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

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MAR 23 1993

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM:

Subject: EPA ID # 067501: Piperonyl butoxide. Review of Developmental Toxicity Study in Rat (MRID # 423808-01)

EPA Submission No. S421535/S432462
Caswell No. 670
P.C. No. 067501
HED Project No. D180523/D186054

From: Guruva B. Reddy, D.V.M., Ph. D. 3/15/93
Section 4
Toxicology Branch I
Health Effects Division (H7509C)

To: Bruce Sidwell/Teung Chin
Project Manager 53
Reregistration Division (H7508W)

Thru: Marion P. Copley, D.V.M., D.A.B.T Marion P. Copley 3/19/93
Section Head
Section 4, Toxicology Branch I
Health Effects Division (H7509C)

1. **CONCLUSIONS:**

The developmental toxicity study in the rat was reviewed and determined to be core-Guideline.

This study satisfy the requirements of Subdivision F Guideline, 83-3 for the developmental toxicity study in the rat.

A copy of the DER is attached.

2. **Action Requested:**

The Piperonyl Butoxide Task Force II has submitted developmental toxicity study in the rat in support of FIFRA 88. The study was reviewed and a copy of the DER is attached.

cc: Doherty

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3. **Study reviewed:** 010108

Study/Classification	TB-I Comments
83-4. Developmental Toxicity Species: rat Bushy Run Res. Center, PA #54-586, Dec. 20, 1991 MRID #: 423808-01 core-Guideline Rangefinding study MRID 425869-01	Doses administered: 0, 200, 500 and 1,000 mg/kg/day, administered by gavage in deionized water to pregnant Sprague-Dawley CD rats from Days 6 through 15 of gestation, inclusive. **Maternal NOEL: 200 mg/kg/day. LEL: 500 mg/kg/day,** based upon statistically significant reduction in body weight gain during treatment and food consumption during Days 6 - 9 of treatment and increased incidence of urogenital red discharge and perinasal encrustation. At 1000 mg/kg/day, in addition to depressed body weight gain and feed consumption, the incidence of urogenital wetness, urine stains, red discharge and perinasal en ustation increased. **Developmental NOEL ≥ 1,000 mg/kg/day.**

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GUIDELINE: 83-3

Review by: Guruva B. Reddy, DVM, PHD 3/5/93
Review Section IV, Toxicology Branch I (7509C)
Secondary Reviewer: Marion P. Copley, DVM, DABT Marion P. Copley 3/9/93
Section Head, Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

TOX. CHEM NO: 670

MRID NO.: 423808-01

PC NUMBER: 067501

TEST MATERIAL: Piperonyl butoxide

SYNONYMS: None

STUDY NUMBER or LAB. PROJECT ID: 54-586

SPONSOR: Piperonyl Butoxide Task Force II

TESTING FACILITY: Bushy Run Research Center
Export, PA 15632-8902

TITLE OF REPORT: Developmental Toxicity Evaluation of Piperonyl Butoxide Administered by Gavage to CD® (Sprague-Dawley) Rats

AUTHOR(S): J. C. Chun and T. L. Neeper-Bradley

REPORT ISSUED: December 20, 1991

CONCLUSION:

Doses administered: 0, 200, 500 and 1,000 mg/kg/day, administered by gavage in deionized water to pregnant Sprague-Dawley CD rats from Days 6 through 15 of gestation, inclusive.

Maternal NOEL: 200 mg/kg/day. LEL: 500 mg/kg/day, based upon statistically significant reduction in body weight gain during treatment and food consumption during Days 6 - 9 of treatment and increased incidence of urogenital red discharge and perinasal encrustation. At 1000 mg/kg/day, in addition to depressed body weight gain and feed consumption, the incidence of urogenital wetness, urine stains, red discharge and perinasal encrustation increased.

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Developmental NOEL ≥ 1,000 mg/kg/day. 010105

Classification: core-Guideline

The information presented for this Developmental Toxicity Study in rats satisfies the criteria set forth in Subdivision F Series, 83-3.

A. Materials

Test Compound: Purity: 90.78 %
Description: Yellow, slightly viscous liquid
Lot No.: FEP-100
Contaminant: Not specified

Vehicle(s): Deionized water

Test Animal(s):
Species: Rats
Strain: CD® (Sprague-Dawley; Crl:CD® BR)
Source: Charles River Breeding Labs., Inc., Portage, MI
Age: ≈ 56 days
Weight: 175 - 200 g (females)

B. Study Design

This study was designed to assess the developmental toxicity potential of piperonyl butoxide when administered by gavage to timed-pregnant CD® (Sprague-Dawley) rats on gestation days 6 through 15, inclusive. Feed (Certified Rodent Chow® #5002, Ralston Purina Co., St. Louis, MO) and water was provided ad libitum. Animals were maintained at temperature of 66 - 77°F, relative humidity of 40 to 70 % and 12 hours of light and dark cycles. On Day 0, a total of 25 plug-positive females each were assigned to the following treatment groups using a weight-stratified randomization procedure.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	200	25
Mid Dose	500	25
High Dose	1,000	25

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C. Methods

Mating

Untreated virgin female rats were mated 1:1 with untreated virgin males of same breed. Animals were cohabited overnight and successful matings identified in the morning by vaginal copulation plugs and dropped vaginal plugs beneath the cages. These females were considered to be fertilized and that day was designated as Day 0 of the study. Pregnant females were housed individually for the duration of the study.

Dosing:

Range Finding Studies (MRID# 425869-01): Dose levels tested - 250, 500, 1000, 2000 and 4000 mg/kg/day. Four of five dams in the 1000 mg/kg/day and 5/5 dams in the 4000 mg/kg/day dose level died or were sacrificed moribund. At the 500 and 1000 mg/kg/day dose levels clinical signs and weight loss were observed.

In the actual study, the test substance was administered, undiluted, by gavage. Controls received deionized water at a dose equivalent to that used in high dose group. The dose volumes were based on the dam's body weight on gestation day 6, the percent active ingredient and specific gravity of the test substance.

Results: The purity of undiluted test compound was reported as 90.78 %. No impurities were listed.

Observations

The animals were checked for mortality or abnormal condition twice daily during dosing period. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of: evaluation of body weight, liver and gravid uterine weight, number of corpora lutea, and number and status of implantation sites (including early and late resorptions, dead fetuses and live fetuses).

All live fetuses were counted, weighed, sexed and examined for external malformations. About one-half of the fetuses were examined for thoracic and abdominal visceral abnormalities according to Staples (Teratology: Detection of Visceral Alterations in Mammalian Fetuses, A37, 1974). These fetuses were decapitated and the heads were fixed in Bouin's solution and examined for soft tissue craniofacial malformations and variations according to the method of Wilson (Teratology: Principles and Techniques p. 251; U. of Chicago Press, 1965). The remaining half of the fetuses were fixed in ethanol, stained with alizarin red S and examined for skeletal malformations and

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variations.

Historical control data were provided from 29 studies on cervical centrum #5 and #6 vertebral malformations and variations to allow comparison with concurrent controls. The report did not specify when the studies were conducted.

Statistical analysis

The data for quantitative continuous variables were intercompared for the three groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA) and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogenous variances, all groups were compared by an ANOVA for unequal variances and followed by a t-test for pairwise comparisons. Nonparametric data were analyzed using Kruskal-Wallis test, followed by the Mann-Whitney U test. Incidence data were evaluated using the Fisher's Exact Test.

Compliance

A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

D. Results

1. Maternal Toxicity

Mortality

No maternal deaths occurred during the study.

Clinical Observations

13 of 24 high dose animals showed urogenital wetness during the study. Of these, 8 animals stained once between days 9 to 15 and 5 animals exhibited wetness for extended periods ranging from 2 to 7 days. In this group, urine stains in 3 animals, red discharge in 1 animal and perinasal encrustation in 2 animals were observed. Perinasal encrustation and red urogenital discharge was observed in 1 and 2 mid dose animals, respectively. These effects were considered treatment-related.

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Body Weight 010106

Maternal body weights were measured on Days 0, 6, 9, 12, 15 18 and 21. Following are the weight gain data for the specified intervals:

TABLE 1. BODY WEIGHT GAIN (GRAMS)*

	PIPERONYL BUTOXIDE MG/KG/DAY			
INTERVAL	0	200	600	1,000
PRETREATMENT: 0 - 6 DAYS	36.0	32.7	30.2	28.3
TREATMENT: 6 - 9 DAYS	11.4	8.8*	9.8	8.8**
9 - 12 DAYS	18.9	17.8	15.4	16.1
12 - 15 DAYS	22.1	21.2	17.4*	17.2*
6 - 15 DAYS	52.4	47.8	42.6**	41.8**
POST-TREATMENT: 15 - 21 DAYS	103.2	100.6	98.7	94.7
GESTATION: 0 - 21 DAYS	180.6	181.0	171.8	172.0

* Data taken from summary Table 3
* $P < 0.05$, ** $P < 0.001$

Maternal body weight gain appeared to be depressed in all treated groups between 6 - 9 days of treatment, but the decrease was only significant in low and high dose groups (Table 1). The decrease was 22.8 and 51%, for the low and high dose animals, respectively, when compared to the controls, and were consistent with decreased food consumption during this period (Table 2). The maternal body weight depression in 200 mg/kg/day dams appear unrelated to treatment since the trend was not seen during the remaining gestation period. Maternal body weight gain was significantly depressed at mid and high dose during gestation days 12 - 15 and during treatment (6 - 15 Days). In addition, weight gains appeared to be depressed for gestation days 6 - 15 in low dose, but it was due to one gravid female (#7525) which bore only one live fetus. When weight of this dam was excluded, the mean body weight gain for this group was comparable to that of the controls. During the treatment period the body weight gain of mid and high dose dams was 18.3 and 21.8 %, respectively, when compared to the controls and was considered treatment-related. Body weight gain during the gestation days 15 - 21 for the treated groups appear depressed but not significantly.

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Food Consumption

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Food consumption was recorded for intervals 0 - 3, 3 - 6, 6 - 9, 9 - 12, 12 - 15, 15 - 18 and 18 - 21 days. Following are the food consumption data:

TABLE 2. FOOD CONSUMPTION (GRAMS/ANIMAL/DAY)*

	PIPERONYL BUTOXIDE MG/KG/DAY			
INTERVAL	0	200	500	1,000
PRETREATMENT: 0 - 6 DAYS	26.0	25.7	25.6	26.1
TREATMENT: 6 - 9 DAYS	25.7	24.1**	22.7**	21.1**
9 - 12 DAYS	23.6	27.4	25.6	25.0
12 - 15 DAYS	29.7	29.1	27.6	28.7
6 - 15 DAYS	26.3	26.8	25.3**	25.1*
POST-TREATMENT: 18 - 21 DAYS	32.6	31.4	31.2	32.6
GESTATION: 0 - 21 DAYS	26.9	27.6	27.0	26.6

* Data taken from summary Table 4
* $P < 0.05$, ** $P < 0.001$

Food consumption significantly decreased in the 200, 500 and 1,000 mg/kg/day groups for Days 6 - 9 of gestation (10, 15 and 21 %, respectively). This decrease in food consumption corresponded with depressed body weight gains during this period (Table 1). Although food consumption was significantly reduced in the 200 mg/kg/day group for Days 6 - 9, but the decreased food consumption did not last through the gestation period and therefore, considered to be of no biological significance. The magnitude of reduction in food consumption in the 500 and 1000 mg/kg/day dams on Days 6 - 9 and 9 - 12 resulted in significant reduction in food consumption during the treatment period (9.2 and 7.8 %, respectively) and was considered to be treatment-related.

Gross Pathological Observations

No treatment-related gross pathological observations were noticed in dams at necropsy among any treatment group.

Absolute and relative liver weights increased by 7.7 and 10.6 % respectively, at 1000 mg/kg/day group, respectively; increased relative liver weights were statistically significant. These increases may be the result of reduced body weights and

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compensatory phenomenon in the treated and therefore of no biological significance.

Cesarean section Observations

No dose-related effects on percent preimplantation loss and numbers of total and viable implants or treatment-related early resorptions, late resorptions, percent live fetuses, fetal weights and sex ratios were observed in dams at necropsy (see the attached Table 7 from study report).

2. Developmental Toxicity

External Examinations:

No treatment-related external malformations/variations were observed in fetuses at necropsy in any treated groups.

Visceral Examinations:

The summary visceral variations are presented below:

Table 3: Visceral Examinations[1]

Observations	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	184(24)	162(22)	170(23)	165(24)
#pups(litters) affected	12(9)	34(16)	11(8)	20(9)
Lateral ventricle-dilated (%)	6.5(37.5)°	21(72.7)*	6.5(35)	12(37.5)

[1] Data extracted from summary Table 9
(°) fetal [litter] incidence

The increased incidence of dilated lateral ventricals was observed in low dose fetuses and the increase was statistically significant. The incidence in the mid dose and high dose fetuses were comparable to that of controls. The incidence of dilated lateral ventricles, at 200 mg/kg/day, lacked dose-relationship, therefore, considered spurious.

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Skeletal Examinations:

Table 4: Skeletal Examinations[1]

Observations*	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	171(24)	149(21)	161(23)	152(24)
#pups(litters) affected				
Unossified Cervical centra, 1-4				
#Pups/litter	111/23	113/21	141/23	123/24
Pups/litter (%)	64.9/95.8	75.8/100	87.6/100	80.9/100
Unossified Cervical centrum #5				
#Pups/litter	16/8	31/13	47/19**	55/19**
Pups/litter (%)	9.4/33.3	20.8/61.9	29.2/82.6	36.2/79.2
(historical control range: Fetus 7.5 - 45.7%; mean 22.6%)				
(Litter 43.5 - 82.6%; mean 61.3%)				
Unossified Cervical centrum #6				
#Pups/litter	17/10	23/11	35/1[illegible]	48/18*
Pups/litter (%)	9.9/41.7	15.4/52.4	21.7/56.5	31.6/75.0
(historical control range: Fetus 6.3 - 32.5%; mean 19.1%)				
(Litter 30.4 - 72%; mean 55.8%)				
Unossified Cervical centrum #7				
#Pups/litter	2/2	0/0	6/4	11/7
Pups/litter (%)	1.2/8.3	0/0	3.7/17.4	7.2/29.2

[1] Data extracted from summary Table 9
* P <0.05
** P <0.01

A dose-related increase in the incidence of unossified cervical centra #5 and 6 was observed in the fetuses. The incidence for the cervical centra #5 was statistically significant in the mid and high dose fetuses, when compared to the controls. A statistically significant increase in the unossified cervical centra #6 was also observed in the high dose fetuses. There were no malformations in the cervical centra 1 - 4 or between 6 and 7. In the controls, the litter incidence of malformations for cervical centrum 1 to 7 was 100%. The authors concluded that the above malformations were unrelated to treatment because of highly variable nature of the skeletal findings and that the incidence of these findings were within the historical control ranges established for this strain and age of rats (Appendix 1). We have further examined individual fetal data with regard to weight differences between the litters affected with incidences and the unaffected litters and the controls. There appears to be no difference between the fetal weights of the aforementioned groups. Therefore, we concur with the study authors' conclusions that these effects were not

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related to treatment and considers 1,000 mg/kg/day the NOEL for skeletal variations (unossified cervical centrum #5).

D. Discussion/Conclusions

The data reporting was thorough and the summary means that were validated were supported by the individual animal data. The doses selected were based upon a rang finding study. The data indicate that maternal toxicity was reached at 500 mg/kg/day based on decreased body weight gain.

a. **Maternal Toxicity:** At 500 and 1,000 mg/kg/day, food consumption was slightly, but significantly reduced during treatment (9.2 and 7.8 %, respectively) and body weight gain was significantly reduced during the treatment period (18.3 and 21.8 %, respectively), when compared to the controls. These decreases were due to significant reduction in food consumption (15 and 21 %, respectively) during 6 - 9 days of treatment and subsequent reductions during the treatment. At 200 mg/kg/day, food consumption was reduced (10 %; P <0.01) significantly, however, the magnitude of reduction in food consumption was not adequate to depress the weight gain (8.8 %) significantly. In addition, an increased incidence of urogenital wetness (13/24; 54 %), urine stains (12.5 %), red discharge (4 %) and perinasal encrustation (8 %) were observed in high dose dams. In the mid dose females perinasal encrustation and red urogenital discharge were observed in 1 of 24 (4 %) and 2 of 24 (8 %) dams, respectively. TB-I agree with the study authors' conclusions that these effects were probably treatment-related and consider **Maternal NOEL: 200 mg/kg/day and LEL: 500 mg/kg/day** for this study. based on statistically significant reduction in body weight gain during treatment and food consumption during Days 6 - 9 treatment and increased incidence of urogenital red discharge and perinasal encrustation.

b. **Developmental Toxicity:** At 500 and 1,000 mg/kg/day, there was significantly increased unossified cervical centrum #5. At 1000 mg/kg/day, the incidence of unossified cervical centrum #6 was also significantly increased. These increased incidences of unossified cervical centra appear to be dose-related, and are within the historical range established for this strain and species of rat. We agree with the study authors' conclusions that these effects were not related to treatment and considers the developmental NOEL to be 1,000 mg/kg/day for this study.

D. Study Deficiencies:

Study dates for historical control data were not provided. While this information should have been furnished in the report, the deficiency is not considered significant enough to invalidate the conclusions of the study.

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E. Core Classification: Core-Guideline.

Maternal NOEL = 200 mg/kg/day
Maternal LOEL = 500 mg/kg/day
Developmental Toxicity NOEL = 1,000 mg/kg/day

As presented, the study satisfies the requirements set forth in Subdivision F Guideline, 83-3 for Developmental Toxicity Study in Rats.

Reddy/piperonyl butoxide/dev.der/2-2-93; final 3-18-93
DP Barcode: D180523

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