DATA EVALUATION RECORD 4/13/1999

PEBULATE (TECHNICAL)

Study Type: DEVELOPMENTAL – RABBIT (83-3B)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit
OPPTS 870.3700 [§83-3b]

DP BARCODE: D247841 SUBMISSION CODE: S546073
P.C. CODE: 041403 TOX. CHEM. NO.: 710
TEST MATERIAL (PURITY): Pebulate (Technical, purity not provided)
SYNONYMS: Tillam®, PEBC, S-Propyl butyl(ethyl)thiocarbamate
CITATION: Sauerhoff, M.W. (1986) A teratology study in rabbits with Tillam® Technical. WIL Research Laboratories, Inc., 1407 Montgomery Township Road 805, Ashland, OH 44805. Study No. WIL-27036, November 24, 1986. MRID 40033201. Unpublished.
SPONSOR: Stauffer Chemical Company, 400 Farmington Avenue, Farmington, CT 06032

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 40033201), 20 pregnant New Zealand White rabbits per group were administered Pebulate (97.1% a.i.) by gavage at doses of 0 (corn oil vehicle), 5, 30, or 150 mg/kg/day on gestation days (GD) 7 through 19. On GD 29, all does were sacrificed and all fetuses were weighed and examined for external malformations/variations. Each fetus was dissected, sexed, and examined for visceral abnormalities/variations. Then, each fetus was eviscerated, stained, and examined for skeletal malformations/variations.

No compound-related deaths occurred throughout the treatment period. No clinical signs of toxicity were observed at any treatment level. There were no significant differences in mean maternal body weight, mean body weight minus mean gravid uterine weight, or food consumption between the treated and control groups at any time during gestation. At the 150 mg/kg/day dose level, body weight gain was statistically significantly ($p \leq 0.05$) greater than the controls during the GD 7-10 interval and slightly greater during the GD 10-13 interval. The increase in weight gain was followed by a significant ($p \leq 0.01$; -22% of control) body weight loss of 27 g by the high dose group compared to a gain of 94 g by the control group during the GD 13-19 treatment interval.

Under the conditions of this study, the maternal toxicity LOAEL is 150 mg/kg/day based on decreased body weight gain during gestation days 13-19 and the maternal toxicity NOAEL is 30 mg/kg/day.

No treatment-related effects were observed for gravid uterine weights, number of fetuses/litter, pre- and postimplantation loss, numbers of corpora lutea/doe, number of implantations/doe,

resorptions/doe, fetal body weights, or fetal sex ratios. No statistically significant differences ($p \leq 0.05$) in the incidence rates of any external visceral, or skeletal malformations/variations were observed in the treated litters as compared to controls.

Under the conditions of this study, the developmental toxicity LOAEL is not established and the developmental NOAEL is 150 mg/kg/day.

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

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Pebulate (Tillam®, Technical; S-Propyl butyl(ethyl)thiocarbamate)
   Description: amber liquid
   Lot No.: WRC #4921-20-18
   Purity: 97.1% (Data extracted from MRID 40033301)
   Stability of compound: stated by registrant to be stable in corn oil for up to one week
   CAS No.: 1114-71-2

2. Vehicle and/or positive control
   Mazola® corn oil (100% pure; Best Foods, CPC International) was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals
   Species: rabbit
   Strain: New Zealand White
   Age and weight at study initiation: ≈6.5 months of age; 3.324-4.130 kg on GD 0
   Source: Hazleton Dutchland, Inc., Denver, PA
   Housing: Animals were housed individually in suspended stainless steel cages with wire bottoms.
   Diet: Purina Certified Rabbit Chow® #5322 and municipal tap water were available *ad libitum*.
   Environmental conditions:
   Temperature: 67±3°F
   Humidity: ≥40%
   Air changes: ≈10 per hour
   Photoperiod: 12-hour light/dark
   Acclimation period: ≥33 days

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Pebulate when administered by gavage to rabbits on gestation days 7 through 19, inclusive.

1. In life dates
   Start: May 20, 1986;.end: June 20, 1986

2. Mating
   Virgin females were impregnated by artificial insemination using semen collected from male New Zealand White rabbits obtained from the same supplier as the females except one male from another source (Buckshire Corporation, Perkasie, PA) which was inadvertently used to inseminate two females per study group. Semen from one male was used to inseminate one or two females of each treat-

ment group. Immediately following insemination, females were injected with human chorionic gonadotropin via the marginal ear vein. The day of insemination was considered GD 0.

3. Animal assignment and dose selection is presented in Table 1. Assignment of mated animals was random using a computer-generated randomization chart.

TABLE 1. Animal assignment

Group	Dose (mg/kg/day)	Number Assigned
Control	0	20
Low Dose	5	20
Mid Dose	30	20
High Dose	150	20

Data taken from page 17, MRID 40033201.

4. Dose selection rationale
The rationale for dose selection was not reported. The protocol suggests that a preliminary study was to be conducted and a teratology range-finding study is listed as Reference 1 on page 29. However the study was not described and no dose selection data were included in the study report (MRID 40033201).

5. Dosing
All doses were administered in a volume of 0.5 mL/kg/day. All doses were based on weight of test material and not on per cent active ingredient. Doses were administered daily for 13 consecutive days (GD 7-19) based on the most recent body weight determinations.

6. Dose solution preparation and analysis
Dosing solutions were prepared separately by mixing Pebulate in corn oil (w/v) to give nominal concentrations of 10.0, 60.0, or 300.0 mg/mL. The dosing solutions were prepared fresh weekly and stored in sealed containers at room temperature between uses. Solutions were stirred constantly during dosing. Prior to initial administration, homogeneity was tested by analyzing 1 mL aliquots taken from the top, middle, and bottom of each dosing solution. Stability was not tested. Concentrations of dosing solutions were verified by analysis of 1 mL aliquots taken from each freshly prepared dosing solution.

Results –
Homogeneity analysis: Samples taken for homogeneity analysis ranged from 100-103.7% of nominal for samples taken from the top, middle and bottom of each test dosing solution; well within the ±10% allowable range.
Stability analysis: Not provided.

Concentration analysis: The actual concentrations of the dosing solutions ranged from 100.0-103.7% of nominal during week 1 and 99.3-102.8% during week 2. These were within the allowable limit of ±10%.
The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were observed daily for clinical signs of toxicity, moribundity, and mortality. Maternal body weights were recorded on GD 0, 7, 10, 13, 19, 24, and 29 and body weight changes were calculated for the same gestation days and for intervals of 7-19, 19-29, and 0-29 GD. Food consumption was measured daily for GD 0-29 and food intake was calculated as g/animal/day and g/kg/day for days corresponding to body weight measurements. On GD 29, does were killed by intravenous injection of T-61® euthanasia solution and cesarean sections were performed as well as examination of the thoracic and abdominal cavities for visceral abnormalities. The uteri and ovaries were removed and the weights of trimmed uteri were recorded. Ovaries were examined for numbers of corpora lutea and uteri were examined for total implantation sites and live, dead, and resorbed fetuses. Uteri without apparent fetuses were opened and checked for early embryolethality by the ammonium sulfide enhancement technique.

2. Fetal evaluations

All fetuses were sexed, weighed, examined for external malformations/variations, and examined for visceral malformations/variations by dissection. Approximately one-half of the fetal heads were removed and placed in Bouin's fixative for subsequent soft tissue examination. The brains of the remaining fetuses were examined by mid-coronal slicing. Each fetus was then eviscerated, fixed in 95% isopropanol, macerated in KOH , stained with Alizarin Red S, cleared, and examined for skeletal malformations/variations.

D. DATA ANALYSIS

1. Statistical analysis

The fetal sex ratios were compared using the Chi-square test with Yates' correction factor. The numbers of fetuses, litters with malformations, and developmental variations were compared using Fisher's Exact test. The numbers of early and late resorptions, dead fetuses, and postimplantation losses were compared using the Mann-Whitney U-test. One-way analysis of variance (ANOVA) and Dunnett's test were used to analyze continuous data such as mean numbers of corpora lutea, total implantations, viable fetuses, fetal body weights and maternal body weights at each interval, maternal body weight gains, and food consumption (g/animal/day, g/kg/day). Analyses were two-tailed with the minimum level of significance set at 5%.

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

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

No treatment-related deaths occurred during the study. Three spontaneous abortions occurred in the control (2) and 5 mg/kg/day (1) groups. There were no treatment- related clinical signs of toxicity in the Pebulate Technical treated groups. Random and sporadic clinical signs included hair loss on body surfaces, decreased defecation, brown anogenital matting and staining, soft stool, and red matter under the cage.

2. Body weight

Selected body weight and body weight gain data are listed in Table 2. There were no statistically significant differences in mean absolute body weights between the treated groups and the control group at any time during gestation.

Body weight gain, at the 150 mg/kg/day dose level, was statistically significantly ($p \leq 0.05$; 277% of controls) greater than the controls during the GD 7-10 interval and slightly greater (N.S.; 143% of controls) during the GD 10-13 interval. The increase in weight gain was followed by a significant ($p \leq 0.01$; -22% of control) body weight loss of 27 g by the high dose group compared to a gain of 94 g by the control group during the GD 13-19 treatment interval. The study author concluded that the decrease in body weight gain at 150 mg/kg/day, during GD 13-19, was a treatment-related effect. The reviewer agreed that there was cumulative effect on body weight gain deficit during later part of treatment period (GD 13-19). Although the corrected body weight gain was comparable with the control, there was lack of effect on fetal body weight. This finding suggests that the reduced body weight gain at 150 mg/kg/day was a maternal effect and therefore, was toxicological significant.

There were no treatment-related changes in body weight or body weight gain at the 5 or 30 mg/kg/day treatment levels.

TABLE 2: Maternal body weights and body weight gains during gestation (g)				
Gestational day	Dose in mg/kg/day (# of does)			
	0 (19)	5 (19)	30 (18)	150 (20)
0	3669±219	3669±198	3693±173	3668±195
7	3841±248	3837±258	3813±179	3826±193
10	3868±235	3862±230	3835±199	3901±206
13	3922±237	3909±246	3880±204	3978±226
19	4016±239	3958±294	3948±249	3951±243
24	4029±246	4069±280	3980±287	3991±276
29	4052±249	4009±337	4002±349	4061±285
Body weight gain 7-10	27±36	25±66	22±46	75±56
Body weight gain 13-19	94±68	50±110	67±101	-27±151
Body weight gain 7-19	175±94	122±146	135±139	125±132
Body weight gain 0-29	387±145	322±225	309±252	393±189
Corrected body weight[a]	3617.1±240.9	3558.5±370.2	3646.5±416.6	3595.4±288.6

Data taken from Tables 3, 4, and 14, pp. 35, 36, and 52-55, MRID 40033201.
[a]Corrected body weight = GD 29 body weight minus gravid uterine weight.

3. Food consumption
There were no significant differences in absolute and relative maternal food consumption between the treated groups and controls throughout the study.

4. Gross pathology
There were no significant ($p \leq 0.05$) or treatment-related gross necropsy observations at maternal sacrifice.

5. Cesarean section data
Cesarean section data are summarized in Table 3. There were no statistically significant differences ($p \leq 0.05$) in cesarean section parameters at any treatment level compared to controls.

TABLE 3. Cesarean section observations				
Observations	**Dose in mg/kg/day**			
	0	**5**	**30**	**150**
No. Animals Assigned	20	20	20	20
No. Animals Pregnant	19	19	18	20
Pregnancy rate (%)	95	95	90	100
Maternal Mortality	0	0	0	0
Delivered Early/Aborted	2	1	0	0
Total Corpora Lutea	178±2.6	199±2.5	168±1.8	205±1.8
Corpora Lutea/Doe[a]	10	11	9	10
Total Implantations	141±2.8	159±2.8	119±2.4	180±2.7
Implantations/Doe	8	9	7	9
Preimplantation Loss (%)	37±2.2	40±3.1	49±2.7	26±2.4
Postimplantation Loss (%)	23±1.7	9±0.9	15±1.3	15±0.9
Gravid Uterine Weight (g)	434.9±136.0	450.0±129.6	355.2±158.2	465.5±113.0
Does with Viable Fetuses	17	18	18	20
Total Live Fetuses	118±2.9	150±3.1	104±3.9	165±2.8
Live Fetuses/Litter[a]	7	8	6	8
Live Mean Fetal Weight (g)	41.9±6.9	39.0±5.9	44.1±5.9	40.7±7.7
Sex Ratio (% Male)[a]	51	55	51	46
Total Resorptions	20	9	15	15
Early Resorptions/Doe	0.7	0.4	0.8	0.4
Late Resorptions/Doe	0.5	0.1	0.0	0.4
Resorptions/Doe[a]	1.2	0.5	0.8	0.8
Total Dead Fetuses	3	0	0	0

Data taken from Tables 7, and 14, pp. 39 and 52-55, respectively, MRID 40033201.
[a]Calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

There were no treatment-related or statistically significant ($p \leq 0.05$) external, visceral, or skeletal malformations/variations in the fetuses or litters. When all major malformations are combined, 4/17, 3/18, 3/16, and 3/20 litters in the 0, 5, 30, and 150 mg/kg/day groups, respectively, contained affected fetuses.

1. External examination

There were no treatment-related external malformations/variations observed in fetuses at any dose level. There were two fetuses in one control litter with carpal and/or tarsal flexure.

2. Visceral examination

No treatment-related visceral malformations were observed in any treatment group, compared to controls. Variations common to both controls and treated does were retrocaval ureters and major blood vessel variations.

3. Skeletal examination

There were no dose- or treatment-related skeletal malformations or variations. Malformations/variations common to control and treated litters were malaligned sternebrae, 13th full ribs, sternebrae #5 and/or #6 unossified, 27 presacral vertebrae, 13th rudimentary ribs, and vertebral anomalies with or without associated rib anomalies.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

No deaths occurred in any treatment group and no treatment-related clinical signs of toxicity were observed. Three abortions occurred during the study; two in the control group and one in the 5 mg/kg/day group. The study authors concluded that treatment of pregnant rabbits with Pebulate significantly affected only a decreased body weight gain of the 150 mg/kg/day test group during GD 13-19 ($p \leq 0.01$). No treatment-related changes in mean absolute weight and in body weight corrected for gravid uterine weight occurred at this dose and there was no significant change in food consumption. No maternal effects were observed at 5 or 30 mg/kg/day. No embryotoxic effects were observed and there was no increase in the incidence of malformations in litters from treated does. On the basis of the study results, the study author set the maternal LOEL at 150 mg/kg/day and the maternal NOEL was at 30 mg/kg/day. The NOEL for developmental toxicity was considered to be 150 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

No mortality occurred in any treatment group. Three does aborted; 2 controls and one 5 mg/kg/day doe. No treatment-related signs of toxicity were observed for any group. The reviewer agrees with the study author that the maternal LOEL is

150 mg/kg/day based on a significant reduction ($p \leq 0.01$) in body weight at this dosage during days 13-19.
Therefore, the maternal toxicity LOAEL is 150 mg/kg/day based on decreased body weight gain at GD 13-19, and the maternal toxicity NOAEL is 30 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions
Treatment with Pebulate did not cause an increase in the number of dead fetuses or in the number of resorptions. There were also no treatment- or dose-related changes in pre- and postimplantation losses, implantation sites, or number of corpora lutea.

b. Altered growth
There were no treatment- or dose-related differences in gravid uterine weights or in fetal weights. There was no evidence of delayed growth or maturation of fetuses in any dose group compared to controls.

c. Developmental variations
No abnormal differences in external, visceral, or skeletal developmental variations were described.

d. Malformations
The total litter incidence rate of visceral malformations was not statistically significantly increased ($p \leq 0.05$) at any treatment level. There were no treatment-related increases in individual specific visceral malformations nor were there significant treatment-related increases in the incidences of external or skeletal malformations.

Therefore, the developmental toxicity LOAEL is not determined and the developmental toxicity NOAEL is 150 mg/kg/day.

C. STUDY DEFICIENCIES

None.

D. CLASSIFICATION
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a developmental toxicity study in rabbits (83-3b).

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

Tables: Number and percent of fetus with malformations or variations.
(Tables extracted from pages 40-42 of the study report, MRID 40033201)

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