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WASHINGTON, DC 20460

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OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Trichlorfon

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and

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TO: Robert Forrest  
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THROUGH: Stephanie R. Irene Ph.D. *Stephanie R. Irene*  
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The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on April 6 and August 31, 1994 to discuss and evaluate the weight-of-the-evidence on trichlorfon with particular reference to its carcinogenic potential. The CPRC concluded that the tumors associated with the administration of trichlorfon to F344 rats occurred only at doses that were determined to be excessively toxic to the rats. Based on these findings, the consensus of the CPRC was that trichlorfon should be classified as a Group E.

## SUMMARY

Administration of trichlorfon in the diet to Fisher 344 (F344) rats in a multi-dose study at doses of 100, 300 or 1750 ppm was associated with a statistically significant increase in the incidence of benign pheochromocytomas in high dose males. The incidence of these tumors was slightly outside of the upper end of the historical control range.

In a second single-dose study, administration of trichlorfon in the diet to F344 rats at a dose of 2500 ppm was associated with increased incidences of lung and renal adenomas in males, and lung carcinomas in female rats. All of these tumor incidences were well outside the upper end of the historical control range.

When the data from these two studies were combined for statistical analyses, there was a statistically significant increase in lung adenomas and combined adenomas/carcinomas and for kidney adenomas in the males, and for lung carcinomas in females, all at the 2500 ppm dose only. There were also statistically significant increased trends for lung adenomas and combined adenomas/carcinomas and kidney adenomas in male rats, and for lung carcinomas in females. The pheochromocytomas which were significantly increased at the HDT (1750 ppm) in the multi-dose study, were not significantly increased in the single higher dose (2500 ppm) study.

The consensus of the CPRC was that the lung and kidney tumors in the rat were related to compound administration. However, since they occurred only at a dose which was considered to be excessively toxic, based on cholinesterase inhibition and other clinical signs of toxicity, these tumors were not considered relevant to a consideration of the potential carcinogenicity of trichlorfon for humans.

Administration of trichlorfon to CD-1 mice in the diet at doses of 300, 900 or 2700 ppm was associated with statistically significant increases in lung adenomas at 300 ppm only, combined adenomas/carcinomas at 300 and 900 ppm and lung carcinomas at the mid-dose only (900 ppm) in female mice. The increased incidence of lung tumors at the low and mid doses was flat, and the increase was not sustained at the high dose; therefore, the CPRC did not consider this response in the female mouse lung to be compound-related. In male mice there was only a statistically significant increased trend for hepatocellular adenomas. The doses tested in the mouse were considered to be adequate for assessing the carcinogenic potential of trichlorfon.

There were both positive and negative data for genotoxicity. Trichlorfon is structurally related to naled (Group E - RfD) and dichlorvos or DDVP (classified as a Group C by CPRC).

A. Individuals in Attendance at one or both meetings:

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

Penny Fenner-Crisp

Penny U. Fenner-Crisp

Stephanie Irene

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

Melba Morrow<sup>1</sup>

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Joycelyn Stewart

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Lucas Brennecke<sup>2</sup>  
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3. Other Attendees: Bernice Fisher, John Doherty, David Anderson, B.H.Chin, Ed Budd and Jane Smith (HED).

<sup>1</sup>Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

<sup>2</sup>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. Melba Morrow, and tables and statistical analysis by Lori Brunzman. The material reviewed is attached to the file copy of this report.

## C. Background Information

Trichlorfon, chemical name, Dimethyl(2,2,2-trichloro-1-hydroxyethyl) phosphonate is an organophosphate compound used as an insecticide on food and non-food crops. (HED was informed in a memo from L. Rossi dated 9/30/92, that the registrant intended to drop all food uses for the compound). The compound is also used as fly bait in and around farm buildings, including dairy barns, milk processing rooms and battery poultry establishments. The compound has been used in the veterinary drug area as a livestock anthelmintic and as an insecticide.

Trichlorfon is a white crystalline solid with a melting point of 75 to 84° C. The compound is readily soluble in water, dichloroethane and 2-propanol. The toxic chemical number (HED) is 385. The CAS Registry Number is 52-68-6.

The structure of trichlorfon is:

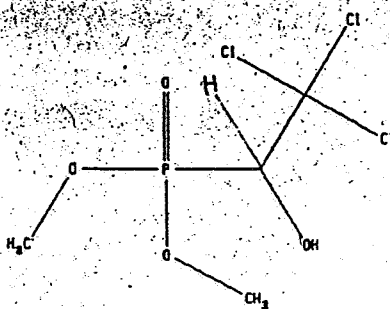


Figure 2 Trichlorfon

D. Evaluation of Carcinogenicity Evidence

1. Multiple Dose Chronic/Carcinogenicity Study in Fischer 344 Rats

Reference: 2-Year Feeding/Oncogenicity study. Mobay Corporation.  
1989 MRID No. 41056201. Hed Doc. No. 009626

a. Experimental Design

Trichlorfon, (technical grade 98.8%) was administered to Fischer 344 rats (70/sex/group for control and high dose; 50/sex/group for low and mid dose groups). The compound was administered at dietary levels of 0, 100, 300 or 1000 ppm (0, 4.4, 13.3 or 75.5 mg/kg for males; 0, 5.8, 17.4 or 93.7 mg/kg for females). At the highest dose tested, no signs of toxicity were present after the first 27 weeks, so the dose was increased to 1250 ppm from weeks 28-32; 1500 ppm from weeks 33-40 and 1750 ppm for weeks 41-106. This resulted in an average dose of 1514 ppm (75.7 mg/kg) for the high dose group for the duration of the study. Animals were housed individually and received food and water ad libitum.

A total of 20 rats/sex of control and high dose animals were killed at the 53 week interim sacrifice. At the interim sacrifice, animals were examined for body weights, organ weights, gross pathology and histopathology. Blood was collected prior to sacrifice for hematology and serum chemistry evaluations.

Animals (50/sex/group) continuing in the main study were sacrificed at week 105. Blood was collected for hematology, serum chemistry and cholinesterase determination at weeks 14, 27, 52, 79 and 105. At the final sacrifice, animals were examined for body weights, organ weights, gross and microscopic pathology.

b. Discussion of Tumor Data

Under the conditions of this study, administration of trichlorfon was associated with an increase in the incidence of benign pheochromocytomas in male rats. The incidence of this tumor in males was 6, 12, 16 and 28 percent in control, low, mid and high dose males, respectively. Only the increase at the highest dose was considered statistically significant.

A statistically significant increase in the incidence of hepatocellular adenomas was observed in 9/50 low dose males (18%), only when compared to 1/50 controls (2%). The tumor incidence was not dose related, nor was it accompanied by a trend. With the

exception of the control group, all of the dosed male animals had incidences of hepatocellular adenomas that were outside of the historical control range.

A statistically significant increase in the incidence of multiple site mononuclear cell leukemia in low and high dose males was also reported. (Incidence was 37, 46, 38 and 52 percent for control, low, mid and high dose groups, respectively). These incidences were within the historical control range and were not associated with a dose-related increasing trend.

c. Non-neoplastic Lesions and Other Observations

No compound related mortality was reported in this study. Survival percent was 60, 68, 62 and 52 percent in control, low-dose, mid-dose and high-dose males, respectively and 68, 70, 78 and 72 percent in control, low-dose, mid-dose and high-dose females, respectively.

A decrease in body weight (4%) was reported in high dose males during the last 32 weeks of dosing. In high dose females, a decrease in body weight (4%) was apparent from weeks 13 through 55 and at termination and corresponded to a decrease in food consumption. Clinical findings included paleness and hunched back in high dose males and rough coats in high dose females.

In high dose animals of both sexes, anemia was reported and was characterized by decreases in hematocrit, hemoglobin, red blood cell counts and mean corpuscle volume. Hypercholesterolemia was also present in mid dose males and in high dose animals. Decreases in red cell, plasma and brain cholinesterase activity  $\geq 20\%$  was reported at various sampling intervals for high dose males and females.

Absolute and relative liver weights in high dose females were significantly increased by 22.1 and 26.6%, respectively. In high dose males, absolute and relative kidney weights were increased by 19.6% and 7.5%, respectively. In high dose females, relative kidney weight was significantly increased by 7.44%.

Gross pathological findings consisted of granular kidneys in high dose animals of both sexes, foci in the lungs of high dose females; thickened, enlarged and/or dilated cranial small intestines and thickened and/or granular non-glandular stomachs were observed grossly in high dose males. These gross findings were correlated with microscopic findings of cranial hyperplasia in the small

intestines, non-glandular gastritis in high dose males and females and chronic inflammation of the lung in high dose females. In the kidneys, chronic nephropathy was increased in incidence in high dose females and increased in severity in high dose males. Renal calcification was also significantly increased in mid and high dose males.

d. Adequacy of Dosing for Determining Carcinogenic Potential

The doses selected were considered adequate based on the occurrence of clinical signs of toxicity, alterations in hematological and serum chemistry parameters and the inhibition of blood, plasma and brain cholinesterase activity in the high dose animals. These animals demonstrated slight anemia, associated with decreases in hematocrit, hemoglobin, erythrocyte counts and mean corpuscular volume. Hypercholesterolemia was also reported and absolute and/or relative increases in kidney and liver weights were recorded. Histological findings included duodenal hyperplasia, non glandular gastritis, chronic nephropathy and inflammation of the lungs, which could be correlated to gross findings of thickened cranial intestines, thickened stomach, granular kidneys and localized lesions on the lungs. There was a 10% reduction in body weight gain in high dose males and an 18% reduction in high dose females as compared to controls at week 13.

2. Single Dose Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats

Reference: Trichlorfon: 2-Year feeding/Oncogenicity Study in Rats. Mobay Corporation. 1989. MRID No. 41973001, 42510301. HED. Doc. No. 008845.

a. Experimental Design

Seventy male and 70 female Fischer 344 rats were administered technical trichlorfon (98.5%) at dietary levels of 0 or 2500 ppm (0 or 129 mg/kg for males and 0 or 159 mg/kg for females). The animals received the test diet for 104 weeks. Satellite groups of 20/sex/dose level were included for interim sacrifice at week 58. At the interim sacrifice and at study termination, animals were examined grossly and histopathologically for lesions that could be related to trichlorfon administration. Organ and terminal body weights were recorded for all animals. Blood was collected at 3, 6, 12, 18 and 24 months for assessment of clinical chemistry and hematology parameters and for evaluation of blood and plasma



cholinesterase activity.

b. Discussion of Tumor Data

The administration of TCF was associated with an increase in the incidence of renal tubular adenomas in males (0/50 in controls vs 3/50 at 2500 ppm). Alveolar/bronchiolar adenomas were also increased in males (0/50 in controls vs 4/50 at 2500 ppm) and alveolar/bronchiolar carcinomas (0/50 in controls vs 3/50 at 2500 ppm) were increased in females. The lung tumors and renal tubular tumors were outside of the historical control range for the specific tumor type (lung or renal) in Fischer 344 rats. None of these tumor types were increased in the multiple dose study conducted in the same strain of rats. The benign pheochromocytomas that were present at a statistically significant level in the high dose males in the initial chronic/oncogenicity study in Fisher 344 rats, were not significantly elevated at 2500 ppm, nor was there a statistically significant increase in the incidence of mononuclear cell leukemia. (See Tables 1 & 2 for combined analyses in male rats, and Table 3 for combined analysis in female rats. The available historical control data are given in Table 4)

Table 1. Trichlorfon - Combined Charles River Fisher 344 Rat Studies

	<u>Dose (ppm)</u>				
	0	100	300	1750 <sup>#</sup>	2500
Alveolar/ Bronchiolar Adenomas (%)	0/96 (0)	0/50 (0)	0/50 (0)	0/50 (0)	4 <sup>a</sup> /49 (8)
p =	0.000 <sup>**</sup>	-	-	-	0.001 <sup>**</sup>
Alveolar/ Bronchiolar Carcinomas (%)	0/59 (0)	1 <sup>b</sup> /34 (3)	0/31 (0)	0/26 (0)	0/20 (0)
p =	0.724	0.094	-	-	-
Combined (%)	0/96 (0)	1/50 (2)	0/50 (0)	0/50 (0)	4/49 (8)
p =	0.001 <sup>**</sup>	0.094	-	-	0.001 <sup>**</sup>

<sup>+</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

<sup>#</sup>Time-weighted average of this dose group is 1514 ppm.

<sup>a</sup>First alveolar/bronchiolar adenoma observed at week 70, 2500 ppm.

<sup>b</sup>First alveolar/bronchiolar carcinoma observed at week 106, 100 ppm.

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.

9

Table 2. Trichlorfon - Combined Charles River Fisher 344 Rat Studies

Male Kidney Tumor Rates<sup>+</sup> and Peto's  
 Prevalence Test Results (p values)

	<u>Dose (ppm)</u>				
	0	100	300	1750 <sup>#</sup>	2500
Adenomas (%)	0/66 (0)	0/42 (0)	0/41 (0)	0/32 (0)	3 <sup>a</sup> /27 (11)
p =	0.001**	-	-	-	0.007**

<sup>+</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

<sup>#</sup>Time-weighted average of this dose group is 1514 ppm.

<sup>a</sup>First adenoma observed at week 101, dose 2500 ppm.

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.

10

Table 3. Trichlorfon - Combined Charles River Fisher 344 Rat Studies

Female Lung Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (ppm)</u>				
	0	100	300	1750 <sup>#</sup>	2500
Alveolar/ Bronchiolar Carcinomas (%)	0/98 (0)	0/49 (0)	0/48 (0)	1/50 (2)	3 <sup>a</sup> /49 (6)
p =	0.004 <sup>**</sup>	1.000	1.000	0.338	0.036 <sup>*</sup>

<sup>+</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

<sup>#</sup>Time-weighted average of this dose group is 1514 ppm.

<sup>a</sup>First alveolar/bronchiolar carcinoma observed at week 97, 2500 ppm.

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

Table 4. Historical Control Data for the F344 Rat

**Lung Tumors**

Mobay historical data (no dates or number of studies provided)

Lesion	Males		Females	
	Range	Number	Range	Number
Alv/bronch. adenoma	0 -2%	4/360	0 -1%	1/360
Alv/bronch. carcinoma	0 -1%	2/360	0 -2%	2/360

(No historical data were provided for renal tubular adenomas.)

**Literature Historical controls (%) from NTP data**

Source	Lesion	# Males	# Females
Hasegan, et al. 1984	Alv/bron. adenoma	35/2350 (1.5)	18/2354 (0.8)
	carcinoma	20/2350 (0.9)	8/2354 (0.4)
Solleveld, et al. 1984	Alv/bron. adenoma	35/2320 (1.5)	18/2370 (0.8)
	carcinoma	20/2320 (0.9)	9/2370 (0.4)
Goodman, et al. 1979	Alv/bron. adenoma	35/1794 (1.9)	21/1754 (1.2)
	carcinoma	16/1794 (0.9)	5/1754 (0.3)

**Kidney Tumors**

Historical control data from Charles River Laboratories, Feb. 1990

Lesion	Male Renal adenoma	# Males	Range
		5/964 (0.5)	(0-3.3%)

c. Non-Neoplastic Observations

There was no treatment related mortality in this study; however, when the results from this study were combined with those from the initial study in rats, a significant increasing trend in mortality in males was observed by statistical analysis.

The administration of the test compound was associated with body weight (9%) decrease and body weight gain decrements (10.5%, M and 18.5%, F). Compound related effects on weight gain became apparent after week 10 of dosing. Food consumption was lower for the dosed males and females for the first 65 and 90 weeks, respectively.

Clinical signs of toxicity included increased incidences of urine staining, enlarged abdomen and tail zones. In males, rough hair coats and ocular paleness were reported. Anemia, characterized by decreases in hematocrit, hemoglobin, red cell count, MCV and MCH, was reported in both sexes of treated animals during the course of the study. Hypercholesterolemia was also present in both sexes and in males, sporadic increases in hepatic enzymes (alkaline phosphatase, AST, ALT and GGPT) were reported. Plasma, red cell and brain cholinesterase activity were depressed to levels  $\geq 20$  percent in both sexes of treated animals. Brain cholinesterase was 58 percent and 54 percent lower than that reported for controls for females and males, respectively.

There were reported increases in liver, kidney and lung weights (percentages not provided in DER), when controls are compared to treated females. Both sexes of animals also had decreased terminal body weights (7% decrease for males and 11% decrease for females).

At 2500 ppm, macroscopic examination demonstrated statistically significant treatment related lesions such as external stains and discharges, tail and skin zones of ulceration in males, lung zones and foci in both sexes, kidney discoloration in both sexes, thickened and dilated cranial small intestines and raised or granular serosal zones in the intestines of males. In females, enlarged spleens were also observed.

Microscopically, renal tubular hyperplasia was present to a greater degree in both sexes of treated animals; however, this finding was not statistically significant. The incidence of this lesion was 3/50 and 7/50 for control and treated males, and 0/50 and 3/50 for control and treated females, respectively. Chronic nephropathy was present at a statistically significant level in dosed females. This lesion was also present in almost all male rats, the incidence being 49/50 for controls and 50/50 for treated males.

Lung lesions in both sexes of treated animals consisted of chronic inflammation, type II pneumocyte hyperplasia, and multifocal distribution of adenomatous hyperplasia. The incidence of duodenal hyperplasia was significantly increased in the treated males and was non-significantly increased in treated females. Gastritis in the non-glandular portion of the stomach was reported in treated males and females and was associated with ulceration, necrosis and reactive inflammation, hyperplasia with overlying hyperkeratosis and active healing (re-epithelialization). Hepatocellular vacuolation was also statistically increased in both sexes of animals.

Renal calcification was significantly increased for males with 33/50 animals affected compared to 9/50 for controls. Hepatocellular hyperplasia was reported in dosed males, only.

d. Adequacy of Selected Dose Levels

The purpose of conducting this single dose rat study was to make sure that trichlorfon was tested for carcinogenicity at an adequate dosage. All findings at 2500 ppm, with the exception of hepatic dilatation and associated increases in hepatic enzymes, were present at the highest dose tested in the previous rat study. There was also a greater depression in blood, brain and plasma cholinesterase activity when the values obtained at the 2500 ppm dose level were compared to the values at 1514 ppm (average high dose in the multiple dose rat study). In males, an increasing trend in mortality, with increasing dose, was present when the results were combined for both studies in rats. Based on the clinical findings (urine staining, enlarged abdomen, rough coats, anemia, increases in hepatic enzymes, decreases in red cell parameters), the gross and microscopic findings (in the gastrointestinal tract, liver, lungs and kidneys), and on the depression of red cell, plasma and brain cholinesterase at levels greater than 20% of controls it was determined that this dose was excessive.

3. 24 Month Feeding/Carcinogenicity Study in Mice

Reference: Trichlorfon: 2-Year Feeding/Oncogenicity Study in Mice. Mobay corporation. 1988. MRID No. 40782401, 40844301. HED Doc. No. 009626.

a. Experimental Design

Trichlorfon was administered in the diet to 50 male and 50 female CD-1 mice at levels of 0, 300, 900 and 2700 ppm (0, 49, 158 or 514 mg/kg in males and 0, 66, 245 or 750 mg/kg in females). Animals were individually housed and food and water were available ad libitum. 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 Tumor Data

Trichlorfon was associated with an increase in the incidence of lung adenomas in low dose females (30%), only. The incidence of lung carcinomas was increased in mid dose females (18%) and the incidence of combined lung tumors was increased for all treated groups of females when compared to controls; however, there was a statistically significant increase for combined adenomas/carcinomas in the low and mid-dose groups only (Table 5). In males, a positive trend was observed in the incidence of hepatocellular adenomas; however, there were no statistically significant increases by pairwise comparison with concurrent controls (Table 6). In male mice there was also an increase in the number of treated animals that were found to have hemangiosarcomas of the liver, muscle and spleen when compared to controls; however, this increase was not associated with a dose response relationship.



Table 5. Trichlorfon - Charles River CD-1 Mouse Study

Female Lung Tumor Rates<sup>+</sup> and  
 Peto's Prevalence Test Results (p values)

	<u>Dose (ppm)</u>			
	0	300	900	2700
Alveolar/Bronchiolar Adenomas (%)	4/44 (9)	13/44 (30)	5 <sup>a</sup> /42 (12)	7/43 (16)
p =	0.442 <sup>n</sup>	0.020*	0.370	0.188
Alveolar/Bronchiolar Carcinomas (%)	2/46 (4)	2/49 (4)	9 <sup>b</sup> /49 (18)	5/47 (11)
p =	0.109	0.439	0.013*	0.088
Combined (%)	6/46 (13)	15/49 (31)	14/49 (29)	10 <sup>c</sup> /47 (21)
p =	0.379 <sup>n</sup>	0.022*	0.022*	0.121

<sup>+</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

<sup>n</sup>Negative trend.

<sup>a</sup>First alveolar/bronchiolar adenoma observed at week 73, 900 ppm.

<sup>b</sup>First alveolar/bronchiolar carcinoma observed at week 62, 900 ppm.

<sup>c</sup>Two animals in the 2700 ppm 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.

16

Table 6. Trichlorfon - Charles River CD-1 Mouse Study

Male Hepatocellular Tumor Rates<sup>+</sup> and Exact Trend  
 Test and Fisher's Exact Test Results (p values)

	<u>Dose (ppm)</u>			
	0	300	900	2700
Adenomas (%)	10/50 (20)	11/49 (22)	16/47 (34)	17 <sup>a</sup> /48 (35)
p =	0.041 <sup>*</sup>	0.479	0.091	0.069
Carcinomas (%)	7/50 (14)	2/49 (4)	5 <sup>b</sup> /47 (11)	4/48 (8)
p =	0.395 <sup>n</sup>	0.085 <sup>n</sup>	0.425 <sup>n</sup>	0.286 <sup>n</sup>
Combined (%)	17/50 (34)	13/49 (27)	21/47 (45)	20 <sup>c</sup> /48 (42)
p =	0.119	0.278 <sup>n</sup>	0.193	0.283

<sup>+</sup>Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

<sup>n</sup>Negative trend or negative change from control.

<sup>a</sup>First adenoma observed at week 70, dose 2700 ppm.

<sup>b</sup>First carcinoma observed at week 68, dose 900 ppm.

<sup>c</sup>One animal in the 2700 ppm 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.

17

c. Other Non-Neoplastic Observations

In female mice, there was a statistically significant increasing trend for mortality that was associated with increasing doses of trichlorfon.

Body weight increases were reported to have occurred sporadically during the study for mid and high dose females. Clinical observations included increased incidences of urine stains and ear lesions in males at the two highest dose levels and vaginal discharges in females at the highest dose tested. Plasma, erythrocyte and brain cholinesterase activity were depressed in all dosed groups at some sampling interval during the study. Depression of cholinesterase activity was reported to be  $\geq 20\%$  for erythrocytes in both sexes at the highest dose tested. For plasma, depression in cholinesterase activity of 20% or greater, occurred in both sexes at 900 and 2700 ppm. Brain cholinesterase activity was depressed at levels greater than 20% in all of the treated groups.

Absolute liver weights were significantly increased (18%) in high dose females and relative liver weights were increased in mid (12%) and high dose (17%) females. Relative adrenal weight was increased in low dose males and absolute heart weight was significantly increased in high dose males. (No figures were provided in the DER which would allow for calculation of percentages).

Grossly, liver foci were present in high dose males at significantly increased incidences. In females, gross lesions included changes in lymph node color and splenic enlargement. Microscopically, the only significantly increased lesions were congested mesenteric lymph nodes and skin ulcers in high dose females and hematopoiesis in high dose males.

d. Adequacy of Dosing for Determining Carcinogenic Potential

Based on the decreases in plasma, brain and erythrocyte cholinesterase activity reported in the 24 month feeding/carcinogenicity study in mice, the doses were adequate for determining carcinogenic potential.

E. Additional Toxicology data on Trichlorfon

1. Ten year Chronic Feeding/Oncogenicity Study in Monkeys

Reference: Trichlorfon: Chronic Feeding/Oncogenicity Study in Monkeys. Mobay Corporation. 1988. MRID 40776001. Hed Doc. No. 009626.

Technical trichlorfon (99.1% and 98.6% purity reported) was fed to Rhesus monkeys (5/sex/dose level) six days per week, for 10 years. The dietary levels were 0, 0.2, 1.0 or 5.0 mg/kg/day and the compound was administered via Tang orange juice.

There were no compound related malignancies reported for any of the monkeys in this study. Benign neoplasms were reported but were infrequent in their occurrence and could not be associated with trichlorfon administration. Additionally, there were no preneoplastic lesions reported after the ten year treatment.

At the highest dose tested, there was a decrease in the body weight for both sexes (6-28%, males, 6 - 33% females). At this same dose level, transitory signs of cholinesterase depression were observed during the first month of dosing in females. These signs included muscle fasciculations and diarrhea. Diarrhea was also observed in the high dose males.

Anemia, that was characterized by decreases in erythrocyte counts, hemoglobin and hematocrit values, was reported in both sexes of high dose animals. Plasma and erythrocyte cholinesterase activity were depressed (>20%) in all treated groups except low dose males and brain cholinesterase was depressed (>20%) in all treated groups except the low dose females.

## 2. Metabolism

In the 1992 IPCS Environmental Health Criteria publication on trichlorfon, it is stated that trichlorfon rearranges to form dichlorvos (DDVP) via dehydrochlorination. After the administration of trichlorfon, DDVP has been found in animal tissues at less than 5% of the total dose of the parent; however, DDVP has not been detected very often and only the degradation products of DDVP were found as evidence that this compound was formed (total dose and circumstances of administration not provided)<sup>3</sup>.

The main metabolites of radiolabelled trichlorfon found in mammals were demethyl trichlorfon, demethyl dichlorvos, dimethyl hydrogen phosphate, methyl hydrogen phosphate and phosphoric acid. The main degradation routes of trichlorfon are demethylation, P-C bond cleavage, and ester hydrolysis.

## 3. Genotoxicity

Data from mutagenicity tests conducted in vivo and in vitro indicate that trichlorfon is positive in some tests and negative in others depending on the system used, the dosage, the purity and source of the test material and the possible effects derived from its degradation products (IARC, 1983). The following results were found in HED's data base (from acceptable studies) for trichlorfon:

- In gene mutation assay (Ames) with *Salmonella typhimurium*, trichlorfon was found to be weakly mutagenic at toxic concentrations with or without activation. (MRID 249535, HED Doc. # 003267).

- In an in vitro gene mutation study in mammalian cells, trichlorfon induced significant increases in mutation frequencies both with and without metabolic activation. (MRID 2456446, HED Doc. # 004509).

- In a gene mutation assay conducted using *S. cerevisiae*, trichlorfon was not found to be mutagenic with or without metabolic activation. (MRID 2546446, HED Doc. # 004509).

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<sup>3</sup>The IPCS document also indicates that dichlorvos (DDVP) is a contaminant of technical trichlorfon and is a degradation product of trichlorfon in neutral and basic solutions. DDVP can also be formed during the analytical detection process [Memo, dated June 13, 1988: Trichlorfon, Gross To Miller, et al., HED Doc. # 006665].

- In an UDS study, trichlorfon was inactive in inducing unscheduled DNA synthesis in rat hepatocytes up to levels of severe cytotoxicity. (MRID 00028625, HED Doc. # 003267).
- In DNA damage and repair studies, trichlorfon was positive for DNA damage and repair in *S. typhimurium*, but negative in relative toxicity assays with *E. coli* and *B. subtilis* strains. (MRID 00028625, HED Doc. # 003267).
- In another DNA damage/repair study conducted with *S. cerevisiae*, trichlorfon was positive for mitotic recombination in the presence or absence of S-9 activation at concentrations from 10 to 50 mg/mL. (MRID 00028625, HED Doc. # 003267).
- At cytotoxic levels, trichlorfon was associated with a marginal but significant increase in sister chromatid exchange in Chinese hamster ovary cells. (GS0104149, HED Doc. # 003267).
- Trichlorfon was demonstrated to be clastogenic in human lymphocytes in the absence of S9 activation at doses of 3, 10 and 30 ug. (HED Doc. # 008481).

#### 4. Structure Activity Relationship

Trichlorfon, naled and dichlorvos (DDVP) are structurally related organophosphate insecticides. DDVP has been classified as a Group C carcinogen based on the presence of forestomach tumors in female B6C3F1 mice and mononuclear cell leukemia in male Fischer 344 rats; naled was classified as a Group E chemical, having no evidence of being a carcinogen in laboratory animals.

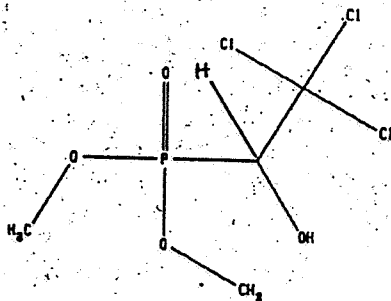


Figure 3 Trichlorfon

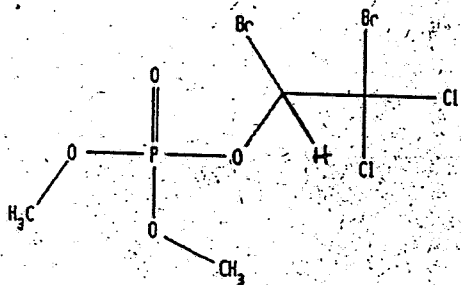


Figure 4 Naled

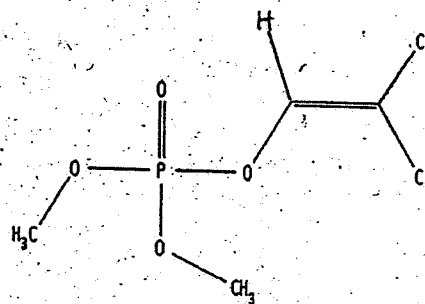


Figure 5 DDVP

**F. Weight of the Evidence Considerations:**

The Committee considered the following facts regarding the toxicology data on trichlorfon in determining the carcinogenic potential:

1. Administration of trichlorfon to male and female Fischer 344 rats at doses of 0, 100, 300 or 1750 ppm (average high dose 1514 ppm) was associated with an increased incidence of benign pheochromocytomas in high dose males which was significant by pairwise comparison with the controls ( $p \leq 0.05$ ) and which was slightly outside of the historical control range. It was also associated with an increased incidence of multiple site mononuclear cell leukemia in male rats which was significant at the low and high doses, but not at the mid-dose. The incidence rate for this tumor type was 37, 46, 38, and 52 percent for the control, low, mid and high dose groups, respectively.

Dosing in this study was considered adequate based on significant depression of cholinesterase activity; (plasma: 23% M, 38% F; RBC: 35% M, 20 F%; brain: 47% M, 46% F) in high dose male and female rats, accompanied by clinical signs of paleness, rough coats, hunched backs, reduction of body weight gain (10.5% M, 18 % F); histopathological lesions of hepatocellular dilatation, renal calcification, intestinal hyperplasia and gastritis in males and inflammation of the lungs and Harderian glands; and chronic nephritis and gastritis in females.

2. Administration of trichlorfon to male and female Fischer 344 rats at doses of 0 and 2500 ppm was associated with increased incidences of lung adenomas and renal tubular adenomas in males and of lung carcinomas in females. All of these tumor incidences were outside of the historical control range. There was no increase in the incidence of mononuclear cell leukemia in male rats in this study (incidence rate 37 percent for control and 43 percent for the trichlorfon treated group). There was also no significant increase in the incidence of pheochromocytomas (8 percent and 12 percent respectively in the control and treated groups).

When the data from these two studies were combined and statistically analyzed male rats had significant increases in the pairwise comparisons of the 2500 ppm group and the controls for lung adenomas and for lung adenomas/carcinomas and for renal adenomas. These increases were accompanied by dose related increasing trends.



Female rats had a significant dose related increasing trend and a significant increase by pairwise comparison of the 2500 ppm dose group with the controls for lung carcinomas.

Dosing in this study was probably excessive based on 63 and 52 percent depression of plasma in males and females, respectively and 58 and 54 percent depression in brain cholinesterase activity in males and females, respectively. The cholinesterase depression was accompanied by clinical signs of urine stain, paleness and rough hair coat, clinical chemistry changes of decreased red cell parameters (red cell count, MCV, hemoglobin and hematocrit), increased cholesterol and increased hepatic enzymes (AP, AST, ALT and GGT), histopathological lesions similar to those reported in the multiple dose study, with additional lesions of hepatocellular vacuolation and adenomatous and pneumocyte hyperplasia in the lungs, and an increasing trend in mortality in males when data from the two studies were combined.

3. In female CD-1 mice administered trichlorfon at doses of 0, 300, 900 or 2700 ppm, there were significant differences in the pair-wise comparisons of the 300 ppm group with controls for alveolar/bronchiolar adenomas and combined alveolar/bronchiolar adenomas and /or carcinomas. There was also a significant difference in pair-wise comparison of the 900 ppm group with controls for alveolar/bronchiolar carcinomas and combined alveolar/bronchiolar adenomas and/or carcinomas. No statistically significant pairwise increases relative to concurrent controls were reported at 2700 ppm for lung adenomas, carcinomas or combined tumors.

Male CD-1 mice had a significant dose-related increasing trend in hepatocellular adenomas; however, there were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Based on the clinical signs of urine stains and vaginal discharge, histopathology (lymph node congestion and skin ulcers) and depression of brain, plasma and red cell cholinesterase > 20% depression in all cholinesterase parameters at the highest dose the doses tested were adequate for determining the carcinogenic potential of trichlorfon.

4. In Rhesus monkeys administered trichlorfon at doses of 0, 0.2, 1.0 or 5.0 mg/kg for ten years, there were no compound associated tumors or preneoplastic lesions. The duration of treatment in monkeys was not representative of life-time exposure and the study is not considered adequate for determination of carcinogenic

potential for this species.

5. Metabolism data demonstrated that the compound is excreted via the urine, feces and expired air, with 80 to 90% of the compound being excreted within 24 hours. The main degradation routes are demethylation, P-C bond cleavage and ester hydrolysis.

6. Positive genotoxicity data are available in the Salmonella assay, in in vitro cytogenetics assay in mammalian cells, DNA damage and repair using S. typhimurium, and clastogenicity to human lymphocytes. Negative genotoxicity data are available for gene mutation in S. cerevisiae, unscheduled DNA synthesis in rat hepatocytes and DNA damage and repair using E. coli and B. subtilis.

7. Trichlorfon is structurally related to DDVP which has been classified as a Group C carcinogen by the Cancer Peer Review Committee and the Scientific Advisory Panel. Trichlorfon is also structurally related to naled which has been classified as a Group E chemical with regard to carcinogenicity.

8. Carcinogenicity in animals -- Trichlorfon

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to trichlorfon resulted in an increased incidence of tumors of the kidney (adenomas) in male rats and tumors of the lungs in both sexes of the rat (adenomas and combined adenomas/carcinomas in males, carcinomas in females) at doses determined to be excessively toxic to the rats. The relevance of the tumor data to an evaluation of trichlorfon's potential for human carcinogenicity is discussed elsewhere in this document.

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 consensus of the CPRC was that trichlorfon should be classified as Group E - no evidence for carcinogenicity in humans. This decision was based on the results of two animal studies in different species (mouse and rat). In the rat, although there were statistically significant increases in tumors (lung, kidney) these occurred only at a dose which the CPRC considered to be excessive. Although tumors of the lung were also seen in female mice, at doses considered to be adequate, the CPRC did not consider these to be compound-related, since the increases seen at the low and mid-doses were flat and not sustained at the high dose. The increase in pheochromocytomas seen in male rats in the multi-dose study was not confirmed in the single higher dose study.

While the majority of the CPRC agreed on the E classification for trichlorfon, some members felt that a Group D classification was more appropriate, based on the following: Although the significant increase in tumors occurred only at an excessive dose in both sexes of the rat, the increases were statistically significant by pairwise comparison with controls ( $p < .01$  in the male<sup>4</sup>), the incidences were well outside of the historical controls, there were statistically significant positive trends ( $p < .01$ ) and these tumors of the lung and kidney are uncommon in the rat (incidence of less than 5%). 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, and that the data from genotoxicity studies and SAR were also equivocal.

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<sup>4</sup>In the female rat, only tumors in the lung (all carcinomas) were significantly increased, with a pair-wise significance of  $p < 0.05$ ; there was also a positive trend,  $p < 0.01$ , for these tumors.