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

004797

NOV 20 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a Teratology Study in Rats with EPTC

TO: Robert Taylor, PM #25
Registration Division (TS-767)

FROM: Chad B. Sandusky, Ph.D.
Pharmacologist, Toxicology Branch
HED (TS-769)

Chad B. Sandusky 11/19/85

THROUGH: Robert P. Zendzian Ph.D., Acting Head
Review Section IV

11/19/85

Theodore M. Farber Ph.D.
Chief, Toxicology Branch
HED (TS-769)

Compound: EPTC

Tox. Chem. #: 435

Registration #: 476-2140/476-2165

Registrant: Stauffer

Accession #: 252322

Action Requested:

Stauffer Chemical Company has submitted for review a rat teratology study with EPTC. These data were submitted in response to a Data-Call-In and the Registration Standard prepared on EPTC.

Summary and Conclusions:

Technical grade EPTC (100%) was administered by gavage in corn oil to Charles-River CD rats on days 6-15 of gestation at doses of 0, 30, 100 or 300 mg/kg/day. Maternal toxicity was observed

at the highest dose tested (LEL=300mkd, NOEL=100mkd) and consisted of excessive mortality (60%), and reductions in body weight gain, adjusted body weight and food consumption. Fetotoxicity was also observed at the highest dose tested (LEL=300mkd, NOEL=100mkd) and consisted of decreased fetal body weights and increased incidence of skeletal retardations. In addition, embryolethal effects (increased incidence of resorptions) were observed at 300mkd, NOEL=100mkd (see DER Addendum).

Due to high maternal mortality at the high dose (60%), there were only 76 fetuses from 6 litters available for teratogical evaluation. Although the incidence of omphalocele in the high dose was higher than controls, other treated groups and historical conrtols (see DER Addendum), the biological significance of this effect cannot be established on these limited data. Therefore, EPTC was not teratogenic under the conditions of this study.

An additional teratology study in a second species is required to complete the data requirements for teratology testing on EPTC.

CORE Classificetion: Minimum

Numerous deficiencies were noted in this study, including:

1) The highest dose level tested caused an excessively high incidence of maternal mortality (60%), which resulted in only six litters and 76 fetuses for examination.

2) The absence of analytical data on dosing mixtures precluded verification of dosage concentrations and stability of the test material in the vehicle. In addition, the Technical material used in this study was "assumed" 100% pure. A technical product of this purity is unusual and analytical data supporting this "assumption" was absent.

DER's:

The following DER and DER ADDENDUM prepared on this teratology study in rats with EPTC is attached:

Tasker, E.J.: A teratology study in rats with EPTAM. (Unpublished study no. T-11753 by WIL Research Laboratories, Inc., Ashland, OH, for Stauffer Chemical Company, Richmond, CA; dated November 3, 1983.) Accession #252322.

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DER ADDENDUM

Chemical: EPTC

Study Type: Rat Teratology

Citation: Tasker, E.J.; A Teratology Study in Rats with EPTAM. (Unpublished study No. T-11753 by WIL Research Laboratories, Inc., Ashland, OH. for Stauffer Chemical Company, Richmond, CA: dated November 3, 1983.) Accession No. 252322.

Addendum Prepared By: Chad B. Sandusky, Ph.D. Chad B Sandusky 11/19/85
Pharmacologist
Toxicology Branch, HED (TS-769)

Addendum Reviewed By: Robert P. Zendzian, Ph.D. 11/19/85
Acting Head, Review Section IV
Toxicology Branch, HED (TS-769)

Original DER: Dynamac

Addendum:

The conclusions reached in the DER prepared at Dynamac have been re-evaluated based on additional analyses of data available in the submitted report, i.e., historical controls and resorption data from the dams that died during the study.

Fifteen dams died at the high dose (300 mkd) during the study on gestation days 11 (3 died), 12 (12 died), 13 (4 died 16 (1 died and 1 sacrificed) and 17 (2 died). The reproduction data available from the postmortum done on these animals is summarized below (mean # per dam ± S.E.):

Resorptions		Corpora Lutea	Normal Implantations
Early	Late		
3.4 ± 0.9	1.2 ± 0.8	11.1 ± 0.5	7.3 ± 0.3

These data demonstrate that the incidence of resorptions in the dams that died at the high dose (300 mkd) was higher than in those that survived to delivery (3.4 vs. 2.1) (see TABLE 3 in the original DER). EPTC was clearly embryotoxic at this dose, although the MTD was exceeded (60% maternal mortality). Although there was an apparent increase in early resorptions at 100 mkd (see TABLE 3 in the original DER), this increase was magnified by one litter in this dose group being completely resorbed. The NOEL for embryotoxicity, based on this additional

analysis, is 100 mkd, LEL = 300 mkd (based on increased resorptions at the high dose).

The historical controls for omphalocele were included in Appendix B of the original report (see below):

	No. Litters Examined	% Litters w/ Omphalocele	No. Fetuses Examined	% Fetuses w/ Omphalocele
Historical Conrtols	359	0.0-4.5	4942	0.0-0.3
Current Study, High Dose (300 mkd)	6	33.3	76	2.6
Current Study, Control	22	0	284	0

As can be discerned from the above data, due to high maternal mortality at the high dose (60%), there were only 76 fetuses from 6 litters available for teratological evaluation. Although the incidence of omphalocele in the high dose was higher than controls, other treated groups and historical controls from the same laboratory in the same species and strain, the biological significance of this effect cannot be established on these limited data. Therefore, EPTC was not teratogenic under the conditions of this study.

EPA: 68-01-6561
TASK: 89
September 3, 1985

DATA EVALUATION RECORD

EPTC/EPTAM

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Tasker, E. J. A teratology study in rats with EPTAM. (Unpublished study No. T-11753 by WIL Research Laboratories, Inc., Ashland, OH, for Stauffer Chemical Company, Richmond, CA; dated November 3, 1983.) Accession No. 252322.

APPROVED BY:

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

Signature: I Cecil Felkner
Date: 9-4-85

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1. CHEMICAL: EPTC/EPTAM.

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2. TEST MATERIAL: EPTAM technical, WRC #4921-4-10. The lot No. of the test material used was CHK0601; it was described as a yellow liquid at room temperature, with an assumed purity of 100 percent.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Tasker, E. J. A teratology study in rats with EPTAM. (Unpublished study No. T-11753 by WIL Research Laboratories, Inc., Ashland, OH, for Stauffer Chemical Company, Richmond, CA; dated November 3, 1983.) Accession No. 252322.

5. REVIEWED BY:

Ronald D. Hood, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: G Millicovsky FOR RDH
Date: 4 Sep 85

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

Signature: James R. Plautz
Date: September 4, 1985

6. APPROVED BY:

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

Signature: G Millicovsky
Date: 4 Sep 85

Chad Sandusky, Ph.D.
EPA Reviewer

Signature: Chad B. Sandusky
Date: October 2, 1985

Robert Zendzian, Ph.D.
EPA Section Head

Signature:
Date: 11/19/85

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7. CONCLUSIONS:

A. EPTAM mixed with corn oil was administered at dosages of 0, 30, 100, or 300 mg/kg/day by gavage to pregnant Charles River COBS CD rats on each of gestation days 6-15. Under the conditions of this study, we assess the NOELs and LOELs to be the following:

	NOEL (mg/kg/day)	LOEL (mg/kg/day)	EFFECTS OBSERVED
Maternal toxicity	100	300	Mortality and reductions in body weight, weight gain, adjusted body weight and food consumption.
Embryolethality	30	100	Increased incidence of resorptions.
Fetotoxicity	100	300	Decreased body weight and increased incidence of skeletal retardations.
Teratogenicity	100	300	Omphalocele.

The observable effect levels for teratogenicity were assessed on the basis of very limited fetal data, resulting from excessive maternal mortality at the highest dose tested.

B. Although the study design and final report had several deficiencies (see section 14C), this study is classified Core Minimum.

Items 8 through 10--see footnote 1.

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

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II. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Male and female Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Portage, MI. Upon receipt, animals were examined, ear tagged for identification, and individually housed in suspended wire-mesh cages. Purina Certified Rodent Chow #5002 and water were available ad libitum. The animal room was maintained at 72 ± 3°F with a relative humidity of greater than 40 percent and a 12/12 hour light/dark cycle.

At the end of a 14-day acclimation period, healthy females within the desired weight range were selected for mating. One female was housed with one male until a copulatory plug or a sperm-positive vaginal smear was observed; this day was designated gestation day 0. Female body weights ranged from 220 to 316 g on day 0 of gestation.

Females with evidence of mating were serially allocated to each of the four dose groups until 25 females were assigned to each group.

2. Dosing mixtures of the test material were prepared daily by dilution of a stock mixture of EPTAM in corn oil at a concentration of 30 mg/mL (w:v); dilutions were made to obtain concentrations of 10 and 3 mg/mL (v:v).

3. The test material was administered once daily by oral intubation to mated females on gestation days 6 through 15 at dosage levels of 0 (vehicle), 30, 100, or 300 mg/kg/day (10 mL/kg body weight). Dosages were based on body weights at gestation days 6, 9, and 12.

Dose levels were reportedly selected on the basis of a preliminary study conducted by WIL Research Laboratories, Inc.; however, no data from this study were reported.

4. All animals were observed daily for mortality/moribundity, appearance, and behavior before dosing and for clinical signs of toxicity after dosing. Body weights and food consumption were recorded on gestation days 0, 6, 9, 12, 16, and 20.

Gross necropsies were performed on females that were found dead and those that delivered early. Fetuses from these dams were examined externally and retained in formalin, as were the maternal tissues having gross lesions.

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Surviving dams were sacrificed by carbon dioxide inhalation on day 20 of gestation and examined internally for gross lesions. Uteri and ovaries were removed, gravid uterine weights were recorded, and the number of corpora lutea were counted. The uteri were excised and the number and distribution of implantation sites, viable and nonviable fetuses, and early and late resorptions were noted and recorded. Uteri that appeared to be nongravid were stained with a 10 percent ammonium sulfide solution to determine pregnancy status.

The fetuses were individually tagged for identification, weighed, sexed, and examined for external abnormalities. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution, sectioned, and examined by methods described by Wilson. The remaining fetuses were fixed in alcohol, cleared in potassium hydroxide, stained with alizarin red-S, and examined for skeletal abnormalities by a variation of Dawson's technique.[2]

Fetal abnormalities were classified as either developmental variations or malformations.

5. All statistical analyses were conducted with a minimum 5 percent probability level using two-tailed tests. The Chi-square test with Yates' correction factor was used to analyze fetal sex ratios. Fisher's Exact test was used to compare the number of litters with abnormal fetuses. Numbers of early and late resorptions, dead fetuses, and post-implantation losses were analyzed by the Mann-Whitney U-test, whereas mean numbers of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weights, maternal body weight gain, and food consumption were analyzed by a one-way ANOVA and Dunnett's test.

   A copy of the CBI, Materials and Methods, is presented in Appendix A.

B. Protocol: No protocol was included with the study report.

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[2] Dawson, A. B. Stain Technol. 1 (1926): 123-124.

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12. REPORTED RESULTS:

Clinical Observations: No deaths occurred in the control, low-, or mid-dose groups; however, 14 deaths occurred between gestation days 11 and 17 in the high-dose group. One of the deaths was attributed to intubation error, whereas the remaining 13 deaths were attributed to a compound-related incidence of "metrorrhagia," severe internal hemorrhage, or cardiorespiratory arrest. One additional high-dose female was sacrificed moribund on gestation day 16.

Results of individual animal clinical observations were not provided. However, the author reported that starting on gestation day 7, all of the high-dose females exhibited "a reaction to treatment with EPTAM"; the signs observed included a wet, matted, stained urogenital region and "red matter in the facial area" and/or "various other body surfaces." Compound-related signs of toxicity were not observed in the mid- and low-dose groups; hair loss was noted in all groups.

One control dam escaped from the cage for an unspecified length of time, and was not dosed on gestation days 14 and 15; in addition, body weight was not recorded for gestation day 16. However, the author claimed that this animal was within normal limits of all parameters measured, and the data from this animal were included in tabulations.

The only necropsy finding observed at terminal sacrifice was hydronephrosis in one low-dose dam and two mid-dose dams.

One control dam and three low-dose dams delivered earlier than expected due to "technical error." Data from these animals were not included in the calculations.

Maternal Body Weights and Food Consumption: High-dose dams exhibited a significant ($p < 0.01$) reduction in mean body weight, body weight change, and in mean food consumption during the dosing period (gestation days 6-16) when compared to controls (Tables 1 and 2).

Body weight gains and food consumption of high-dose females were similar to controls before commencement of dosing (gestation days 0-6) and recovered to control levels after the dosing period (gestation days 16-20). Animals in the mid- and low-dose groups had mean body weights, mean body weight changes, and mean food consumption that were comparable to control values (Tables 1 and 2) during all intervals measured except for a significant ($p < 0.01$) elevation in food consumption for mid-dose animals after the dosing period (gestation days 16-20).

TABLE 1. Effects of EPTAM on Maternal Body Weight and Maternal Body Weight Change in Rats

Gestation Day	Group Mean Maternal Body Weight (g)			
	Dosage (mg/kg/day)			
	0	30	100	300
0	268 ± 19[a]	274 ± 15	266 ± 21	268 ± 17
6	288 ± 18	290 ± 16	288 ± 17	289 ± 19
16	323 ± 19	326 ± 14	325 ± 23	265 ± 31**
20	371 ± 21	377 ± 17	374 ± 31	326 ± 34**

Study Period	Group Mean Maternal Body Weight Change (g)			
	Dosage (mg/kg/day)			
	0	30	100	300
0-6 (pre-dosing)	20 ± 8	16 ± 10	21 ± 10	21 ± 9
6-16 (dosing)	34 ± 10	36 ± 11	37 ± 15	-17 ± 27**
16-20 (post-dosing)	49 ± 10	51 ± 13	49 ± 14	45 ± 25
0-20 (gestation)	103 ± 17	103 ± 19	108 ± 29	64 ± 36**

	Group Mean Adjusted[b] Maternal Body Weight Change (g)			
	Dosage (mg/kg/day)			
	0	30	100	300
0-20	33.8 ± 14.5	36.1 ± 16.0	46.4 ± 16.1	13.6 ± 10.9*

[a] All values represent group mean ± SD.

[b] Adjusted by subtracting gravid uterine weight from maternal body weight at sacrifice.

*Significantly different from control ($p \leq 0.05$) by ANOVA and Kruskall-Wallis analysis conducted by our reviewers.

**Significantly different from control ($p \leq 0.01$).

TABLE 2. Effect of EPTAM on Mean Group Maternal Food Consumption (g/rat/day)

Gestational Period	Dosage (mg/kg/day)			
	0	30	100	300
0-6	19 ± 2	18 ± 4	19 ± 3	19 ± 3
6-9	14 ± 2	14 ± 4	13 ± 3	6 ± 3**
9-12	13 ± 2	14 ± 1	14 ± 4	3 ± 3**
12-16	14 ± 2	15 ± 2	15 ± 3	6 ± 4**
16-20	21 ± 3	24 ± 3	25 ± 4**	20 ± 5

**Statistically different from control value ($p \leq 0.01$).

Reproductive Indexes: The reproductive data are summarized in Table 3. A high incidence of maternal deaths in the high-dose group resulted in 6 litters with viable fetuses surviving to scheduled sacrifice compared with 22, 19, and 21 litters in the control, low-, and mid-dose groups, respectively.

Post-implantation loss and the subsequent reduction in the number of viable fetuses in the mid- and high-dose groups were attributed primarily to one high-dose female with complete (early) litter resorption and two mid-dose females--one with total (early) litter resorption and one with several early resorptions. The only statistically significant ($p < 0.05$) difference reported by the authors was the decreased fetal mean body weight in the high-dose group litters (Table 3).

Teratogenicity Assessment: The 76 fetuses in six high-dose litters were excluded from "meaningful assessment of the teratogenic potential of EPTAM" due to the 15 deaths among 25 pregnant animals in this group and due to the increased "postimplantation loss" resulting in an inadequate sample size for comparison. Therefore, the significantly ($p < 0.05$) higher incidence of high-dose litters having fetuses with omphalocele "was not considered biologically significant." There was also a significant ($p < 0.05$) increase in the number of high-dose litters with fetuses having unossified sternebrae Nos. 1, 2, 3, and/or 4, which may have been related to reduced fetal body weight. The malformations and developmental variations observed in the low- and mid-dose groups were "unremarkable in frequency and nature" and were comparable to controls.

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

A. The author concluded that administration of the test material throughout organogenesis in Charles River COBS CD rats "produced an embryotoxic effect at a dose level of 300 mg/kg", "may have produced embryotoxicity" at 100 mg/kg, "but produced no evidence of teratogenicity in the 30 or 100 mg/kg/day groups."

B. The Quality Assurance Unit of the testing laboratory inspected the final report, but the dates and frequency were not indicated.

TABLE 3. Effects of EPTAM on Selected Reproductive and Fetal Parameters in Rats

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Parameter	Dosage Level (mg/kg/day)			
	0	30	100	300
No. Mated females	25	25	25	25
No. Aborted or Delivered Early	1	2	0	0
Mortality (%)	0	0	0	15 (60)
No. C-sectioned	24	23	25	10
No. Pregnant (%)	22 (92)	19 (83)	22 (88)[a]	7 (70)[a]
No. Corpora Lutea/Litter[b]	14.5±2.6	16.0±2.8	14.4±3.1	14.9±2.2
No. Implantations/Litter[b]	13.4±2.6	12.8±3.9	12.7±4.0	13.0±3.1
No. Early Resorptions/ Litter[b,c]	0.5±0.8	0.6±1.6	1.2±2.0[a]	2.1±4.5[a]
No. Live Fetuses/Litters[b]	12.9±2.8	12.2±4.3	11.5±4.3	10.9±5.5
Fetal Body Weight[b]	3.4±0.2	3.6±0.6	3.4±0.2	2.8±0.4*
(%) Malformed Fetuses	0.0	0.4[d]	0.4[e]	2.6[d]
(%) Litters with Malformed Fetuses	0.0	5.3	4.8	33.3*

[a] One litter completely resorbed.

[b] Mean ± SD.

[c] Only one late resorption in the 100 mg/kg group was noted for all groups examined.

[d] Omphalocele.

[e] Great vessel anomaly including absent ductus arteriosus and abnormal origin of great vessels.

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

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS: 004797

A. Our assessment of the reported data indicates that no maternal effects were associated with the compound at 30 and 100 mg/kg/day. However, 300 mg/kg/day produced a 60 percent incidence of maternal mortality, statistically significant reductions in maternal body weight, body weight gain, adjusted body weight, and food consumption.

The group mean number of embryonic resorptions per litter were 0.5, 0.6, 1.2, and 2.1 for the 0, 30, 100, and 300 mg/kg/day dosages, respectively. These data indicate a slight embryolethal effect at 100 mg/kg/day and a more pronounced effect at 300 mg/kg/day; however these effects were not statistically significant. Fetotoxic effects were noted only at the 300 mg/kg/day, based on a statistically significant decrease in fetal body weights and on the increase in the incidence of skeletal retardations. No malformations were reported for the control group, whereas the percentage of malformed fetuses was 0.4, 0.4, and 2.6 for the 30, 100, and 300 mg/kg/day groups, respectively; the percentage of affected litters was 5.3, 4.8, and 33.3 (a statistically significant increase), respectively. All of the reported terata were visceral malformations; however, those in the 30 and 300 mg/kg/day groups were omphaloceles (umbilical hernias), and the one malformation noted in the 100 mg/kg/day group was a great vessel anomaly; involving the aorta, pulmonary arteries, and the ductus artercosus. While it is difficult to assess the teratogenic effect of the test material due to the loss of critical fetal data resulting from the maternal lethal effects of the high-dose treatments, we could not rule out the teratogenic potential of the test article (particularly at 300 mg/kg/day) due to the occurrence of identical abdominal defects in the 30 and 300 mg/kg/day groups.

B. The study author concluded that a meaningful assessment of the teratogenic potential of the test material was precluded and that the malformations observed at 300 mg/kg/day were not biologically significant due to the elevated incidence of maternal mortality in this group; however, based on the limited data available, we assessed that EPTAM has fetotoxic and teratogenic potentials at 300 mg/kg/day; this dosage level is also capable of producing marked maternal toxic effects. In fact, it is possibile that the teratogenic peak exists between 100 and 300 mg/kg/day because a dosage level lower than 300 mg/kg/day may provide a greater opportunity for malformed fetuses to be noted at term if more pregnant animals (and their fetuses) survive.

C. Two deficiencies negatively impacted on the scientific validity of the study:

1. The highest dosage level tested caused an excessively high incidence of maternal mortality. As a result, only six litters were available for examination at this dosage level.

2. The absence of analytical data on dosing mixtures precluded our verification of dosage concentrations and stability of the test material in the vehicle.

Items 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 5-10.

APPENDIX A

Materials and Methods
(CBI pp. 5-10)

Eptam

Page _____ is not included in this copy.

Pages _18_ through _23_ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

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_____ 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.

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__X__ FIFRA registration data.

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