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OFFICE OF
PREVENTION, PESTICIDES AN
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Trichlorfon - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division

TO: Thurston Morton, Risk Assessor
Reregistration Branch 4
Health Effects Division (7509C)

The Cancer Assessment Review Committee met on February 17, 1999 to evaluate the carcinogenic potential of Trichlorfon. Attached please find the Final Cancer Assessment Document.

cc: Karl Baetcke
Luke Brennecke
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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

TRICHLORFON

FINAL REPORT

16-JUN-1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Linda Lee Taylor
Linda Taylor, Toxicologist

DOCUMENT PREPARATION: Sanjivani Diwan
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam	
Kerry Dearfield	Kerry Dearfield
Virginia Dobozy	Virginia Dobozy
Yiannakis Ioannou	
Esther Rinde	Esther Rinde
Joycelyn Stewart	Joycelyn Stewart
Clark Swentzel	Clark Swentzel
Linda Taylor	Linda Lee Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of ~~data~~, respectively)

Lucas Brennecke,	Pathology Consultant	Lucas J. Brennecke
Lori Brunsman,	Statistical Analysis	Lori L. Brunsman

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EXECUTIVE SUMMARY

On February 17, 1999, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate additional information regarding the carcinogenicity of Trichlorfon.

A previous review by the Cancer Peer Review Committee (CPRC; Document No. 011437, dated February 28, 1995) concluded that administration of Trichlorfon to female CD-1 mice in the diet at doses of 0, 300, 900 or 2700 ppm (equivalent to 0, 66, 245, or 750 mg/kg in females) was associated with statistically significant increases in lung tumors at 300 and 900 ppm but this increase was not sustained at the 2700 ppm dose. The CPRC concluded that the doses tested in CD-1 mice were adequate to assess the carcinogenic potential of Trichlorfon, and did not consider lung tumors in female mice to be treatment related.

Combined results of two studies in F344 rats revealed that dietary administration of Trichlorfon at doses of 0, 100, 300, 1750 (equivalent to 0, 4.4, 13.3, 75.5 in males and 5.8, 17.4 and 93.7 mg/kg/day in females in a multi-dose level study) or 2500 ppm (equivalent to 129 and 159 mg/kg/day for males and females, respectively in a single dose study) was associated with a statistically significant increase in lung adenomas, combined lung adenomas/carcinomas and kidney adenomas in the males, and lung carcinomas in females. An increased incidence of pheochromocytomas at 1750 ppm in the multi-dose study was not observed at 2500 ppm in a single dose study. The Committee determined that, although the lung and kidney tumors in F344 rats were treatment related, they occurred only at doses considered to be excessively toxic based on cholinesterase inhibition and other clinical signs. The lung and kidney tumors in rats seen at excessive dosing were concluded by the CPRC to be not relevant for consideration of potential human carcinogenicity. While the majority of the CPRC classified trichlorfon as Group E, some members felt that a Group D classification was more appropriate because (1) there were significant increasing trends and statistically significant differences by pair wise comparison with controls, 2) the incidences were outside the historical controls, and 3) these tumors are uncommon in the rat. It was also noted that the response seen in the lungs of female mice, as well as the pheochromocytomas in male rats, could be considered as equivocal evidence. The evidence for genotoxicity/mutagenicity was both positive and negative and data for structure-activity relationship were equivocal.

On February 17, 1999, the Cancer Assessment Review Committee (CARC) evaluated additional data submitted by the registrant on the mammary gland tumors since the 1995 meeting. The CARC concluded that administration of Trichlorfon was associated with increasing significant trends for mammary gland adenocarcinomas, adenoacanthomas, and combined adenomas, adenocarcinomas, and adenoacanthomas in female CD-1 mice. There was also a significant difference in the pair-wise comparison of the high-dose group with controls for mammary gland combined adenomas, adenocarcinomas, and adenoacanthomas. Additionally, the incidence was outside the historical control range. However, the highest dose was considered excessive because of significant cholinesterase inhibition and increased mortality. Also, the increase in tumor incidence was seen only at the high-dose level, there was no dose response, no decrease in

previous CPRC assessment of the rat and the other mouse tumor data.

The Committee classified Trichlorfon as **"not likely to be carcinogenic to humans at low doses, but is likely to be carcinogenic at high doses".**

I. INTRODUCTION

On April 6, 1994 and August 31, 1994, the Health Effects Division's Cancer Peer Review Committee (CPRC) evaluated the carcinogenic potential of Trichlorfon (CPRC, 1995; Doc. No. 011437). The CPRC concluded that the lung and kidney tumors in the rat were treatment related. However, since they occurred only at a dose that was considered to be excessively toxic based on cholinesterase inhibition and clinical signs of toxicity, they were not considered to be relevant for assessing risk to humans. In mice, the increase in lung tumor incidence seen at low and mid dose females was not sustained at the high dose. The CPRC did not consider this response to be compound related. The doses tested in mice were considered to be adequate by the CPRC at that time.

On February 17, 1999, the Cancer Assessment Review Committee (CARC) agreed and reaffirmed CPRC's decision regarding the earlier mouse tumor evaluation . The previous assessment of the carcinogenic potential of Trichlorfon in mice presented to the CPRC did not include an evaluation of the incidence of mammary tumors.

II. EVALUATION OF CARCINOGENICITY EVIDENCE

1. 24-Month Feeding/Carcinogenicity Study in Mice

Reference: Trichlorfon: 2-Year Feeding/Oncogenicity Study in Mice. Mobay Corporation. 1988. MRID No. 40782401; 40844301.

a. Experimental Design

In a two-year feeding/oncogenicity study, Trichlorfon was administered to 50 male and 50 female CD-1 mice in diet at dose levels of 0, 300, 900 or 2700 ppm (0, 49, 158 or 514 mg/kg in males and 0, 66, 245, or 750 mg/kg in females). Hematology examinations were performed on 10 males and 10 females per dose level at weeks 80 and 104 and at the termination of the study. Cholinesterase activity was assessed at weeks 80 and 104. No interim sacrifices were performed in this study.

b. Discussion of Mouse Mammary Tumor Data

The CARC analyzed the additional information submitted by the Registrant subsequent to the 1995 evaluation. The additional information consisted of statistical analyses [MRID 44627701] of survival and tumor incidence by Peto's survival-adjusted trend test methods for the mouse carcinogenicity study. The Registrant concluded that the new assessment indicated that mammary gland tumors in the high-dose female mice were statistically significantly increased when analyzed together; i.e., combined mammary gland epithelial neoplasia and adenocarcinoma/ adenoacanthoma [memo from L.Taylor to B.Tarplee, dated 2/2/99].

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The assessment of the new statistical evaluation by Science Analysis Branch [SAB memo dated January 28, 1999], considered **only** mammary tumors in female mice. It concluded that female mice had significant trends for mammary gland adenocarcinomas and combined adenomas, adenocarcinomas and adenoacanthomas, both at $p < 0.01$. There was also a significant trend for mammary gland adenoacanthomas at $p < 0.05$. Female mice showed a significant difference in the pair-wise comparison of the 2700 ppm dose group with the controls for mammary gland combined adenomas, adenocarcinomas and adenoacanthomas at $p < 0.05$ (Table 1). This incidence (20%) was outside the historical control range of the testing laboratory (0-6%) and that reported in the published literature (0%-12.5%; Table 2).

Table 1. Female Mouse Mammary Gland Tumor Rates+ and Peto's Prevalence Test Results (p values)- Brunsman (1999)

	Dose (ppm)			
	0[1]	300	900	2700
Adenomas[a]	0/36	1/39	0/29	2/32
(%)	(0)	(3)	(0)	(6)
p =	0.068	0.296	-	0.088
Adenocarcinomas[b]	1/42	1/41	0/41	4/41
(%)	(2)	(2)	(0)	(10)
p =	0.008**	-	-	0.121
Adenoacanthomas[c]	0/32	0/33	0/26	2/24
(%)	(0)	(0)	(0)	(8)
p =	0.010*	-	-	0.159
Combined	1/42	2/41	0/41	8/41
(%)	(2)	(5)	(0)	(20)
p =	0.000**	-	-	0.014 *

[1]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor

.a First mammary gland adenoma observed at week 91, 2700 ppm.

b First mammary gland adenocarcinoma observed at week 76, 2700 ppm

c First mammary gland adenoacanthoma observed at week 101, 2700 ppm

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

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Table 2. Comparison of Mammary Tumor Incidence [% based on Brunsman, 1999 memo] to Historical Control Data						
Trichlorfon [ppm]					Laboratory HC	Literature HC
Tumor type/Dose	0	300	900	2700	N/A	N/A
adenoma	0	1 [3%]	0	2 [6%]		0%-2% [1995]
adenocarcinoma♪	1 [2%]	1 [2%]	0	4 [10%]		0%-12.2% [1995]
adenoacanthoma♪	0	0	0	2 [8%]		0%-4.2% [1995]
combined	1 [2%]	2 [5%]	0	8 [20%]	0%-6% {9/647}	3.8%-12.5% [1988] 0%-12.2% [1995] 2.8% (20/725) [1992]

♪ literature for adenocarcinoma/adenoacanthoma 0%-13.2%, 5/38 mice [1971] cited in a memo by L. Taylor (1999)

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c. Non-neoplastic lesions

In female mice, there was a statistically significant increasing trend for mortality that was associated with increasing doses of Trichlorfon [60%, 54%, 44%, and 42% survival for the control, low-, mid-, and high-dose females, respectively]. Clinical observations included increased incidences of urine stains and ear lesions in males at the two highest dose levels and vaginal discharge in females at the highest dose tested. Plasma, erythrocyte and brain cholinesterase activity were depressed in all dosed groups at some sampling intervals during the study as follows: (1) decrease in plasma cholinesterase activity in females at week 80 at the mid- [20%] and high-dose [70%] levels and at week 104 at all dose levels [20%, 31%, and 74%], (2) decrease in RBC cholinesterase activity at all dose levels at both week 80 [14%, 19%, 38%] and week 104 [10%, 13%, 38%], and (3) decrease in brain cholinesterase activity at all dose levels [26%, 44%, 71%] at study termination.

Absolute liver weights were increased 18% in high dose females and relative liver weights were increased in mid and high dose females (12% and 17%, respectively). Grossly, increased number of liver foci were present in high dose males. There were no precursor lesions observed in the mammary gland.

d. Adequacy of Dosing for Determining Carcinogenic Potential

Although the CPRC concluded that the doses were adequate for determining the carcinogenic potential of trichlorfon in CD-1 mice, the CARC considered the high dose to be excessive because (1) based on recent analysis of survival data there was increased mortality in females; in addition, there was (2) decrease in plasma, RBC, and brain cholinesterase levels.

II. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC reaffirmed the previous assessment by the CPRC regarding the lung and liver tumors in mice and adrenal, lung and kidney tumors in rats (CPRC, Document No. 011437). However, based on recent statistical analysis (Brunsman, 1999), the CARC concluded that the tumors in mice were induced only at an excessively toxic dosing based on increasing trend in mortality and decrease in cholinesterase inhibition.

(a) CD-1 Mice

(i) Mammary Gland

Tumors of the mammary gland were observed in females at 2700 ppm (750mg/kg/day).

- The previous assessment of the tumor incidence in mice that was presented to the CPRC did not include an evaluation of the incidence of mammary tumors.

- The analyses by the Registrant of survival and tumor incidence indicated no statistically-significant differences in mortality rates among the groups [both sexes], and the Peto analysis of tumor data in females showed that the combined mammary gland epithelial neoplasia and adenocarcinoma/adenoacanthoma were statistically significant. The mammary tumors in the high-dose females were statistically significant only when analyzed together. The combined incidence of mammary epithelial tumors in female mice was 1/42, 2/41, 0/41, and 8/41 at 0, 300, 900, and 2700 ppm, respectively. The registrant's overall conclusion was that the borderline incidence of mammary gland [epithelial] tumors in the high-dose females was an incidental finding for the following reasons: (1) Peto analysis did not indicate statistical significance for individual mammary tumors; (2) there was no linear dose-related response as there were no tumors in the mid-dose group; (3) latency for the tumors in the high-dose group was not decreased; (4) there was no associated increase in epithelial hyperplasia or preexisting chronic inflammatory changes in these mice; and (5) the combined mammary epithelial tumor incidence was near the range reported in the literature.

- Re-analyses of these tumors by the Science Analysis Branch (Brunsman, 1999) indicated that female mice had significant ($p<0.01$) trends for mammary gland adenocarcinomas and combined adenomas, adenocarcinomas and adenoacanthomas. There was also a significant ($p<0.05$) trend for mammary gland adenoacanthomas. Female mice showed a significant difference in the pair-wise comparison of the 2700 ppm dose group with the controls for mammary gland combined adenomas, adenocarcinomas and adenoacanthomas (8/41; 20%; $p=0.014$ vs 1/42; 2% in controls).

  The CARC attributed the mammary tumors to the treatment because the combined incidence of these tumors was outside the historical control range reported by the testing laboratory (0%-6%) and in the published literature (0%-12.5%) but did not consider them relevant for human cancer risk assessment because they were seen at doses that were excessively toxic. This was based on the following: (1) although the Registrant's analysis did not indicate a difference in mortality rates,

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SAB's analysis found a significant increasing trend for mortality in females with increasing dose [60%, 54%, 44%, and 42% survival for the control, low-, mid-, and high-dose females, respectively], (2) there was statistically significant decrease in plasma cholinesterase activity in females at week 80 at the mid- [20%] and high-dose [70%] levels and at week 104 at all dose levels [20%, 31%, and 74%], (3) decrease in RBC cholinesterase activity was noted at all dose levels at both week 80 [14%, 19%, 38%] and week 104 [10%, 13%, 38%], and (4) decrease in brain cholinesterase activity was evident at all dose levels at study termination [26%, 44%, 71%].

(ii) Lung

Increased incidence of alveolar/bronchiolar adenomas, combined alveolar/bronchiolar adenomas /carcinomas and carcinomas alone were seen only in female mice at the low (300 ppm) and/or mid dose (900 ppm). This increase, however, was not sustained at the high dose (2700 ppm) and therefore, was not attributed to treatment.

- Females had significant differences in pair-wise comparison with controls for adenomas at 300 ppm and carcinomas at 900 ppm (adenomas 13/44; 30%; p=0.02 and 9/49; 18%; p=0.013, respectively; controls: 4/44; 9% and 2/46; 4%, respectively). A significant difference in pair-wise comparison was also evident at 300 ppm and 900 ppm (15/49; 31%; p=0.022 and 14/49; 29%; p=0.022, respectively) for the combined adenomas and carcinomas compared to controls (6/46; 13%). This increased incidence was not sustained at the high dose (10/47; 21%).

The CARC concurred with CPRC's decision that the lung tumors in female mice were not treatment related.

(iii) Liver

In males only a positive trend was observed in the incidence of hepatocellular adenomas.

- In males, a significant (p=0.041) positive trend was observed in the incidence of adenomas at 300, 900 and 2700 ppm (22%, 34% and 35%, respectively, vs 20% on controls); however, there were no statistically significant increases by pair-wise comparison with concurrent controls.

- The Registrant's recent assessment confirmed the nonstatistical increase in hepatocellular adenoma in males discussed in the original report; however, there was no overall combined increase in proliferative

hepatocellular lesions.

The CARC reaffirmed the CPRC's assessment that the liver tumors were not treatment related.

(b) Fisher 344 Rat

(i) Adrenal Gland

The pheochromocytomas of the adrenal gland observed in high dose (1750 ppm or 75.5 mg/kg/day) males in a multi-dose study were not seen at 2500 ppm (129 mg/kg/day) in another study.

- There were significant (p=0.05) differences in pair-wise comparison of the 1750 ppm group with the controls for pheochromocytomas. The incidence of this tumor was outside the historical control range.

The CPRC noted that the significant increase in the incidence of pheochromocytomas at 1750 ppm in a multi-dose level study was not confirmed at a higher dose (2500 ppm) in a single dose study. The CARC agreed with CPRC's previous conclusion and considered the evidence for pheochromocytomas in male rats as equivocal.

(ii) Lung

In a single dose study Trichlorfon at 2500 ppm (129 and 159 mg/kg/day, for males and females, respectively) induced lung adenomas in male rats and and lung carcinomas in female rats.

- When the data from the two rat studies were combined, there was significant (p= 0.004) increasing trend for lung adenomas in females and significant differences in the pair-wise comparisons of the 2500 ppm group with the controls for lung adenomas and combined lung adenomas/carcinomas in males (Adenomas: 4/49; 8%; p=0.001; combined 4/49, 8%; p=0.001) and carcinomas in females (3/49, 6%; p=0.036)

- The incidences of these tumors were outside the historical control range (0% -2%) for both sexes.

The CPRC attributed the lung tumors to the treatment. However, did not consider them relevant for human risk assessment because the lung adenomas in males and lung carcinomas in females occurred only at a dose level considered excessive based on cholinesterase inhibition [males 64%/females 52% (plasma); males 58%/females 54% (brain)] and other clinical signs [urine stains, paleness and rough coat, clinical chemistry

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CARC concurred with CPRC's assessment of lung tumors in rats.

(iii) Kidney

In a single dose study, renal tubular adenomas were seen at 2500 ppm (129 mg/kg/day)in male rats. No such tumors were seen in female rats.

- There was a significant positive trend in high dose males (p=0.001). When the data from the two rat studies were combined there was significant difference in the pair-wise comparison of the 2500 ppm group with the controls (3/27; 11%; p=0.007 vs 0/66 in controls) for renal adenomas in males.

- The incidences of these tumors were outside the historical control range (0% -3.3%).

The CPRC attributed the renal cortical tumors to the treatment. However, they were not relevant for human risk assessment because the kidney tumors in males occurred only at a dose level considered excessive based on cholinesterase inhibition [males 64%/females 52% (plasma); males 58%/females 54% (brain)] and other clinical signs [urine stains, paleness and rough coat, clinical chemistry changes (↓RBC, HCT, HGB), ↑ hepatic enzymes, ↑ trend in mortality in males. The CARC concurred with CPRC's assessment of renal tumors in rats.

2. **Mutagenicity/Other Considerations**

The CARC noted that in the previous CPRC assessment (Document No. 011437) of Trichlorfon both positive and negative evidence for genotoxicity/mutagenicity were found. Based on two additional studies in the literature, a Hungarian study, which reported an occurrence of Down Syndrome in babies born to mothers who had consumed fish grown on farms where Trichlorfon was extensively used (Czeizel *et al.*, 1993) and a report of an aneugenic effect on male mouse germ cells in Trichlorfon-treated mice (Bulsiewicz *el al.*, 1976), the CARC concluded that Trichlorfon may have a potential to cause gene mutations in humans.

Also discussed was Dichlorvos (DDVP), a metabolite of Trichlorfon, which has mutagenic activity. A concern was raised that *in vivo* (or possibly in situ) conversion to DDVP may be a possible mode of action for Trichlorfon mutagenic activity.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified Trichlorfon as **"not likely to be carcinogenic to humans at low doses, but is likely to be carcinogenic at high doses"** based on the following weight-of-the-evidence considerations:

1. Exposure to Trichlorfon resulted in an increased incidence of tumors of the kidneys (adenomas) in male rats and an increased incidence of tumors of the lungs in both sexes of rat (adenomas/carcinomas in males; carcinomas in females) only at dose levels determined to be excessively toxic to the rats (increased mortality, cholinesterase inhibition, non-neoplastic histopathological changes in these organs). None of these tumors was considered to be relevant for human risk assessment because they were seen only at doses that were excessively toxic.

2. Mammary tumors were induced by Trichlorfon in female CD-1 mice only at a dose level that was considered excessively toxic (increased mortality and cholinesterase inhibition). These tumors were not considered relevant for human risk assessment because they were seen only at a dose that was excessively toxic.

3. The structurally-related compounds, Naled and Dichlorvos, were classified as Group E and Group C, respectively.

IV. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Not required.

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