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Subject: ADDENDUM I TO MITRE'S REVIEW. CHRONIC ONCOGENIC EVALUATION OF ETHOPROP WITH B6C3F1 MICE. (78 weeks).

Test Compound: Ethoprop Technical Grade, MCTR-60-78, Lot No. 2225-62

Accession Number: 263797, -798, -799, -800

Testing facility: Food and Drug Research Labs

Study Number: FDRL Study No. 5-5849

Testing Period: December 28, 1978 - July 3, 1980

Report Submitted to Sponsor: January 26, 1983

Purity of Test Material: Technical Grade MCTR-60-78, Lot No. 2225-62

Contractor Classification: Supplementary

Toxicology Branch Classification: Supplementary

The reader is referred to the following review.

Toxicology Branch position on the MITRE review of the Chronic Oncogenic Evaluation of Ethoprop Technical conducted with B6C3F1 mice supplied by Harlan Industries of Indianapolis, Indiana.

The reviewer at the MITRE Corporation classified this chronic feeding oncogenicity study in mice as supplementary for the following two reasons:

- the inadvertant one time administration of a ten (10) fold excess of Ethoprop^{R} in the diet at week 54, and

- the high incidence of ocular disease in females for the duration of this 78 week study.

the reviewer at MITRE therefore concluded that an unequivocal evaluation of Ethoprop related effects could not be made and recommended that the study be repeated using accurately prepared diets and healthy animals such that the quality and the integrity of the data would not be compromised.

This Toxicology Branch reviewer agrees with the Supplementary classification assigned to this study but for reasons different than those presented by the MITRE reviewer. The rationale for this is as follows:

The purpose of this study was to determine whether or not Ethoprop produces an oncogenic response in mice. The reviewer at MITRE indicated that there was no direct evidence that Ethoprop is oncogenic in this strain of mouse. This Toxicology Branch reviewer in consultation with the Toxicology Branch pathologist (Dr. Louis Kasza) also agree that there is no evidence that Ethoprop is oncogenic under the test conditions. We point out here that subsequent to the inadvertant one time administration of a ten (10) fold increase in dose at week 54 and the nearly immediate deaths of several mice of both sexes, there was no increased death rate at any dose level for the remaining duration of the experiment. We also point out that a sufficient number of animals were available at the terminal sacrifice to give an evaluation of the oncogenicity of Ethoprop in this study. The evaluation of animals sacrificed at termination as well as animals sacrificed and found dead intercurrently gave no indication that the test compound was oncogenic. Furthermore, with the exception of the findings related to the eye, which may have been a localized non-systemic effect, the histopathological examination as well as the examination of other parameters indicated that the animals were in generally good health and relatively unaffected by compound administration.

This Toxicology Branch reviewer therefore concludes that:

- Despite the one (1) time ten (10) fold increase in dose and immediate subsequent deaths, it was still possible to evaluate the oncogenicity of Ethoprop in this study (at the doses tested).

- The gross and microscopic findings as related to the eye did not compromise the oncogenic evaluation of this study.

- There is no evidence to suggest that Ethoprop was an oncogen in this study at the doses tested. However, as a result, the study will not be upgraded from Core Supplementary because a Maximum Tolerated Dose (MTD) could not be ascertained (for discussion see Toxicology Branch Addendum II).

We are also concerned with respect to the findings in the 78 week study as they relate to the eye. We are therefore requesting the following additional study to be conducted in an attempt to resolve this issue as to whether or not these noted eye effects were systemically induced. We therefore are asking that:

- an oral gavage study be conducted with 30 male and 30 female mice in the control group and 30 male and 30 female mice in the treated group.

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° that the same strain of mouse be used (B6C3F1),

° that the strain of mouse used be obtained from a supplier other than Harlan Industries of Indianapolis,

° that a single daily dose be administered for a period of 60 days and be sufficient to give biologically meaningful RBC and plasma cholinesterase depression,

° that the test material used be Ethoprop technical, MCTR-60-78; Lot No. 2225-62,

° that all animals be observed daily and that five animals/sex/group be sacrificed on days 5, 10, 15, 20, 30, 60 and examined for gross and microscopic findings of the eye only and address those adverse findings previously noted,

° that the results of the study be reported in full to the Agency with a discussion to the findings and a conclusion drawn.

We are also requesting that a separate but concurrent study be conducted under the following conditions:

° A dietary study be conducted with 30 male and 30 female mice in the control group and 30 male and 30 female mice in the treated group.

° that the possibility of cross contamination be eliminated between treated and control in this study and the gavage study.

° that a strain of mouse be used other than the B6C3F1 strain and be obtained from a supplier other than Harlan Industries,

° that the dose to be administered in feed for a period of 60 days be sufficient to give biologically meaningful RBC and plasma cholinesterase inhibition,

° that the test material administered be Ethoprop technical MCTR-60-78; Lot No. 2225-62,

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° that all animals be observed daily and that 5 animals/sex/group be sacrificed on days 5, 10, 15, 20, 30 and 60 and examined for gross and microscopic finds of the eye only and address those adverse findings previously noted.

° that at 60 days the experiment be terminated.

° that the results of the study be reported in full to the Agency with a discussion of the findings and a conclusion drawn.

We also ask that the results of the separate studies be integrated in any disucssion and conclusion of the registrants position with regard to the eye effects in general.

Lastly, if the results of the gavage study are negative and those of the dietary study are positive we ask the registrant to present a scientifically sound argument as to the causation of the adverse eye effects previously noted and the probability of those effects occuring from topical exposure to Ethoprop via the eye with time.

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TOXICOLOGY BRANCH ADDENDUM II TO MITRE'S REVIEW. CHRONIC ONCOGENIC EVALUATION OF ETHOPROP WITH B6C3F1 MICE.

In the 78-week mouse oncogenicity study, under the planned dosing regimen, a clear cut toxicolgical basis upon which to determine if a Maximum Tolerated Dose (MTD) had been reached appeared to be lacking (see rationale below):

78-Week B6C3F1 Mouse Oncogenicity Study

Ethoprop was administered at doses of 0, 15, 30 and 60 ppm. The only findings reported which could definitely be attributed to the administration of the test compound were inhibition of erythrocyte (RBC) and plasma cholinesterase activities, relative to controls, at study weeks 52 and 78.* Although statistically and biologically significant enzyme depression was observed at all doses tested and in both sexes at both time points (see Table I), for the most part, inhibition did not appear to be dose dependent. In fact, for the most part, at each time point, the magnitude of RBC and plasma cholinesterase inhibition was similar in the high, mid, and low dose groups of either sex and the variability in cholinesterase activity measurements tended to be large (see actual data Table I).

While the cholinesterase inhibition was taking place, there did not appear to be any significant concommitant changes in body weight, food consumption, clinical chemistry, severity of clinical signs, changes in organ weights, or histopathologic changes, etc. (as the data was reported by the study authors). Hence, an MTD could not be ascertained in this study.

Apparent Lack of Clear-Cut Toxicological Endpoint(s) in Subchronic Study

It was not indicated in the study report which subchronic study was used as a basis to select dose levels in the 78-week mouse study. However, a six month toxicity study using Ethoprop was performed in B6C3F1 mice by Gulf South Research Inst., New Iberia, Louisianna, July 28, 1978 (see attached Toxicology Branch review). The study was conducted using the following dose levels: 0, 30.5, 61, 122 ppm. Although the study design was deficient (i.e. cholinesterase activity, clinical chemistries and other parameters were not measured), Ethoprop had no clear effects on mortality, food consumption, body weight gain, nor did it appear to cause histopathological changes in tissues examined. Clinical observations were reported to have been of low incidence. Thus at 122 ppm, two times the highest dose tested in the 78-week study, a clear toxicological endpoint by which might be used to establish or estimate an MTD was not apparent in the six-month study.

Rat Chronic/Oncogenicity Study

A chronic toxicity/oncogenicity study in rats performed by Gulf South Research Institute, report dated 1/83, was also submitted for review but at this point it cannot be concluded that there were no neoplastic effects resulting from test compound administration since additional data/information from the sponsor are necessary to evaluate this concern.

*For Cholinesterase determinations, animals were fasted overnight prior to collection of blood samples from periorbital sinus.

Conclusion

Since it does not appear that an MTD or an apparent MTD can be determined based on the data described above, it is the judgement of this reviewer that the mouse oncogenicity study be repeated using a protocol (based on the current Pesticide Guidelines) which incorporates the concept of MTD. This requirement for a new study may be reconsidered if the sponsor can provide a clear, concise, and convincing counter-argument.

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TABLE 1*

Cholinesterase Inhibition In Ethoprop 78-Week Mouse Oncogenicity Study
(% decrease compared to controls)

	Week 52		Week 78	
	Males		Males	
Dose	RBC	Plasma	RBC	Plasma
15 ppm	-62	-66	-69	-37
30 ppm	-69	-71	-80	-43
60 ppm	-61	-71	-80	-50
	Females		Females	
15 ppm	-70	-58	-66	-30
30 ppm	-66	-69	-66	-38
60 ppm	-83	-72	-78	-39

Actual Data*
(Group Mean ± S.D.)
FDRL-Michel Units

	Males		Males	
Control	191 ±43	2008 ±188	321 ±79	2656 ± 25
15 ppm	72[b] ±48	676[b] ±105	99[b] ±48	1686[b] ±100
30 ppm	59[b] ±48	578[b] ±78	63[b] ±39	1519[b] ±236
60 ppm	74[b] ±72	592[b] ±79	64[b] ±29	1326[b] ±371
	Females		Females	
Control	286 ±108	2297 ±117	289 ±33	2778 ± 49
15 ppm	86[b] ±46	975[b] ±153	97[b] ±30	1936[b] ±142
30 ppm	97[b] ±98	710[b] ±90 (4)	98[b] ±88	1729[b] ±221
60 ppm	47[b] ±22	654[b] ±26	65[b] ±27	1690[b] ±223

[b] Significantly different from respective control, $p \leq 0.05$.

*Abstracted from study report

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MITRE CONTROL NO.
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* CONFIDENTIAL BUSINESS INFORMATION *

MRID: Not reported

Study Type: Chronic Oncogenic Evaluation In Mice

Accession Number: ~~Not reported~~ ~~252364, -65, -66, -67~~ 263797, -798, -799, -800

MRID Number: Not reported

Sponsor: Rhone-Poulenc, Inc.

Contracting Lab: Food & Drug Research Laboratories, Inc. (FDRL)

FDRL Study # 5-5849

Date: January 26, 1983

Reviewed by: _____ Date: 9/25/84
The MITRE Corporation

Approved by: _____ Date: _____
EPA

Test Material: Ethoprop®

Protocol:

The following descriptions of the materials and methods used for this study were abstracted and paraphrased from the original report.

1. Test Substance and Purity: Ethoprop®, technical grade MCTR-60-78, Lot No. 2225-62, assigned FDRL Identification No. 78-0254.

2. Species of Animals: Male and female B6C3F$_1$ mice (four to six weeks old) from Harlan Industries, Indianapolis, IN were used.

3. Dosing Schedule: The mice were acclimated for four weeks, randomly allocated into test and control groups, and individually housed. Groups of 60 animals per sex were

* CONFIDENTIAL BUSINESS INFORMATION *

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provided Ethoprop® in the diet for 78 weeks at nominal concentrations of 0, 15, 30, and 60 ppm in Charles River RHM 3200 Meal. The diets were freshly prepared weekly and provided ad libitum. Samples were reserved for analysis every other week the first six months and at least once each month thereafter.

4. Parameters to be examined:

- Mortality, general health, and behavior daily.
- Palpable masses weekly.
- Body weight and food consumption weekly the first 13 weeks and bi-weekly thereafter.
- Water consumption in months 14, 16, and 18.
- Clinical studies in weeks 52 (interim) and 78 (final).
- Organ weights at necropsy.
- Macroscopic and microscopic examination on all animals.

5. Statistics Used: Body weight, food and water consumption, food utilization efficiency, clinical, and organ weight data were analyzed by one-way analysis of variance. "Differences between groups were established with the Least Significant Differences Test. Mortality and pathology incidence data were analyzed by Chi-square with Yates' Correction for 2 x 2 contingency tables. Urine analysis

score data were analyzed by an extention of the Mantel-Haenszel Procedure."

Results

Diet Analysis: The average Ethoprop® concentrations in diets analyzed throughout the study excluding week 54 were 14.8 ± 2.4 ppm, 29.0 ± 3.6 ppm, and 58.3 ± 9.4 ppm for nominal concentrations in test groups designated 15, 30, and 60 ppm, respectively. The average recovery ranged from 83.8% at 60 ppm to 90.8% at 30 ppm. Due to a weighing error diets prepared in week 54 contained a tenfold excess of Ethoprop® and were analyzed to be 163.0 ± 3.5 ppm, 283.6 ± 15.6 ppm, and 602.1 ± 8.4 ppm, rather than the intended 15, 30, and 60 ppm, respectively.

Mortality: No statistically significant differences ($p>0.05$) in mortality occurred between the control, 15 ppm, and 30 ppm groups of both sexes during the entire 78 week study or between the control and 60 ppm groups of both sexes during the initial 54 weeks of the study (Table 1). Subsequent to the administration of greater than 600 ppm Ethoprop® in week 54, the mortality in the males and females of the high dose group was significantly ($p<0.05$) increased. During weeks 55 and 56 eighteen males and nine females died. No additional deaths occurred in the high dose animals after returning to 60 ppm Ethoprop® through termination at 78 weeks. Overall, higher mortality was

TABLE 1

MORTALITY OF CONTROL AND ETHOPROP® TREATED MICE

Sex	Dose (ppm)	Cumulative Deaths At Week[a]										
		5	13	21	29	37	45	54	56	61	69	78
Males	0	0	2	7	7	9	9	10	10	10	10	10
	15	1	5	5	5	5	5	5	5	5	5	5
	30	1	3	3	3	3	4	4	5	5	7	8
	60	3	5	5	5	5	5	6	24*	26*	26*	26*
Females	0	1	5	9	11	11	11	11	11	11	12	12
	15	4	8	13	13	15	15	15	16	16	17	17
	30	1	7	12	13	13	13	13	14	15	15	15
	60	1	9	13	14	14	14	14	23*	23*	23*	23*

[a] Values are the number of dead animals. Initially there were 60 animals per group.

* Significantly different from control value ($p < 0.05$)

observed in females than in males and this was possibly related to the higher incidence of eye diseases in females than in males (discussed below). As of week 54 in the study, there were no significant effects on mortality in either sex at any dose. At week 56, a significant increase in mortality was observed in both sexes at the high dose; however, we cannot establish an LEL, because of the accidental administration of 602.1 ppm Ethoprop® in the diet of both sexes in week 54.

General Health and Behavior: Daily observations revealed ocular disease. The ocular disease was prevalent in control and treated mice throughout the study and was not caused by Ethoprop® treatment. Because it is possible that the ocular disease might have influenced the incidence or extent of other diseases that were related to Ethoprop treatment, the incidence and description of the ocular disease are summarized below.

Of the 480 mice placed on study, 115 developed ocular disease as early as the initial two weeks. The disease progressed to clinical blindness. More females than males were affected. The disease descriptions included periocular alopecia, corneal ulceration and endophthalmitis, periophthalmitis with draining exudate, perforated eyes with an orbital cellulitis, inflammation of cornea, iris, and

ciliary body, necrosis, ulceration, fibrosis, and orbital systemic disease. Because the disease was unusual and severe and occured in high incidence, cultures for infectious fungal, viral, and bacterial etiological agents were taken. No fungi or viruses were detected. Infectious bacteria were isolated as early as two months after the initial observations of eye problems and were identified as follows: Alpha streptococcus veridans, GpD enterococcus, rare to occasional Pasteurella pneumotropica and Clostridium species. The presence of these bacteria is indicative of poor housing conditions for the animals. During the 78 week study the microscopic pathology of eyes from animals found dead or sacrificed in moribund condition revealed general severe inflammation in all layers. At necropsy 177 mice (149 females and 28 males) had lost one or both eyes. The general appearance otnerwise, and behavior were normal. Because the eye diseases affected control and treated animals, they are not considered to be treatment related.

Body Weight: Significant differences between control and treated groups occurred sporadically for both sexes during the study but were not observed at termination of the study. Ethoprop® was not considered to adversely affect body weight in either sex. For chronic administration of Ethoprop® in both sexes the NOEL is 60 ppm and LEL was not established.

Food Consumption: There were numerous statistically significant differences in food consumption between control and treated groups in both sexes. No sustained increase or decrease in food consumption and no dose-response relationships were observed with treatment. Food consumption was not reported in week 54. Food consumption effects were not considered to be biologically significant. The calculated food utilization efficiency increases and decreases between control and treated groups that were statistically significant were not considered to be biologically significant.

Water Consumption: Water consumption was significantly ($p<0.05$) reduced in 30 and 60 ppm males in weeks 59, 68, and 76 and in 60 ppm females in weeks 59 and 76. This is a difficult parameter to measure with accurancy and was measured only three times during the study. The toxicological significance of decreased water consumption cannot be ascertained with the paucity of data available.

Clinical Studies: Hematological data were evaluated at 52 and 78 treatment weeks. The only statistically significant ($p < 0.05$) difference between control and treated groups was a decreased eosinophil count. Because this decrease was observed in the 15 and 30 ppm females at 52 weeks, but was not sustained through 78 weeks, and the decreased value was within the normal range in mice for eosinophil counts, the decreased eosinopnil count is not considered

to be toxicologically significant. The decreased eosinophil count suggests the bacteria identified (page 6) were incidental to and not likely etiological agents of the ocular diseases.

Significant findings from clinical chemistry evaluations at 52 and 78 weeks are summarized in Table 2. Males and females treated with 15, 30, and 60 ppm Ethoprop® had significantly ($p \leq 0.05$) decreased RBC and plasma cholinesterase levels compared with their respective controls at both 52 and 78 weeks. Cholinesterase activity was determined by the modified Michel method (Table 2). For reduction in cholinesterase activity by Ethoprop® in males and females the LEL is 15 ppm and the NOEL is not established.

The serum cholesterol level (Table 2) was significantly ($p \leq 0.05$) elevated at 52 weeks in females at 60 ppm and at 78 weeks in males at 15, 30, and 60 ppm. Overall, for cholesterol effects, the LEL is 15 ppm and the NOEL was not establisned.

Total protein (Table 2) was significantly increased ($p \leq 0.05$) in 60 ppm males at 78 weeks. This protein level was within the normal range for mice and was not considered to be biologically significant. In males and females for total protein the LEL is not established.

The alkaline phosphatase (Table 2) level was significantly reduced ($p \leq 0.05$) in one isolated incidence (15 ppm males at 52 weeks)

TABLE 2

SUMMARY OF CLINICAL CHEMISTRY DATA

Sex	Dose (ppm)	Week	Cholinesterase In RBC (FDRL - Michel Units)[a]		Cholinesterase In Plasma (FDRL - Michel Units)[a]		Cholesterol (mg/dl)		Total Protein (gm/dl)		Alkaline Phosphatase (IU/L)	
			52	78	52	78	52	78	52	78	52	78
Males	0		191	321	2008	2656	138	83	6.3	5.4	68	44
	15		72*	99*	676*	1686*	155	98*	6.8	5.4	46*	44
	30		59*	63*	578*	1519*	158	100*	6.1	5.7	54	41
	60		74*	64*	592*	1326*	181	128*	6.5	5.8*	56	39
Females	0		286	289	2297	2778	131	82	6.7	5.7	112	254
	15		86*	97*	975*	1936*	161	94	6.6	5.9	159	234
	30		97*	98*	710*	1729*	145	95	6.1	5.6	119	291
	60		47*	65*	654*	1690*	173*	112	6.2	5.7	173	181

* Significantly different from control value ($p \leq 0.05$)

[a] $pH_1 - pH_2 \times 1000$ = FDRL - Michel units (Wills, J.H., "CRC Critical Reviews in Toxicology" March 1972, p. 165-166)

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and was not reduced by higher doses in either sex at 52 and 78 weeks. This reduction was not considered to be a biologically significant effect of Ethoprop®. For alkaline phosphatase activity in both sexes the LEL was not established.

The urine analysis data did not differ significantly between control and treatment groups for either sex.

Organ Weights:

Table 3 summarizes the absolute and relative organ weight data for liver and adrenals. A significant increase in the relative liver weight (% BWT = 4.79 $\pm$ 0.41) was observed in 60 ppm males at 52 weeks. A significant increase in the relative adrenal weight (% BWT x 10^2 = 2.3 $\pm$ 0.4) was observed in 15 ppm females at 78 weeks. Because significant increases in the absolute liver and adrenal weights in the corresponding groups were not observed and because significant increases in absolute and relative liver and adrenal weights were not observed at both time intervals in both sexes and in all doses, the above isolated observations are not considered to be biologically significant or treatment related (LEL not established).

Pathology

Histopathological examinations were performed by Mobil Oil Corporation. The incidence tabulations of the gross and micropscopic data plus the text of the pathology report were prepared by FDRL.

TABLE 3

SUMMARY OF LIVER AND ADRENAL ABSOLUTE AND RELATIVE WEIGHT DATA

Sex	Dose (ppm)	52 Week[a] Body Wt. g ± SD	52 Week[a] Liver g ± SD	52 Week[a] Liver % BWT ± SD	52 Week[a] Adrenals mg ± SD	52 Week[a] Adrenals % BWT x 10^2 ± SD	78 Week[b] Body Wt g ± SD	78 Week[b] Liver g ± SD	78 Week[b] Liver % BWT ± SD	78 Week[b] Adrenals mg ± SD	78 Week[b] Adrenals % BWT x 10^2 ± SD
Males	0	26 ±4	1.11 ±0.17	4.30 ±0.48	5.3 ±1.2	2.0 ±0.3	25 ±2	1.19 ±0.27	4.72 ±0.98	5.2 ±0.9	2.1 ±0.4
	15	25 ±2	1.17 ±0.12	4.63 ±0.36	5.7 ±1.1	2.3 ±0.4	24 ±2	1.16 ±0.27	4.75 ±0.86	5.5 ±1.1	2.3* ±0.4
	30	27 ±3	1.22 ±0.11	4.46 ±0.36	6.0 ±0.9	2.2 ±0.4	25 ±2	1.16 ±0.18	4.63 ±0.63	5.4 ±0.9	2.2 ±0.4
	60	27 ±4	1.27 ±0.13	4.79* ±0.41	5.6 ±0.6	2.1 ±0.4	26 ±3	1.16 ±0.16	4.49 ±0.49	5.1 ±1.4	2.0 ±0.5
Females	0	24 ±5	1.14 ±0.23	4.76 ±0.52	8.6 ±2.1	3.6 ±0.7	22 ±2	1.04 ±0.13	4.70 ±0.50	7.0 ±0.8	3.2 ±0.4
	15	24 ±4	1.31 ±0.16	4.78 ±0.20	8.5 ±0.9	3.6 ±0.6	22 ±2	1.05 ±0.16	4.69 ±0.51	7.0 ±1.1	3.1 ±0.5
	30	23 ±3	1.07 ±0.16	4.67 ±0.54	8.4 ±1.0	3.7 ±0.6	23 ±2	1.12 ±0.31	4.89 ±0.93	7.2 ±1.1	6.2 ±0.6
	60	22 ±1	1.06 ±0.05	4.75 ±0.28	8.0 ±1.6	3.6 ±0.7	24* ±2	1.18 ±0.42	5.00 ±1.90	7.5 ±3.2	3.2 ±1.2

[a] Group means ± SD for 10-11 observations per group
[b] Group means ± SD are for 27-38 observations per group
* Significantly different from control value ($p \leq 0.05$)

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TABLE 4

INCIDENCE OF GROSS PATHOLOGY

Location / Finding	Ethoprop® (ppm)							
	Males				Females			
	0	15	30	60	0	15	30	60
Eyes (No. Examined)	59	60	60	58	59	58	60	59
Color Alterations	0	2	2	1	3	3	6	2
Missing	4	12	9	4	40	35	36	38
Recessed	4	4	2	4	10	13	11	10
Size Alteration	4	5	2	3	11	13	13	9
Liver (No. Examined)	59	60	60	58	59	58	60	59
Color Alterations	3	4	3	7	6	7	9	4
Nodule/Mass	4	1	2	3	0	0	2	1
Lung (No. Examined)	59	60	60	58	59	58	60	59
Color Alteration	3	5	3	14*	11	5	8	10
Nodule/Mass	2	1	4	2	3	1	1	2
Lymph Nodes (No. Examined)	59	60	60	58	59	58	60	59
Color Alteration	15	21	25	19	5	4	11	9
Swollen	25	25	31	21	28	29	29	23

* Significantly different from control value ($p \leq 0.05$)

TABLE 5

INCIDENCE OF NONNEOPLASTIC MICROSCOPIC PATHOLOGY

Location / Finding	Ethoprop® (ppm)							
	Males				Females			
	0	15	30	60	0	15	30	60
Adrenals (No. Examined)	59	60	60	58	59	58	60	59
Cortex-Hyperplasia	0	0	1	1	4	2	0	1
Bladder (No. Examined)	59	60	60	58	58	56	58	59
Lymphocytic Aggregates	6	4	0	1	15	12	10	10
Eyes (No. Examined)	58	60	60	58	59	57	60	58
Phthisis Bulbi	4	7	3	4	16	22	17	21
Optic Nerve Gliosis	6	4	4	2	3	10	15*	22*
Cornea-Inflammation	5	4	1	1	2	5	2	2
Lacrimal Gland (No. Examined)	56	59	58	57	55	55	57	57
Lymphocytic Aggregates	6	8	7	11	29	22	25	19
Liver (No. Examined)	59	60	60	58	59	58	60	59
Focal Necrosis	1	1	0	0	1	4	2	1
Lymphocytic Aggregates	0	1	1	0	3	2	5	11*
Lungs (No. Examined)	59	60	60	60	59	57	60	59
Inflammation								
Acute	7	7	6	6	9	10	12	18
Chronic	8	12	3	7	9	6	11	12
Lymph Nodes (No. Examined)	57	60	58	58	58	58	60	58
Histiocytosis	23	23	32	25	15	23	30*	19
Lymphoid Hyperplasia	6	13	15	9	17	20	22	14
Ovaries (No. Examined)					59	58	58	59
Cyst, Follicular					3	9	6	8
Interstitial Gland Formation					1	9*	10*	7
Salivary Gland (No. Examined)	59	60	60	58	59	58	60	59
Lymphocytic Aggregates	10	15	7	13	12	6	2*	6
Spleen (No. Examined)	59	59	60	56	59	58	60	59
Hematopoiesis	0	8*	6	3	3	4	9	3
Lymphoid Depletion	2	2	2	13*	1	3	2	9*
Sternum/Bone Marrow (No. Examined)	59	60	60	57	59	58	60	59
Fibrosis	0	0	0	0	42	43	42	44
Uterus (No. Examined)					59	58	60	59
Cystic Endometrial Hyperplasia					51	46	49	45

* Significantly different from control value ($p \leq 0.05$)

TABLE 6

INCIDENCE OF NEOPLASTIC MICROSCOPIC PATHOLOGY

Location Finding	Ethoprop® (ppm)							
	Males				Females			
	0	15	30	60	0	15	30	60
Lymph Nodes (No. Examined)	57	60	58	58	58	58	60	58
Lymphosarcoma	0	3	5	0	0	1	2	1
Thymus (No. Examined)	49	53	55	50	56	52	58	52
Thymic Lymphoma	0	0	1	0	0	0	1	0
Liver (No. Examined)	59	60	60	58	59	58	60	59
Hepatocellular Carcinoma	0	0	1	0	0	0	0	1
Lungs (No. Examined)	59	60	60	60	59	57	60	59
Bronchioloalveolar Cell Adenoma	3	0	2	1	2	1	0	1
Bronchioloalveolar Cell Carcinoma	0	0	2	0	0	1	0	0
Thyroid (No. Examined)	57	59	60	58	56	58	60	58
Follicular Adenoma	0	0	0	0	0	1	0	1

Summaries of the gross pathology (Table 4) and nonneoplastic (Table 5) and neoplastic (Table 6) microscopic pathology are presented for the primary findings in control and treated animals. Gross pathological findings (Table 4) in the eyes occurred in high incidence, especially in females. Gross findings included alterations in color and size (e.g. atrophy), missing eyes, and recessed eyes. These occurred in similar incidences in control and treated animals. Color alteration in lung from 60 ppm males was the only statistically significant ($p \leq 0.05$) gross pathological finding (Table 4).

Although numerous nonneoplastic findings are summarized in Table 5, few are statistically significant and none can be conclusively related to Ethoprop® treatment. In 30 and 60 ppm females the incidences of optic nerve gliosis are significantly ($p \leq 0.05$) higher than the female control. The incidence of phthisis bulbi is high in control and all treated female groups. The etiology of the ocular disease is unknown and cannot be unequivocally related to Ethoprop® treatment.

Other nonneoplastic pathology of significance ($p \leq 0.05$) included lymphocytic aggregates in liver in 60 ppm females, lymphoid depletion in spleen of 60 ppm males and females, histiocytosis in lymph nodes and lymphocytic aggregates in salivary glands in 30 ppm females,

interstitial gland formation in ovaries of 15 and 30 ppm females, and hematopoiesis in spleen of 15 ppm males. Because the lymphoid depletion in spleen occurred in both sexes at high dose, this condition may be Ethoprop® induced. Based on the occurence of lymphoid depletion in spleen, for pathological effects of Ethoprop the LEL is 60 ppm and tne NOEL is 30 ppm.

In Table 6 lymphosarcoma was diagnosed in the highest incidence for neoplastic diseases, but was not significantly increased over controls in either sex. No neoplastic conditions were related to Ethoprop® treatment.

Conclusions

Groups of male and female B6C3F_1 mice were fed 0, 15, 30, and 60 ppm Ethoprop® in the diet for periods up to 78 weeks.

- Due to a weighing error diets prepared in week 54 contained a tenfold excess of Ethoprop® and were analyzed to be 163.0, 283.6 and 602.1 ppm rather than the intended 15, 30, and 60 ppm, respectively.
- A significant (p 0.05) increase in mortality in high dose males (18 deaths) and females (9 deaths) occured in weeks 55 and 56 following administration of tenfold higher than tne nominal concentration (602.1 ppm) of Ethoprop®. No other significant differences in mortality between control and low or medium dose groups were observed. Overall, the mortality was nigher in females than in males.

- Ocular disease was prevalent in control and treated mice from two weeks after study initiation to termination.
- No adverse effects on body weight were treatment related (NOEL = 60 ppm).
- No biologically significant effects on food consumption were observed between control and treated groups.
- All doses of Ethoprop® significantly reduced RBC and plasma cholinesterase levels in both sexes at 52 and 78 weeks (LEL = 15 ppm and NOEL not established).
- In males all doses of Ethoprop® significantly elevated serum cholesterol at 78 weeks and in females the only significant increase in cholesterol was with 60 ppm. Overall, the LEL is 15 ppm and the NOEL was not established
- No biologically significant effect on total protein and alkaline phosphatase was caused in either sex of mice administered 15, 30, or 60 ppm Ethoprop® (LEL not established).
- Increases in the relative weights were observed for liver in 60 ppm males at 52 weeks and for adrenals in 15 ppm females

at 78 weeks and were not considered to be biologically significant (LEL not established).

- In females phthisis bulbi occurred in high incidence in control and all treated groups and optic nerve gliosis was significantly increased in 30 and 60 ppm Ethoprop® groups.
- Lymphoid depletion in spleen in 60 ppm males and females was significantly increased over respective controls and may be treatment related (LEL = 60 ppm and NOEL = 30 ppm).
- There was no direct evidence for oncogenicity of Ethoprop®.

CORE Classification: Supplementary. Because of the administration of a 10-fold excess Ethoprop® in the diet in week 54 and the high incidence of ocular disease especially in females for the duration of the 78 week study, an unequivocal evaluation for Ethoprop® related effects cannot be made. The study should be repeated using accurately prepared diets and healthy animals such that the quality and integrity of the data are not compromised.

phthisis bulbi = shrinkage and wasting of the eyeball.