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

008809

NOV 12 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: EPA ID No.: 055801. Guideline 83-3. Naphthalene: Evaluation of a Developmental Toxicity Study with Rabbits

Submission No.: S393297
HED Project No.: 1-0909
Tox. Chem. No.: 587

FROM: Krystyna K. Locke, Toxicologist Krystyna K. Locke 10/1/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

TO: Jay Ellenberger/Karen Samek, PM 50
Generic Chemical Support Branch
Special Review and Reregistration Division (H7508W)

THRU: Roger Gardner, Section Head
Section I, Toxicology Branch I Roger Gardner 1-4-91 KB 11/5/91
Health Effects Division (H7509C)

Toxicology Branch/HED has completed an evaluation of the following study:

Margitich, D.J. Development toxicity study in rabbits. (Unpublished study No. PH 329-TX-001-85 conducted by Pharmakon Research International, Inc., Waverly, PA, and submitted by Texaco, Inc., Deacon, NY; dated February 17, 1986.)
MRID No. 00157145

Naphthalene (another name: 5601-56-1) was administered by gavage to 18 artificially inseminated New Zealand white rabbits per group at doses of 0, 40, 200 and 400 mg/kg/day during gestation days 6 through 18. The dose levels used were based on the results of a range-finding study in rabbits (Study No. 329DR-TX-001-85; MRID No. 00152835 ; not reviewed separately).

Maternal Effects:

NOEL: < 40 mg/kg (Not clearly established)
LOEL: ≤ 40 mg/kg (aggressive behavior, diarrhea, dyspnea, decreased activity, and ocular and nasal discharges in 1 or 2 rabbits).

Developmental Effects

NOEL: ≥ 400 mg/kg

Core Classification: Supplementary (upgradeable). Data submission is incomplete. The identity, purity, and analysis for test material stability, homogeneity and concentration in dosing medium (methylcellulose) were not submitted. Also, no signed Statement of No Data Confidentiality Claim was provided.

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EPA No.: 68D80056
DYNAMAC No.: 367-D
TASK No.: 3-67D
September 20, 1991

# DATA EVALUATION RECORD

## NAPHTHALENE

## Developmental Toxicity Study in Rabbits

STUDY IDENTIFICATION: Margitich, D.J. Developmental toxicity study in rabbits. (Unpublished study No. PH 329-TX-001-85 conducted by Pharmakon Research International, Inc., Waverly, PA, and submitted by Texaco, Inc., Beacon, NY; dated February 17, 1986.) MRID No. 00157145.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir
Date: 9/20/91

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DATA EVALUATION RECORD

1. CHEMICAL: Naphthalene.

2. TEST MATERIAL: 5601-56-1 (naphthalene), purity not stated; white crystalline flake; order No. J-277.

3. STUDY/ACTION TYPE: Developmental toxicity study in rabbits.

4. STUDY IDENTIFICATION: Margitich, D.J. Developmental toxicity study in rabbits. (Unpublished study No. PH 329-TX-001-85 conducted by Pharmakon Research International, Inc., Waverly, PA, and submitted by Texaco, Inc., Beacon, NY; dated February 17, 1986.) MRID No. 470144-082.

5. REVIEWED BY:

Pia Lindström, DPH
Principal Reviewer
Dynamac Corporation

Signature: Pia Lindström
Date: September 20, 91

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

Signature: James R. Plautz
Date: September 20, 1991



6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: September 20, 1991

Krystyna Locke, Ph.D.
EPA Reviewer
Review Section I
Toxicology Branch I
(H-7509C)

Signature: Krystyna R. Locke
Date: 11/7/91

Karl Baetcke, Ph.D.
Branch Chief
Toxicology Branch I
(H-7509C)

Signature: Roger Gardner for
Date: 11-7-91

STUDY TYPE: Developmental toxicity. Guideline §83-3.

MRID NUMBER: 470144-082. MRID 00157145

TEST MATERIAL: 5601-56-1 (naphthalene), purity not stated; white crystalline flake; order No. J-277.

SYNONYMS: None reported.

STUDY NUMBER: PH 329-TX-001-85.

SPONSOR: Texaco, Inc., Beacon, NY.

TESTING FACILITY: Pharmakon Research International, Inc., Waverly, PA.

TITLE OF REPORT: Developmental Toxicity Study in Rabbits.

AUTHOR: Margitich, D.J.

REPORT ISSUED: February 17, 1986.

CONCLUSIONS: A developmental toxicity study was conducted in which New Zealand White rabbits were administered 5601-56-1 via gavage at 0, 40, 200, or 400 mg/kg/day during gestational days (GD) 6 through 18. Maternal toxicity was evidenced as an increased incidence of clinical signs at all treatment levels and an increased rate of abortions and decreased food consumption during the dosing period at the highest dose level. Maternal NOEL was, therefore, not determined in this study. Toxic signs, observed in some rabbits in the 40-mg/kg group (LDT), included aggressive behavior, diarrhea, dyspnea, decreased activity, and white ocular and nasal discharges.

Developmental toxicity was not observed in this study.

Classification: CORE Supplementary Data. Because of the lack of information on the test material, this study does not meet the minimum requirements set forth under EPA Guideline §83-3 for a developmental toxicity study in rabbits. However, this classification may be upgraded to Core Minimum upon submission and acceptance of the missing data (listed under Study and Reporting Deficiencies).

A. MATERIALS:

Test Compound:
Purity: Not reported.
Description: White crystalline flake.
Order No.: J-277.
Contaminants: Not reported.

Vehicle: 1% methylcellulose (source: Fischer Scientific, Lot No. 745522).

Test Animals:
Species: Rabbit.
Strain: New Zealand White.
Source: Hare Marland, Hewitt, NJ.
Age: Minimum 24 weeks at initiation.
Weight: 3358-4741 g on GD 0.

B. STUDY DESIGN:

This study was designed to assess the potential of 5601-56-1 to cause developmental toxicity in rabbits when administered daily via gavage from GD 6 through 18, inclusive.

Mating: Following a minimum of 10 days of acclimation, females were artificially inseminated using semen from proven males. The day of insemination was designated day 0 of gestation.

Group Arrangement: Animals were randomly (based on body weight) assigned to dose groups as follows:

| Test Group | Nominal Dose Level (mg/kg/day) | Number Assigned per Group |
|---|---|---|
| Control | 0 | 18 |
| Low dose | 40 | 18 |
| Mid dose | 200 | 18 |
| High dose | 400 | 18 |

Dosing: Doses were administered daily via gavage on GD 6 through 18 in a volume of 2 mL/kg. The most recently recorded individual body weights were used to calculate the concentration of the doses. Dose suspensions were prepared daily in 1% methylcellulose. No information was provided regarding the concentrations, stability, and homogeneity of the test compound in the vehicle.

The selection of dose levels was based on the results of a range-finding study in rabbits (study No. PH 329DR-TX-001-85; MRID No. 470103-010), in which rabbits were dosed at 0, 50, 250, 630, or 1000 mg/kg/day via gavage on GD 6-18. In that study, maternal toxicity was observed at 630 mg/kg/day; no developmental toxicity was noted.

Observations: Animals were observed once daily for mortality and overt signs of toxicity. Animals found dead were examined macroscopically. Gross maternal findings and fetuses from these animals were preserved in 10% neutral buffered formalin for possible microscopic examination. Body weight was recorded on GD 0, 6, 12, 18, 24, and 29. Food consumption was recorded for 2-day intervals during the entire gestation. Females were sacrificed on GD 29 by sodium pentobarbital injection, and litters were delivered by cesarean section. Examination of the dams at sacrifice included the following:

- Gross pathological observations;
- Number of corpora lutea;
- Gravid uterine weight;
- Number of implantation sites; and
- Number and location of resorptions (early and late) and live and dead fetuses.

All fetuses were examined in the following manner:

- Individual fetuses were weighed and sexed;
- External anomalies were recorded, including the palate and the eyes;
- Visceral anomalies were evaluated by dissection; and
- Skeletal anomalies were evaluated after staining with Alizarin Red S.

Statistical Analysis: The following methods were used.

- Sex distribution and number of litters with malformations--Chi-square test criterion with Yate's correction for 2x2 contingency tables;
- Numbers of early resorptions and postimplantation loss--Mann-Whitney U-test; and
- Numbers of viable fetuses, implantations, and corpora lutea, and fetal body weight--ANOVA and Dunnett's test.

The level of significance was set at $p \leq 0.05$.

Compliance:

- No signed Statement of No Data Confidentiality Claim was provided;
- A signed Statement of Compliance with FDA, EPA, and OECD GLPs, dated February 17, 1986, was provided; and
- A signed Quality Assurance Statement, dated February 17, 1986, was provided.

C. RESULTS:

The following results were reported by the study author:

1. Test Material Analysis: No information was provided.
2. Maternal Toxicity:

   Mortality: Two animals in the 400-mg/kg/day group died on GD 15 and 18; necropsy confirmed gavage errors for both animals.



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Abortion: Two animals in the 400-mg/kg/day group aborted on GD 23 and 27. One premature delivery was noted on GD 29 in the 40-mg/kg/day group.

Clinical Observations: The following clinical signs were observed: excessive facial grooming in one animal at 40 mg/kg/day and in several animals at 200 and 400 mg/kg/day; vocalization immediately after dosing in several animals at 200 and 400 mg/kg/day; agressive behavior and loose stools in one animal at 40 mg/kg/day; diarrhea, dyspnea, decreased activity, and a white ocular and nasal discharge in one animal at 40 mg/kg/day, one animal at 200 mg/kg/day, and two animals at 400 mg/kg/day; alopecia in two animals at 400 mg/kg/day; and dyspnea, cyanosis, body drop, decreased activity and salivation in several animals at 200 and 400 mg/kg/day. (Individual data were not submitted for any animals but were reported only as above.)

Body Weight: A summary of maternal body weight gain and corrected weight gain data is presented in Table 1. Body weight (data not shown), body weight gain, and corrected body weight were similar in all dose groups, including the control group.

Food Consumption: A summary of food consumption is presented in Table 2. Significant decreases were observed on GD 7-15 in the 400-mg/kg/day group, while significant increases were observed on GD 23-25 and 27-29 (data not shown).

Gross Pathological Observations: No gross observations were reported.

Cesarean Section Observations: A summary of cesarean section data is presented in Table 3. No significant differences were observed in any parameter.

3. Developmental Toxicity:

A summary of incidences of malformations is presented in Table 4.

External Examinations: No external malformations were observed. One fetus in the control group, two fetuses in the 40-mg/kg/day group, and two fetuses in the 200-mg/kg per day group exhibited abnormal curvature of a rear appendage (variation; data not shown).

Visceral Examinations: One fetus in the control group exhibited umbilical herniation (Table 4). No variations were noted.

TABLE 1. Mean Body Weight Gain (g ± S.D.)[a,b]

| Dose Group (mg/kg/day) | Prior to Dosing Period (GD 0-6) | Dosing Period (GD 6-18) | Post-dosing Period[b] (GD 18-29) | Entire Gestation Period (GD 0-29) | Corrected Body Weight Gain[c] |
|---|---|---|---|---|---|
| 0 | 55 ± 73 | 154 ± 134 | 167 ± 101 | 376 ± 253 | -186 ± 239 |
| 40 | 64 ± 86 | 99 ± 123 | 186 ± 98 | 360 ± 152 | -101 ± 237 |
| 200 | 104 ± 106 | 33 ± 196 | 204 ± 123 | 342 ± 239 | -173 ± 245 |
| 400 | 96 ± 98 | -120 ± 242 | 284 ± 193 | 293 ± 184 | -207 ± 174 |

[a]Data were extracted from Study No. PH 329-TX-001-85, Table 2.

[b]Calculated by the reviewers.

[c]Calculated as GD 29 body weight - GD 0 body weight - gravid uterine weight.

TABLE 2. Mean Food Consumption (g/animal/day ± S.D.)[a,b]

| Dose Group (mg/kg/day) | Prior to Dosing Period (GD 0-7) | Dosing Period (GD 7-19) | Post-dosing Period[b] (GD 19-29) | Entire Gestation Period (GD 0-29) |
|---|---|---|---|---|
| 0 | 77 ± 22 | 92 ± 34 | 78 ± 16 | 86 ± 25 |
| 40 | 96 ± 15 | 92 ± 26 | 85 ± 26 | 88 ± 24 |
| 200 | 93 ± 29 | 85 ± 36 | 93 ± 16 | 89 ± 24 |
| 400 | 95 ± 21 | 55 ± 25* | 80 ± 46 | 66 ± 30 |

[a]Data were extracted from Study No. PH 329-TX-001-85, Table 4.

[b]Calculated by the reviewers using ANOVA and Dunnett's test.

*Significantly different from control ($p < 0.05$).

## TABLE 3. Cesarean Section Observations[a]

| Parameter | Dose Level (mg/kg/day) 0 | 40 | 200 | 400 |
|---|---|---|---|---|
| No. animals assigned | 18 | 18 | 18 | 18 |
| No. animals pregnant | 13 | 16 | 17 | 15 |
| Pregnancy rate (%) | 72 | 90 | 94 | 83 |
| Maternal wastage | | | | |
| No. died/pregnant | 0 | 0 | 0 | 2 |
| No. died/nonpregnant | 0 | 1 | 0 | 0 |
| No. nonpregnant | 5 | 2 | 1 | 3 |
| No. aborted | 0 | 0 | 0 | 0 |
| Uterine weight/doe | | | | |
| Total No. corpora lutea | 167 | 206 | 205 | 155 |
| Corpora lutea/doe | 12.9 ± 2.4[b] | 13.7 ± 3.2 | 12.1 ± 2.8 | 14.1 ± 2.5 |
| Total No. implantations | 118 | 110 | 148 | 100 |
| Implantations/doe | 9.1 ± 1.5 | 7.3 ± 3.9 | 8.7 ± 2.7 | 9.1 ± 2.7 |
| Total No. live fetuses | 107 | 103 | 129 | 84 |
| Live fetuses/doe | 8.2 ± 1.7 | 6.9 ± 3.7 | 7.6 ± 2.8 | 7.6 ± 2.3 |
| Total No. resorptions | 9 | 6 | 16 | 13 |
| Early | 6 | 5 | 12 | 10 |
| Late | 3 | 1 | 4 | 3 |
| Resorptions/doe[c] | 0.7 ± 1.0 | 0.4 ± 0.7 | 0.9 ± 1.4 | 1.2 ± 1.3 |
| Total No. dead fetuses | 2 | 1 | 3 | 3 |
| Fetal weight/litter (g) | 44.0 ± 5.3 | 47.4 ± 6.7 | 44.5 ± 5.8 | 42.0 ± 6.3 |
| Preimplantation loss (%) | 29.3 | 46.6 | 27.8 | 35.5 |
| Postimplantation loss/doe | 0.9 ± 1.1 | 0.5 ± 0.7 | 1.1 ± 1.5 | 1.5 ± 1.4 |
| Postimplantation loss (%) | 9.3 | 6.4 | 12.8 | 16.0 |
| Sex ratio (% male) | 54 | 57 | 63 | 49 |

[a]Data were extracted from Study No. PH 329-TX-001-85, Tables 5 and 6.

[b]Mean ± S.D.

[c]Calculated by the reviewers.

## TABLE 4. Summary of Fetal Malformations[a]

| Finding[b] | Dose Level (mg/kg/day) 0 | 40 | 200 | 400 |
|---|---|---|---|---|
| No. fetuses (litters) | 109 (13) | 104 (15) | 132 (17) | 87 (11) |
| VISCERAL OBSERVATIONS: | | | | |
| Umbilical herniation | 1 | 0 | 0 | 0 |
| SKELETAL OBSERVATIONS: | | | | |
| Fused sternebrae | 2 (2) | 0 | 1 | 3 (3) |
| TOTAL NO. FETUSES (LITTERS) WITH MALFORMATIONS | 3 (3) | 0 (0) | 1 (1) | 3 (3) |

[a]Data were extracted from Study No. PH 329-TX-001-85, Tables 7, 8, and 9.

[b]More than one finding may be observed in one fetus.

Skeletal Examinations: Fused sternebrae were observed in two fetuses (two litters) in the control group, one fetus in the 200-mg/kg/day group, and three fetuses (three litters) in the 400-mg/kg/day group (Table 4). Variations were noted in all groups at similar incidences and included delayed ossification in the skull, sternebrae, and ribs, enlarged/diminished fontanelle, bipartite sternebrae, and extra/rudimentary/floating rib (data not shown).

D. REVIEWERS' DISCUSSION/CONCLUSIONS:

1. Acceptance Criteria: The reviewers have completed an Acceptance Criteria checklist (Attachment I) that is included with this evaluation. Criteria 1 (purity of test material) and 6 (analytical chemistry data on test material) were not satisfied. Criteria 7 (individual daily observations) and 10 (individual necropsy observations) were carried out; however, only results for the affected animals (identified by their ID numbers) were submitted, which is acceptable.

2. Test Material Analyses: The report did not state the purity of the test compound. In addition, no data were submitted to support the stability, homogeneity and concentrations of the test compound in the vehicle.

3. Maternal Toxicity: Maternal toxicity was evidenced by clinical signs at 40, 200, and 400 mg/kg/day, an increased rate of abortions at 400 mg/kg/day, and significantly decreased food consumption during dosing at 400 mg/kg/day.

   Although very few animals exhibited clinical signs at 40 mg/kg/day, the signs were the same as those observed at the higher dose levels, and therefore, they were considered to be compound-related.

   Two animals aborted at 400 mg/kg/day; no animals aborted in the other dose groups. Since historical data were not submitted to show that the present rate was within the normal range, this increased abortion rate was considered to be a compound-related effect.

   Based on these results, maternal toxicity was observed at all dose levels and the NOEL was, therefore, not determined.

4. Developmental Toxicity:

   a. Deaths/Resorptions: Postimplantation loss was increased from 9.3% in the control group to 16.0% in the 400-mg/kg/day group (0.9/doe in the control group

to 1.5/doe in the 400-mg/kg/day group). This increase was not statistically significant and does not appear to be biologically significant.

b. Altered Growth: A slight (5%) decrease was observed in fetal body weight in the group dosed at 400 mg/kg/day. This decrease was not statistically significant and appears to be a normal variation rather than a compound-related effect.

c. Developmental Anomalies: No compound-related effects were observed.

Based on these results, developmental toxicity was not observed in this study.

5. Study and Reporting Deficiencies:

a. Purity of the test compound was not reported.

b. Analytical chemistry data on the test compound were not reported.

c. No signed Confidentiality Claim was submitted.

E. CLASSIFICATION: CORE Supplementary data. This classification may be upgraded to Core Minimum upon submission and acceptance (by TB/HED) of the missing information.

Although maternal NOEL was not determined in this study (clinical signs were observed in the low-dose group), the study does not have to be repeated. Toxic signs, observed in this study in the low-dose group, were similar to those observed in other studies with pregnant rats and rabbits, which are in EPA files. In other words, maternal toxicity data may readily be obtained from other studies. Also, very few low-dose rabbits (only one or two) exhibited toxic signs in the current study.

Maternal NOEL = <40 mg/kg/day (LDT; aggressive behavior, diarrhea, dyspnea, decreased activity, and ocular and nasal discharges).

Developmental Toxicity NOEL = ≥400 mg/kg/day (HDT).

F. RISK ASSESSMENT: Not applicable.

MRID NO. 470144082 008809

Study NO. PH 329-TX-001-85

ATTACHMENT I Study Date : 2/17/86

83-3 Teratology Studies (Rabbit)

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

| | | |
|---|---|---|
| 1. | ? | Technical form of the active ingredient tested. |
| 2. | YES | At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group). |
| 3. | YES | At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1000 mg/kg). |
| 4.* | YES | At the low dose, no developmental toxicity is reported. |
| 5. | YES | Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term. |
| 6.* | NO | Analysis for test material stability, homogeneity, and concentration in dosing medium. |
| 7. | Y/N | Individual daily observations. |
| 8. | YES | Individual body weights. |
| 9. | YES | Individual food consumption. |
| 10. | Y/N | Necropsy on all animals. |
| 11. | YES | Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex. |
| 12. | YES | All ovaries examined to determine number of corpora lutea. |
| 13. | YES | Individual litter weights and/or individual fetal weights/sex/litter. |
| 14. | YES | Individual fetal external examination. |
| 15. | YES | Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits. |
| 16. | YES | Individual fetal soft tissue examination. |

Criteria marked with an asterisk are supplemental; they may not be required for every study.

- Only findings for affected animals (with their ID numbers) were submitted, which is acceptable. However, it was stated, that all animals were observed daily and all were necropsied.