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OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ethoprop - Report of the Cancer Assessment Review Committee

FROM: Jess Rowland *Jess Rowland 10/2/97*  
Executive Secretary  
Cancer Assessment Review Committee  
Health Effects Division (7509C)

Through: William Burnam  
Chairman  
Cancer Assessment Review Committee  
Health Effects Division (7509C)

To: Mike Metzger  
Chief, Reregistration Action Branch 1  
Health Effects Division (7509C)

And

Walter Waldrop  
Chief, Reregistration Branch 111  
Special Review and Reregistration Division (7508W)

The Cancer Assessment Review Committee met on June 25 and August 20, 1997 to evaluate the carcinogenic potential of Ethoprop. Attached please find the Cancer Assessment Document.

cc: Stephanie Irene  
Randy Perfetti  
Margaret Stasikowski

1935



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Randy Perfetti  
Margaret Stasikowski

2835

**FINAL**

**FINAL**

**CANCER ASSESSMENT DOCUMENT**

**EVALUATION OF THE CARCINOGENIC POTENTIAL OF  
*ETHOPROP***

**FINAL REPORT**

**September 25, 1997**

**CANCER ASSESSMENT REVIEW COMMITTEE  
HEALTH EFFECTS DIVISION  
OFFICE OF PESTICIDE PROGRAMS**

3835

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DATA PRESENTATION:

Kit Farwell  
Kit Farwell, D.V.M., Toxicologist

## DOCUMENT PREPARATION:

Jess Rowland  
Jess Rowland, M.S., Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE  
AT ONE OR BOTH MEETINGS

(Signature indicates concurrence with the  
assessment unless otherwise stated).

William Burnam, Chairman

Wm Burnam  
*not in attendance  
at 2nd meeting*

Karl Baetcke

Kerry Dearfield

Kerry Dearfield

Virginia Dobozy

Virginia Dobozy

Mike Ioannou

M. Ioannou

Hugh Pettigrew

Hugh Pettigrew

Esther Rinde

Esther Rinde

Joycelyn Stewart

Joycelyn Stewart

Linda Taylor

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE

(Signature indicates concurrence with  
the pathology report and statistical  
analysis of data, respectively)

Luke Brennecke, Pathology Consultant

Lori Brunsman, Statistical Analysis

Hugh Pettigrew for Lori Brunsman

OTHER ATTENDEES: Ed Budd

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## EXECUTIVE SUMMARY

On June 25 and August 20, 1997, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Ethoprop.

Dr. Kit Farwell introduced the chronic toxicity/carcinogenicity studies in Sprague-Dawley rats, Fischer 344 rats and B6C3F1 mice by: describing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested; and presenting the weight of the evidence for the carcinogenicity of Ethoprop. Dr. Farwell also discussed the toxicology, metabolism and mutagenicity studies as well as structure activity relationships.

Ethoprop was administered in the diet to male and female Sprague-Dawley rats at 0, 1, 60, or 400 ppm and male and female Fischer 344 rats at 0, 1, 10 or 100 ppm for 24 months as well as to male and female B6C3F1 mice at 0, 0.2, 2 or 30 ppm for 24 months. In another study with Fischer 344 rats, parental animals were fed diets containing Ethoprop at 0, 60.5, 131 or 262 ppm; after weaning, the F<sub>1</sub> pups were fed diets containing Ethoprop at 0, 4.5, 9.0 or 18 ppm for 12 weeks and then at 0, 49, 98 or 196 ppm for 97 weeks.

The Committee concluded that the dose levels tested in the Sprague-Dawley rat study and the two Fischer 344 rat studies as well as the B6C3F1 mouse study were adequate to assess the carcinogenic potential of Ethoprop. In all four studies, the principal toxicological effects were inhibition of plasma, red blood cell and/or brain cholinesterase activity as well as decreases in body weight gains. The degree of cholinesterase inhibition observed at most doses was considered to constitute "excessive" toxicity in the rats; however, the absence of clinical cholinergic signs of toxicity along with little, if any, frank pathology and increased or comparable survival in treated rats was inconsistent.

Tumors were seen in the adrenal and thyroid glands of male rats, the uterus of female rats and the liver of female mice. The Committee's primary concern was the occurrence of malignant pheochromocytomas of the adrenal gland in male Sprague-Dawley rats, a rare life-threatening tumor in this species. This tumor type was seen at all dose levels tested. The incidence at the high dose (400 ppm) reached pair-wise statistical significance when compared to concurrent controls and exceeded the historical control range. Malignant pheochromocytomas were also seen at the mid-(60 ppm) and low-(1 ppm) dose groups. However, since the adrenals at the low- and mid-dose groups were examined only in rats that died or were sacrificed moribund, the true dose-responsive nature of the tumor incidence at these doses could not be ascertained. The Committee noted that in contrast to the mid- and high-dose groups where tumors were seen in the presence of cholinesterase inhibition, at the low-dose, tumors were seen in the absence of cholinesterase inhibition.

The Committee also had concern for the occurrence of C-cell tumors of the thyroid glands in male Sprague-Dawley rats and in two different studies in male Fischer 344 rats. In Sprague-Dawley rats, the incidences of C-cell carcinomas at the high dose (400 ppm) showed a positive trend, reached pair-wise significance when compared to controls, and exceeded the historical control range. In Fischer 344 rats, while positive trends were seen for C-cell adenomas, carcinomas, and combined adenomas/carcinomas, there was a non-statistically significant increase in C-cell carcinomas at the high dose (100 ppm) in males when compared to none in the concurrent controls. The increase in C-cell carcinomas in this strain of rats is supportive of the same target organ/tumor type in the Sprague-Dawley rats.

In the *in utero* study with Fischer 344 rats, only the incidences of C-cell adenomas showed a positive trend and a pair-wise statistical significance at the high dose (196 ppm) when compared to controls. Although only adenomas were seen in this study, again, the presence of C-cell tumors is supportive of the studies discussed above.

Thus, the Committee noted that the thyroid was the target organ in rats of two different strains (Sprague-Dawley and Fischer 344) with C-cell tumors occurring in all three studies. Even though tumors were observed at doses with cholinesterase inhibition, the Committee agreed the tumors were compound related and not a secondary consequence of the cholinesterase inhibition.

The Committee had lesser concern for the follicular cell tumors seen in male Sprague-Dawley rats since the combined adenomas/ carcinomas showed only a borderline significance at the high dose. Again, the presence of this tumor also confirms the thyroid being the target organ for Ethoprop-induced carcinogenicity.

The Committee discussed the presence of uterine tumors in Sprague-Dawley and Fischer 344 rats. These tumors (endometrial polyps, endometrial stromal polyps or stromal polyps) which occur spontaneously within the uterus, are considered benign, and there is no evidence to indicate that they would transform into a more aggressive form with time. In Sprague-Dawley rats, the incidence of endometrial stromal polyps at the high dose (400 ppm), while significantly increased when compared to concurrent controls, was within the historical control range. In the *in utero* Fischer 344 rat study, the incidence of endometrial polyps was significantly increased at the mid-(98 ppm) and high-(196 ppm) dose groups, but the combined tumors (endometrial/stromal polyps) showed increasing trends and were significantly increased but only at the high dose. In both studies, these tumors were seen only at doses that caused cholinesterase inhibition. In contrast, no uterine tumors were seen in the other Fischer 344 rat study.

The Committee did not consider the hepatocellular adenomas, carcinomas and combined adenomas/ carcinomas seen at all dose levels, including the controls, in female B6C3F1 mice to be treatment related. There was no pair-wise significance for any of the three tumor measures.

In mutagenicity testing, Ethoprop exhibited clastogenic activity *in vitro* in the presence of metabolic activation. This activity provided support for a mutagenic component of the carcinogenicity concern. Clastogenicity in the whole animal, however, could not be evaluated because the severe acute toxicity precluded testing at doses high enough to possibly affect the target organ.

Of the four compounds that Ethoprop is structurally-related to (Ebufos, Disulfoton, Terbufos and Tribufos), only Tribufos was carcinogenic in mice. The tumor type and site, however, were different from that of Ethoprop. Tribufos induced adenocarcinomas of the small intestines and hemangiosarcomas of the liver in male CD-1 mice as well as alveolar/bronchiolar adenomas in female CD-1 mice. None of the other compounds exhibited any carcinogenic activity.

In accordance with the Agency's *Proposed Guidelines for Carcinogen Risk Assessment (April 10, 1996)*, the Committee classified Ethoprop as a "likely" human carcinogen. This classification is based on the following factors:

- (i) presence of a rare and life-threatening (malignant) tumor (pheochromocytoma of the adrenal glands) in male Sprague -Dawley rats at the low dose in the absence of cholinesterase inhibition;
- (ii) occurrence of another type of tumor (C-cell carcinomas of the thyroid glands) in male rats in two strains (Sprague-Dawley and Fischer 344) in three different studies at doses that did cause cholinesterase inhibition; and
- (iii) evidence of clastogenicity *in vitro* mutagenicity testing.

The Committee recommended a linear low-dose approach for human risk characterization and extrapolation of risk should be based on the occurrence of malignant pheochromocytomas of the adrenal glands in male rats at all dose levels tested. This extrapolation is supported by: (I) lack of mode of action, (ii) evidence from the total data base [i.e., occurrence of other tumor types (C-cell carcinomas of the thyroid glands) at doses that caused cholinesterase inhibition], and (iii) confirmation of clastogenic activity in mutagenicity testing.

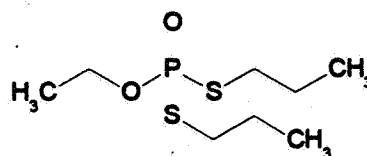


## I. INTRODUCTION

On June 25 and August 20, 1997, the Health Effects Division's Cancer Assessment Review Committee evaluated the carcinogenic potential of Ethoprop. The Committee evaluated a chronic toxicity/carcinogenicity study in CD rats, two separate chronic toxicity/carcinogenicity studies in Fischer 344 rats and a carcinogenicity study in B6C3F1 mice. Dr. Kit Farwell of the Reregistration Branch-1 presented the experimental design and results of these studies, statistical analysis of the tumor data, weight of the evidence considerations, as well as the toxicology, metabolism, mutagenicity and structure-activity data of Ethoprop.

## II. BACKGROUND INFORMATION

### Structure



Ethoprop

Synonyms: O-ethyl S,S-dipropylphosphorodithioate; Ethoprophos; Mocap; VCP-104

PC Code: 041101

CAS # 13194-48-4

Ethoprop, an organophosphate insecticide/fungicide, is a List A chemical. Tolerances have been established for a variety of raw agricultural commodities (40 CFR 180.262). Ethoprop is acutely toxic with a narrow margin between cholinesterase inhibition and death. The acute oral LD<sub>50</sub> in rats is 32 mg/kg, the acute dermal LD<sub>50</sub> in rats is 25 mg/kg, and the acute inhalation LC<sub>50</sub> in rats is 0.123 mg/L. Ethoprop was lethal at a dose of 0.1 mL when instilled in the eyes of rabbits and at a dose of 0.5 mL when applied dermally to rabbits.

On May 8, 1996, the HED Reference Dose Peer Review Committee recommended that the carcinogenic potential of Ethoprop should be evaluated by the Cancer Assessment Review Committee. This recommendation was based on the increased incidences of: malignant pheochromocytomas of the adrenal glands and follicular cell tumors of the thyroid glands in male Sprague Dawley rats.

### III. EVALUATION OF CARCINOGENICITY STUDIES

#### 1. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Crl:CD Rats

Reference: KD Williams: "104-Week Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Rats". Report Date: 9/10/92. **MRID 42530201**. Study # HLA 6224-151. Testing Facility: Hazleton Laboratories, Madison, WI.

A. Experimental Design: Technical Ethoprop (95.6%) was administered in the diet to groups of Crl:CD(SD)BR VAF/Plus rats at dietary concentrations of 0, 1, 60, or 400 ppm (400 ppm rats received 600 ppm the first 2 weeks of the study). There were 70 rats/group/sex in the main study with an additional 10/sex/group sacrificed at week 52. An additional 10/sex/group in control and high dose tested (HDT) groups were sacrificed at 56 weeks after discontinuance of dosing for 4 weeks. The study was classified Minimum.

B. Discussion of Tumor Data: As shown in Tables 1 and 2, there were decreasing trends for mortality with increasing doses of Ethoprop in males and females. Male mortality rates for the duration of the study were 50/70, 42/70, 42/70, and 30/71 and female mortality rates were 42/71, 47/70, 37/70 and 27/71 for control, low-, mid-, and high-dose groups, respectively.

As shown in Table 3, male rats had significant increasing trends ( $p < 0.01$ ) and males in the high-dose group of 400 ppm had a significant pair-wise comparison to controls for adrenal gland malignant pheochromocytomas (0/41, 2/16, 2/18, 5/60;  $p < 0.01$ ). Low- and mid-dose animals had considerably fewer adrenal glands examined, so essentially the only 2 groups available for statistical comparison were the control and high-dose groups. The frequency of malignant adrenal gland pheochromocytomas in the high-dose group of 400 ppm (8%) exceeded the historical control range (0-1.7%).

As shown in Table 4, male rats had an increasing trend for thyroid C-cell carcinomas (0/61, 0/63, 1/64, 3/66;  $p < 0.05$ ) as well for pair-wise comparison of the 400 ppm group with controls ( $p < 0.05$ ). The frequency of thyroid C-cell carcinomas in the high-dose group (5%) exceeded the historical control range for "all death codes". Of the 7 studies conducted at the testing laboratory, no C-cell carcinomas were seen in 5 studies consisting of 330 rats, but were seen in 1/50 rats (2%) in one study and in 1/60 rats (1.7%) in another study. The incidence at the high dose, however, was within the historical control range for rats surviving to terminal sacrifice due to the occurrence of 1 tumor out of 18 rats (5.6%) in one study.

As shown in Table 5, males at all dose groups had significant pair-wise comparisons to controls for thyroid follicular cell adenomas (0/53, 4/56, 4/56, 5/63; each at  $p < 0.05$ ). There was a borderline significant difference for pair-wise comparison of the 400 ppm group with controls for combined thyroid follicular cell adenomas/carcinomas ( $p = 0.05$ ). Historical control data were not submitted.

Female rats had increasing trends for endometrial stromal polyps (1/78, 2/52, 3/44, 7/78;  $p < 0.05$ ) as well as for pair-wise comparison of the 400 ppm group with controls ( $p < 0.05$ ). The frequency for endometrial stromal polyps in the high-dose group (9%) was within the historical control range (0-19%) for female rats surviving to terminal sacrifice. The reviewer for this study attributed the increase in polyps to increased survival among treated females.

C. Non-neoplastic Lesions: The incidence of non-neoplastic lesions was similar between control and treatment groups. A high incidence of age-related renal disease occurred in control and treatment groups and was one of the major causes of death.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential: Dosing was judged adequate for assessment of carcinogenic potential due to toxicity in the study. Cholinesterase inhibition occurred in mid- and high-dose males and females at various time points. Body weight decrements and anemia occurred in the high-dose male and female groups. No cholinergic signs were noted in any group.

At the mid-dose, there was statistically significant inhibition of cholinesterase activity compared to control values for plasma (-64% males and -77% females), red blood cell (-44% males, -50% females), and brain (-35% males, -32% females).

At the high-dose, cholinesterase activity was significantly inhibited in comparison to controls for plasma (-77% males, -82% females), red blood cell (-46% males, -51% females), and brain (-64% males, -66% females). In addition, at the high-dose, there were also reductions in body weight at various time points (-20% males, -18% females). Anemia was also evident at the high-dose with reductions in hematocrit compared to controls occurring at various time points (-10% in males, -12% in females).

**Table 1. Mortality Rate<sup>+</sup> in Male Sprague-Dawley Rats Following Dietary Administration of Ethoprop<sup>a</sup>**

<u>Weeks</u>						
<u>Dose</u>	<u>1-26</u>	<u>27-52</u>	<u>53<sup>i</sup></u>	<u>53-78</u>	<u>79-106<sup>f</sup></u>	<u>Total</u>
(ppm)						
0	2/80	0/78	10/78	12/68	36/56	50/70 (71) <sup>**n</sup>
1	1/80	2/79	10/77	9/67	30/58	42/70 (60) <sup>n</sup>
60	1/80	2/79	10/77	9/67	30/58	42/70 (60) <sup>n</sup>
400	2/80	1/78	9/77	5/68	22/63	30/71 (42) <sup>**n</sup>

<sup>a</sup> Mortality Rates and Cox or Generalized K/W Test Results

<sup>+</sup> Number of animals that died during interval/Number of animals alive at the beginning of the interval.

<sup>i</sup> Interim sacrifice at week 53.

<sup>f</sup> Final sacrifice at week 105.

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

( ) Percent.

Note: Time intervals were selected for display purposes only.

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. Mortality Rate<sup>+</sup> in Female Sprague-Dawley Rats Following Dietary Administration of Ethoprop<sup>a</sup>**

<u>Dose</u> (ppm)	<u>Weeks</u>					<u>Total</u>
	<u>1-26</u>	<u>27-52</u>	<u>53<sup>i</sup></u>	<u>53-78</u>	<u>79-106<sup>f</sup></u>	
0	0/81	4/81	10/77	13/67	25/54	42/71 (59) <sup>**n</sup>
1	1/80	5/79	10/74	10/64	31/54	47/70 (67)
60	0/80	2/80	10/78	5/68	30/63	37/70 (53) <sup>n</sup>
400	2/81	2/79	10/77	7/67	16/60	27/71 (38) <sup>*n</sup>

<sup>a</sup> Mortality Rates and Cox or Generalized K/W Test Results

+ Number of animals that died during interval/Number of animals alive at the beginning of the interval.

<sup>i</sup> Interim sacrifice at week 53.

<sup>f</sup> Final sacrifice at week 105.

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

( ) Percent.

Note: Time intervals were selected for display purposes only.

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 3. Pheochromocytomas of the Adrenal Glands in Male Sprague-Dawley Rats. Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results.**

<b>Tumor Type/ Incidence</b>	<b>0 ppm</b>	<b>1 ppm<sup>#</sup></b>	<b>60 ppm<sup>#</sup></b>	<b>400 ppm</b>
Benign % p <sup>S</sup> =	14 <sup>a</sup> /49 29 0.991 <sup>n</sup>	7/26 27 --	7/28 25 --	5/61 8 0.991 <sup>n</sup>
Malignant % p <sup>S</sup> =	0/41 0 0.005 <sup>**</sup>	2 <sup>b</sup> /16 12 --	2/18 11 --	5/60 8 0.005 <sup>**</sup>
Combined % p <sup>S</sup> =	14/49 29 0.837 <sup>n</sup>	8 <sup>c</sup> /26 31 --	9/28 32 --	10/61 16 0.837 <sup>n</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. Also excludes Week 53 interim sacrifice animals.

<sup>=</sup> Not all animals in the 1 and 60 ppm dose groups were examined microscopically for adrenal gland pheochromocytomas. Therefore, no statistical comparisons of these dose groups with the control could be made.

<sup>a</sup> First benign pheochromocytoma observed at week 88, dose 0 ppm.

<sup>b</sup> First malignant pheochromocytoma observed at week 94, dose 1 ppm.

<sup>c</sup> One animal in the 1 ppm dose group had both a benign and a malignant pheochromocytoma.

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

<sup>S</sup> The p-values for the trend and pair-wise comparisons are the same since there are essentially only a control and one dose group.

Note: Interim sacrifice animals are not included in this analysis.

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 4. C-Cell Tumors of the Thyroid Glands in Male Sprague-Dawley Rats.  
Tumor Rates<sup>+</sup> and Peto's Prevalence Test Results.**

<b>Tumor Type/ Incidence</b>	<b>0 ppm</b>	<b>1 ppm</b>	<b>60 ppm</b>	<b>400 ppm</b>
Adenomas % p =	8 <sup>a</sup> /66 12 0.258	6/67 9 0.775 <sup>n</sup>	9/67 13 0.452	12/68 18 0.491
Carcinomas % p =	0/61 0 0.014*	0/63 0 --	1/64 2 0.199	3 <sup>b</sup> /66 5 0.042*
Combined % p =	8/66 12 0.089	6/67 9 0.776 <sup>n</sup>	10/67 15 0.366	15/68 22 0.249

<sup>+</sup> Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Also excludes Week 53 interim sacrifice animals.

<sup>a</sup> First adenoma observed at week 60, dose 0 ppm.

<sup>b</sup> First carcinoma observed at week 71, dose 400 ppm.

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

Note: Interim sacrifice animals are not included in this analysis.

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 5. Follicular Cell Tumors of the Thyroid Glands in Male Sprague-Dawley Rats. Tumor Rates <sup>+</sup> and Peto's Prevalence Test Results.**

<b>Tumor Type/ Incidence</b>	<b>0 ppm</b>	<b>1 ppm</b>	<b>60 ppm</b>	<b>400 ppm</b>
Adenomas	0/53	4/56	4 <sup>a</sup> /56	5/63
%	0	7	7	8
p =	0.203	0.040*	0.020*	0.022*
Carcinomas	1/34	0/31	1/36	1 <sup>b</sup> /51
%	3	0	3	2
Combined	1/53	4/56	5/56	6/63
%	2	7	9	10
p =	0.165	0.152	0.089	0.050

<sup>+</sup> Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor. Also excludes Week 53 interim sacrifice animals.

<sup>a</sup> First adenoma observed at week 82, dose 60 ppm.

<sup>b</sup> First carcinoma observed at week 100, dose 400 ppm.

Note: Interim sacrifice animals are not included in this analysis.

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 6. Uterine Tumors in Female Sprague-Dawley Rats. - Tumor Rates <sup>+</sup> and Peto's Prevalence Test Results.**

<b>Tumor Type/ Incidence</b>	<b>0 ppm</b>	<b>1 ppm<sup>#</sup></b>	<b>60 ppm<sup>#</sup></b>	<b>400 ppm</b>
Endometrial	1/78	2 <sup>a</sup> /52	3/44	7/78
Stromal	(1%)	(4%)	(7%)	(9%)
Polyps				
p <sup>S</sup> =	0.044 <sup>*</sup>	--	--	0.044 <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. Also excludes Week 53 interim sacrifice animals.

<sup>#</sup> Not all animals in the 1 and 60 ppm dose groups were examined microscopically for uterine endometrial stromal polyps. Therefore, no statistical comparisons of these dose groups with the control could be made.

<sup>S</sup> The p-values for the trend and pair-wise comparisons are the same since there are essentially only a control and one dose group.

<sup>a</sup> First endometrial stromal polyp observed at week 45, dose 1 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  $p < 0.01$ .

2. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Fischer 344 Rats-1985

Reference: EJ Spicer: "Lifetime Dietary Toxicity and Oncogenicity Study in Rats".  
Report Date: 4.30.85. **MRID 40291801**. Study # 347-029. Testing Facility:  
International Research and Development Corporation, Mattawan, MI.

A. Experimental Design: Technical Ethoprop (94-96%) was administered in the diet to 50 F344 rats/sex/group at dosage levels of 0, 1, 10, or 100 ppm for 2 years. An additional 10 rats/sex/group were sacrificed at 12 and at 18 months.

This study was classified Supplementary, upgradeable pending receipt of requested data which were never received. Among the deficiencies were clarification of mortality tables, requested description of methodology for cholinesterase determination, criteria for scoring monocytic leukemia, and requested historical control data including parafollicular cell adenomas and carcinomas.

B. Discussion of Tumor Data: No significant effect of treatment upon mortality was noted. Female rats had no statistically significant increase in tumors. This study refers to thyroid parafollicular tumors. The thyroid parafollicular cell is synonymous with the thyroid C-cell.

As shown in Table 7, male rats had increasing trends ( $p < 0.05$ ) for thyroid C-cell adenomas (8/49, 5/48, 5/50, 12/50;  $P < 0.05$ ) as well as increasing trends for thyroid C-cell carcinomas (0/49, 0/48, 1/50, 3/50;  $< 0.05$ ) and increasing trends for combined adenomas/carcinomas ( $p < 0.01$ ).

No historical control data from this testing facility were submitted. However, thyroid C-cell adenomas and carcinomas exceeded the historical control range from other laboratories. The historical control values reported for other laboratories may not be relevant to this study.

C. Non-neoplastic Lesions: No changes in gross or microscopic non-neoplastic lesions were attributed to treatment.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential: This study was classified Supplementary as noted above, but could have been upgraded had requested data been submitted. Toxicity in the study included plasma and red blood cell cholinesterase inhibition in mid-dose males and females and, in addition, brain cholinesterase inhibition in high-dose males and females at various time points. The doses tested were judged to be adequate to assess the carcinogenic potential of Ethoprop.

At the mid-dose, there was significant inhibition of cholinesterase activity in comparison to control values for plasma (-60% males, -62% females) and red blood cell (-18% males, -27% females).

At the high-dose, cholinesterase activity was significantly inhibited compared to controls for plasma (-87% males, -94% females), red blood cell (-45% males, -44% females), and brain (-35% males, -48% females).

**Table 7. C-Cell Tumors of the Thyroid Glands in Male Fischer-344 Rats. Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results.**

Tumor Type/ Incidence	0 ppm	1 ppm	10 ppm	100 ppm
Adenomas % p =	8 <sup>a</sup> /49 16 0.036*	5/48 10 0.290 <sup>n</sup>	5/50 10 0.264 <sup>n</sup>	12/50 24 0.242
Carcinomas % p =	0/49 0 0.020*	0/48 0 1.000	1/50 2 0.505	3 <sup>b</sup> /50 6 0.125
Combined % p =	8/49 16 0.005**	5/48 10 0.290 <sup>n</sup>	6/50 12 0.371 <sup>n</sup>	15/50 30 0.085

Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54. Also excludes week 53 and week 79 interim sacrifice animals.

<sup>a</sup> First adenoma observed at week 97, dose 0 ppm.

<sup>b</sup> First carcinoma observed at week 88, dose 100 ppm.

<sup>n</sup> Negative change from control.

Note: Interim sacrifice animals are not included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

3. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Fischer 344 Rats by Feeding, in utero, and by Lactational Exposure, 1983

Reference: J Barnett, et al: "Evaluation of the Chronic Toxicity and Oncogenic Potential of Ethoprop in Fischer 344 Rats. Report Date: 1/20/83. **MRID 00138636**. Report # 413-858-41. Testing Facility: Gulf South Research Institute, New Iberia, LA.

A. Experimental Design: Test animals ( $F_1$  generation) were exposed to Ethoprop *in utero*, during lactation, and then by feeding. This was accomplished by administering technical Ethoprop (95.3%) in the diet to parental F344 rats ( $F_0$  generation) at dietary concentrations of 0, 60.5, 131, or 262 ppm. After weaning, 60  $F_1$  pups/sex/group were fed diets containing 0, 4.5, 9.0, or 18 ppm for 12 weeks and then placed on diets containing 0, 49, 98, or 196 ppm of Ethoprop. Ten of the 60  $F_1$  rats/group/sex were sacrificed at 53 weeks. This study was classified Supplementary because a NOEL was not established and the number of tissues examined microscopically was not evident to the Agency reviewer. Other data requested included more data on analytical testing of diet, information on statistical methods used, and breeding and litter data.

B. Discussion of Tumor Data: Mortality in control and treatment groups was similar. No historical control data were submitted for this study.

As shown in Table 8, male rats had increasing trends for thyroid C-cell adenomas (2/46, 4/43, 1/41, 10/40;  $p < 0.01$ ) as well for the pair wise comparison of the 196 ppm group with controls ( $p < 0.01$ ).

Female rats had increasing trends for uterine endometrial polyps (0/44, 4/45, 8/37, 13/42;  $p < 0.01$ ) as well as for pair wise comparison of mid- and high-dose rats compared to controls ( $p < 0.01$ ). Combined endometrial and stromal polyps showed increasing trends (8/44, 6/45, 10/37, 16/42;  $p < 0.01$ ) as well as by pair-wise comparison of the high-dose group with controls ( $p < 0.05$ ) (Table 9).

C. Non-neoplastic Lesions: No treatment-related non-neoplastic lesions were seen.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential: The doses tested were judged to be adequate to assess the carcinogenic potential of Ethoprop. Treatment related effects included significant inhibition of serum and brain cholinesterase (all dose groups), decreased body weight gain (mid- and high-dose groups), and clinical signs (emaciation and rough hair coat) in both sexes. Red blood cell activity was not decreased in any dose group. Decreased mean thyroid weight (absolute and relative) was noted for high-dose females.

At the low-dose, cholinesterase activity was decreased in comparison to control values for serum (-81% males, -89% females) and brain (-36% males, -30% females).

At the mid-dose cholinesterase activity was decreased in comparison to controls for serum (-84% males, -91% females) and brain (-45% males, -50% females). At the mid-dose, there were also body weight decrements in comparison to controls at various time points. (-8% males, -7% females).

At the high-dose, cholinesterase activity was decreased in comparison to controls for serum (-86% males, -93% females) and brain (-68% males, -65% females). In addition, there were more severe body weight decrements at the high-dose (-15% males, -16% females). High-dose females also had decreased mean absolute and relative thyroid weights.

**Table 8. C-Cell Tumors of the Thyroid Glands in Male Fischer-344 Rats  
Tumor Rates<sup>†</sup> and Exact Trend Test and Fisher's Exact Test Results.**

Tumor Type <sup>S</sup> / Incidence	0 ppm	49 ppm	98 ppm	196 ppm
Adenomas	2/46	4 <sup>a</sup> /43	1/41	10/40
%	4	9	2	25
p =	0.001*	0.307	0.544 <sup>n</sup>	0.007**

<sup>S</sup> No carcinomas were observed.

<sup>†</sup> Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53. Also excludes week 52 interim sacrifice animals.

<sup>a</sup> First adenoma observed at week 95, dose 49 ppm.

<sup>n</sup> Negative change from control.

Note: Interim sacrifice animals are not included in this analysis.

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 9. Uterine Tumors in Female Fischer-344 Rats - Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results.**

<b>Tumor Type/ Incidence</b>	<b>0 ppm</b>	<b>49 ppm</b>	<b>98 ppm</b>	<b>196 ppm</b>
Endometrial polyps % p =	0/44 0 0.000**	4 <sup>a</sup> /45 9 0.061	8/37 22 0.001**	13/42 31 0.000**
Stromal polyps % p =	8 <sup>b</sup> /44 18 0.098 <sup>n</sup>	2/45 4 0.041 <sup>n</sup>	2/37 5 0.078	3/42 7 0.113
Combined p =	8/44 18 0.006**	6/45 13 0.368 <sup>n</sup>	10/37 27 0.246	16/42 38 0.034*

<sup>+</sup> Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54. Also excludes week 53 and week 79 interim sacrifice animals.

<sup>a</sup> First endometrial polyp observed at week 109, dose 49 ppm

<sup>b</sup> First stromal polyp observed at week 88, dose 0 ppm.

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

Note: Interim sacrifice animals are not included in this analysis. There were no uterine tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If \*, then  $p < 0.05$ . If \*\*, then  $p < 0.01$ .

#### 4. Oncogenicity Study with Ethoprop in B6C3F1 Mice

Reference: Hiroyuki Inoue: "Chronic Feeding and Oncogenicity Studies in Mice with Ethoprop". Report Date: 12/29/84. **MRID Nos 40356301 and 43326001**. Study # 94. Report # 497 An-Pyo Center. Testing Facility: Biosafety Research Center, Foods, Drugs & Pesticides, Japan.

A. Experimental Design: Technical grade Ethoprop (94.6%) was administered in the diet to 50 B6C3F1 mice/sex/group at dosage levels of 0, 0.2, 2.0, or 30.0 ppm for 2 years. An additional 10 mice/sex/group were sacrificed at weeks 26, 52, and 78.

B. Discussion of Tumor Data: Mortality was similar between control and treatment groups. Male mice showed no statistically significant increase in tumors.

Female mice had increasing trends for liver carcinomas (4/49, 3/49, 1/50, and 9/49;  $p < 0.01$ ) and in combined liver adenomas/carcinomas (9/49, 5/49, 8/50, 13/49;  $p < 0.05$ ). There were no significant differences in pair-wise comparisons of treated female groups with controls.

As shown in Table 10, hepatocellular carcinoma incidence in the high-dose group (18%) exceeded mean historical control values (range, 0-10%; mean 6.5%).

C. Non-neoplastic Lesions: Treatment-related gross or microscopic lesions were not seen.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential: Dosing was adequate for assessment of carcinogenicity. Plasma and red blood cell cholinesterase inhibition occurred at the mid-dose in males and females. In addition, at the high-dose, there was inhibition of brain cholinesterase and reductions in body weight gain and food efficiency.

At the mid-dose, cholinesterase activity was significantly inhibited in comparison to control values at various time points for plasma (-35% males, -20% females) and red blood cell (-13% males, -17% females).

At the high-dose, cholinesterase activity was significantly inhibited in comparison to control values at various time points for plasma (-77% males, -71% females). Body weight gain in the high-dose group was decreased in comparison to controls (-13% males, -15% females), mostly in the first year of the study.

**Table 10. Liver Tumors Female B6C3F1 Mice - Tumor Rates<sup>+</sup> and Exact Trend Test and Fisher's Exact Test Results.**

<b>Tumor Type/ Incidence<sup>+</sup></b>	<b>0 ppm</b>	<b>0.2 ppm</b>	<b>2 ppm</b>	<b>30 ppm</b>
Adenomas % p =	5 <sup>a</sup> /49 10 0.510 <sup>n</sup>	2/49 4 0.218 <sup>n</sup>	7/50 14 0.394	4/49 8 0.500 <sup>n</sup>
Carcinomas % p =	4/49 8 0.009 <sup>**</sup>	3/49 6 0.500 <sup>n</sup>	1/50 2 0.175 <sup>n</sup>	9 <sup>b</sup> /49 18 0.116
Combined % p =	9/49 18 0.038 <sup>*</sup>	5/49 10 0.194 <sup>n</sup>	8/50 16 0.482 <sup>n</sup>	13/49 27 0.234

<sup>+</sup> Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53. Also excludes week 26, week 52 and week 78 interim sacrifice animals.

<sup>a</sup> First adenoma not in an interim sacrifice or accidental death animal seen at week 104, dose 0 ppm.

<sup>b</sup> First carcinoma observed at week 77, dose 30 ppm.

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

**Note:** Two animals in the control group, and one animal in each of the 0.2 and 2 ppm dose groups, of the week 78 interim sacrifice group had liver adenomas. One accidental death animal in the 0.2 ppm dose group had a liver adenoma. Week 26, week 52 and week 78 interim sacrifice animals and accidental death animals are not included in this analysis.

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  $p < 0.01$ .



#### IV. TOXICOLOGY

- 1. **Metabolism:** In a 1990 metabolism study (MRID 41804301), Ethoprop was administered to Crl:CD(SD)BR rats as a single i.v bolus (males and females); single oral bolus (females, metabolism and pharmacokinetic studies; males, metabolism only); or by multiple oral doses. Ethoprop was completely metabolized following administration and completely absorbed when given orally. Excretion was by urinary ( $\geq 50\%$  administered dose), fecal (7-16%), and respiratory (11-19%) routes and was essentially complete by 48 hours. Terminal elimination  $t_{1/2}$  in blood was 92-135 hours. Metabolism was by dealkylation of one or both S-propyl groups, followed by hydroxylation and probably conjugation. Two urinary metabolites were identified by HPLC while 3 others were believed to be possible conjugates of those metabolites. The TLC profiles of fecal metabolites were similar to the profiles for urinary metabolites.

2. **Mutagenicity:** The acceptable studies for Ethoprop satisfy both the pre-1991 and the current mutagenicity initial testing battery guidelines. Ethoprop was shown to be an in vitro clastogen, with exogenous metabolic activation required to express genotoxicity. However, clastogenic potential in the whole animal could not be evaluated because severe systemic toxicity precluded using doses high enough to reach the target cells. It was concluded that additional mutagenicity testing was not warranted due to the limitations posed by toxicity to the test animal and the acceptable dominant lethal study. Followed are summaries of the mutagenicity studies.

a) Salmonella typhimurium reverse gene mutation assay (MRID 00160180). This acceptable test was negative with and without activation when tested to solubility and cytotoxicity limits.

b) CHO cell HGPRT gene mutation assay (MRID 00160181). This acceptable test was negative when tested to cytotoxic concentrations both in the absence and presence of activation.

c) Mouse lymphoma L5178Y forward gene mutation assay (MRID 44065001). This acceptable test was negative when tested to cytotoxic concentration both in the absence and presence of activation.

d) In vitro CHO cell chromosome aberration assay (MRID 00160183). This acceptable test was positive with S9 activation: reproducible and significant but not clearly dose-related clastogenic effects were observed over a narrow dose range approaching cytotoxicity limits. Nonactivated Ethoprop was negative up to a level (300  $\mu\text{g/mL}$ ) that interfered with cell cycle kinetics.

e) In vivo bone marrow cytogenetic assay (MRID 41211202). This acceptable test was negative in rats administered oral gavage doses. Lethality and other signs of severe toxicity occurred at the highest dose tested but there was no evidence of compound interaction with the target tissue.

f) Rat dominant lethal assay (MRID 40386901). This acceptable test was negative in rats after oral gavage doses. Severe toxicity occurred at the highest dose tested but there was no evidence that Ethoprop reached the target tissue.

g) In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID 00160182). This acceptable test was negative up to cytotoxic levels.

h) In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID 44038702). This acceptable test was negative up to cytotoxic levels.

i) In vitro CHO cell sister chromatid exchange assay (MRID 00160184). This acceptable test was positive with activation; reproducible, significant and dose-related genotoxic effects were observed over a narrow dose range approaching the cytotoxicity limits of the test material. Nonactivated Ethoprop was negative up to concentrations causing severe cell cycle delay. These findings support the previously discussed positive results from the in vitro chromosome aberration test.

j) In addition to the above acceptable studies, an unacceptable dominant lethal assay (MRID 44038701) reported inconclusive results. However, an acceptable dominant lethal assay (summarized above), was subsequently submitted, and fulfilled this data requirement.

**3. Structure-Activity Relationship:** The following observations were made in a recent review on the carcinogenicity of organophosphates (Yin-tak Woo, et al; *Carcinogenicity of Organophosphorous Pesticides/Compounds: an Analysis of their Structure-Activity Relationships*; Environ. Carcino. & Ecotox. Revs., C14(1): 1-42; 1996).

"Organophosphorus compounds represent one of the most difficult structural classes of chemicals for predicting or assessing carcinogenic potential because of the complex interplay of multiple physicochemical and biological factors." Also, according to the review, since most organophosphates are not very potent alkylating agents, high doses may be needed to demonstrate carcinogenesis. However, the toxicity of many organophosphates may limit dosing at high enough doses to clearly show carcinogenesis. Bioactivation and detoxification processes further complicate the prediction of carcinogenic potential by structure activity analysis".

The review article gave results of carcinogenic testing for several organophosphates with structures similar to Ethoprop. Ebufos, terbufos, and disulfoton were negative when tested for carcinogenesis in rat and mouse studies. Tribufos treatment was associated with liver hemangiosarcomas and adenocarcinomas of the small intestine in male CD-1 mice and alveolar bronchiolar adenomas in female CD-1 mice.

**4. Acute Toxicity:** Ethoprop is an acutely toxic organophosphate with a narrow margin between cholinesterase inhibition and death. Minimal toxicity occurred in several studies, however toxicity prevented testing at higher doses. The acute oral LD<sub>50</sub> in rats is 32 mg/kg, the acute dermal LD<sub>50</sub> in rabbits is 25 mg/kg, and the acute inhalation LC<sub>50</sub> in rats is 0.123 mg/L. In the primary eye irritation study, a dose of 0.1 mL was lethal to all rabbits. In the primary dermal irritation study, all rabbits receiving doses of 0.5 mL died.

## V. WEIGHT-OF-THE EVIDENCE CONSIDERATIONS

### 1. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Crl:CD Rats.

There were significant decreasing trends for mortality with increasing doses of Ethoprop in both males and females.

(A) Adrenal gland malignant pheochromocytomas in males showed significant increasing trends as well as a significant ( $p < 0.05$ ) pair-wise comparison of the high-dose group (5/60) with the controls (0/41). The frequency in high-dose males (8%) exceeded the historical control range (0-1.7%). An increasing trend was also seen for this tumor type.

(B) Thyroid C-cell carcinomas in males showed significant increasing trends (0/61, 0/63, 1/64, 3/66;  $p < 0.05$ ) as well as a significant pair-wise comparison of the high-dose group with controls ( $p < 0.05$ ). The frequency of C-cell carcinomas at the high-dose group (5%) exceeded the historical control range. Of the 7 studies conducted at the testing laboratory, no C-cell carcinomas were seen in 5 studies consisting of 330 rats; C-cell carcinomas were seen in 1/50 (2%) in one study and in 1/60 (1.7%) in another study."

(C) Thyroid follicular cell adenomas in males had significant pair-wise comparisons to controls for all dose groups (0/53, 4/56, 4/56, 5/63; each at  $p < 0.05$ ). Combined thyroid follicular cell adenomas/carcinomas ( $p = 0.05$ ) showed borderline significance for comparison to controls ( $p = 0.05$ ). Historical control data were not submitted.

(D) Endometrial stromal polyps showed significant increasing trends in females (1/78, 2/52, 3/44, 7/78;  $p < 0.05$ ) as well as by pair-wise comparison of the high-dose group with controls ( $p < 0.05$ ). The frequency for endometrial stromal polyps in the high-dose group (9%) was within the historical control range (1.1-19%).

## **2. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Fischer 344 Rats - (1985).**

There was no significant effect of treatment upon mortality. No historical control data from the testing facility were submitted.

Thyroid C-cell adenomas showed significant increasing trends in males (8/49, 5/48, 5/50, 12/50;  $p < 0.05$ ) as did carcinomas (0/49, 0/48, 1/50, 3/50;  $p < 0.05$ ) and combined thyroid C-cell adenomas/carcinomas ( $p < 0.01$ ).

## **3. Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Fischer 344 Rats by Feeding and in utero Exposure - (1983).**

Mortality in control and treated groups was similar. No historical control data were submitted.

(A) Thyroid C-cell adenomas showed significant increasing trends for males (2/46, 4/43, 1/41, 10/40;  $p < 0.01$ ) and pair wise comparison of the high-dose group (196 ppm) with controls ( $p < 0.01$ ).

(B) Uterine endometrial polyps showed increasing trends in females (0/44, 4/45, 8/37, 13/42;  $p < 0.01$ ) as well as by pair wise comparison of mid- and high-dose rats compared to controls ( $p < 0.01$ ). Combined endometrial and stromal polyps showed increasing trends (8/44, 6/45, 10/37, 16/42;  $p < 0.01$ ) as well as by pair-wise comparison of the high-dose group with controls ( $p < 0.05$ ).

## **4. Carcinogenicity Study with Ethoprop in B6C3F1 Mice.**

There were no statistically significant differences in mortality between control and treatment groups.

Liver carcinomas in females showed increasing trends (4/49, 3/49, 1/50, and 9/49;  $p < 0.01$ ) as did combined liver adenomas/carcinomas (9/49, 5/49, 8/50, 13/49;  $p < 0.05$ ). Hepatocellular carcinoma incidence in the high-dose group (18%) exceeded the mean historical control value (10.0%).

**5. Mutagenicity:** Ethoprop was shown to be an in vitro clastogen, with exogenous metabolic activation required to express genotoxicity. However, clastogenic potential in the whole animal could not be evaluated because severe systemic toxicity precluded doses that may reach the target cells.

Both an in vitro CHO cell chromosome aberration assay and an in vitro CHO cell sister chromatid exchange assay were positive with bioactivation; non-activated Ethoprop was negative in both tests.

Two in vivo tests, an in vivo bone marrow cytogenetic assay and a rat dominant lethal assay, were negative, but there was no evidence that the compound reached the target tissue. Severe toxicity to the test animal prevented testing at higher doses.

Other tests showed no indication of mutagenicity when tested with or without activation. Negative tests included a Salmonella typhimurium reverse gene mutation assay, a CHO cell HGPRT gene mutation assay, a mouse lymphoma L5178Y forward gene mutation assay, and in two in vitro unscheduled DNA synthesis assays in primary rat hepatocytes.

**6. Metabolism:** Ethoprop was completely metabolized following administration and completely absorbed when given orally. Excretion was by urinary ( $\geq 50\%$  administered dose), fecal (7-16%), and respiratory (11-19%) routes and was essentially complete by 48 hours. Metabolism was by dealkylation of one or both s-propyl groups, followed by hydroxylation and probably conjugation. Several metabolites were identified.

**7. Structure-Activity Relationships:** A compound structurally similar to Ethoprop, Tribufos, was associated with liver hemangiosarcomas and adenocarcinomas of the small intestine in male CD-1 mice and alveolar/bronchiolar adenomas in female CD-1 mice. Other compounds structurally similar to Ethoprop (Ebufos, Terbufos, and Disulfoton) were negative when tested for carcinogenesis in rat and mouse studies.

## VI. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

A discussion of the Committee's assessment of the weight-of-the-evidence for the carcinogenic potential of Ethoprop is presented below:

### 1. Carcinogenicity

Evidence for carcinogenicity was seen in the adrenal and thyroid glands of male rats, the uterus of female rats and the liver of female mice.

#### A. Adrenal

The primary concern was the occurrence of malignant pheochromocytomas of the adrenal glands in male Sprague-Dawley rats at all dose levels tested.

At the high dose (400 ppm), the incidence (5/60, 8%) was significantly ( $p=0.005$ ) higher than the concurrent controls (0/41, 0%) and exceeded the historical control range (0-1.7%).

Malignant pheochromocytomas were seen in 2/16 males at the low-dose (1 ppm) and in 2/18 males at the mid dose (60 ppm). The Committee noted that while the adrenals of all high dose group rats were examined microscopically, only the adrenals of rats that died or were sacrificed moribund were examined from the low and mid dose groups. Because of this variation, the true dose-responsive nature of the tumor incidence could not be ascertained.

The Committee attributed this tumor to treatment because: (i) it was seen at all dose levels, even at the low-dose (1 ppm) in the absence of cholinesterase inhibition; (ii) this malignant tumor is rare in rats and is life-threatening; and (iii) the incidences at the high dose exceeded both the concurrent control and the historical control range.

#### B. Thyroid

The Committee also had concern for the occurrence of C-cell tumors of the thyroid glands in two strains of male rats: Sprague-Dawley and Fisher 344 (in two different studies).

In Sprague-Dawley rats, the incidences of C-cell carcinomas at the high dose (3/66, 5%) showed a positive trend ( $p = 0.014$ ), pair-wise significance ( $p=0.042$ ) when compared to controls (0/61, 0%) and exceeded the historical control range. Of the 7 studies conducted at the testing laboratory, C-cell carcinomas were seen in 1/50 (2%) rats in one study and in 1/60 (1.7%) rats in another study but none in 5 studies consisting of 330 rats. Thus, the incidence (5%) of this tumor seen in the present study was more than twice that seen in seven previous studies.

In the 1985 Fischer 344 rat study, a positive trend was seen for C-cell adenomas ( $p=0.036$ ), carcinomas ( $p=0.020$ ), and combined adenomas /carcinomas ( $p=0.005$ ) of the thyroid glands. The Committee noted the presence of C-cell carcinomas in 3 of 50 rats (6%) at the high dose (100 ppm) when compared to none in the concurrent controls. Historical control incidences from the testing laboratory were not available. The increase in C-cell carcinomas seen in this strain of rats is supportive of the same target organ/tumor type in the Sprague-Dawley rats.

In the 1983 Fischer 344 rat study, only the incidences of C-cell adenomas showed a positive trend ( $p=0.001$ ) and a pair-wise statistical significance ( $p=0.007$ ) at the high dose (10/40, 25%) when compared to controls (2/46, 4%). The Committee noted that although only adenomas were seen in this study, the thyroid was again indicated as the target organ.

The Committee had lesser concern for the follicular cell tumors seen in male Sprague-Dawley rats. Although there was a pair-wise significance for adenomas at all dose levels when compared to controls, evaluation of this tumor type is most relevant for combined adenomas/ carcinomas in which case there was only a borderline significance ( $p = 0.050$ ) for pair-wise comparison at the high-dose (6/63, 10%) compared to controls (1/53, 2%).

### C. Uterus

The Committee discussed the presence of uterine tumors in the Sprague-Dawley and the *in utero* Fischer 344 rat studies. These tumors (endometrial polyps, endometrial stromal polyps or stromal polyps) which occur spontaneously within the uterus, are considered benign, and there is no evidence to indicate that they would transform into a more aggressive form with time. These tumors were seen only at doses that cause cholinesterase inhibition at the high dose in Sprague-Dawley and at the mid-and high doses in Fischer-344 rats.

In Sprague-Dawley rats, the incidence of endometrial stromal polyps at the high dose (7/78, 9%) showed both a positive trend ( $p=0.044$ ) and pair-wise significance ( $p = 0.044$ ) when compared to controls (1/78, 1%) but was within the historical control range (0-19%).

In the *in utero* Fischer 344 rat study, a positive trend was seen for endometrial polyps ( $p=0.000$ ) as well as the combined tumors (endometrial polyps and stromal polyps) ( $p=0.006$ ) but not for stromal polyps. While the incidence of endometrial polyps was significantly increased at the mid (8/37, 22%,  $p = 0.001$ ) and high doses (13/42, 31%,  $p = 0.000$ ) when compared to controls (0/44), the combined tumors (endometrial polyps and stromal polyps) showed pair-wise significance only at the high dose (16/42, 38%, 0.034) when compared to controls (8/44, 18%). No historical control data were available in this strain of rats.

Uterine tumors were not seen in the carcinogenicity Fischer 344 rat study.

#### **D. Liver**

The Committee did not consider the hepatocellular adenomas, carcinomas and combined adenomas/carcinomas seen at all dose levels, including the controls, in female B6C3F1 mice to be treatment-related. Although there were positive trends for carcinomas ( $p=0.009$ ) and combined adenomas/carcinomas ( $p=0.038$ ), none of the three types showed pair-wise significance when compared to concurrent controls.

### **2. Mutagenicity**

The Committee noted that although Ethoprop was clastogenic *in vitro* with metabolic activation, its clastogenic potential in the whole animal can not be ascertained because of the severe acute toxicity which precluded testing at a dose high enough to affect the target organ.

### **3. Structure Activity Relationship**

Structure activity relationship was not helpful in evaluating the possible carcinogenicity of Ethoprop. Of the four structurally related compounds (Ebufos, Disulfoton, Terbufos and Tribufos), only Tribufos exhibited carcinogenic activity. The tumor type and tumor site, however, were different from that seen with Ethoprop. Tribufos induced liver hemangiosarcomas and adenocarcinomas of the small intestine in male and alveolar/bronchiolar adenomas in female CD-1 mice.



## VII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's *Proposed Revisions to the Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the Committee classified Ethoprop as a "likely" human carcinogen. This classification was based on the following factors:

- (i) presence of a rare and life-threatening (malignant) tumor (pheochromocytomas of the adrenal glands) in male Sprague-Dawley rats at the low dose in the absence of cholinesterase inhibition;
- (ii) occurrence of another type of tumor (C-cell carcinomas of the thyroid glands) in male rats in two strains (Sprague-Dawley and Fischer 344) in three different studies at doses that did cause cholinesterase inhibition; and
- (iii) evidence of clastogenicity in *in vitro* mutagenicity testing.

## VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose approach for human risk characterization and an extrapolation based on malignant pheochromocytomas of the adrenal glands in male rats at all dose levels tested. The linear dose extrapolation is supported by: (i) lack of mode of action, (ii) evidence from the total data base [i.e., occurrence of an other tumor type (C-cell carcinomas of the thyroid glands) at doses that caused cholinesterase inhibition], and (iii) confirmation of clastogenic activity in mutagenicity testing.

## IX. BIBLIOGRAPHY

MRID No.	CITATION
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