DATA EVALUATION RECORD 4/13/1999

PEBULATE (TILLAM® TECHNICAL)

STUDY TYPE: DEVELOPMENTAL TOXICITY– RAT (83-3a)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat
OPPTS 870.3700 [§83-3a]

DP BARCODE: D247841 SUBMISSION CODE: S546073
P.C. CODE: 041403 TOX. CHEM. NO.: 710
TEST MATERIAL (PURITY): Pebulate Technical (97.1%)
SYNONYMS: Tillam®; *S*-propyl butyl(ethyl)thiocarbamate
CITATION: Sauerhoff, M.W. (1986) A teratology study in rats with Tillam® technical. WIL Research Laboratories, Inc., 1407 Montgomery Township Road 805, Ashland, OH. Laboratory Project ID. WIL-27034. November 12, 1986. MRID 40033301. Unpublished.

SPONSOR: Stauffer Chemical Company, 400 Farmington Avenue, Farmington, CT 06032

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 40033301), Pebulate Technical (97.1%, a.i.) was administered by gavage to groups of 25 pregnant Crl:CD®(SD)BR rats at doses of 0, 5, 30, or 200 mg/kg/day on gestation days (GD) 6 through 15, inclusive. On GD 20, all dams were sacrificed and all fetuses were weighed and examined for external malformation/variations. Approximately one-half of each litter was processed for visceral examination and the remainder stained for skeletal evaluation.

All animals survived to terminal sacrifice. No treatment-related clinical signs of toxicity were observed in any animal at any dose. No significant differences in absolute body weights were noted between the treated and control groups. Body weight gains by the high-dose group were significantly ($p \leq 0.01$) less than the controls during the dosing interval (GD 6-16; 75% of control) and overall (GD 0-20; 86% of control). The corrected body weight gains (body weight changes exclusive of the gravid uterine weight) at the high dose were lower than the control (86% of the control). The most pronounced effect on body weight gain occurred towards the end of the dosing interval and continued post-dosing with significantly lower weight gains by the high-dose group as compared with the control group on GD 12-16 ($p \leq 0.01$; 69% of control) and GD 16-20 ($p \leq 0.05$; 88% of control). Food consumption was comparable between the low- and mid-dose groups and the control group throughout the study. The high-dose group had significantly ($p \leq 0.01$; 93% of control) lower food consumption than the controls only during the GD 16-20 interval.

The maternal toxicity LOAEL is 200 mg/kg/day based on lower body weight gains and the NOAEL is 30 mg/kg/day.

Fetal body weights in the high-dose group were significantly ($p \leq 0.01$) less than the controls. No differences were observed between the treated and control groups for number of corpora lutea/dam, implantations/dam, postimplantation loss, fetuses/litter, or fetal sex ratios. One high-dose dam had whole litter resorption consisting of early resorption of two implantation sites; no other treated or control dam had whole litter resorption.

The developmental toxicity LOAEL is 200 mg/kg/day based on lower fetal body weights and increased incidence of unossified sternebrae #5 and #6, and the NOAEL is 30 mg/kg/day.

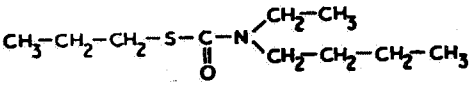
This study is classified as **Acceptable (guideline)** and satisfies the requirements for a developmental toxicity study (83-3a) in rats.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, and Data Confidentially statements were included. A Flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pebulate Technical
   Description: amber liquid
   Batch No.: WRC# 4921-20-18
   Purity: 97.1%
   Stability of compound: stable at room temperature
   CAS No.: 1114-71-2
   Structure:



2. Vehicle and/or positive control
   The vehicle and negative control was Mazola® 100% pure corn oil. No positive control was used in this study.

3. Test animals
   Species: rat
   Strain: Crl:CD®(SD)BR
   Age and weight at study initiation: approx. 12 weeks; 224-290 g
   Source: Charles River Breeding Laboratories, Inc., Portage, MI
   Housing: Females were individually housed in wire mesh cages suspended above cage-board.
   Diet: Purina® Certified Rodent Chow® #5002, meal form, and tap water were available *ad libitum*.
   Environmental conditions:
   Temperature: 72±3°F
   Humidity: minimum of 40%
   Air changes: 10/hr
   Photoperiod: 12 hours light/dark
   Acclimation period: minimum of 18 days

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity of Pebulate Technical when administered by gavage to rats on gestation days 6 through 15, inclusive.

1. In life dates
   Start: May 6, 1986; end: May 30, 1986

2. Mating
Female rats were placed in the cage of a resident male of the same strain and source for breeding. Positive evidence of mating was confirmed by the presence of a copulatory plug or a vaginal smear for sperm. The day evidence of mating was observed was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Bred females were assigned in a block design to treatment groups by the following randomization procedure. On a given day, the first mated female was assigned to group 1, the second mated female was assigned to group 2, the third to group 3, etc. This process was continued daily until each group had sufficient numbers of animals.

TABLE 1. Animal assignment

Group number	Test group	Dose (mg/kg/day)	Number assigned
1	Control	0	25
2	Low Dose	5	25
3	Mid Dose	30	25
4	High Dose	200	25

Data taken from Figure 2, p. 18, MRID 40033301.

4. Dose selection rationale
Doses were selected based on the results of a range-finding teratology study in rats with the test article conducted by the performing laboratory (WIL-27033). Details of this study were not included in the main report.

5. Dosing
All doses were administered in a volume of 5 mL/kg of body weight/day prepared weekly during the dosing period. Dosing was based on the most recent body weight.

6. Dose solution preparation and analysis
A specified amount of test article was weighed and dissolved in corn oil in a volumetric flask. Vehicle was added in sufficient quantity to achieve the appropriate concentration for each dose group. The flasks were inverted several times and shaken by hand to ensure dissolution. The solutions were stirred continuously for 10 minutes using a magnetic stir plate and bars, transferred to amber storage jars, and stored at room temperature. Dosing solutions were prepared fresh weekly. Samples from the top, middle, and bottom of the dosing solutions were analyzed for homogeneity prior to the initiation of treatment. Each week, samples from the dosing solutions were analyzed for concentration. The test article had

been determined to be stable in the vehicle for up to one week by the sponsor; therefore, stability was not measured.

Absence of test article was confirmed in the vehicle. Test article concentrations in samples from the top, middle, and bottom of the 5, 30, and 200 mg/kg/day solutions ranged from 99.0-100%, 93.8-96.3%, and 95.0-97.0% of nominal, respectively. Concentrations of the three dosing solutions ranged from 96.0% to 106.5% of nominal. The analytical results showed that the test article could be adequately mixed in the vehicle and the variation between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1. Maternal observations and evaluations
The animals were checked daily for appearance and behavior prior to dosing, and for signs of toxicity after dosing, as well as for mortality and moribundity. Maternal body weights and food consumption were recorded on GD 0, 6, 9, 12, 16, and 20. Dams were sacrificed on GD 20 by carbon dioxide asphyxiation and subjected to gross necropsy. Gravid uteri were weighed. Ovaries were examined for number of corpora lutea and uteri were examined for numbers of live and dead fetuses and early and late resorptions. Uteri of apparently nonpregnant animals were stained with 10% ammonium sulfide for detection of early implantation sites.

2. Fetal evaluations
Each fetus was weighed, sexed, and examined for external malformations/variations. The crown-rump length of late resorptions was recorded. Approximately one-half of the fetuses from each dam were fixed in Bouin's solution for subsequent visceral examination including grading of renal papillae development. The remaining one-half of the fetuses were eviscerated and processed for skeletal examination.

D. DATA ANALYSIS

1. Statistical analysis
All analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing the treatment group to the control group. Fetal sex ratios were analyzed by the Chi-square test with Yate's correction factor. The numbers of litters with malformations/variations were compared by Fisher's Exact test and the numbers of early and late resorptions, dead fetuses, and postimplantation losses were analyzed by the Mann-Whitney U-test. Mean numbers of corpora lutea, number of implantations, viable fetuses, fetal body weights, maternal body weights and body weight gains, and maternal food consumption were analyzed by a one-way ANOVA and Dunnett's test.

2. Historical control data were provided to allow comparison with current controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All animals survived to terminal sacrifice. No treatment-related clinical signs of toxicity were observed in any animal at any dose.

2. Body weight

Selected maternal body weights and body weight changes during gestation are listed in Table 2. No significant differences in absolute body weights were noted between the treated and control groups. However, body weight gains by the high-dose group were significantly ($p \leq 0.01$) less than the controls during the dosing interval (GD 6-16; 75% of control) and overall (GD 0-20; 86% of control). The corrected body weight gains (body weight changes exclusive of the gravid uterine weight) at the high dose were lower than the control (86% of the control). The most pronounced effect on body weight gain occurred towards the end of the dosing interval and continued post-dosing with significantly lower weight gains by the high-dose group as compared with the control group on GD 12-16 ($p \leq 0.01$; 69% of control) and GD 16-20 ($p \leq 0.05$; 88% of control).

TABLE 2. Selected maternal body weights and body weight changes (g)				
Day of gestation	**0 mg/kg/day**	**5 mg/kg/day**	**30 mg/kg/day**	**200 mg/kg/day**
0	251 ± 13	255 ± 10	252 ± 12	255 ± 11
6	286 ± 14	287 ± 15	286 ± 11	290 ± 11
9	287 ± 15	293 ± 14	292 ± 14	291 ± 14
16	339 ± 18	340 ± 18	337 ± 21	329 ± 18
20	411 ± 17	409 ± 22	404 ± 29	392 ± 28
Wt. Change GD 12-16 (% control)	32 ± 7	29 ± 7 (91%)	28 ± 11 (88%)	22 ± 10** (69%)
Wt. Change GD 16-20 (% control)	72 ± 8	69 ± 8 (96%)	67 ± 11 93%)	63 ± 13** (88%)
Wt. change GD 6-16 (% control)	53 ± 11	53 ± 6 (100%)	51 ± 16 (96%)	40 ± 13** (75%)
Wt. change GD 0-20 (% control)	159 ± 14	154 ± 16 (97%)	152 ± 23 (96%)	137 ± 25** (86%)
Corrected BW gain♧ GD 0-20; (% control)	68 ± 14.5	69 ± 12.7 (101%)	69.7 ± 12.1 (103%)	58.7 ± 11 (86%)

Data taken from Tables 3, 4 and 14, pp. 34, 35 and 51-54, respectively, MRID 40033301.
Significantly different from control: **p ≤ 0.01.
♧ Days 0-20, body weight change minus the gravid uterine weight.

3. Food consumption

Food consumption was comparable between the low- and mid-dose groups and the control group throughout the study. The high-dose group had significantly ($p \leq 0.01$; 93% of control) lower food consumption than the controls during the postdosing interval, GD 16-20. Otherwise, food consumption by the high-dose group was similar to the controls.

4. Gross pathology

No abnormalities were noted in any dam at necropsy.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 3. Fetal body weights in the high-dose group were significantly ($p \leq 0.01$) less than the controls. No differences were observed between the treated and control groups for number of corpora lutea/dam, implantations/dam, postimplantation loss, fetuses/litter, or fetal sex ratios. Preimplantation loss by the mid- and high-dose groups was slightly higher than the controls and correlated with slightly fewer fetuses/litter (not

significant) in these treated groups. One high-dose dam had whole litter resorption consisting of early resorption of two implantation sites.

TABLE 3. Cesarean section observations

Observation	0 mg/kg/day	5 mg/kg/day	30 mg/kg/day	200 mg/kg/day
No. Animals Assigned	25	25	25	25
No. Pregnant	20	23	24	25
Pregnancy Rate (%)	80	92	96	100
Maternal Mortality	0	0	0	0
Total Corpora Lutea	352	396	427	460
Corpora Lutea/Dam	17.6	17.2	17.8	18.4
Total Implantation	331	365	362	388
Implantation/Dam	16.6	15.9	15.1	15.5
Preimplantation loss (%)[a]	6	8	15	16
Postimplantation loss (%)[a]	4	6	5	6
Total Live Fetuses	317	342	345	366
Live Fetuses/litter	15.9	14.9	14.4	14.6
Mean Fetal Weight (g)	3.7	3.7	3.7	3.5**
Sex Ratio (% Male)	53	49	52	52
Total Resorptions	14	23	17	22
Early	13	22	17	22
Early resorptions/dam	0.7	1.0	0.7	0.9
Late	1	1	0	0
Total Dead Fetuses	0	0	0	0
Dams with all resorptions	0	0	0	1

Data extracted from Tables 7 and 18, p. 38 and 68-71, respectively, MRID 40033301.
Significantly different from control, **$p \leq 0.01$.
[a]Calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

No treatment-related malformations/variations were observed in any fetus from any dose group. One control fetus had a thread-like tail, one fetus in each of two low-dose litters had multiple malformations of the head and retroesophageal aortic arch,

respectively, one mid-dose fetus had a dome-shaped head with hydrocephaly, and one high-dose fetus had severely misaligned sternebrae. The study author emphasized a slight increase in the percentage of fetuses in the high-dose group (14.4%) with sternebrae #5 and/or #6 unossified as compared with the control (5.7%). The number of fetuses affected was 9/159, 11/170, 7/167, and 27/188 and the number of litters affected was 7/20, 9/23, 5/23, and 12/24 in the control, 5, 30, and 200 mg/kg/day groups, respectively. The litter incidence rates for the treated groups were not significantly different from the controls and were within the range of historical control data. Common variations in treated and control fetuses included unossified and moderately misaligned sternebrae and 14th rudimentary ribs.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that maternal toxicity was evident as reduced body weight gains during the GD 12-16 interval in animals given 200 mg/kg/day. Fetotoxicity also occurred at this dose as lower mean fetal body weights. The percentage of high-dose fetuses with sternebrae #5 and/or #6 unossified (14.4% vs 5.7% of the controls) was considered a sequelae to reduced fetal body weights. These fetal effects were considered secondary to maternal toxicity. A "teratogenic response" was not observed. In conclusion, the author stated that the NOEL for maternal toxicity and fetotoxicity was 30 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. Maternal toxicity

Maternal toxicity was evident at the high-dose as reduced body weight gains (corrected) at the end of the dosing interval which continued postdosing. During the dosing interval, the lower body weight gains cannot be attributed to reduced food consumption and are considered treatment-related. Although the lower body weight gains by the high-dose dams were pronounced, especially towards the end of the dosing interval, this did not result in significantly lower absolute body weights at any time during the study. The reviewer feels dosing may have been inadequate because the animals could have tolerated higher doses of Pebulate Technical. Details of preliminary studies used for the dose selection rationale would be useful in predicting the toxicity of higher doses.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

The slightly fewer numbers of live fetuses/litter in the mid- and high-dose groups as compared with the control group were probably due to slight increases in preimplantation losses by these treated groups. No dead fetuses were observed at cesarean section to account for the smaller litter sizes. However, the numbers of implantations/dam and fetuses/litter for both treated

groups were within the historical control range. On the other hand, these parameters in the control group exceeded the range of historical control data. Therefore, due to unusually high values in the control group, the fact that implantation occurred prior to the initiation of treatment, and lack of a dose-related trend, these effects are not considered due to treatment with Pebulate Technical.

b. Altered growth
Mean fetal body weights from high-dose dams were significantly less than the controls.

c. Developmental variations
A slight reduction in the ossification rate of sternebrae in fetuses from the high-dose dams correlated with the lower fetal body weights.

d. Malformations
No major fetal malformations could be attributed to maternal treatment with Pebulate Technical.

C. STUDY DEFICIENCIES

A minor deficiency is the omission of details of the dose selection rationale.

D. CLASSIFICATION

This study is classified as **Acceptable (guideline)** and does satisfy the requirements for a developmental toxicity study (83-3a) in rats.

1. Maternal NOAEL = 30 mg/kg/day
2. Maternal LOAEL = 200 mg/kg/day based on decreased body weight gains
3. Developmental toxicity NOAEL = 30 mg/kg/day
4. Developmental toxicity LOAEL = 200 mg/kg/day based on lower fetal body weights and increased incidence of unossified sternebrae #5 and #6.

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
SEE THE FILE COPY.

TABLES 8-11: Numbers of fetus and litters with malformations or variations.
(Tables extracted from pages 39-42 of the study report, MRID 40033301.)

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Toxicology Branch:	TOX1