attributed to an outbreak of sialodacryoadenitis [SDA] virus. Rats of both sexes displayed cervical swelling and eye lesions and were generally debilitated for ≈2 weeks. Because of this, the scheduled 18-month study was postponed for ≈1 month. Females were less affected than males except for the eye lesions, which were more frequent. It was concluded by the original reviewer that Thiodicarb was negative for carcinogenicity under the conditions of this study [HDT was 10 mg/kg/day].

E. Additional Toxicology Data on Thiodicarb

1. Metabolism

Reference: The Metabolism of Thiodicarb (Acetyl-1-^{14}C) in Albino Rats; Phase I - t_{max} of Plasma, Whole Blood, and Red Blood Cell Levels of ^{14}C and Urine Collections; Phase II - Material Balance; Phase III - Urine, Plasma, Red Blood Cell, and Tissue Levels of Thiodicarb and Selected Metabolites; and Amendment No. 1. [Study # HLA 6224-100; MRID # 41250006 and 41250007; Document No. 011436].

The absorption, distribution, elimination, and biotransformation of [Acetyl-1-^{14}C] Thiodicarb have been studied in Charles River Crl:CD®(SD)BR rats following oral [single low (2 mg/kg) and single high (16 mg/kg) doses] administration. The major routes of elimination were expired CO_2 [13-24%], expired acetonitrile [13-38%], and urine [21-34%]. Tissue residues were 7-9% of the dose at 7 days post dose and may reflect the metabolism of ^{14}C-Acetonitrile into the body's C-2 and C-1 pools and subsequent interaction with, or incorporation into natural products. Material balance was ≈88% at 7 days. The major metabolites of Thiodicarb in the rat are CO_2 and Acetonitrile. The major urinary metabolite is a labile unknown that represents ≈50% of the urinary radiolabel. It decomposed to ≈50% materials that were not condensed by dry-ice acetone, 25% volatiles that were condensed by dry-ice acetone, and 25% nonvolatile residues. The tissue residues consisted mainly of water-soluble materials and some insoluble residue. Hydrolysis of the residue resulted in solubilization of the residue in water but not in the formation of discrete metabolites. No acetamide was detected in any of the tissues. The RBCs contained only residue that cannot be extracted by organic solvents or water, indicating the presence of radiolabel incorporated into natural products or of material tightly bound to hemoglobin.

In a metabolism study in monkeys, Thiodicarb [syn, syn-isomer] undergoes in vivo metabolism to syn-Methomyl and subsequent isomerization to anti-Methomyl, with ≈0.8-1.0% [lower limit] to 2.6-3.3% by weight [upper limit] of Thiodicarb being converted to Acetamide and excreted in the urine.

2. Mutagenicity

References: 1. **1985 Studies**: (a) Mutagenicity of Thiodicarb in a Mouse Lymphoma Assay [Study # LBI 20989; dated 7/85; MRID# 00151574; Document No. 005515]. (b) Clastogenic Evaluation of 91.48% a.i. Thiodicarb ... in an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells [Study # LBI 20990; dated 7/85; MRID# 005151572; Document No. 005515]. (c) Evaluation of

Thiodicarb in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay [Study # LBI 20991; dated 7/85; MRID# 005151573; Document No. 005515].

a. Mouse lymphoma assay. Positive Under the conditions of two independent experiments, Thiodicarb at doses ranging from 5 to 10 μg/mL with and without metabolic activation induced dose-related increased mutant frequencies in mouse lymphoma $TK^{+/-}$ cells. Thiodicarb is considered to have an equivocal weak effect in the mouse lymphoma forward mutation assay.

b. Chromosome aberration assay. Negative Under the conditions of the assay, doses of Thiodicarb ranging from 0.5 to 30 μg/mL without S9 and 5 to 100 μg/mL with S9 activation did not cause a clastogenic response in the chromosomes of Chinese hamster ovary cells harvested ≈ 10 hours post treatment. Thiodicarb was adequately tested up to cytotoxic doses without activation [≥30 μg/mL] and with S9 activation [≥60 μg/mL].

c. UDS assay. Negative Under the conditions of the assay, doses of Thiodicarb ranging from 0.5 to 250 μg/mL did not induce an appreciable change in the pattern of nuclear labeling of rat hepatocytes. These doses resulted in a cell survival range of 24.2 to 105.1 percent. Thiodicarb is considered inactive in the primary rat hepatocyte unscheduled DNA synthesis [UDS] assay.

2. **1979 Studies:** (a) Mutagenicity Evaluation of UC-51762 in the Ames Salmonella/Microsome Plate Test. [LBI Project No. 20838; dated 4/78; MRID# 00044872; Document No. 003706]. (b) Micronucleus Test [Pharmakon Study No. PH-309-UC001-79; dated 5/22/79; MRID# 00044873; Document No. 003706]. (c) UC-51762 technical: Reverse Mutation in Saccharomyces cerevisiae. [Pharmakon Study No. PH-303UC-001-79; dated 6/1/79; MRID# 00044874; Document No. 003706]. (d) UC-51762 technical: Mitotic Crossing Over in Saccharomyces cerevisiae. [Pharmakon Study No. PH-302-UC-001-79; dated 6/1/79; MRID# 00044875; Document No. 003706]. (e) UC-51762 technical: Mitotic Gene Conversion in Saccharomyces cerevisiae. [Pharmakon Study No. PH-304-UC-001-79; dated 6/1/79 MRID# 00044876; Document No. 003706]. (f) UC-51762 technical: Primary DNA Damage [Pharmakon Study No. PH-305-AM-002-79; dated 5/14/79; MRID# 0044877; Document # 003706]. (g) UC-51762 technical: Inclusion in the Diet of Rats for Three-Generations and **Dominant Lethal Mutagenesis** Studies. [Carnegie-Mellon Institute of Research Project Report 42-65; dated 7/2/77; MRID# 00044871; Document No. 003706].

a. Salmonella typhimurium/microsome mutagenicity assay. Negative for inducing reverse gene mutation with Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 with or without metabolic activation; Thiodicarb levels up to 1000 μg/plate.

b. Micronucleus assay in mice. Negative for the induction of micronuclei in the bone marrow cells of male and female CF-1 mice at 6 hours after the second daily i.p. administration at dose levels of 5 or 10 mg/kg Thiodicarb. This study is unacceptable; doses were not adequate.

c. Reverse Mutation in Saccharomyces cerevisiae. Negative in inducing eukaryotic reverse mutation in the homoallelic ilv I-92/ilv I-92 diploid strain D_7 of Saccharomyces cerevisiae at dose levels of technical Thiodicarb 0.0025, 006625, 0.025, 0.0625, and 0.25 mg/mL.

d. Mitotic Crossing Over in Saccharomyces cerevisiae. Negative in inducing mitotic crossing over in the heteroallelic ade 2-40/ade 2-119 diploid strain D_7 of Saccharomyces cerevisiae at the same concentrations as in (c) above.

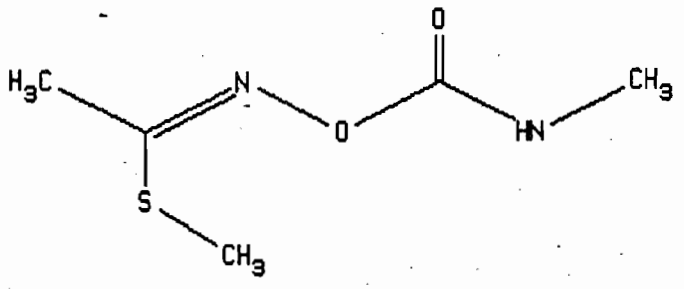
e. Gene Conversion in Saccharomyces cerevisiae. A significant increase in mitotic gene conversion was observed in the heteroallelic trp 5-12/trp 5-27 diploid strain D_7 of Saccharomyces cerevisiae at the 0.25, 0.0625, and 0.025 dose levels compared to the negative control.

(f) DNA Damage Test. Thiodicarb was tested to determine whether it can modify the DNA of the Escherichia coli repair-deficient strain p.3478 differentially than the DNA repair-competent strain W3110. Negative in producing a substantial zone of inhibition in either strain.

(g) Dominant Lethal Test. Negative. This test was run off a 3-generation reproduction study in which there were no signs of toxicity. The dose levels are not adequate. This study is unacceptable.

3. Structure-Activity Relationship

Thiodicarb is a carbamate insecticide. (1) **METHOMYL** (S-methyl N-[(methylcarbamoyl)oxy]-thioacetimidate [metabolite]. Synonyms: Lannate, Lanox, Nudrin; CAS No. 16752-77-5; an insecticide; a cholinesterase inhibitor. Chronic rat study NOEL is 100 ppm [5 mg/kg/day, LOEL is 400 ppm [10 mg/kg/day, based on spleen effects [♀♀], cholinesterase inhibition, growth retardation; negative for carcinogenicity. Mouse carcinogenicity study NOEL is 50 ppm [7.5 mg/kg/day], the LOEL 75 ppm [11.25 mg/kg/day], based on ↓ HCT, HGB, ↑ adrenal weight; negative for carcinogenicity. Mutagenicity: negative for genotoxicity in a number of test systems that have assessed the ability to cause gene mutations, structural chromosomal defects, and direct DNA damage. A single positive result for sister chromatid exchange (SCE) was reported in an abstract. HED RfD Peer Review Committee [4/13/94] concluded that the consumption of residues of Methomyl in the diet should not represent a significant carcinogenic hazard, based on the following: (a) the conversion rate of Methomyl to Acetamide is low [2-3%]; therefore, residue levels of Acetamide should be low; (b) carcinogenicity studies with Methomyl were negative in two rodent species; (c) the product is comprised of 98.7% syn-isomer and 0.92% anti-isomer; syn-isomer must be converted to anti-isomer before Acetamide is formed; (d) Acetamide induced liver tumors in rats only when administered at very high doses; i.e., > 1000 mg/kg/day. (2) **ACETAMIDE** [metabolite]. The HED Peer Review Committee concluded that Acetamide was a Group C [possible human] carcinogen, without quantitation, based on an association between liver tumors in rats and Acetamide dosing. **ACETONE OXIME [ACETOXIME]** induced hepatocellular adenomas or liver hemangiomas in MRC Wistar rats of both sexes following exposure at a high [1 gram/L] dose level in drinking water for 18 months. Methomyl oxime is an intermediate metabolite in Thiodicarb metabolism.



Structure of Methomyl

4. Acute, Subchronic, and Chronic Toxicity

Reference: RPA 051762 Oral LD_{50} in the Mouse. [Study #'s SA 95154, SA 95187, SA 95221; MRID # 43784501; Document No. 011698].

This study was submitted by the Registrant to support their contention that the mid-dose level utilized in the mouse study [70 mg/kg/day] is adequate for assessing the carcinogenic potential of Thiodicarb. They conclude that the 1000 mg/kg/day dose level exceeds the MTD, and that feeding an LD_{50} dose daily for the lifetime of the mouse should be adequately high for assessing carcinogenic potential. It is to be noted that the strain of mouse used in the LD_{50} study is different that the strain used in the carcinogenicity study. Under the conditions of the study [MRID # 43784501], **the acute oral lethal dose (LD_{50}) of Thiodicarb [96%; in methylcellulose in distilled water] to OF1 mice [5/sex/dose; 6-7 weeks old; apparently not fasted; 14-day observation period] was calculated to be 73 mg/kg [55-98] for male mice and 79 mg/kg [62-101] for female mice.**

Reference: Twenty-Eight Day Rat Cholinesterase Study. [Bushy Run Research Center Project Report # 45-10; dated 4/6/82; Accession # 070764; Document No. 001820 and 003062].

This study was performed to assess the effects of Thiodicarb on cholinesterase activity in the rat, since the subchronic and chronic studies had data for this parameter only from animals removed from the test diets and placed on control diets 24 hours prior to the collection of samples for analysis. Fischer 344 rats [10/sex/group] were fed diets containing 0, 1.0, 3.0, 10.0, or 30.0 mg Thiodicarb/ kg/day for 28 days. Analyses for plasma, erythrocyte, and brain cholinesterase were performed. The results are summarized in Tables 11 and 12. Inhibition of plasma and erythrocyte cholinesterase activity was observed in both sexes at the highest dose only, with the greatest inhibition of plasma cholinesterase in males and erythrocyte cholinesterase in both sexes being observed at the first sampling, following 7 days of dosing. Thereafter, with continued dosing, these activities were increased by day 29. Recovery of plasma and erythrocyte cholinesterase activity in males was of sufficient magnitude that no significant inhibition was detected by day 29. Reduced food intake and body-weight gains were observed in females at the 10 and 30 mg/kg/day dose levels. High-dose males displayed decreased body-weight gains, but statistical significance was not attained. Packed cell volumes and total protein concentrations were unaffected in either sex at any dose level.

Table 11. Summary of Results of 28-Day Cholinesterase Study in FEMALE Rats

Parameter/Dose	0 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10.0 mg/kg/day	30.0 mg/kg/day
food intake	12.5	12.0	12.2	11.6*(93)♦	11.5**(92)
body-weight gain [g]	17.0	13.4	16.7	8.2***(48)	7.9***(46)
Cholinesterase, [U/mL]					
PLASMA					
Day 8	3.92	3.78	4.13	3.84	3.45(88)
Day 15	4.38	3.90	3.82	4.29	3.56**(81)
Day 29	4.13	4.26	4.22	4.32	3.86(93)
RED BLOOD CELL					
Day 8	2.79	2.80	2.86	2.61	2.03***(73)
Day 15	2.52	2.74	2.56	2.38	2.06*(82)
Day 29	2.70	2.84	2.82	2.80	2.43*(90)
Brain cholinesterase, [U/mL]					
Day 29	3.82	3.78	3.90	3.89	3.50(92)
Mean Body-Weight Gains [g]					
Day 7	7.1	5.3	6.0	3.98(56)	1.9**(27)
Day 14	8.2	5.8	9.1	3.7*(45)	2.1**(25)
Day 21	13.2	9.2	12.7	4.2***(32)	5.1***(39)
Day 28	17.0	13.4	16.7	8.3***(48)	7.9***(46)

Table 12. Summary of Results of 28-Day Cholinesterase Study in MALE Rats

Parameter/Dose	0 mg/kg/day	1.0 mg/kg/day	3.0 mg/kg/day	10.0 mg/kg/day	30.0 mg/kg/day
food intake	16.6	16.4	16.2	16.7	16.0
body-weight gain [g]	26.0	26.2(92)♦	23.9(92)	26.7	21.9(84)
Cholinesterase, [U/mL]					
PLASMA					
Day 8	0.90	0.93	0.95	0.83(92)	0.69**(77)
Day 15	1.00	1.02	1.02	1.04	0.85***(85)
Day 29	0.91	0.91	0.93	0.94	0.88
RED BLOOD CELL					
Day 8	2.55	2.62	2.64	2.42	1.76***(69)
Day 15	2.47	2.42	2.79	2.36	1.98***(80)
Day 29	2.72	2.70	2.64	2.69	2.66
Brain cholinesterase, [U/mL]					
Day 29	3.86	3.92	3.82	3.75	3.86
Mean Body-Weight Gains [g]					
Day 7	8.7	8.4	8.6	9.6	3.3**(38)
Day 14	14.7	14.5	13.8	15.9	9.6*(65)
Day 21	18.9	20.0	17.4	20.5	14.1(75)
Day 28	26.0	26.2	23.9	26.7	21.9(84)

Reference: Thiodicarb 4 Week Dietary Dose Range Finding Study in Mice. [IRI Project # 450148; Report # 7430; dated 3/8/91; MRID # 43611701; Document No. 011590].

This study was submitted by the Registrant to support their contention that the mid-dose level utilized in the mouse study [70 mg/kg/day] is adequate for assessing the carcinogenic potential of Thiodicarb. Under the conditions of the study, oral administration of Thiodicarb [96%] to CD-1 mice [10/sex/group] for 4 weeks via the

diet at dose levels of 0, 30 ppm [♂♂ 6.2/♀♀ 8.3 mg/kg], 1750 ppm [♂♂ 346/♀♀ 491 mg/kg], 3500 ppm [♂♂ 734/♀♀ 954 mg/kg], and 7000 ppm [♂♂ 1538/♀♀ 2030 mg/kg] resulted in decreased body weight at the 7000 ppm dose level [♂♂ 80%/♀♀ 88% of control], a negative body-weight gain [-1.6 grams] overall in high-dose males, a decreased body-weight gain overall in the high-dose females [47% of control], and a decreased overall body-weight gain in males at the 3500 ppm dose level [76% of control]. Overall food consumption was slightly decreased in both sexes at 7000 ppm, which might be due to a palatability problem. There were no treatment-related effects observed on cholinesterase activity in plasma, RBC, or brain in either sex. There was a dose-related increase in (1) liver weight in females and (2) spleen weight in males. The high-dose females also displayed a decrease in ovarian weight. **The NOEL can be set at 30 ppm (♂♂ 6.2/♀♀ 8.3 mg/kg), the LOEL at 1750 ppm (♂♂ 346/♀♀ 491 mg/kg), based on increased liver weight in females and increased spleen weight in both sexes.**

F. Weight of Evidence Considerations

The Committee considered the following facts regarding the toxicology data on **Thiodicarb** in the Weight-of-the-Evidence determination of its carcinogenic potential:

1. **Male and female CD-1 mice were fed 0, 5, 70, and 1000 mg/kg/day of Thiodicarb for 97 weeks. The highest dose tested is the LIMIT dose. Although body weight/gains at the high dose for the males was definitely decreased relative to the control, there was no adverse effect on survival. The high-dose females displayed comparable body weight/gains to their controls, but there was a significant increasing trend for mortality, although adequate numbers of high-dose females survived to study termination. Neoplastic findings were observed only at the high dose in both sexes compared with the controls.**

Male and female mice had significant increasing trends and significant differences in the pair-wise comparisons of the 1000 mg/kg/day dose group with the controls for hepatocellular adenomas, carcinomas, and adenomas and/or carcinomas combined [$p < 0.01$].

In male mice, both the incidence of adenomas and carcinoma in the **high-dose** males are greater than the historical control incidence, and the incidence of adenomas in the **mid-dose** males [70 mg/kg/day] also exceeds the historical control incidence.

In female mice, the incidence of both hepatocellular adenomas and carcinomas in the **high-dose** females exceeds the historical control incidence.

2. **Male and female Sprague-Dawley rats were fed 0, 60 [♂♂ 3.3/♀♀ 4.5 mg/kg/day], 200 [♂♂ 12/♀♀ 15 mg/kg/day], and 900 ppm [♂♂ 60/♀♀ 80 mg/kg/day] Thiodicarb for 104 weeks. Neoplastic findings were observed only in males at the high-dose level.**

Male rats had a significant increasing trend [$p < 0.01$] and a significant difference in the pair-wise comparisons [$p < 0.05$] of the 900 ppm dose group with the controls for testicular interstitial cell tumors.

The incidence of these tumors is greater than the historical control incidence.

In female rats, there was no increase in any neoplastic lesion attributable to treatment.

3. From the submitted studies, Thiodicarb was weak to equivocally positive in the mouse lymphoma forward mutation assay, negative in both the chromosome aberration and UDS assays, negative in several 1977-1979 assays [Ames, micronucleus assay in mice bone marrow cells, dominant lethal assay, DNA

damage test, reverse mutation and mitotic crossing over in Saccharomyces cerevisiae], but there was a significant increase in mitotic gene conversion in Saccharomyces cerevisiae. Overall there was a low concern for mutagenicity.

4. Structure-Activity. Thiodicarb is metabolized to Methomyl, Methomyl oxime [acetone oxime shown to induce liver tumors at 1 g/L drinking water], acetamide [Group C carcinogen without quantitation], and acetonitrile [equivocal evidence of carcinogenic activity; increased incidence of hepatocellular adenomas/carcinomas in male rats].

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Carcinogenicity Peer Review Committee agreed that Thiodicarb should be a Group B2 - probable human carcinogen, based on increases in liver tumors in both sexes of the CD-1 mouse, statistically significant by both pair-wise and trend analysis and statistically significant increases in testicular interstitial tumors in the male Sprague-Dawley rat. While there was low concern for mutagenicity, data from related structural analogs provided additional support.

The CPRC noted that while the highest dose in mice may have been excessive, the overall dose selection was improper with the highest dose more than 10 fold that of the mid-dose. The CPRC also noticed that there was a suggestive tumor response in the male mouse liver, even at the mid-dose, and the incidence exceeded that of historical controls. Also, the tumor incidences were unusually high (hepatocellular combined adenoma/carcinoma at the highest dose: 76% vs 18% in controls for males; 62% vs 2% in controls for females.)

The CPRC felt it was inappropriate to apply a linear low-dose extrapolation (Q*) to the animal data because the increased incidences of tumors were statistically significant only at the highest dose in both species; in the case of the mice, the highest dose may even have been excessive. In addition there was no evidence of genotoxicity. Therefore, for the purpose of risk characterization, the CPRC recommended that a non-linear methodology (MOE) be applied for the estimation of human risk, based on the hepatocellular combined adenoma/carcinoma in male mice, with the point of departure set at the 5 mg/kg/day dose (NOEL).

H. Induces Cancer Call -- Thiodicarb

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to Thiodicarb resulted in an increased incidence of liver tumors (malignant and benign) in both sexes of the CD-1 mouse and testicular interstitial cell tumors in the male Sprague-Dawley rat. Acetamide, a metabolite of Thiodicarb is associated with liver tumors in rodents and Methomyl-oxime, another metabolite of Thiodicarb is an analog of acetone-oxime, which is also carcinogenic to the rodent liver.

The Committee agrees that Thiodicarb induces cancer in animals.