UNDATED

ETHOPROP: ADDENDUM TO DYNAMAC'S REVIEW OF A THREE-GENERATION REPRODUCTION STUDY IN RATS (study conducted by Gulf South Research Institute, New Iberia, LA, report dated 12/3/80, Study No. 413-858-41, EPA Record No. 103099. Accession No. 263796.

Introduction

This reviewer is in basic agreement with the major conclusions reached in Dynamac's review. The purpose of this addendum is to augment or clarify, if considered necessary, the rationale behind the conclusions with which Toxicology Branch is in agreement, to supply the core grade for this study since Dynamac did not provide one in the review, to list some additional concerns, and to list data which the sponsor needs to provide before this study can be evaluated further.

1. Issue of pneumonia. Pneumonia was present in the majority of animals in this study. Frank histopathologic findings of enzootic pneumonia were observed in parents and splenic extramedullary hematopoiesis noted in offspring would point to the presence of an ongoing infectious process. Of particular concern is whether the presence of the infection and the resulting immune system response may have led to inconsistancies in effects from generation to generation. For instance, greater differences between controls and treated groups for some findings were noted for the F2 generation than for other generations of offspring (i.e. incidences of lenticular opacities and incidences of blue and lethargic pups). In addition, atypical and unexplained behavioral changes were noted in one generation and not in others (i.e. dose-related cannabolism of pups in F1 litters). The study authors imply that because effects were not perpetuated or were not found to similar degrees in more than one generation that they were not treatment related. However, this explanation cannot be accepted so readily since it is not clear whether the effects of the test material, if any, were potentiated, decreased, or unchanged by the infection. Therefore, the sponsor is asked to show that the study was not compromised by the pneumonia.

2. Issue of culling. Neither the procedure, nor the identities and the physical condition of the pups that were culled were specified. The concern here is that the least viable pups may have been culled which may have thus created a bias in the study or led to inconsistancies in effects from generation to generation.

3. Food consumption. Why wasn't food consumption measured in this study? If it was, the data should be provided. Dose-related effects on parental body weights were noted in this study and food consumption data would help to determine if these effects were due, at least in part, to a palatability problem or to a direct action of the test chemical.

4. Pathology and clinical findings. Some difficulty arose in trying to correlate parent and offspring clinical, litter, and pathological findings. In addition, there appeared to be some descrepancies (see "Descrepancies") between data tables and appendices in the study report. Therefore, a complete individual data profile for all litters in the study is requested. Mating pairs should be identified as well as any clinical signs or pathological findings in these parents. Also requested for this profile are any clinical and pathological findings in the offspring produced by the pair. Methods for animal identification should be kept consistant. This information will be helpful in evaluating one pathological finding in particular for which a treatment related etiology cannot be dismissed at the present i.e. eye pathology.

5. Diet Analysis. Analysis data for the concentration of Ethoprop in the test diets were submitted for about the first 5 months of the study. At subsequent times, the "results of analysis on individual feed mixes was not available". Why were these data unavailable? If there are homogeneity data, please submit them. Some stability data was presented (Appendix III), but they appeared to be inconsistant. A stability test dated 6/21/77 indicated that by 45 days post-mixing, Ethoprop technical was breaking down in the diet. However, in another report dated 7/26/77, Ethoprop was apparently shown to be stable in the feed for 81 days. This inconsistancy should be addressed by the sponsor.

6. Reproductive organ data . As part of the reproductive system of the male and female rat respectively, the epididymus and the vagina should have been included in the organs that were examined grossly and histologically. Please indicate why they were not. In addition, by way of the tabulated data requested in point (4) of this addendum, the results of examination (gross, histo-) of the following organs and tissues should be indicated (vagina, uterus, ovaries, testes, epididymus, seminal vesicles, prostate).

7. Statistics. ANOVA was not used jointly with a test for multiple comparisons in the study report, therefore a group that was statistically significantly different from controls could not be identified.

8 . Other Issues. The sponsor is asked to address the following questions/ issues.

a. Why were certain pups killed on day 17 or day 20 rather than day 21? How were the data for this animals handled in statistical calculations?

b. How were breeding pairs selected?

c. Why were only 18 rather than 20 F2 low dose parental females mated to produce the F3 generation?

d. Please explain why three ways were used to report litter weight data.

e. Please submit individual parental body weight data.

f. Please provide historical control data for the eye lesions observed in this study.

g. Please provide a summary table for the litter data contained in Appendix IV.

h. The histopath finding in Table 13 for low dose male F2B15 is incomplete (i.e. Testis +3,). The sponsor should provide it.

i. Individual animal data Appendix IV photocopied poorly, particularly the check marks. It should be checked with the raw data for accuracy (see examples of "Descrepancies" below) and resubmitted.

j. Appendix V indicated that 3 mid dose FO24B litter pups were cannalbalized on 12/17/77. Since the delivery date for the litter was 12/5/77, it is difficult to believe that such large pups were eaten by the mother.

k. Were slides and organ and tissue specimens which had been subjected to histopathological examination preserved?

9. Descrepancies. The following are examples of descrepancies found in study report tables and appendices. These findings should be addressed by the sponsor. Some of these descrepancies were also mentioned in Appendix VIII in a GSRI quality assuarance report. The sponsor should justify why the final report for the reproduction study was released containing descrepancies pointed out by its own quality assurance team.

a. In Table 6, 10 animals are listed as having had litters. 11 animals are listed in Appendix IV.

b. In Appendix IV, litter data of Control group female F2B6A - only 1 pup was alive on day 4 after birth, yet 3 more pups died between day 4 and day 20 post-birth.

c. In Appendix IV, litter data of Mid group female F2B27B - the number of pups alive on day 4 after birth was 6, yet 7 were still alive on day 21.

d. In Table 10 the number of F2A Control group litters analyzed was 15, but Appendix IV lists 17.

• EPA Registration Number _____

Page _____ is not included in this copy of the registration file for the product.

Pages 4 through 10 are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_1_ FIFRA registration data (*)

1 Test methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

4,5,6,7,8,9,10

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

DRAFT

EPA: 68-01-6561
TASK: 88
April 26, 1985

Correction Sheet 005741

DATA EVALUATION RECORD

ETHOPROP TECHNICAL

Three-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Fletcher, M.J., et al. Evaluation of effects of Ethoprop on reproductive performance by a three generation study in Fischer 344 rats. (Unpublished report, project No. 413-858-41 ["Second Final Report"] prepared by Gulf South Research Institute, New Iberia, LA for Mobil Chemical Company, Edison, NJ; dated December 3, 1980.) ~~No~~ Accession Number ~~was supplied.~~ 263796.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

1. CHEMICAL: Ethoprop (MOCAP), O-ethyl-S,S-Dipropyl phosphorodithioate.

2. TEST MATERIAL: The test material was ethoprop technical, lot no. MCTR 15977; 95.3% active ingredient. The compound was a colorless liquid concentrate.

3. STUDY/ACTION TYPE: Three-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Fletcher, M.J., et al. Evaluation of effects of Ethoprop on reproductive performance by a three generation study in Fischer 344 rats. (Unpublished report, project No. 413-858-41 ["Second Final Report"] prepared by Gulf South Research Institute, New Iberia, LA for Mobil Chemical Company, Edison, NJ; dated December 3, 1980.) No accession number was supplied.

5. REVIEWED BY:

Paul Wennerberg, D.V.M., M.S.
Principal Author
Dynamac Corporation

Signature: _____
Date: _____

James R. Plautz, M.S.
Independent Reviewer
Dynamac Corporation

Signature: _____
Date: _____

6. APPROVED BY:

Guillermo Millicovsky, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: _____
Date: _____

A. Kocialski, Ph.D.
EPA Reviewer and Section Head

Signature: _____
Date: _____

12

7. CONCLUSIONS:

Determination of the NOEL and LOEL could not be made due to the following deficiencies in this study. Most of the parent rats in all three generations had pneumonia. This disease may have impacted on reported decreases in mean litter size, adult and pup body weight gain, and lactation indices (pup survivability). In addition, the report did not include food consumption data, or sufficient individual animal data to permit the validation and assessment of reproductive parameters. Also, the procedures implemented for culling pups may have produced biased results (by artificially manipulating the populations).

8. RECOMMENDATIONS:

This study could be upgraded by providing the following: food consumption data, individual adult body weights, pup body weights reported in consistent format, and individual animal data for all parameters. The study authors need to demonstrate that 1) the pneumonia reported was not severe, and 2) it did not have an adverse impact on the outcome of this study.

Items 9 and 10 - see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

The complete materials and methods section of the study report is included in Appendix 1 of this review.

A. Materials and Methods:

1. Ethoprop technical was mixed with corn oil and then added to powdered feed to obtain the highest dose level (262 ppm). This mixture was diluted with untreated feed to produce the other two dose levels (60.5 and 131 ppm). Diets were prepared weekly, and samples were obtained for analysis of homogeneity and concentration of the test material. Dosage diets were replaced with fresh diets every week.

2. The animals were 30-day old (weanling) Fischer 344 rats obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts.

3. The F_0 generation was randomly assigned to test groups on the basis of body weights. Animals were fed their corresponding diets for 8 weeks before mating. The F_{1A} pups were sacrificed and necropsied at the end of the weaning period.

---

[1] Only items appropriate to this DER have been included.

13

Following this weaning, the F_0 parents were rebred. Ten males and 20 females were randomly selected from the F_{1B} pups to become F_1 adults. The procedure for the F_0 parents was repeated with the F_{2B} pups being used for the next mating. The F_{3A} pups were killed at weaning and the F_{3B} pups were killed shortly after weaning.

4. Parents were examined daily for adverse clinical signs. Litter data included: litter size, number of stillborn, number of live births and their sex, the number of live pups at days 4 and 21, and daily examinations for clinical signs. A complete gross necropsy was performed on all F_{1A}, F_{2A}, F_{3A}, and F_{3B} weanlings, and all three generations of parents. Approximately 40 tissues were examined for histopathology from 5 animals/sex/group of F_{1A}, F_{2A}, F_{3A} and F_{3B} weanlings and from all F_1 and F_2 generation parents.

5. Pregnancy rates and the number of live pups were analyzed using chi-square procedures. Litter size and weights were analyzed by ANOVA.

12. REPORTED RESULTS:

A. Analyses of dietary samples showed that, for the reported test dates, the actual concentrations were within 10% of the expected concentrations. Ethoprop technical at the concentration of 10 ppm in the feed was stable at room temperature for 7 days.

B. One parental rat (F_{1B}, control female) died during the study. The gross and histopathological examinations revealed, "Lung-left lobe enlarged with areas of red consolidation, hemorrhage and necrosis. Lung + 1, enzootic pneumonia; Cavernous hemangiosarcoma + 3; Thyroid - dysplasia + 2." Two parental rats were moribund and sacrificed during the study. One of these animals (a high-dose, F_0 female) had stomach ulcers and kidney lesions, while the other (a high-dose, F_{1B} female) had a corneal ulceration and hemorrhage plus nephritis.

No adverse compound-related clinical signs were observed except for the dose-related increase in the incidence of abnormal parental behavior during nesting, and cannibalization of pups in the second litters by the F_0 females. The number of F_0 females cannibalizing pups was 1, 2, 4, and 8 for the control, low- mid-, and high-dose groups, respectively. This dose-related increase was not seen in the other generations.

Parental body weight gains were significantly lower for the dosed groups compared to controls (Table 1). The lack of weight gain appeared to be dose dependent.

TABLE 1. Selected Mean Parental Body Weights (g) for Rats Fed Ethoprop

Concentration (ppm)	Sex	Generation	Study Week 1[a,b]	5	9	13	18
0	M	F_0	163	306	313	sacrifice	
60.5	M	F_0	152	291	311		
131	M	F_0	155	291	293		
262	M	F_0	159	276	288		
0	F	F_0	124	186	218	sacrifice	
60.5	F	F_0	123	189	220		
131	F	F_0	123	176	211		
262	F	F_0	120	165	190		
0	M	F_1	106	212	262	355	sacrifice
60.5	M	F_1	114	211	260	351	
131	M	F_1	105	183	240	327	
262	M	F_1	101	178	222	277	
0	F	F_1	90	139	158	172	sacrifice
60.5	F	F_1	94	142	160	163	
131	F	F_1	86	133	148	165	
262	F	F_1	75	119	133	164	
0	M	F_2	117	232	267	317	353
60.5	M	F_2	123	250	282	322	363
131	M	F_2	104	218	250	278	320
262	M	F_2	107	209	241	277	312
0	F	F_2	99	151	172	191	201
60.5	F	F_2	100	155	181	182	203
131	F	F_2	89	144	167	178	191
262	F	F_2	82	133	162	164	170

[a] Study week 1 = F_0 at 6 weeks of age
F_1 at 4 weeks of age
F_2 at 4 weeks of age

[b] The study authors performed statistical analyses using ANOVA and reported significant differences ($p < 0.01$) for both "dose" and "time" for males and females of all generations. However, the individual means within each sex group and generation associated with these differences could not be identified since the study authors did not perform a test for multiple comparisons.

15

The analyses of pregnancy rates (fertility index) were stated as being nonsignificantly different between dosed and control groups. The high-dose groups, however, for the F_{3A} and F_{3B} generations were reported as being markedly lower than their "corresponding population averages." The table referenced in the study report was absent.

Although the mean litter sizes for all dosage groups were not significantly lower than controls during any generation, this parameter was always lower in the high-dose group compared to the control values for all generations (Table 2).

No compound-related clinical signs in the pups were reported for any dosed group compared to controls for any generation. Four pups died during the study. One F_{2A} control male and three F_{1A} males from the high-dose group died of unknown reasons; it was not stated whether the high-dose males were litter mates.

The viability indices (the number of pups alive between birth and 4-days postpartum) and the lactation indices (the number of pups alive between days 4 and 21 postpartum) were both analyzed by the study authors on the basis of litters with 100% pup survival relative to those with less than 100% survival. These analyses showed that there were no significant differences between dosed groups and controls for the viability indices; however, there were significant decreases in the lactation indices in F_{1B}, F_{2A}, and F_{2B} offspring. It was stated that "there was some inverse relationship between the index and the dietary level of Ethoprop." An additional page (CBI p. 15-A) was added to the study report that showed these two indices calculated based on the number of pups per group. The results appeared (no statistics were performed) to support the study authors' conclusions (Table 2). It appears as if this page was added by the sponsor of the study.

Pup body weight for F_{2A}, F_{2B}, F_{3A}, and F_{3B} litters were recorded at 21 days of age (Table 3). ANOVA revealed significant differences between dosage groups and controls. The high-dose group was lower than controls in all but the F_{3B} litters. It was not clear which dosed group was significantly different from controls, but it was stated that "there appears to be an inverse relationship between body weight of litters and the dietary level of Ethoprop except for F_{3B} generation litters where the trend seems to be reverse."

Gross necropsy examinations revealed no compound-related effects. The eye was the organ with the greatest number of pathological observations. The major finding was bilateral lenticular opacity in the high-dose F_{2A} rats which had an incidence of 28.8% (28/97), versus 0% in the controls. This difference was not statistically analyzed by the study authors.

TABLE 2. Summary of Reproductive Values and Indices for Rats[a] Fed Ethoprop

Diet Concentration (ppm)	Mean Litter Size — Generation No.						No. of Litters with Live Young — Generation No.						No. of Pups Born Alive/No. of Total Pups Born[d] — Generation No.					
	1A	1B	2A[c]	2B	3A	3B	1A	1B	2A	2B	3A	3B	1A	1B	2A	2B	3A	3B
0	8.5[b]	9.7	9.9	9.9	8.6	9.4	15	19	15	17	14	17	1.0	.99	1.0	.99	1.0	1.0
	3.02	2.62	2.66	3.24	3.05	4.25												
60.5	7.8	8.0	11.4	9.1	10.4	10.1	11	18	17	19	17	17	1.0	1.0	.99	1.0	1.0	1.0
	2.64	3.69	1.69	2.47	1.46	2.60												
131	9.0	8.8	10.3	8.9	9.0	9.4	11	17	17	16	15	17	1.0	.96	1.0	1.0	1.0	.99
	2.36	1.99	1.40	2.43	2.48	2.74												
262	7.2	7.8	7.7	8.0	8.4	8.6	11	17	17	16	12	13	1.0	.94	.99	1.0	1.0	1.0
	2.57	1.59	2.28	2.53	2.23	1.66												

Diet Concentration (ppm)	Viability Index[e] (%) — Generation No.						Lactation Index[f] (%) — Generation No.					
	1A	1B	2A	2B	3A	3B	1A	1B	2A	2B	3A	3B
0	91	99	98	100	98	99	86 (87)[g]	93 (94)	100	98	95	95
60.5	95	99	98	99	100	100	99 (100)	98	99	99	99	98
131	100	99	97	100	100	99	100	89 (91)	98	99	96	99
262	98	100	100	99	100	100	100	73 (75)*	82*	86 (82)*	98	100

[a] 20 females mated/group.

[b] Upper value is the mean, lower value is the standard deviation.

[c] ANOVA by author was significant ($p < 0.01$). When analyzed by these reviewers, using Duncan's test for multiple comparisons ($p < 0.05$) and using the number of live pups per litter, the high-dose group value was significantly less than control.

[d] Calculated by finding the no. of pups born alive/total no. born per litter, and then finding the group mean. A value of 1.0 means that the no. born alive = the total no. born. When analyzed by these reviewers using ANOVA, then Duncan's Multiple Range test ($p < 0.05$), the only significant change was the high-dose group value for the 1B generation.

[e] The viability index was defined as the number of pups alive on day 4 divided by the number of pups born alive.

[f] The lactation index was defined as the number of pups alive on day 21 divided by the number alive on day 4.

[g] The value in parentheses is the corrected value obtained during validation by these reviewers and the value used in the analysis using Fischer's Exact test ($p < 0.05$).

* Significantly less than control, $p < 0.001$.

17

TABLE 3. Mean ~~Pup~~ Litter ~~Body~~ Weights at Day 21 of Age for Rats Fed Ethoprop

Generation	Test-Dose Group (ppm) 0	60.5	131	262
F_{2A}	23.0[a,b] 4.29	22.3 2.21	21.3 2.34	18.1* 2.79
F_{2B}	29.2 2.27	29.2 3.66	25.9* 4.65	24.5* 3.99
F_{3A}	36.0 5.30	35.9 3.13	34.7 3.89	31.8* 4.27
F_{3B}	35.5 5.98	32.5 4.25	34.2 3.65	42.6* 5.74

[a] Upper value is the mean, the lower value is the standard deviation.

[b] ANOVA performed by the author showed there was significant differences ($p < 0.01$) in all generations. These values were analyzed by the reviewers using Duncan's Multiple Range Test for multiple comparisons ($p < 0.05$).

* Significantly different from controls.

Histopathological examinations were performed on the F_1 and F_2 parents and F_{1A}, F_{2A}, F_{3A}, and F_{3B} weanlings. Enzootic pneumonia in "most animals and at all dose levels" was seen in the adults, and at "much lower frequency...which was also mild in its severity" in the weanlings.

One adult F_1 from the female control group had a hemangiosarcoma of the lung and one adult high-dose F_2 female had a transitional cell carcinoma of the urinary bladder. Mesenteric lymph node granulomas were found in the following incidence in adult rats.

Mesenteric Lymph Node Granulomas/No. of Examined Rats

	(ppm Ethoprop in the diet)			
Generation	0	60.5	131	262
F_1	0/30	0/30	5/30[a]	2/30
F_2	0/(10) 30	2/30	1/30	9/30[a,b]

[a] Significantly different from control when calculated by these reviewers using Fischer's Exact test, $p < 0.05$.

[b] Significant trend when calculated by these reviewers using the Cochran-Armitage Trend test, $p < 0.05$.

One high-dose F_{1A} weanling had lymphosarcoma of the mesenteric lymph node.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that, "Administration of Ethoprop to Fischer 344 rats at concentrations of 0, 60.5, 131, or 262 ppm in dosed feed over a three generation period produced moderate toxicity as evidenced by dose dependent body weight depression in parental and weanling rats and decreased lactation index in F_{1B}, F_{2A} and F_{2B} generations. Neither the parents nor their offspring exhibited any significant pharmacotoxic signs associated with the Ethoprop exposures. The fertility index of the high dose groups of F_{3A} and F_{3B} generations was markedly lower than the respective population averages. However, there were no significant differences in the indices among the test groups of the three generations. Similarly, the mean litter size of the high dose group was consistently smaller than those of the controls and other test groups in all generations, but statistically significant

19

only in F_{2A} generation. Gross necropsy and histopathologic examinations of parental and weanling rats found no marked changes in any tissues or organs that can be associated with dietary levels of Ethoprop. In summary, at the three dose levels tested, Ethoprop did not produce any significant effects on reproductive performance, but caused moderate toxicity."

B. A quality assurance statement was signed and dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. 1. Over 90% of all dosed and control parental rats (F_{1B} and F_{2B} generations) with reported histological findings were reported as having enzootic pneumonia. An example of the data for each of the two generations is in Appendix 2 of this review.

Although the group of F_{1A} and F_{2A} weanlings selected for histological examination (5/sex/group) did not show direct signs of enzootic pneumonia at histological examination, this finding may have resulted from maternal antibodies inhibiting the disease. However, of the F_3 weanlings with histopathological examinations, pneumonia was noted for 15 percent (6/40) of the F_{3A} and 78 percent (50/66) of the F_{3B} weanlings. An example of the F_{3B} weanling data is presented in Appendix 2.

The parental clinical observation data confirmed the histological evaluations. Almost all of the reported clinical observations for the F_0, F_1, and F_2 parental generations support a diagnosis of pneumonia (see Appendix 3). Furthermore, positive clinical signs of pneumonia (mucus discharge from nose, lacrimation, etc.) were observed during the premating, gestation, and lactation periods.

It appears, from the presented data, that the majority of rats were ill and that, as a consequence, the study results may have been compromised. Parameters such as ovulation, mating, implantation, in utero fetal development, survival and growth, the number of live pups born (fertility index), and pup survivability (viability and lactation indices), may be affected by poor maternal health.

Because of the reported ill health of the rats and its possible adverse impact on parental and fetal parameters, it is very difficult to evaluate whether the reported significant changes in adult and pup body weight gain, mean litter size and the lactation index (pup survivability) are compound or disease related. The main impact on these pup parameters would have resulted from decreased maternal milk production and altered maternal behavior.

2. No food consumption data were provided. It was therefore impossible to evaluate the decreases in body weight gains seen in high-dose parents and 21-day old pups compared to controls. Without food consumption data, it was not possible to determine if the decreased body weight in the pups or in the parents (and, therefore, perhaps indirectly in the pups) was due to a toxic manifestation of the test material or due to a lack of palatability of the dosed diets. Illness, palatability problems, or toxic effects of the test material, all or individually may or may not have contributed to the decreased body weight gains reported. The deficiencies of data in the study report did not allow us to determine causal relationships for the above noted body weight reductions.

3. Culling of pups was done before day 4 of lactation, at a time and in a manner not specified. Specifically, we could not determine if the pups were chosen at random or if only the sick ones were removed. Therefore, the day 4 pup survival data were of limited value and the viability and lactation indices derived from these data could not be interpreted, since compound-related effects could have been masked if only ill or non-normal pups were selectively culled instead of using a random selection method. The viability indices were recalculated by our reviewers using the number of pups alive on day 4 divided by the number alive after culling. We also recalculated the lactation indices using corrected values obtained during our data validation. The results of our analyses of these indices were in agreement with those of the study authors.

4. The only individual animal data available were the clinical observations for adults, histopathological findings for F_1 and F_2 adults and selected offspring, litter survivability data at days 0, 4, and 21 of lactation (litter values), and litter body weights at day 21 of age for F_{2A} through F_{3B} litters. The remainder of the presented data were in summary form and could not be validated; likewise, the statistical comparisons could not be verified.

5. Pup body weights at weaning were given in as many as 3 different forms for the same reporting period: individual pup by sex, individual pup without designation of sex, and by total litter weight. See representative example in Appendix 4.

   This reporting inconsistency did not allow a complete evaluation of pup body weight changes because the specific location of the body weight changes could not be identified. This is an important reproductive parameter as it may be affected by the dam's lactation ability and/or maternal care of the pups.

   Similar conclusions for the pup body weight and culling problems were discussed in a review of the study attached to the study report (see Appendix 5). It is not clear if this report was written by the study authors or the sponsor.

21

6. Where results of statistical analyses were presented (such as the parental body weight table, litter size, number of pups alive at day 4, number of pups alive at day 21, and litter body weights at day 21; see Appendix 6), only overall p-values from ANOVA were presented with no multiple comparisons to distinguish between groups and to identify where the differences were located. We could not recalculate all of the statistics since the individual animal data in the study report were deficient.

7. The table on "Effects of Ethoprop on Fertility Index" was not presented. This summary table was important since individual animal data were not presented and fertility indices are important parameters in reproductive studies. The available data would allow an approximation of this value. This value could have been calculated by dividing the number of females delivering live litters by the number of females that were pregnant.

   Our calculations and statistical analyses indicate that there were no statistically significant changes between dosage and control groups. However, the high-dose group was lower than controls in 5 of the 6 generations (see following table).

No. of Litters with Live Young
(No. of Females Mated = 20)

Concentration (ppm)	Generation					
	F_{1A}	F_{1B}	F_{2A}	F_{2B}	F_{3A}	F_{3B}
0	15	19	15	17	14	17
60.5	11	18	17	19	17	17
131	11	17	17	16	15	17
262	11	17	17	16	12	13

8. The following organs were not examined histologically: vagina, epididymus, and target organs (in this study, the eye).

9. There were 3 numbers listed as the study or project number: M1593-77, 413-858-41, and 413-858-40.

B. 1. The study authors stated that there were no compound-related clinical observation changes between dosage groups and

22

controls. However, the following number of females in the F_{2A} generation had cold, blue, or lethargic pups: control, 1/20; low dose, 0/20; mid dose, 4/20; and high dose, 8/20. When we analyzed these values with the Fischer Exact test the high-dose findings were significantly ($p < 0.05$) larger than control, and the mid-dose findings p-value was 0.0536. There was also a positive trend with the Cochran-Armitage Trend test ($p < 0.05$). The lenticular opacity seen grossly in the high-dose F_{2A} rats mentioned in section 12 of this review (28.8% - 28/97) was significantly ($p < 0.05$) greater than that of controls (0%) when analyzed by these reviewers using Fischer's Exact test.

Item 15 - see footnote 1.

16. CBI APPENDIX:

Appendix 1 - Materials and Methods
Appendix 2 - Parental and pup histopathological findings
Appendix 3 - Parental clinical observations
Appendix 4 - Pup body weights
Appendix 5 - Study review from CBI
Appendix 6 - Statistical analyses from CBI

23

APPENDIX 1

C.B.I. pp. 6-12

• EPA Registration Number 2116-87

Page 15 is not included in this copy of the registration file for the product.

Pages _____ through _____ are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data (*)

1 Test Methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

APPENDIX 2

C.B.I. pages 51, 56, 71
Table 13

EPA Registration Number 2116-87

Page _____ is not included in this copy of the registration file for the product.

Pages 27 through 29 are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data (*)

Test Methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

27, 28, 29

APPENDIX 3

C.B.I. pages 20, 22, 24
Table 2

• EPA Registration Number 2116-87

Page _____ is not included in this copy of the registration file for the product.

Pages 31 (21) through 33 (23) are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ FIFRA registration data (*)

I Test Methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

31, 32, 33

APPENDIX 4

C.B.I. page 161

Appendix V

• EPA Registration Number 2116-87

Page 35 25 is not included in this copy of the registration file for the product.

Pages _____ through _____ are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

__/__ FIFRA registration data (*)

/ Test Methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

APPENDIX 5

C.B.I. Report Release (p 1 and 2)

TOXICOLOGY DIVISION

REPORT RELEASE

Date Revised:

EHSL LIAISON: C. R. Mackerer

DATE: June 11, 1981

SUBJECT: Release of Report Entitled EVALUATION OF EFFECTS OF ETHOPROP ON REPRODUCTIVE PERFORMANCE BY A THREE GENERATION STUDY IN FISCHER 344 RATS

REFERENCE: Request No. _____ Study No. M1593-77 Requestor - _____

MATERIAL TESTED: Ethoprop Technical (95.3% active ingredient)

TESTING LABORATORY: Gulf South Research Institute

RESULTS:

The effects of Ethoprop on the reproductive performance of Fischer 344 rats were evaluated by administering the test material at levels of 0, 60.5, 131, or 262 ppm in their diet over a period of three generations. Clinical observations of parental and offspring generations for pharmacotoxic effects revealed no marked effects that can be associated with the administration of Ethoprop. One parental control female (9A of F_{1B} generation) and four rats from the offspring (high dose males #330, 333 and 334 of F_{1A} and control male #13 of F_{2A} generations) died during the study. A significant dose dependent depression of body weight gain was noted in all parental generations and in the 21 day body weights of weanlings. The fertility index (percentage of pregnancies) of the high dose group was lower than the control in 5 of the matings; the overall fertility rate was 12% lower than the control (72% and 82%). The other groups were not affected. Similarly, the mean litter size of the high dose group was consistently smaller (average 15%) than those of the controls and other dose groups in all generations; however, was statistically significant only in the F_{2A} generation. There were no notable differences in the viability indices of the four test groups in any generations. However, there was a significant decrease in the lactation index of F_{1B}, F_{2A} and F_{2B} litters, and the index appeared to be inversely related to the dietary level of Ethoprop. There were no physical or behavioral abnormalities in the pups as a result of feeding Ethoprop. Gross necropsy examinations of the parental and weanling generation rats showed no tissue organ changes that can be clearly attributed to the test material. Similarly, histopathologic evaluation of tissues from parental (F_{1B}, F_{2B}) and offspring (F_{1A}, F_{2A}, F_{3A}, and F_{3B}) generations showed no changes that are related to dietary exposures to Ethoprop. At a concentration of 262 ppm in the feed Ethoprop exhibited toxicity to the parents and offspring during three generations of reproduction and growth based on body weight depression and decreased survival of pups during lactation. In addition, at this dose there was slightly decreased fertility and average litter size. A dose of 131 ppm in the feed was a subthreshold (no observable effect) level.

G-700 & 8

continued.....

Approvals:

Study Monitor

Section Manager

Director, Toxicology Division

Distribution:
above
Central File
J. Chasar
D. Hodges
M. A. Naro
23 10/80

JUN 26 :

OPPTS 42W00099

DATE 6-54 COPY 0

27

37

REPORT RELEASE (Continued)

Comments on the study and report:

The report presents viability after birth and survival of lactation in a non-standard manner that confuses the high dose effect (Tables 8 and 9). They present the percent of litters with 100% survival; normal description is the percent of total pups that survive. This data is presented in the standard format in the attached table. There clearly is an effect on the lactation index of the high dose but not on lower doses. However, due to their method of culling, the significance is questionable. Since they culled the least viable from each litter and the control litters were larger, did they cull more from the controls or the high dose that might have died? In other words, the effect on the lactation index could have been greater or possibly less.

Although it does not effect the average weaning weight of the pups, the testing facility in some cases weighed each pup of a litter at weaning and in other cases obtained only a total weight of the litter. This precludes analysis of whether the reduced pup weights resulted from a uniform effect of Ethoprop on lactation and pup growth or from some pups being severely affected and others relatively normal.

APPENDIX 6

C.B.I. pages 35, 36, 44, 45, 46

• EPA Registration Number 2116-97

Page _____ is not included in this copy of the registration file for the product.

Pages 30 through 34 are not included in this copy of the registration file for the product.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commerical/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

___ FIFRA registration data (*)

✓ Test Methods

The information not included generally is considered confidential by product registrants. If you wish to obtain the information deleted, please contact the individual who prepared this response to your request.

(*) FIFRA registration data can be released to individuals who submit an Affirmation of Non-Multinational Status.

30,31,32,33,34