ATTACHMENT 3

US EPA (US Environmental Protection Agency). 2020. Cancer Assessment Document for Committee Deliberation Re-evaluation of the Carcinogenic Potential of Ethoprop. Cancer Assessment Review Committee. Health Effects Division. Office of Pesticide Programs. March 16, 2020.

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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: March 16, 2020

SUBJECT: **Ethoprop**: Cancer Assessment Review Committee's Re-Evaluation of Carcinogenic Potential

PC Code: 041101
Decision No.: 528747
Petition No.: N/A
Risk Assessment Type: Cancer Reassessment
TXR No.: 0058008
MRID No.: N/A

DP Barcode: D440371
Registration No.: 5481-9043
Regulatory Action: N/A
Case No.: N/A
CAS No.: 13194-48-4
40 CFR: N/A

FROM: Melinda Wilson, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

THROUGH: Gregory Akerman, Ph.D., Chair
Sarah Dobreniecki, Ph.D., Co-Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: John Liccione, Ph.D., Toxicologist
Risk Assessment Branch V/VII
Health Effects Division (7509P)

The Cancer Assessment Review Committee met on February 26, 2020 to re-evaluate the cancer classification of ethoprop in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

FOR COMMITTEE DELIBERATION

RE-EVALUATION OF THE CARCINOGENIC POTENTIAL of

ETHOPROP

PC: 041101

March 16, 2020

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

CONTENTS

I. EXECUTIVE SUMMARY

The Cancer Assessment Review Committee (CARC) met on February 26, 2020 to re-evaluate the cancer classification of ethoprop in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005). Previously, on June 25 and August 20, 1997 and April 1, 1998, the CARC evaluated the carcinogenic potential of ethoprop and in accordance with the 1996 Proposed Draft Guidelines for Carcinogen Risk Assessment, the Committee classified ethoprop as a "likely" human carcinogen, based on malignant adrenal pheochromocytomas, thyroid C-cell tumors in male rats and evidence of clastogenic activity *in vitro*. The Q_1* for ethoprop is 2.81×10^{-2} mg/kg/day^{-1} is based on the adrenal pheochromocytomas.

AMVAC, the registrant for ethoprop, recently submitted a carcinogenic re-assessment of ethoprop in which they propose a reclassification of its cancer potential. AMVAC does not believe the data justify the current classification of "likely" human carcinogen and that a Q_1* approach for human risk characterization is not warranted. AMVAC submitted Pathology Working Group (PWG) reports on the re-evaluation of adrenal pheochromocytoma and thyroid C-cell tumors in male rats. The CARC evaluated the totality of the data in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005) and concluded the following:

The CARC accepted the findings of the PWG re-evaluation of the histopathology of the adrenal and thyroid tumors in rats. In addition, the agency performed new statistical analyses based on the incidence values from the PWG re-evaluation. The new analyses indicated that the incidence of benign adrenal pheochromocytomas in male Sprague-Dawley rats in the Williams 1992 study was within the range of appropriate historical control data. In contrast, the incidence of malignant adrenal pheochromocytomas was outside the range of appropriate historical control data. Male rats had a statistically significant ($p < 0.05$) pair-wise comparison of the low-dose (1 ppm) group with the controls for malignant pheochromocytomas. The Committee noted that there was no dose response relationship for this tumor type. In addition, there were no pre-neoplastic effects including a lack of adrenal medullary hyperplasia to support a treatment-related effect. The CARC determined that the adrenal pheochromocytomas in male rats were not treatment related at any dose tested.

In Sprague-Dawley rats (Williams 1992 study), male rats had a statistically significant trend for thyroid C-cell carcinomas. The Committee noted that the incidence of thyroid C-cell carcinomas at the high dose (400 ppm) was outside the range of appropriate historical control data, but there was no pairwise statistically significant difference in comparison to concurrent controls. In addition, toxicity at the high dose (400 ppm) was considered excessive based on several factors: (1) the high dose had to be reduced from 600 ppm to 400 ppm after 2 weeks of dosing due to excessive toxicity in females (tremor, ataxia, and 2 deaths); (2) brain ChE activity in 400 ppm males was decreased 64% compared to controls at study termination; (3) there were body weight decrements of 20% compared to controls during the first half of the study; (4) neurotoxicity was noted in a subchronic neurotoxicity study at the same dose (400 ppm); (5) the 400 ppm dose is about 2/3 of the LD_{50} dose for ethoprop; and (6) ethoprop has a steep dose-response curve with death occurring at dose levels slightly above those causing clinical signs. In addition, there were

no effects on adrenal pre-neoplastic lesions such as adrenal hyperplasia that would support a carcinogenic effect. Therefore, the CARC placed less weight on these tumors in the overall weight of evidence evaluation.

In the F344 rats (Spicer 1985 study), male rats had statistically significant trends for both thyroid C-cell adenomas and thyroid C-cell adenomas and/or carcinomas combined at a dose that was not considered excessive. The significant trend for the combined adenomas/carcinomas was driven by the incidence of adenomas. There was also a statistically significant pair-wise comparison of the high-dose (100-ppm) group with the controls for thyroid C-cell adenomas and/or carcinomas combined. The incidences of thyroid C-cell adenomas and carcinomas were within the historical control range. However, the thyroid C-cell carcinomas were outside the historical control range. There were no significant thyroid pre-neoplastic effects (e.g., hyperplasia) that would support a carcinogenic effect. However, the CARC concluded that the increases in thyroid C-cell tumors (driven by adenomas) in male F344 rats are considered treatment-related at 100 ppm based on statistical significance.

In accordance with *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified ethoprop as "Suggestive Evidence of Carcinogenic Potential." This was based on the presence of one tumor type, thyroid C-cell tumors, which occurred in only one sex (males) and one species (F344 rat) and no mutagenicity concerns. The chronic RfD would adequately protect for all the chronic toxicity, including carcinogenicity, that could potentially result from exposure to ethoprop.

II. BACKGROUND

Ethoprop (Figure 1) is an organophosphate (OP) insecticide/nematicide currently registered as an emulsifiable concentrate (EC) and two granular formulations (15% and 20% active ingredient (ai)) for use on various agricultural crops. Ethoprop products are classified as "Restricted Use Pesticide" (RUP) and may be purchased and used only by certified applicators (or persons under their direct supervision). Like other OPs, the initiating event in the adverse outcome pathway (AOP)/ mode of action (MOA) for ethoprop involves inhibition of the enzyme acetylcholinesterase (AChE) via phosphorylation of the serine residue at the active site of the enzyme. This inhibition leads to accumulation of acetylcholine and ultimately to neurotoxicity in the central and/or peripheral nervous system. For ethoprop, AChE inhibition is the most sensitive endpoint in the toxicology database across multiple species, durations, lifestages, and routes.

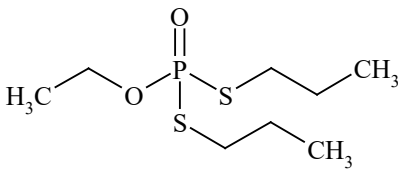

Figure 1. Structure of Ethoprop

On June 25 and August 20, 1997, the Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of ethoprop and in accordance with the 1996 Proposed Draft Guidelines for Carcinogen Risk Assessment the Committee classified ethoprop as a "likely"

human carcinogen (Jess Rowland, TXR 0012564, October 2, 1997). The classification was 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 (1 ppm) in the absence of cholinesterase inhibition (MRID 42530201);
>
> (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 (≥ 10 ppm) that did cause cholinesterase inhibition (MRIDs 42530201, 40291801, 00138636) and;
>
> (iii) evidence of clastogenicity in *in vitro* mutagenicity testing.

The Committee recommended a linear low-dose (Q_1*) approach for human risk characterization and determined that extrapolation of risk should be based on the occurrence of malignant pheochromocytomas of the adrenal glands in male rats at all dose levels tested. The Committee recommended this extrapolation based on the (i) lack of mode of action data, (ii) evidence of carcinogenicity [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. In a subsequent CARC meeting on April 1, 1998, the CARC evaluated historical control data submitted by the registrant and reaffirmed that ethoprop remains classified as a "likely" human carcinogen with a linear low dose (Q_1*) approach for human risk characterization (Jess Rowland and Kit Farwell, TXR 0012890, October 7, 1998).
The CARC 1997 meeting also discussed the presence of uterine tumors in Sprague-Dawley and Fischer 344 rats. The CARC concluded the following: *"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 uterine tumors did not impact the basis for the classification of the carcinogenic potential of ethoprop, which was based on the presence of adrenal pheochromocytomas and C-cell carcinomas of the thyroid glands. Consequently, in this CARC report, uterine tumors will not be discussed further.

AMVAC, the registrant for ethoprop, recently submitted a carcinogenic re-assessment of ethoprop in which they propose a reclassification of its cancer potential (MRID 50259101). AMVAC does not believe the data justify the current classification of "likely to be carcinogenic to humans" and that a Q_1* approach for human risk characterization is not warranted. Dr.

Charles Wood, a pathologist formerly with the National Health and Environmental Effects Research Laboratory of the US EPA, reviewed AMVAC's carcinogenic re-assessment of ethoprop and provided the Office of Pesticide Programs/Health Effects Division (OPP/HED) with a list of critical issues that he identified with the re-assessment. The primary recommendations from Dr. Wood were that AMVAC specify pathological criteria clearly and the need for a formal PWG. These issues were communicated to AMVAC, and in response to these issues, AMVAC submitted comments (MRID 50352001), three PWG reports (MRIDs 50390801; 50390802; and appendices 5-7 of MRID 50259101) and a review and statistical analyses of tumor incidences in the chronic toxicity and carcinogenicity studies in rats (MRID 50400701). More recently, the current HED CARC pathologist, Dr. Jerrold Ward, reviewed Dr. Wood's comments, AMVAC's responses to comments, the PWG reports and the review and statistical analyses of tumor incidences in the chronic toxicity and carcinogenicity studies in rats. Dr. Ward provided HED with his assessment of his review (email communication).

On February 26, 2020, the CARC convened to evaluate AMVAC's proposed re-classification, the PWG reports, the review and new statistical analyses of rat tumor incidences, and Dr. Jerrold Ward's conclusions. The results of the Committee's evaluation are discussed in this report.

This report covers the following topics in this order:

i. Previous CARC reviews of malignant adrenal pheochromocytomas in Sprague-Dawley rats (Williams, 1992– MRID 42530201), the registrant's proposed conclusions on adrenal tumors, results of the PWG slide review, and Dr. Ward's conclusion regarding adrenal pheochromocytomas in male Sprague-Dawley rats.

ii. Previous CARC reviews of thyroid C-cell and follicular cell tumors in male Sprague-Dawley rats (Williams, 1992– MRID 42530201) and in Fisher 344 rats (Spicer, 1985– MRID 40291801), the registrant's proposed conclusions on thyroid tumors, results of the PWG slide review, Dr. Ward's conclusion regarding thyroid tumors in rats.

iii. HED qualitative reanalysis of PWG pathology slides on adrenal medulla and thyroid tumors (Spicer, 1985 MRID 40291801 and Williams, 1992 MRID 42530201).

III. REVIEW OF AMVAC'S PROPOSED RE-CLASSIFICATION RATIONALE

1. Malignant Adrenal Pheochromocytomas in Male Sprague-Dawley 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) Previous CARC Assessments

The primary concern of the 1997 CARC assessment was the occurrence of malignant pheochromocytomas of the adrenal gland in male Sprague-Dawley rats (Table 1) at all doses tested (Jess Rowland, TXR 0012564, October 2, 1997). At the high dose (400 ppm), the

incidence (5/60, 8%) was statistically (trend and pairwise comparison) significantly ($p<0.01$) higher than the concurrent controls (0/41, 0%) and exceeded the available 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.

Table 1. Adrenal Tumors and Mortality in Male Sprague Dawley Rats (Williams 1992 Study: MRID 42530201)[b]

	DOSE (ppm)[c]			
	0	1	60	400
Mortality Rate (weeks 1-106)	50/70 (71%)	42/70 (60%)[n]	42/70 (60%)[n]	30/71 (42%)**[n]
Tumor Type / Incidence				
Benign Pheochromocytomas	14/49 (29%)[n]	7/26[a]	7/28[a]	5/61 (8%)[n]
Malignant Pheochromocytomas	0/41 (0%)**	2/16[a]	2/18[a]	5/60 (8%)**
Combined Pheochromocytomas	14/49 (29%)[n]	8/26[a]	9/28[a]	10/61 (16%)[n]

n. Negative trend or change from control.
a. Not all animals examined.
b. Table from the 1998 CARC report.
c. Doses corresponded to 0, 0.04, 2.44, or 18.38 mg/kg/day in males.
** $p<0.01$. Significance of trend denoted at control. Significance of pairwise comparison with control denoted at dose level.

In the CARC's 1998 review of the historical control data and the registrant's comments on re-classifying ethoprop as "not likely" to be a human carcinogen, the CARC concluded the following:

"*The Committee's primary concern is still the occurrence of malignant adrenal pheochromocytomas which was seen at all dose levels tested. Incidence at the high-dose (400 ppm) reached pair-wise statistical significance when compared to concurrent controls. Malignant pheochromocytomas were also seen at the low- (1 ppm) and mid-dose groups (60 ppm). However, not all adrenals in the low- and mid-dose group were examined, so the true dose-responsive nature of tumor incidence could not be determined.*

Malignant pheochromocytomas exceeded the historical control range for the period of approximately 5 years preceding this study. However, the registrant submitted new historical control data from the testing lab for approximately 5 years after the study. In the new historical control data, pheochromocytomas in the present study had a similar incidence for 1 study while another study had an incidence above the current study. One study had decreased incidence compared to the present study and another study had zero malignant pheochromocytomas.

The criteria for distinguishing between benign and malignant pheochromocytomas has changed over the years. The present criteria for classification of malignancy is tissue invasion or metastasis. It could not be determined which criteria were used in the 1992 study and which criteria were used in the historical control studies. ***The registrant needs to describe the criteria used for classification of pheochromocytomas in the historical control studies.***

Malignant adrenal pheochromocytomas are classified as "uncommon" tumors by the Society of Toxicologic Pathologists but should not be described as "life-threatening" as mentioned in the preceding Cancer Peer Review (CPR) document (5/9 animals with these tumors survived to study termination). Benign pheochromocytomas cannot be stated as progressing to malignancy, according to HED consulting pathologist, Dr. Luke Brennecke.

In addition, in this study there were fewer surviving control animals compared to all male treatment groups at study termination (20, 28, 28, and 41 survivors in control, low-, mid-, and high-dose groups respectively). The Registrant argued that increased survival in the high-dose group allowed more time for benign pheochromocytomas to progress to malignancy. However, this argument was rejected because benign pheochromocytomas cannot be stated as progressing to malignancy.

Although the Registrant submitted data showing that the increase in malignant pheochromocytomas was not significantly increased using logistic regression analysis, HED uses the Peto prevalence test which also accounts for differential survival among groups and is more sensitive than logistic regression analysis."

"The Committee evaluated doses tested in the study and concluded that dosing was excessive and was close to a lethal dose based on the following factors:

> *1) The high dose had to be reduced from 600 ppm to 400 ppm after 2 weeks of dosing due to excessive toxicity in females (tremor, ataxia, and 2 deaths).*
>
> *2) Brain ChE activity in 400 ppm males was decreased 64% compared to controls at study termination.*
>
> *3) There were body weight decrements of approximately -20% compared to controls during the first half of the study.*
>
> *4) Neurotoxicity was noted in a subchronic neurotoxicity study (MRID 43442401) at the same dose as the high dose in the 1992 chronic rat study (400 ppm). Neurotoxicity*

included cholinergic signs, decreased analgesic response, decreased motor activity, and decreased hindlimb grip strength. These signs of neurotoxicity are more likely to be detected in the neurotoxicity study than in a chronic study.

5) The high-dose group of 400 ppm was equivalent to 20 mg/kg/day. This dose is approximately 2/3 of the LD_{50} oral dose for ethoprop (32.8 mg/kg).

6) Ethoprop has a steep dose-response curve with death occurring at dose levels slightly above those causing clinical signs."

Overall Previous CARC Conclusion on Adrenal Pheochromocytomas

The 1998 CARC assessment concluded that, "*Ethoprop remains classified as a "likely" human carcinogen with a linear low dose (Q_1^*) approach for human risk characterization (Jess Rowland and Kit Farwell, TXR 0012890, October 7, 1998).*"

(b) Registrant's Proposed Cancer Re-assessment and the PWG Experimental Pathology Lab's (EPL) Slide Review of Adrenal Pheochromocytomas

To address the issue of the diagnostic differentiation between benign and malignant pheochromocytomas, the registrant provided a contemporary re-reading of the pheochromocytomas present in the carcinogenicity study. Table 2 below is a summary of the data obtained from the contemporary pathology review of the adrenal glands from the Williams (1992) study (MRID 42530201). The registrant stated that the review indicated that some of the adrenal medullary neoplasms were wrongly diagnosed and one malignant pheochromocytoma was missed from a control animal in the original report. In addition, a statistical analysis of the malignant pheochromocytoma incidence following the PWG EPL review using a Cochran-Armitage trend test and poly k test to account for the differing survival between the control and treated rats has shown that the increased incidences were not statistically significant.
In addition, the registrant stated that the overall weight of evidence supported a hypothesis that the adrenal pheochromocytomas seen in the male Sprague-Dawley carcinogenicity study (MRID 42530201) are a reflection of the high and variable spontaneous incidence of this tumor type in control rats and are not a product of treatment with ethoprop. The registrant provided the following rationale:

a. Ethoprop is not genotoxic.
b. Pheochromocytomas are common endocrine neoplasms found in untreated control male Sprague-Dawley rats with reported incidences for malignant forms of the neoplasm being up to 6.7%. This compares with an incidence of 7% present in the top dose level, and 2% at each of the lower dose levels of the ethoprop study. Table 3 summarizes incidences of spontaneous pheochromocytomas in male Sprague-Dawley rats.
c. Because of the diagnostic difficulties in distinguishing benign from malignant pheochromocytomas a contemporary pathology review of the neoplasms was undertaken by EPL and a statistical analysis of this data showed the increased incidences present were not statistically significant.
d. There were no increased incidences of pheochromocytomas recorded in the

other two, 2-year rat carcinogenicity studies.

e. There was no increased incidence of pheochromocytomas in the female rat adrenal glands in the same study as that showing the increases in the male rats.
f. There was no increased incidence of pheochromocytomas in the equivalent mouse carcinogenicity study of 104 weeks duration conducted with ethoprop, which was not carcinogenic in this species.
g. Survival in the top dose level of ethoprop (which showed the increases in incidence of pheochromocytomas) was approximately twice that present in the concurrent control group. There is a known positive relationship between the incidence of certain spontaneous neoplasms, such as pheochromocytomas, and increasing age in rats.
h. There was a decreased incidence of benign pheochromocytomas at the top dose level suggesting a greater conversion to a malignant phenotype produced by the better survival seen at this dose level in comparison to the control rats.
i. Other cholinesterase inhibiting chemicals have not shown increased incidences of this tumor type in lifetime rat or mouse carcinogenicity studies.
j. Ethoprop has undergone the full series of the Endocrine Disruptor Screening Program (EDSP) Tier 1 assays as part of a response to a US Environmental Protection Agency's question regarding its potential for interacting with the endocrine system and does not activate any of the parameters assessed in any of the assays.
k. There are five known modes of action for inducing pheochromocytomas in the rat including chemicals that alter calcium metabolism, excessive food intake, uncouplers of oxidative phosphorylation, chemicals that induce hypoxia/mitochondrial dysfunction, and inhibitors of catecholamine uptake and storage. There is no evidence that ethoprop could act to induce any of these modes for inducing increased pheochromocytomas.
l. Following a World Health Organization/International Programme on Chemical Safety (WHO/IPCS) approach to considering possible alternative explanations for the development of pheochromocytomas in the rat, it is concluded that there are no plausible explanations supporting an ethoprop induced increase in this tumor type.

Table 2. Adrenal Medulla: Tumor Incidences in Sprague-Dawley rats from the Original and EPL Review Evaluations (Williams 1992 Study – MRID 42530201)

Evaluation	Sex	Males				Females			
	Group	1	2	3	4	1	2	3	4
	Dose (ppm)[a]	0	1	60	400	0	1	60	400
Original	N[d]	90	45	48	90	90	61	48	90
	(B) Pheochromocytoma	13(14) 14%(16%)	7[b] 16%	7 15%	5 6%	3 3%	2 3%	1 2%	2 2%
	(M) Pheochromocytoma	0	2 4%	2 4%	4(5)[c] 4% (6%)	0	0	0	0
PWG EPL Review	N[d]	88	46	48	90	90	61	48	90
	(B) Pheochromocytoma	14 (16%)	**4** (9%)	**6** (13%)	5 (6%)	3 (3%)	2 (3%)	**0**	**3** (3%)
	(M) Pheochromocytoma	**1** (1%)	**4** (9%)	2 (4%)	5 (6%)	0	0	0	0

Differences in incidence from original evaluation are highlighted in **bold.**

a. Doses of 0, 1, 60 or 600/400 ppm (600 ppm during the first 2 weeks of treatment) of ethoprop were fed to rats (80/sex/group) for 105 weeks. Doses corresponded to 0, 0.04, 2.44, or 18.38 mg/kg/day in males and 0, 0.06, 3.56, or 23.98 mg/kg/day in females. Groups of 10 rats/sex were sacrificed at 52 weeks. An additional 10 rats/sex in the control and high-dose groups were treated for 52 weeks and allowed a 4-week recovery period before being sacrificed.

b. For one rat of this group, animal no. C39512, a unilateral malignant pheochromocytoma was not available for review

(B) = benign; (M) = malignant

c**. ( )** = original number documented on the original Final Report, Table 78, "Incidence of Microscopic Observations". The numbers not in parentheses are those that have been corrected by the PWG.

d. The Pathology Report indicated that the pathologist examined the exact same slides as was done previously.

Table 3: Incidences of Spontaneous Pheochromocytomas in Male Control Sprague-Dawley Rat Studies in Comparison to Ethoprop Data from the Williams study (MRID 42530201)[a]

Strain	Years covered	Benign pheochromocytomas	Malignant pheochromocytomas	Reference
Crl:CDBR	1991-2002	15.3% (4.0% - 24.0%)	1.9% (0% - 6.7%)	Baldrick 2005
Crl:CDBr	1984-1991	19.0% (10.2%-30.0%)	1.9% (0.0%-6.0%)	McMartin et al 1992
Sprague-Dawley	1992 (i)	4.0% (range not specified)	0.82% (range not specified)	Chandra et al 1992
Incidence in control group in Ethoprop Study	**1992**	**16%**	**1%**	Williams 1992
Incidence at 400 ppm in Ethoprop study	**1992**	**6.0%**	**6.0%**	Williams 1992

a. Per EPA Cancer Guidelines, animals should be fed ad libitum. The CARC considered only the historical control data from studies that included ad libitum feeding, and that encompassed the actual study date i.e., conducted within 5 years prior to or 5 years after the study being evaluated.

Notes: (i) = date of publication

(c) Responses from CARC Pathologists

Based on a review of the PWG reports, AMVAC's response to Dr. Wood, and the PWG EPL re-read of the carcinogenicity studies, Dr. Ward agreed with the procedure used by the PWG for reviewing the slides/lesions. Dr. Ward also agreed with the criteria used by ELP for diagnoses of adrenal lesions. In addition, Dr. Ward agreed with the registrant's overall conclusion that adrenal pheochromocytomas are a "reflection of the high and variable spontaneous incidence of this tumor type in control rats and are not a product of treatment with ethoprop."

(d) Incidences of Adrenal Medullary Hyperplasia in the available Combined Chronic Toxicity Studies in the Rat

In both the Spicer (1985) and Williams (1992) studies, there were no dose-related increases in incidences of adrenal medullary hyperplasia (tabulated below) that would support the conclusion of a treatment-related effect on adrenal pheochromocytomas (Table 4).

Table 4. Incidences of Adrenal Medullary Hyperplasia in the Spicer (1985) and Williams (1992) Studies

Spicer (1985)	**Male**				**Female**			
	Dose level (ppm)				Dose level (ppm)			
	0	1	10	100	0	1	10	100
Total animals Examined	49	46	48	48	47	49	45	49
Medullary Hyperplasia	18	10	17	15	6	5	8	6
Williams (1992)								
	Dose level (ppm)				Dose level (ppm)			
	0	1	60	400	0	1	60	400
Total animals Examined	90	80	80	90	90	80	80	90
Medullary Hyperplasia	15	13	13	6	4	1	2	2

2. <u>Thyroid C-Cell and Follicular Tumors in Male Sprague-Dawley Rats</u>

Like the 1997 CARC, the 1998 CARC also had concern for the occurrence of C-cell tumors of the thyroid glands in male Sprague-Dawley rats (Williams 1992; MRID 42530201) as well as in two different studies in male Fisher 344 rats (Spicer 1985, MRID 40291801; Barnett et al. 1983, MRID 00138636). These studies are discussed in more detail below.

(a) Previous CARC Assessments

<u>Reference:</u> Williams 1992: "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.

The CARC 1998 assessment noted that the incidence of C-cell carcinomas at the high dose (400 ppm) in SD rats showed a positive trend, reached pair-wise significance when compared to controls, and exceeded the historical control range (Table 5). The CARC 1998 report did not present the historical control data but stated that the historical control data are in a file copy. The CARC also noted that in the Spicer (1985) study (MRID 40291801) 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 zero in the concurrent controls (Table 7). The CARC concluded that 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.

Table 5. Thyroid C-Cell Tumors in Male Sprague-Dawley Rats (Williams 1992 - MRID 42530201).

Tumor Type / Incidence	DOSE (ppm)			
	0	1	60	400
C-Cell Adenomas	8/66 (12%)	6/67 (9%)[n]	9/67 (13%)	12/68 (18%)
C-Cell Carcinomas	0/61 (0%)*	0/63 (0%)	1/64 (2%)	3/66 (5%)*
Combined C-Cell Tumors	8/66 (12%)	6/67 (9%)[n]	10/67 (15%)	15/68 (22%)

n. Negative trend.
* $p<0.05$ ** $p<0.01$. Trend denoted at control. Pairwise significance noted at dose. Tumor rates exclude rats dying before first tumor. Statistical analyses were based upon Peto's Prevalence Test.

The 1998 CARC assessment had lesser concern for thyroid follicular cell tumors in male Sprague Dawley rats since combined adenomas/carcinomas had only borderline significance at the high dose (Table 6) but was supporting evidence for the thyroid being a target organ for ethoprop-induced carcinogenicity.

Table 6. Thyroid Follicular Cell Tumors in Male Sprague Dawley Rats (Williams 1992 - MRID 42530201).

Tumor Type / Incidence	DOSE (ppm)			
	0	1	60	400
Follicular Cell Adenomas	0/53 (0%)	4/56 (7%)*	4/56 (7%)*	5/63 (8%)*
Follicular Cell Carcinomas	1/34 (3%)	0/31 (0%)	1/36 (3%)	1/51 (2%)
Combined Follicular Cell Tumors	1/53 (2%)	4/56 (7%)	5/56 (9%)	6/63 (10%)

* $p<0.05$ Tumor rates exclude rats dying before first tumor. Statistical analyses were based upon Peto's Prevalence Test.

References: ***Spicer 1985 – "Lifetime dietary toxicity and oncogenicity study in rats". (MRID 40291801). IRDC Study Number 347-029.***

The CARC (1998) assessment noted that male rats in the Spicer (1985) feeding study (MRID 40291801) exhibited a statistically significantly increasing trend for thyroid C-cell adenomas, carcinomas, and combined tumors (Table 7).

Table 7. Thyroid C-Cell Tumors in Male F344 Rats (Spicer et al. 1985 - MRID 40291801).

Tumor Type / Incidence	DOSE (ppm)[a]			
	0	1	10	100
C-Cell Adenomas	8/49 (16%)*	5/48 (10%)[n]	5/50 (10%)[n]	12/50 (24%)
C-Cell Carcinomas	0/49 (0%)*	0/48 (0%)	1/50 (2%)	3/50 (6%)
C-Cell Combined Tumors	8/49 (16%)**	5/48 (10%)[n]	6/50 (12%)[n]	15/50 (30%)

n. Negative trend.
* $p<0.05$ ** $p<0.01$. Statistical analyses performed by the Exact Trend Test and Fisher's Exact Test.
a. Doses correspond to 0, 0.041, 0.40, or 4.19 mg/kg/day in males.

The previous CARCs also considered thyroid C-cell adenomas in the Barnett et al. (1983) *in utero* study in which F344 rats were exposed to ethoprop *in utero*, during lactation and then by diets. However, the study was unacceptable and not designed to assess carcinogenicity. Therefore, the Barnett et al. (1983) study was not considered by the current CARC.

Overall Previous CARC Conclusion on Thyroid tumors

The 1998 CARC assessment had lesser concern for thyroid C-cell and follicular tumors which occurred in several chronic rat studies. Both tumor types are common.

Thyroid C-cell carcinomas in the Williams (1992) rat study showed an increasing trend as well as significant pair-wise comparison of the high-dose male group with controls (3/61 in the high-dose group compared to 0/61 in controls) (Table 5). Thyroid follicular cell adenomas in the same study were increased by pair-wise comparison for all treatment groups (Table 6). The CARC Committee felt that these were small increases and that the maximum tolerated dose had been exceeded on the basis of several factors: (1) the high dose had to be reduced from 600 ppm to 400 ppm after 2 weeks of dosing due to excessive toxicity in females (tremor, ataxia, and 2 deaths); (2) brain ChE activity in 400 ppm males was decreased -64% compared to controls at study termination; (3) there were body weight decrements of -20% compared to controls during the first half of the study; (4) neurotoxicity was noted in a subchronic neurotoxicity study at the same dose (400 ppm); (5) the 400 ppm dose is about 2/3 of the LD_{50} dose for ethoprop; and (6) ethoprop has a steep dose-response curve with death occurring at dose levels slightly above those causing clinical signs.

Thyroid C-cell adenomas and carcinomas showed increasing trends in the Spicer (1985) rat study (Table 7). Again, the Committee felt that these were small increases. For further details, refer to the Cancer Assessment Document dated October 2, 1997 (Jess Rowland, TXR 0012564, October 2, 1997).

(b) Registrant's Cancer Re-assessment and Experimental Pathology Lab's Slide Review of Thyroid C-cell Neoplasms

To address the issue of the diagnostic differentiation between thyroid hyperplasia from thyroid neoplasms, the registrant provided a contemporary re-reading of all the thyroid C-cell neoplasms present in two carcinogenicity studies (Williams 1992 study – MRID 42530201; Spicer 1985 study – MRID 40291801). Tables 8 and 9 summarize the data obtained from the contemporary pathology review of the thyroid gland from the Williams (1992) and Spicer (1985) studies using the most up to date criteria for differentiating benign from malignant, and hyperplasia from benign C-cell proliferations. The registrant stated that the review indicated the re-read showed some of the neoplasms had been misdiagnosed and statistical analysis of the incidence data, using Cochran-Armitage trend test and the poly k test has revealed that the incidences of adenomas and carcinomas in the Spicer (1985) study show a statistically significant trend in male rats ($p=0.002$ and $p=0.035$ for adenoma and carcinoma respectively), while the incidences of male thyroid malignant carcinomas were also statistically significant ($p=0.015$) (statistics not presented in tables).

The registrant concluded that C-cell neoplasms are one of the most common neoplasms found in control groups of both sexes in rat carcinogenicity studies. They have a high but variable spontaneous incidence rate that makes interpretation of incidences in treated groups extremely difficult when the concurrent control group is the only reference for incidence (Table 10 summarizes the incidences of spontaneous C-cell neoplasms in rats). The registrant stated there is no plausible explanation for why ethoprop should have induced C cell neoplasia and a critical review of the three rat studies affected, together with a review of the biology and function of the affected tissue, leads to a conclusion that the most likely explanation is that the apparent increases observed in the studies reflect the variable spontaneous rate of the tumor in the control population. The increased incidence at the top dose levels over the concurrent controls is a product of the abnormally low incidence present in the concurrent controls. The registrant provided the following rationale in their weight-of-evidence evaluation:

a. Ethoprop is not genotoxic.
b. Thyroid C-cell adenomas showed a marginal numerical, but not statistically significant, increase in all three rat carcinogenicity studies in male rats.
c. Following a pathology review of the neoplasms by EPL, and a current statistical analysis of the incidences in the Spicer (1985) and Williams (1992) studies, there was a statistically significant increase in thyroid C-cell adenomas and carcinomas in the Spicer study and a statistically significant increase in thyroid C-cell carcinomas in the Williams study.
d. Thyroid C-cell neoplasms are the most common endocrine neoplasms found in untreated control male F344 and Sprague-Dawley rats.

e. The incidences of both benign and malignant neoplasms found in the three ethoprop rat carcinogenicity studies all lie well within the ranges reported for the spontaneous incidences of these tumors in the published literature, with reported control incidence ranges for benign and malignant forms of the neoplasm being up to 45% and 25%, respectively. Incidences of spontaneous C-cell neoplasms in male and female controls from F344 and Sprague-Dawley rat carcinogenicity studies are summarized in Table 10.
f. There was no increased incidence of thyroid C-cell neoplasms in the female rat thyroid gland.
g. There was no increased incidence of C-cell neoplasms in the equivalent mouse carcinogenicity study of 104 weeks duration conducted with ethoprop, which was not carcinogenic in this species.
h. Other cholinesterase inhibiting chemicals have not shown increased incidences of this tumor type in lifetime rat or mouse carcinogenicity studies.
i. Ethoprop has undergone the full series of EDSP Tier 1 assays as part of a response to a US Environmental Protection Agency's question regarding its potential for interacting with the endocrine system and does not activate any of the parameters assessed in any of the assays.
j. There are two accepted modes of action for the chemical induction of thyroid C-cell neoplasms in the rat including chronic stimulation of calcitonin secretion, and activation of the glucagon-like peptide-1 (GLP-1) receptor. There is no evidence that ethoprop could act to induce any of these modes for inducing increased incidences of thyroid C-cell neoplasms.
k. While there are a small number of chemicals that have been shown to induce thyroid C-cell neoplasms in rats, there are no common pharmacological modes of action or chemical structure relationships that would permit the formulation of a hypothesis for their production from ethoprop, or that would support the interaction of ethoprop with the thyroid C-cells.
l. Following a WHO/IPCS approach to considering possible alternative explanations for the development of thyroid C-cell neoplasms in the rat, it is concluded that there are no plausible explanations supporting an ethoprop induced increase in this tumor type.
m. The overall weight of evidence therefore supports a hypothesis that the thyroid C-cell neoplasms, seen in the male ethoprop carcinogenicity studies, are a reflection of the high and variable spontaneous incidence of this tumor type in control rats and are not a product of treatment with ethoprop.

Table 8. Thyroid C-cells: Comparison of Tumor Incidences from the Original and EPL Review Evaluations (Williams Study – MRID 42530201)

Evaluation	Sex	Males				Females			
	Group	1	2	3	4	1	2	3	4
	Dose (ppm)[a]	0	1	60	400	0	1	60	400
Original	N	90	80	80	90	90	80	80	90
	(B) C-cell Adenoma	9 (10%)	6 (8%)	9 (11%)	13 (14)[b] 14% (16%)	9 (10) 10% (11%)	8 (10%)	11 (14%)	12 (13%)
	(M) C-cell Carcinoma	0	0	1 (1%)	3 (3%)	1 (1%)	1 (1%)	1 (1%)	2 (2%)
Review	N	89	80	80	90	90	80	80	90
	(B) C-cell Adenoma	**11** (12%)	6 (8%)	**10** (13%)	**15** (17%)	**12** (13%)	**11** (14%)	11 (14%)	**14** (16%)
	(M) C-cell Carcinoma	**0**	**0**	1 (1%)	3 (3%)	1 (1%)	1 (1%)	1 (1%)	2 (2%)

Differences in incidence from original evaluation are highlighted in **bold.**

a. Doses of 0, 1, 60 or 600/400 ppm (600 ppm during the first 2 weeks of treatment) of ethoprop were fed to rats (80/sex/group) for 105 weeks. Doses corresponded to 0, 0.04, 2.44, or 18.38 mg/kg/day in males and 0, 0.06, 3.56, or 23.98 mg/kg/day in females. Groups of 10 rats/sex were sacrificed at 52 weeks. An additional 10 rats/sex in the control and high-dose groups were treated for 52 weeks and allowed a 4-week recovery period before being sacrificed.

(B) = benign; (M) = malignant

b. ( ) = number documented on the original Final Report, Table 78, "Incidence of Microscopic Observations The numbers not in parentheses are those that have been corrected by the PWG.

Table 9. Thyroid C-cells: Comparison of Tumor Incidences from the Original and EPL Review Evaluations (Spicer Study – MRID 40291801)

Evaluation	Sex	Males				Females			
	Group	1	2	3	4	1	2	3	4
	Dose (ppm)[a]	0	1	10	100	0	1	10	100
Original	N	70	70	70	70	70	69	70	69
	(B) C-cell Adenoma	8 (11%)	5 (7%)	5 (7%)	12 (17%)	2 (3%)	4 (6%)	0	6 (9%)
	(M) C-cell Carcinoma	0	0	1 (1%)	3 (4%)	0	1 (1%)	0	1 (1%)
Review	N	70	68	70	70	68	69	70	65
	(B) C-cell Adenoma	**5** (7%)	**3** (4%)	5 (7%)	12 (17%)	**1** (1%)	**3** (4%)	**0**	**4** (6%)
	(M) C-cell Carcinoma	**1** (1%)	0	1 (1%)	3 (4%)	0	**0**	**1** (1%)	**0**

Differences in incidence from original evaluation are highlighted in **bold**

Table 10: Incidences of Spontaneous C-cell Neoplasms in Male and Female Controls from F344 and Sprague-Dawley Rat Carcinogenicity Studies in Comparison to Ethoprop Carcinogenicity Studies (Williams 1992; Spicer 1985; Barnett et al. 1983)[a]

Strain	Years covered	C-cell adenoma		C-cell carcinoma		Reference
		Males	Females	Males	Females	
Crl:CDBR	1991-2002	13.4% (7.7% - 20.0%)	9.5% (2.0% - 20.0%)	1.01% (0 – 3.4%)	0.44% (0% - 2.0%)	Baldrick 2005
Crl:CDBr	1984-1991	6.5% (2.9%-8.7%)	5.9% (1.4% - 11.7%)	0.9% (0.0% - 2.9%)	1.7% (0.0% - 4.3%)	McMartin et al 1992
Sprague-Dawley NOS	1992 (i)	3.6% (range not specified)	2.86% (range not specified)	0.82% (range not specified)		Chandra et al 1992
Incidence in 400 ppm group in Ethoprop study	**1992**	**15.5%**	**13.3%**	**3.3%**	**2.2%**	**Williams 1992**
F344/N	1971-1997	13% (2% - 35%)	1.8% (0% - 6%)	11.7% (4% - 22%)	1.9% (0% - 4%)	Haseman et al 1998
CDF® (F-344) CrIBR	1980-1986	2.2% (0%-6.4%)	5.2% (0% - 13.3%)	0.6% (0% - 2.8%)	0.9% (0% - 4.2%)	Lang 1990
Incidence in 100 ppm group in Ethoprop	1985	25%	10%	6.3%	2%	Spicer 1985

a. Per EPA Cancer Guidelines, animals should be fed *ad libitum*. The CARC considered only the historical control data from studies that included ad libitum feeding, and that encompassed the actual study date (i.e., conducted within 5 years prior to or 5 years after the study being evaluated).
(i) = date of publication. Notes: NOS = not otherwise specified.

(c) CARC Pathologist Response

Dr. Ward agreed with the procedure used by the PWG for reviewing the slides/lesions, as well as the criteria used by the PWG ELP for diagnoses of thyroid lesions. Dr. Ward recommended that the CARC should evaluate statistically the one high-dose male rat group in the Spicer (1998) study (MRID 425302021) which showed a statistically significant (statistics completed by registrant) result for thyroid C-cell adenoma or carcinoma (6 vs. 15). However, Dr. Ward noted that the lack of consistency among experiments would suggest that the normal variation in tumor incidences in the thyroid would explain the findings. Dr. Ward concurred with the registrant's overall conclusion that the thyroid tumors are a "reflection of the high and variable spontaneous incidence of this tumor type in control rats and are not a product of treatment with ethoprop."

3. HED Qualitative Reanalysis of PWG Pathology Slides on Adrenal Medulla and Thyroid Tumors – Spicer 1985 and Williams 1992 Studies

Spicer 1985 – "Lifetime dietary toxicity and oncogenicity study in rats". (MRID 40291801). IRDC Study Number 347-029.

HED reanalyzed statistically the thyroid C-cell tumors in male rats based on the 2017 PWG of the 1985 Spicer study with ethoprop (Lori Brunsman; TXR 0057990; January 9, 2020). Results of the analyses are presented below.

Background

A chronic oral toxicity and oncogenicity study in Fischer F344 rats was conducted by International Research and Development Company, Mattawan, Michigan, for Rhone-Poulenc Agrochemie, Mt. Pleasant, Tennessee, and issued April 30, 1985 (amended June 5, 1985, IRDC Study No. 347-029, MRID 40291801; Spicer 1985 study). The study design allocated groups of 50 rats per sex to dose levels of 0, 1, 10 or 100 ppm of ethoprop for 105 weeks. Doses correspond to 0, 0.041, 0.40, or 4.19 mg/kg/day in males. An additional 20 rats per sex per dose were designated for interim sacrifices, 10 per sex per dose at 53 and 79 weeks. A statistical reanalyzes (L. Brunsman, TXR 0012720, November 6, 1996) was prepared based on the findings of this study (Tables 11-15, below).

Survival Analyses

There were no statistically significant survival disparities between the dose groups in male rats (Table 11).

Tumor Analyses

Male rats had statistically significant trends at $p < 0.01$ for both thyroid C-cell adenomas and thyroid C-cell adenomas and/or carcinomas combined. There was also a statistically significant pair-wise comparison of the 100-ppm dose group with the controls for thyroid C-cell adenomas and/or carcinomas combined at $p < 0.05$. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (Table 12).

Table 11. Ethoprop – 1985 Fischer F344 Rat Study
2017 PWG Re-read (MRID 50390802)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Weeks

Dose (ppm)	1-26	27-52	53[i]	53-78	79[i]	79-105[f]	Total
0	0/70	1/70	10/69	0/59	10/49	13/49	14/50 (28)
1	1/70	1/69	10/68	2/58	10/56	7/46	11/50 (22)
10	0/70	0/70	10/70	2/60	10/58	13/48	15/50 (30)
100	0/70	0/70	10/70	2/60	10/58	9/48	11/50 (22)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifices at weeks 53 and 79.
[f]Final sacrifice at week 105.

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 12. Ethoprop – 1985 Fischer F344 Rat Study
2017 PWG Re-read (MRID 50390802)

Male Thyroid C-Cell Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	1	10	100
Adenomas	**5[a]/59**	3/56	5/60	12/60
(%)	**(8)**	(5)	(8)	(20)
P =	**0.00525****	0.84664	0.63920	0.06148
Carcinomas	1/59	0/56	1/60	3[b]/60
(%)	(2)	(0)	(2)	(5)
P =	0.06772	1.00000	0.75630	0.31566
Combined	**5[c]/59**	3/56	6/60	**15/60**
(%)	**(8)**	(5)	(10)	**(25)**
P =	**0.00051****	0.84664	0.51189	**0.01420***

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 99 in the control group.
[b]First carcinoma observed at week 87 in the 100-ppm dose group.
[c]One animal in the control 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 $p < 0.01$.

Williams 1992: "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.

HED also prepared a statistical reanalyzes of adrenal medulla and thyroid C-cell tumors in male rats based on the 2017 PWG of the 1992 Williams study with ethoprop (Lori Brunsman, TXR 0057993, January 21, 2020). Results of the statistical reanalyzes are presented below.

Background

A chronic oral toxicity and oncogenicity study in Crl:CD(SD)BR rats was conducted by Hazleton Laboratories America, Inc., Madison, Wisconsin, for Rhone-Poulenc Ag Company, Research Triangle Park, North Carolina, and completed September 10, 1992 (Laboratory Project ID No. HLA 6224-151, MRID 42530201). The study design allocated groups of 70 rats per sex to dose levels of 0, 1, 60 or 400 ppm of ethoprop for 105 weeks. Doses corresponded to 0, 0.04,

2.44, or 18.38 mg/kg/day in males. An additional 10 rats per sex per dose were designated for interim sacrifice at week 53 and an additional 10 rats per sex were assigned to the control and high dose groups and designated for a recovery phase (52 weeks of exposure to the test diet followed by an additional 4 weeks of exposure to the basal diet). Due to the toxicity of the original high dose of 600 ppm, group 4 animals were reduced to a dose of 400 ppm during week 3 of the study and continued to receive 400 ppm of test diet throughout the remainder of the study.

Survival Analyses

There was a significant decreasing trend, and a significant negative pairwise comparison of the high dose with the control, for survival among the male rats, both at $p<0.01$ (Table 13).

Tumor Analyses

Male rats had a statistically significant trend for thyroid C-cell carcinomas and a statistically significant pair-wise comparison of the 1 ppm dose group with the controls for adrenal medulla malignant pheochromocytomas, both at $p < 0.05$. The statistical analyses of the tumors in male rats were based upon Peto's Prevalence Test (Tables 14 and 15).

Table 13. Ethoprop – 1992 Crl:CD(SD)BR Rat Study
2017 PWG Re-read (MRID 50390801)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results
Weeks

Dose (ppm)	1-26	27-52	53[i]	53-57	57[i]	58-78	79-105[f]	Total
0	2/90	0/88	10/88	1/78	10/77	11/67	36/56	**50/70 (71)[n]****
1	1/80	2/79	10/77	0/67	0/67	9/67	30/58	42/70 (60)
60	1/80	2/79	10/77	0/67	0/67	9/67	30/58	42/70 (60)
400	2/90	1/88	9/87	0/78	10/68	5/68	22/63	**30/71 (42)[n]****

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifices at weeks 53 and 57.
[f]Final sacrifice at week 105.
[n]Negative trend or negative pair-wise comparison with the controls.
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 14. Ethoprop – 1992 Crl:CD(SD)BR Rat Study
2017 PWG Re-read (MRID 50390801)

Male Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	1	60	400
Adenomas	11[a]/76	6/67	10/67	15[a]/78
(%)	(14)	(9)	(15)	(19)
P =	0.25699	0.90706	0.63729	0.56335
Carcinomas	**0/60**	0/63	1/64	3[b]/66
(%)	**(0)**	(0)	(2)	(5)
P =	**0.02111***	-	0.20504	0.06137
Combined	11/76	6/67	11/67	18/78
(%)	(14)	(9)	(16)	(23)
P =	0.09672	0.90706	0.55681	0.34062

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

[a]First adenoma observed in the week 57 interim sacrifice, simultaneously in the control and 400 ppm dose groups.

[b]First carcinoma observed at week 70 in the 400 ppm dose group.

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

Table 15. Ethoprop – 1992 Crl:CD(SD)BR Rat Study
2017 PWG Re-read (MRID 50390801)

Male Adrenal Medulla Pheochromocytoma Tumor Rates[+]
and Peto's Prevalence Test Results

	Dose (ppm)			
	0	1	60	400
Benign	14[a]/47	4/26	6/28	5/61
(%)	(30)	(15)	(21)	(8)
P =	0.99844	0.81821	0.61634	0.99824
Malignant	1[b]/40	**4/19**	2/20	5/60
(%)	(3)	**(21)**	(10)	(8)
P =	0.52750	**0.01420***	0.08487	0.05509
Combined	15/47	8/26	8/28	10/61
(%)	(32)	(31)	(29)	(16)
P =	0.99453	0.38273	0.37255	0.94990

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.
[a]First adenoma observed at week 88 in the control group.
[b]First carcinoma observed at week 93 in the control group.
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 $p < 0.01$.

(e) Non-neoplastic Thyroid Lesions in the Pubertal Assays and Combined Chronic Carcinogenicity Studies

Pubertal assays

In the male pubertal assay (MRID 48683901), serum T_4 levels were increased by 28%, and serum TSH levels were decreased by 16% following treatment with 6 mg/kg/day (highest dose tested) from PND 23 to 53. In addition, thyroid weights were increased by 17%, and there was a shift toward lower thyroid follicular height to colloidal area ratio scores.

No treatment-related thyroid effects were reported in the female pubertal assay (MRID 48683901).

Williams (1992). 104-Week Combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Rats. MRID 42530201.

At week 53, high-dose males had decreased left absolute thyroid/parathyroid weights; this decrease was significant after a 4-week recovery period at week 57. At the end of the study at week 105, there was no difference in absolute thyroid weight between high-dose males and

controls. In high-dose females, relative thyroid/parathyroid weights were significantly lower at week 53, week 57, but were higher by the end of the study. There were no nonneoplastic lesions in the thyroid in either sex.

Spicer (1985). Lifetime dietary toxicity and oncogenicity study in rats. MRID 40291801.
There were no treatment-related changes in thyroid weights or nonneoplastic lesions in the thyroid.

IV. OTHER TOXICOLOGICAL CONSIDERATIONS

The metabolism and structure activity relationships, of ethoprop have been discussed in previous 1997 and 1998 CARC reports (refer to CARC 1997 and CARC 1998).

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-EVIDENCE-CONSIDERATIONS

1. Carcinogenicity

Adrenal Pheochromocytomas

In Sprague-Dawley rats (Williams 1992; MRID 42530201), the results of HED's statistical reassessment of PWG tumor rates using the Peto's prevalence test showed that male rats had a statistically significant ($p < 0.05$) pair-wise comparison only of the low (1 ppm) dose group with the controls for malignant adrenal pheochromocytomas. HED applied the Peto's Prevalence test because it adjusts for mortality unlike the statistics used by the registrant (e.g., Cochran-Armitage test). The Committee noted that the PWG only evaluated the low- and mid-dose animals that were available, not the total animals. The Committee also noted that there was no dose response relationship for this tumor type. The incidence of benign adrenal pheochromocytomas was within the range of appropriate historical control data. The CARC considered only the historical control data from studies that included *ad libitum* feeding, and that encompassed the actual study date (i.e., conducted within 5 years prior to or 5 years after the study being evaluated). These studies included Baldrick (2005), McMartin et al. (1992), and Chandra et. al. (1992). In contrast, the incidence of malignant adrenal pheochromocytomas was outside the range of appropriate historical control data for all doses tested (i.e., 0 – 6.7% reported by Baldrick 2005; 0 – 6% reported by McMartin et al. 1992). In addition, the incidences of pre-neoplastic lesions (e.g. adrenal medullary hyperplasia) do not support a treatment-related effect. The CARC did not consider the adrenal tumors to be treatment-related.

Thyroid C-cell Tumors

In Sprague-Dawley rats (Williams 1992; MRID 42530201), the results of the CARC's reassessment of the PWG tumor rates indicated that male rats had a statistically significant trend for thyroid C-cell carcinomas. The Committee noted that the incidence of thyroid C-cell carcinomas at the high dose (400 ppm) was outside the range of appropriate historical control data (i.e., Baldrick 2005; McMartin et al. 1992; Chandra et

al. 1992), but there was no pairwise statistically significant difference in comparison to concurrent controls. For thyroid C-cell carcinomas, Baldrick (2005) reported an historical control range of 0 – 3.4%; whereas the range report by McMartin et al. (1992) was 0 – 2.9%). Chandra et al. (1992) did not present a range of historical control data but did provide a mean rate of 0.82%. Also noted was that the high dose (400 ppm) was excessive based on several factors: (1) the high dose had to be reduced from 600 ppm to 400 ppm after 2 weeks of dosing due to excessive toxicity in females (tremor, ataxia, and 2 deaths); (2) brain ChE activity in 400 ppm males was decreased 64% compared to controls at study termination; (3) there were body weight decrements of -20% compared to controls during the first half of the study; (4) neurotoxicity was noted in a subchronic neurotoxicity study at the same dose (400 ppm); (5) the 400 ppm dose is about 2/3 of the LD_{50} dose for ethoprop; and (6) ethoprop has a steep dose-response curve with death occurring at dose levels slightly above those causing clinical signs. Therefore, the CARC did not consider these tumors treatment related at any dose tested.

In the F344 rats (Spicer 1985; MRID 40291801), the results of the CARC's reassessment of tumor rates revealed that male rats had statistically significant trends for both thyroid C-cell adenomas and thyroid C-cell adenomas and/or carcinomas combined. There was also a statistically significant pair-wise comparison of high dose (100-ppm) group with the controls for thyroid C-cell adenomas and/or carcinomas combined. The Committee commented that the significant trend and pairwise comparison at the high dose for the combined adenomas/carcinomas was driven by the incidence of adenomas. The Committee noted that the incidences of thyroid C-cell adenomas and carcinomas were within the historical control range reported by Haseman (1998). Haseman (1998) reported the mean rates and range of historical control data for thyroid C-cell adenoma as 13 (range: 2 – 35%) and for thyroid C-cell carcinoma as 1.8 (range: 0 – 6%). However, the thyroid C-cell carcinomas were outside the historical control range (0 – 2.8% for carcinomas; 0 – 6.4% for adenomas) in the Lang (1990) study. The Committee also discussed the Spicer (1985) study and concluded that the 100 ppm (high dose) was not excessive; cholinesterase activity was decreased by 68% (in brain) and by 86% (in red blood cell) but there was no mortality or serious clinical signs of toxicity. The Committee also mentioned that in this case there were no significant thyroid non-neoplastic effects (e.g., hyperplasia) that would support a carcinogenic effect. However, the CARC concluded that the statistically significant increase in thyroid C-cell tumors in the high-dose male F344 rats is treatment-related.

2. Mutagenicity

The mutagenic potential of ethoprop was previously assessed by CARC in 1997 (Jess Rowland; TXR 0012564; October 2, 1997). At that time the CARC concluded that ethoprop was an *in vitro* clastogen based on positive findings in an *in vitro* cell chromosomal aberration (CA) assay in Chinese Hamster Ovary (CHO) cells (MRID 00160183). However, the incidences of CAs in that study were not concentration-related and occurred over a narrow concentration range approaching the limits of cytotoxicity. Similarly, a positive finding was reported in a sister chromatid exchange assay in CHO cells at concentrations approaching cytotoxic levels (MRID 00160184). In both studies,

the positive *in vitro* findings occurred only in the presence of S9-activation. When tested *in vivo*, ethoprop did not induce chromosomal aberrations in bone marrow of rats up to doses that resulted in severe toxicity or death (MRID 41211202). Given that the positive *in vitro* findings occurred near cytotoxic levels and there were no treatment-related increases in CAs observed *in vivo* at doses causing marked toxicity, the overall mutagenic concern for ethoprop is low. This is further supported by negative findings in the *in vitro* unscheduled DNA assay in primary rat hepatocytes (MRID 00160182 and MRID 44038702), gene mutation test in bacteria (MRID 00160180), gene mutation assay in mammalian cells *in vitro* (MRID 001660181), mouse lymphoma cells *in vitro* (MRID 44065001), and the *in vivo* rodent dominant lethal assay (MRID 40386901).

3. Mode of Action

No tumor mode of action data were available for ethoprop.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified ethoprop as "Suggestive Evidence of Carcinogenic Potential." This was based on the presence of one tumor type, thyroid C-cell tumors, which occurred in only one sex (males) and one species (F344 rat) and had no mutagenicity concerns. The chronic RfD would adequately protect for all the chronic toxicity, including any potential carcinogenicity, that could result from exposure to ethoprop.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification of carcinogenic risk is not required for ethoprop.

VIII. BIBLIOGRAPHY

Baldrick, P. (2005). Carcinogenicity Evaluation: Comparison of Tumor Data from Dual Control Groups in the Sprague–Dawley Rat. *Toxicol. Pathol.* 33: 283–291.

Barfknecht, T. (1985). Ames *Salmonella*/Microsome Plate Test: Ethoprop. Pharmacon Research International, Inc. Study # PH 301-RP-01-85. Report Date: August 9, 1985. MRID 00160180.

Barfknecht, T. (1985). Rat Hepatocytes Primary Culture/DNA Repair Test: Ethoprop. Pharmacon Research International, Inc. Study # PH 311-RP-01-85. Report Date: August 9, 1985. MRID 00160182.

Blackburn, G. (1981). A Murine Lymphoma Mutagenesis Assay. Heterozygous at the Thymidine Kinase Locus for the Determination of the Potential Mutagenicity of Ethoprop. Lab Project No. 2474-80. Mobil Environmental and Health, 1981. MRID 44065001.

CARC 1997. Ethoprop – Report of the Cancer Assessment Review Committee (dated 10/2/97). TXR 0012564.

CARC 1998. Ethoprop – Review of Registrant's Rebuttal to HED's Cancer Classification (dated 10/7/98). TXR 0012890.

Chandra, M., Riley, M.G.I. and Johnson, D.E. (1992). Spontaneous neoplasms in aged Sprague-Dawley rats. *Arch.Toxicol.* 66: 496-502.

Dearlove, G. (1987). Dominant Lethal Study of Ethoprop Technical Administered Orally Via Gavage to Crl:COBS CD (SD)BR Male Rats. Argus Research Laboratories. Study # 218-004. Report Date: July 28, 1987. MRID 40386901.

Dinse, G.E., Peddada, S.D., Harris, S.F. and Elmore, S.A. (2010). Comparison of NTP Historical Control Tumor Incidence Rates in Female Harlan Sprague Dawley and Fischer 344/N Rats. *Toxicol. Pathol.* 38; 765-775.

Giknis, M.L.A. and Clifford, C.B. (1998). Spontaneous Neoplastic Lesions and Survival in CrI:CD®(SD)BR Rats Maintained on Dietary Restriction. Charles River Laboratories, Data Sheet.

Giknis, M.L.A. and Clifford, C.B. (2013). Compilation of spontaneous neoplastic Lesions and Survival in CrI:CD®(SD)BR Rats from control groups. Charles River Laboratories, Data Sheet.

Haseman, J. K., Huff, J., and Boorman, G. A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol Pathol* 12; 126–135.

Haseman, J. K., Hailey, J.R. and Morris, R.W. (1998). Spontaneous Neoplasm Incidences in Fischer 344 Rats and B6C3F, Mice in Two-Year Carcinogenicity Studies: A National Toxicology Program Update. *Toxicol. Pathol*. 26; 428-441.

Ivett, JL. (1989). Mutagenicity Test on Ethoprop Technical in the Rat Bone Marrow Cytogenetic Assay. Hazleton Laboratories. Study # HLA 10750-0-452, Report Date: August 9, 1989. MRID 41211202.

Keenan, K.P., Soper, K.A., Smith, P.F., Ballam, G.C. and Clark, R.L. (1995). Diet, Overfeeding, and Moderate Dietary Restriction in Control Sprague-Dawley Rats: I. Effects on Spontaneous Neoplasms. *Toxicol. Pathol.* 23; 269-286.

Kuroiwa, Y., Ando, R., Kasahara, K., Nagatani, M., Yamakawa, S., Okazaki, S. (2013). Transition of historical control data for high incidence tumors in F344 rats. *J. Toxicol. Pathol.* 26, 227-230.

Lang, P.L. (1990). Spontaneous Neoplastic Lesions in the CDF (F344)/CrlBR rat. Charles River Laboratories.

McMartin, D., Sahota, P., Gunson, D.E., Hsu, H.H., and Spaet, R. (1992). Neoplasms and Related Proliferative Lesions in Control Sprague-Dawley Rats from Carcinogenicity Studies. Historical Data and Diagnostic Considerations. *Toxicol. Pathol.* 20; 212-221.

MRID 50259101. Foster, J. (2017). Ethoprop: Summary of Response to EPA Draft Human Health Risk for Carcinogenesis in the Rat. Report No: RSA/AMV012-4105_EtHOPROP_004. Prepared by Regulatory Science Associates, United Kingdom.

MRID 50352001. AMVAC response to written questions from the EPA and those arising from conference call discussion regarding the carcinogenesis of Ethoprop. Undated document from AMVAC.

MRID 50390801. Hardisty, J. (2017). Pathology Working Group: Review of Thyroid and Adrenal Glands from a 104-Week combined Chronic Toxicity and Carcinogenicity Study with Ethoprop in Rats. Williams 1992.

MRID 50390802. Hardisty, J. (2017). Pathology Working Group: Review of Thyroid and Adrenal Glands from a Lifetime Dietary Toxicity and Oncogenicity Study in Rats. Spicer 1985.

MRID 50400701. AMVAC. September 25, 2017. Chronic Toxicity and Carcinogenicity Studies with Ethoprop in Rats. Review and statistical analysis of tumor incidence in recoded slides for Spicer (1985) and Williams (1992) studies following Pathology Working Group Review (presented as Appendix 4 of Foster, J. 2017).

Pace, V., Perentes, E. and Germann, P-G. (2002). Pheochromocytomas and Ganglioneuromas in the Aging Rats: Morphological and Immunohistochemical Characterization. *Toxicol. Pathol*. 30; 492–500.

Prejean, J.D., Peckham, J.C., Casey, A.E., Griswold, D.P., Weisburger, E.K. and Weisburger, J.H. (1973). Spontaneous Tumors in Sprague-Dawley Rats and Swiss Mice. *Can. Res*. 33; 2768-2773.

Putnam, D. (1981) Activity of T1688 in the Dominant Lethal Assay in Rodents. LabProject No. T1688.116: Microbiological Associates, 1981. MRID 44038701.

Rao, G.N., Haseman, J.K., Grumbein, S., Crawford, D.D., Eustis, S.L., (1990). Growth, body weight, survival, and tumour trends in F344/N rats during an eleven-year period. *Toxicol. Pathol*. 18, 61-70.

SanSebastian, J. (1985). *In Vitro* Chromosome Aberration Analysis in Chinese Hamster Ovary (CHO) Cells: Ethoprop. Pharmacon Research International, Inc. Study # PH 320-RP-01-85. Report Date: October 26, 1985. MRID 00160183.

SanSebastian, J. (1986). *In Vitro* Sister Chromatid Exchange in Chinese Hamster Ovary (CHO) Cells: Ethoprop. Pharmacon Research International, Inc. Study # PH 319-RP-01-85. Report Date: April 23, 1986. MRID 00160184.

Stankowski, L. (1985). CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay: Ethoprop. Pharmacon Research International, Inc. Study # PH 314-RP01-85. Report Date: August 9, 1985. MRID 00160181.

Spicer, E.J.F. (1985). Ethoprophos technical: Lifetime dietary toxicity and oncogenicity study in rats. Document No. R009010. IRDC Study No. 347-029. MRID 40291801.

Williams, K.D. (1992a). 104 week combined chronic toxicity and carcinogenicity study with Ethoprop in rats. Document R009812. MRID42530201.

Williams, K.D. (1992b). Supplemental historical control data requested for the 104 week combined chronic toxicity and carcinogenicity study with Ethoprop in rats. Document R008864.