

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

DRAFT

005741

EPA: 68-01-6561
TASK: 88
May 8, 1985

468-

2500

Jim Plautz

DATA EVALUATION RECORD

ETHOPROP

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Wolfe, G. W. Rabbit teratology study. (Unpublished Study No. 230-233 by Hazleton Laboratories America, Inc., Vienna, VA for Mobil Oil Corporation, Princeton, NJ; dated August 10, 1981.)
Accession No. ~~not available~~. 263801

APPROVED BY:

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

Signature: _____

Date: _____

1. CHEMICAL: Ethoprop.
2. TEST MATERIAL: Ethoprop Technical, 01238101, a clear colorless liquid containing 95.7% active ingredient.
3. STUDY/ACTION TYPE: Teratogenicity Study in Rabbits.
4. STUDY IDENTIFICATION: Wolfe, G. W. Rabbit teratology study. (Unpublished Study No. 230-233 by Hazleton Laboratories America, Inc., Vienna, VA for Mobil Oil Corporation, Princeton, NJ; dated August 10, 1981). Accession No. not available.

5. REVIEWED BY:

Guillermo Millicovsky, Ph.D.
Principal Author
Dynamac Corporation

Signature: _____

Date: _____

James Plautz, M.S.
Independent Reviewer
Dynamac Corporation

Signature: _____

Date: _____

6. APPROVED BY:

I Cecil Felkner, Ph.D.,
Teratogenicity and Reproductive
Effects
Technical Quality Control
Dynamac Corporation

Signature: _____

Date: _____

Albin Kocialski, Ph.D.,
EPA Reviewer

Signature: _____

Date: _____

Edwin Budd, Ph.D.,
EPA Section Head

Signature: _____

Date: _____

7. CONCLUSIONS:

Ethoprop administered daily to pregnant New Zealand white rabbits by oral intubation from day 6 through 18 of gestation at dose levels of 0.125, 0.500, and 2.000 mg/kg produced no maternal toxic effects of biological significance. The test material may have produced embryotoxic effects as evidenced by an increase in the incidence of resorptions. Fetotoxicity was also demonstrated by an increase in the incidence of developmental variations. In addition, several malformations were observed with a sporadic frequency, but only in groups dosed with the test material.

Since no toxic effects of biological significance were observed in pregnant animals, even at the highest level tested, the maternal LOEL could not be established.

In the absence of more definitive data, the LOEL for embryotoxicity and teratogenesis was estimated to be 0.125 mg/kg/day.

8. RECOMMENDATIONS:

The teratogenic potential of Ethoprop should be tested at higher dose levels capable of producing more significant maternal effects. The additional data should be useful in further assessing the embryotoxic and teratogenic activity of the test material, and in determining if the terata observed in the present study was related to the test material. It is also recommended that individual maternal and fetal data be included in the study report for the reviewers' evaluation.

9. BACKGROUND:

The testing laboratory conducted a pilot study in rabbits (their sex or pregnancy status were not reported) dosed at 10, 5, or 1 mg/kg/day. The reported mortality was 3 out of 4, 2 out of 5, and 2 out of 4, respectively.

Item 10 - see footnote 1.

11. MATERIALS AND METHODS:

A. Materials and Methods:

The test material, identified as Ethoprop technical - 01238101, was a clear colorless liquid containing 95.7% active ingredient (according to the sponsor). The testing laboratory assumed the liquid to be 100% active ingredient for the preparation of dosing solutions. Corn oil was used as vehicle and control material.

¹Only items appropriate to this DER have been included.

Female New Zealand white rabbits from Dutchland Laboratory Animals, Inc., Denver, Pennsylvania were artificially inseminated after an acclimatization period of approximately 2 months. Sperm was obtained from breeding New Zealand white rabbits from the testing laboratory.

Four groups of animals consisting of 17 females (at study initiation) were dosed by oral intubation once daily from day 6 through 18 of gestation. Dosages were adjusted on days 6, 11, and 15 of gestation. Dose levels of 0, 0.125, 0.500, or 2.00 mg/kg/day were administered to control, low, middle, and high groups, respectively.

Study animals were observed for toxicologic effects from the onset of treatment until sacrifice. Maternal body weights were recorded on days 0, 6, 11, 15, 18, and 29 of gestation. Food and water consumption was checked but not measured.

Surviving females were sacrificed on gestational day 29 and their abdominal cavities were examined grossly, corpora lutea were counted and the uterine contents were observed for number, type and placement of implantations. Fetuses were examined externally, measured, and weighed. The technique of Staples was implemented for visceral examinations. The craniofacial structures of approximately one third of the fetuses were examined using Wilson's technique. Skeletal structures were stained with Alizarin red and examined under magnification.

For complete details of the methodology used, the materials and methods section of the study report has been included in Appendix A of this review.

12. REPORTED RESULTS:

- A. Test Material: It is not known if tests were performed to determine the concentration, homogeneity, or stability of the test material. Results were not reported.
- B. Maternal Effects: No compound-related deaths were observed in this study. One control and one mid-dose animal died of undetermined causes, but no pathological changes were observed in these animals during the necropsies. Increases in the incidence of anorexia were noted in Ethoprop-treated animals during and after the dosing period. The study author reported a dose-related decreases in body weight changes during the dosing period, but these effects were not statistically significant. Mean body weight changes for the periods of gestational days 18-29 and 0-29 were similar for all groups.

There were no differences among groups in pregnancy rates, mean number of corpora lutea or implantations, and uterine weights.

- C. Embryonic and Fetal Effects: The test material had no effects on the number of resorptions, fetal viability, sex ratio, and fetal weights or lengths.

Malformations were present in four fetuses from the low-dose group (two fetuses had ectopic kidneys, a third fetus had umbilical hernia, and a fourth fetus had a cardiac septal defect). One mid-dose fetus had multiple visceral and skeletal malformations involving the heart, lungs, spleen, kidneys, liver, vertebral column, and limbs. One high-dose fetus had cranial defects. In addition, there was an increase in the incidence of skeletal variations in Ethoprop-treated groups, but no dose response or statistical significance for this parameter was noted by the study author.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The study report does not contain the author's conclusions. The summary section presents only a synopsis of the study methods and results.

A quality assurance statement was signed and dated by the manager of the Quality Assurance Office of the testing laboratory.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. The test material did not affect maternal survival, but it may have produced very mild reductions in maternal body weight gain in the mid- and high-dose groups during the dosing period. After treatment was discontinued on day 18 of gestation, the animals in the high-dose group showed a compensatory weight gain. These weight changes are of very little biological significance since the group mean loss of 52.5 grams during the dosing period and of 149.7 grams gained in the post dosing days by animals in the high-dose group represent only -1.4% and 3.9% of their group mean body weight measured on day 0 gestation, respectively. Maternal body weight and body weight changes are summarized in Table 1.

TABLE 1. Effects of Ethoprop on Maternal Body Weight and Maternal Body Weight Change in the Rabbit

Gest. Days	Dose Level (mg/kg/day)			
	0	0.125	0.500	2.000
Mean Maternal Body Weight (g)				
0	3763.3	3743.2	3766.6	3802.2
6	3814.6	3796.2	3798.4	3893.4
18	3882.2	3837.6	3805.0	3840.9
29	3981.4	3962.4	3924.6	3990.6

Gest. Days	Study Period	Dose Level (mg/kg/day)			
		0	0.125	0.500	2.000
Mean Maternal Body Weight Change (g)					
(0-6)	Pre-dosing	51.1	52.9	31.9	91.3
(6-18)	Dosing	67.6	41.5	-1.0	-52.5
(18-29)	Post-dosing	99.2	124.8	107.5	149.7

The reported test material effects on food consumption were difficult to interpret since this parameter was not actually measured by the testing laboratory, but rather inspected grossly. The study report indicated slight increases in the incidences of anorexia in Ethoprop-treated groups during the dosing and post-dosing periods. However, our interpretation of the incidences of anorexia was that there were no biologically significant differences between dosage groups and controls during the dosing period. Although mild increases in the incidence of anorexia were reported in Ethoprop-treated animals during the post-dosing period, this apparent reduction in food intake was also considered of little biological significance since animals treated with Ethoprop demonstrated the highest increases in body weight gains during this same period of gestation. The incidences of maternal anorexia for the dosing and post-dosing periods are summarized in Table 2.

TABLE 2. Effects of Ethoprop on the Incidence of Maternal Anorexia in Rabbits

Gest. Days	Study Period	Dose Level (mg/kg/day)			
		0	0.125	0.500	2.000
(6-18)	Dosing	4/17(24%)	6/17(35%)	5/16(31%)	5/16(31%)
(18-29)	Post-Dosing	4/15(27%)	9/17(53%)	10/15(67%)	9/17(53%)

There were no compound-related effects on fecundity, number of corpora lutea, number of implantations, or uterine weights. Intrauterine examinations, revealed a slight increase in the mean number of resorptions and a decrease in the mean number of viable fetuses for the low- and high-dose groups when compared with controls. Since the reported number of dead fetuses was comparable for all groups, we concluded that the increased incidence of resorptions were the result of intrauterine deaths during the early (perhaps embryonic) developmental stages. Details are presented in Table 3.

TABLE 3. Effects of Ethoprop on Resorptions and Fetal Viability in Rabbits

Parameter (mean per litter)	Dose Level (mg/kg/day)			
	0	0.125	0.500	2.000
Resorptions	19.05%	27.05%	5.65%	22.24%
Dead fetuses	0.89%	0.00%	0.00%	0.00%
Live fetuses	80.06%	72.95%	93.94%	77.76%

Fetal sex ratio and size (weight and length) were not affected by the test material. Several fetuses from Ethoprop-treated groups had developmental malformations while no anomalies were observed among controls. Four fetuses from 3 litters in the low-dose group had visceral malformations involving cardiovascular, renal and/or gastrointestinal structures. One fetus from a mid-dose litter had multiple anomalies involving cardiovascular, pulmonary, splenic, renal, hepatic, vertebral and hindlimb structures. In the high-dose group, one fetus had an abnormally shaped cranium associated with incomplete closure of the bones in the calvarium. In addition, fetuses from Ethoprop-treated dams had higher incidences of reported visceral and skeletal variations than concurrent controls. Table 4 summarizes the incidence of malformations and variations as reported by the study author.

TABLE 4. Effect of Ethoprop on the Mean Group Incidences of Malformations and Variations per Litter

	Dose Level (mg/kg/day)			
	0	0.125	0.500	2.000
Visceral malformations (%)	0.0	4.2	0.9	0.0
Visceral variations (%)	0.0	4.8	0.0	6.7
Skeletal malformations (%)	0.0	0.0	0.9	6.7
Skeletal variations (%)	6.1	14.1	13.0	17.0

B. The major differences between the study author and the reviewers in the interpretation of the results are:

1. The reviewers assess the changes in maternal body weights associated with the test material to be of very minor biological significance. The largest decrease in group-mean weights during the dosing period reported for the high-dose animals (52.5 g) represented only a 1.4% decrease. This apparent decrease occurred in the group of animals which gained the most weight during the pre-dosing period suggesting that this group of animals had reached a body weight plateau earlier than other groups. In addition, the reviewers calculations indicate that there were no statistical differences among groups in mean body weights recorded on gestational days 0, 6, 18, and 29.
2. We do not agree with the study author's conclusion that the test material had an adverse effect on maternal food consumption. No inferences should have been made to test material effects on food intake unless food consumption had been systematically quantitated. Interpretations of the data was further complicated by the reported increases in the incidences of anorexia and body weight gains during the post-dosing period.
3. We assess that the data do not necessarily support the study author's conclusions that the test material had no effect on intra-uterine deaths. The mean incidences of resorptions in the low- and high-dose groups are slightly higher than that of the controls. We also conclude that there is a possible relationship between malformations observed in this study and the test material, although no dose-related response could be established. This conclusion is supported by the absence of malformations in concurrent control fetuses.

C. The following problems with the study report may adversely affect the validity of the results or of their interpretation.

1. The study report presented no description of tests (or their results) performed to determine the purity, concentration, and stability of the test material solutions. Without these data it was not possible to determine if dosing solutions were acceptable.
2. The materials and methods section of the study report did not indicate the method (if any) implemented to determine the pregnancy status of uteri with no implantations visible during gross inspection (immersion of uteri in a solution of ammonium sulfide is one of the acceptable methods). In the absence of such information, the accuracy in quantitation of resorption parameters is considered questionable.
3. Individual clinical observations of female animals were not presented. These data are needed in order for the reviewers to evaluate maternal responses to treatments at the various dosage levels, and to correlate them with individual necropsy findings (which were also not available).
4. Individual data for fetal malformations and variations were not reported. Therefore, we were unable to correlate the data presented in summary Tables 5, 6, 7, and 8 of the study report with individual fetal data.

Item 15 - see footnote 1.

16. APPENDIX: Appendix A, Materials and Methods, CBI pp. 5-9.

005741

APPENDIX A
Materials and Methods

• EPA Registration Number 2416-87

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

Pages 11 through 15 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 commercial/financial information
 - ☐ A draft product label
 - ☐ The product confidential statement of formula
 - ☐ Information about a pending registration action
 - ☐ FIFRA registration data (*)
-

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.