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

APR 20 1999

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Amine Oxide: review of a developmental toxicity study in rats.

EPA Identification Numbers:

P.C. Code: 000439
Submissions: S538885
ID# 005382-00016

DP Barcode: D253846
MRID: 44762401

TO: Velma Noble / Jackie Campbell
PM Team # 31
Regulatory Management Branch I
Antimicrobials Division (7510C)

FROM: Timothy F. McMahon, Ph.D. 4/15/99
Senior Toxicologist, RASSB
Antimicrobials Division (7510C)

THRU: Laura Morris for 4/16/99
Team Leader, Team Two
RASSB/AD (7510C)

And

Norm Cook, Chief 04/20/99
RASSB/AD (7510C)

Registrant: Procter and Gamble Company

Action Requested: Review of a developmental toxicity study in rats submitted for amine oxide.

Background

The registrant (Proctor and Gamble Company) submitted a developmental toxicity study with the active ingredient amine oxide. The results of review by the Risk Assessment and Science Support Branch, Antimicrobials Division, is summarized below:

CITATION: York, Ramond G. (1996) Oral (Gavage) Developmental Toxicity Study of SIO801.01 in Rats. Study performed by Argus Research Laboratories, Inc. for Proctor and Gamble. Laboratory Project No. 916-025. Submitted under MRID # 44762401. Unpublished

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44762401), amine oxide (32% w/v) was administered by oral gavage to 25 Crl:CD ®BR VAF/Pplus® Sprague-Dawley female rats/dose at dose levels of 0, 25, 100, and 200 mg/kg/day from days 6 through 19 of gestation. On day 20 of gestation , all surviving dams were sacrificed and necropsied. At both the 100 and 200 mg/kg dose levels, a significant number of maternal animals were observed with increased incidence of excessive salivation, rales, and urine stained abdominal fur. Observations of labored breathing, gasping, brown or red perivaginal substance, chromorhinorrhea, emaciation, brown perianal substance, brown perinasal substance, and dehydration were also observed. Mortality occurred in two maternal animals at the 200 mg/kg dose. Statistically significant decreases in maternal body weight were observed from day 11 of dosing to study termination at the 200 mg/kg dose. At the 200 mg/kg dose level, weight gain in maternal rats was decreased by 78%, 48%, 48%, and 37% , respectively, for the study periods days 6-9, 15-18, 6-20, and 0-20. At the 100 mg/kg/day dose level, group mean maternal body weight gain was decreased as well for the time periods days 18-20 and 6-20 by 31% and 17% respectively. Cesarean section observations showed an increase in resorptions (mainly early resorptions), and decreased mean litter weight as well as decreased mean weight of male and female pups at the 200 mg/kg dose. Examination of fetuses for soft tissue and skeletal abnormalities showed an in creased incidence (fetal and litter) of skeletal variations at the 100 and 200 mg/kg dose levels.

Based on the results of this study, the **Maternal NOAEL is 25 mg/kg/day, and the Maternal LOAEL is 100 mg/kg/day,** based on decreased body weight gain, clinical signs, and decreased food consumption at 100 mg/kg/day. The **Developmental NOAEL is 25 mg/kg/day, and the Developmental LOAEL is 100 mg/kg/day**, based on increased incidence of skeletal variations (delayed ossification). There was no evidence of increased susceptibility to fetuses from administration of amine oxide in this study.

This study is classified as **acceptable** (guideline) and satisfies the data requirement for a developmental toxicity study (OPPTS 870.3700) in rats.

EPA Reviewer: T. McMahon, Ph.D. _____ Date: 7/15/99
Senior Toxicologist, RASSB
Antimicrobials Division (7510C)

EPA Secondary Reviewer: Jonathan Chen, Ph.D. Jonathan Chen Date: 7/15/99
Toxicologist, RASSB
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - rat
OPPTS Number: 870.3700
DP BARCODE: D253846
P.C. CODE: 000439

OPP Guideline Number: §83-3a
SUBMISSION: S538885

TEST MATERIAL (PURITY): Amine Oxide
SYNONYMS: Amines, C10-C16 alkyldimethyl, N-oxides; SIO801.01

CITATION: York, Ramond G. (1996) Oral (Gavage) Developmental Toxicity Study of SIO801.01 in Rats. Study performed by Argus Research Laboratories, Inc. for Proctor and Gamble. Laboratory Project No. 916-025. Submitted under MRID # 44762401. Unpublished

SPONSOR: The Proctor & Gamble Company, Cincinnati, OH

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44762401), amine oxide (32% w/v) was administered by oral gavage to 25 Crl:CD ®BR VAF/Pplus® Sprague-Dawley female rats/dose at dose levels of 0, 25, 100, and 200 mg/kg/day from days 6 through 19 of gestation. On day 20 of gestation , all surviving dams were sacrificed and necropsied. At both the 100 and 200 mg/kg dose levels, a significant number of maternal animals were observed with increased incidence of excessive salivation, rales, and urine stained abdominal fur. Observations of labored breathing, gasping, brown or red perivaginal substance, chromorhinorrhea, emaciation, brown perianal substance, brown perinasal substance, and dehydration were also observed. Mortality occurred in two maternal animals at the 200 mg/kg dose. Statistically significant decreases in maternal body weight were observed from day 11 of dosing to study termination at the 200 mg/kg dose. At the 200 mg/kg dose level, weight gain in maternal rats was decreased by 78%, 48%, 48%, and 37% , respectively, for the study periods days 6-9, 15-18, 6-20, and 0-20. At the 100 mg/kg/day dose level, group mean maternal body weight gain was decreased as well for the time periods days 18-20 and 6-20 by 31% and 17% respectively. Cesarean section observations showed an increase in resorptions (mainly early resorptions), and decreased mean litter weight as well as decreased mean weight of male and female pups at the 200 mg/kg dose.
Examination of fetuses for soft tissue and skeletal abnormalities showed an in creased incidence (fetal and litter) of skeletal variations at the 100 and 200 mg/kg dose levels.

Based on the results of this study, the Maternal NOAEL is 25 mg/kg/day, and the Maternal LOAEL is 100 mg/kg/day, based on decreased body weight gain at 100 mg/kg/day. The Developmental NOAEL is 25 mg/kg/day, and the Developmental LOAEL is 100 mg/kg/day, based on increased incidence of skeletal variations. There was no evidence of increased susceptibility to fetuses from administration of amine oxide in this study.

This study is classified as **acceptable** (guideline) and satisfies the data requirement for a developmental toxicity study (OPPTS 870.3700) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. Flagging statements were not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Amine Oxide
   Description: light, straw-colored liquid
   Lot/Batch #: .01
   Purity: Not specified

2. Vehicle: Sterile water

3. Test animals: Species: Rat
   Strain: Crl:CD ®BR VAF/Plus®
   Age at arrival: 65 days
   Weight at mating:218-262 g
   Source: Charles River Laboratories, Raleigh, North Carolina
   Housing: Individually, except during mating periods. During mating, each pair of male and female rats were housed together.
   Diet: Purina Certified Rodent Chow #5002, ad libitum
   Water: Tap water, purified by reverse osmosis, with added chlorine, ad libitum
   Environmental conditions:
   Temperature: 18-26 °C
   Humidity: 30-70%
   Air changes: minimum of 10 air changes per hour
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: stated, but exact duration not specified

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: June 9, 1998 end: July 9, 1998

2. Mating: After acclimation, virgin female rats were placed with male rats in a 1:1 ratio. Gestation day 0 was assumed after observation of a copulatory plug or spermatozoa from a vaginal smear and females were returned to individual cages.

3. Animal Assignment: Female rats were assigned to dosage groups using a computer-based randomization procedure based on body weight. Table 1 indicates animal assignment:

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Number of Females
I - Control	0	25
II- Low	25	25
III-Mid	100	25
IV - High	200	25

4. Dose selection rationale: Doses for this study were selected on the basis of a dose range-finding study (Argus study # 916-025P), in which doses of 0, 32.5, 100, 325, and 650 mg/kg/day were studies. From this preliminary study, it was observed that maternal effects (excess salivation, decreased maternal body weight gain and food consumption) were evident at 32.5 and 100 mg/kg/day, while no developmental effects were observed at these doses. Doses of 325 and 650 mg/kg/day were excessively toxic to the dams.

5. Dosage preparation and analysis: Solutions of the test material were prepared weekly at a final volume of 5 mg/kg, with adjustments made for the 32% w/v concentration of the test substance. Prepared formulations were stored at room temperature and stirred continuously during dose administration. Alkyl trimethyl ammonium bromide was mixed with distilled water to 0.54% and serially diluted to achieve the lower concentrations. Solutions were prepared fresh daily. It is not known with certainty whether homogeneity and stability analyses were performed. The report noted (page 17) that these analyses are "on file with the sponsor." No data were presented in the report to substantiate this claim.

6. Dosage administration: All animals received a dose volume of 5 ml/kg bodyweight by gavage. All doses were administered once daily on gestation days 6 through 19. Dosing volume was adjusted daily for body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - Rats were observed at least twice daily for viability and for general appearance once weekly. Immediately before and approximately 60 mintues after dosing and on the day of sacrifice, the maternal rats were also examined for clinical observations, abortions, premature deliveries, and mortality.

On gestation day 20, all surviving rats were sacrificed by carbon dioxide asphyxiation, and a gross necropsy of the thoracic, abdominal, and pelvic viscera performed. Cesarean section observations

6

and fetal observations were performed in a blinded manner to minimize bias.

The uterus of each rat was excised and examined for pregnancy, number and distribution of implantations, live and dead fetuses, and early and late resorptions. The weight of the gravid uterus was recorded.

2. Fetal Evaluations - Each fetus was weighed, sexed, and examined for gross external alterations. Approximately one-half of the fetusesin each litter were examined for soft tissue alterations, while the remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S, and examined for skeletal alterations.

D. DATA ANALYSIS

1. Statistical analyses: Both parametric (Bartlett's test, ANOVA) and non-parametric (Kruskal-Wallis, Dunn's test) were employed in this study as appropriate.

2. Indices: The following indices were calculated by the reviewer:

Pre-implantation loss:
corpora lutea - # implantations/# corpora lutea x 100

Post-implantation loss:
implantations - # viable progeny/# implantations x 100

3. Historical control data: Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations - At the 200 mg/kg dose, a statistically significant number of maternal animals were observed with increased incidence of excessive salivation, rales, and urine stained abdominal fur. Observations of labored breathing, gasping, brown or red perivaginal substance, chromorhinorrhea, emaciation, brown perianal substance, brown perinasal substance, and dehydration were also observed. Mortality occurred in two maternal animals at the 200 mg/kg dose. The first rat was found dead on day 19 of gestation, after 13 daily doses. No gross lesions were observed in this rat upon necropsy, but 17 early resorptions were observed *in utero*. The second rat was found dead on gestation day 18 after 12 daily doses. Gross necropsy revealed a tear in the esophagus, and 14 fetuses were observed *in utero*. This death was attributed to gavage error.

At the 100 mg/kg dose, increased incidence of excessive salivation and rales was observed, but did not reach statistical significance, as at the 200 mg/kg dose. These observations are sumarized in the following Table.

Table 1. Clinical Observations in Maternal animals dosed with Amine Oxide on days 6 through 19 of gestation.[a]

Observation	Dosage (mg/kg/day)			
	0	25	100	200
Found dead	0	0	0	2
Excessive salivation	0/0	0/0	4/3	48/22
Rales	0/0	0/0	21/9	89/21
Urine stained abdominal fur	0/0	0/0	2/1	45/15
Brown or red perioral substance	0/0	0/0	3/2	16/10
Gasping	0/0	0/0	1/1	15/6
Labored breathing	0/0	0/0	1/1	26/9

aData taken from pages 39-40 of the report. N/N = total number of observations/number of rats with observation. Maternal N = 25/dose group.

2. Bodyweight - A summary of body weight changes observed in maternal rats in this study is summarized in the following Table (Table 2):

Table 2. Mean Maternal Bodyweight (g) in animals dosed with Amine Oxide on days 6 through 19 of gestation.[a]

Interval	Dosage (mg/kg/day)			
	0	25	100	200
Day 0	234.2±9.3	235.3±10.8	237.7±11.4	235.8±10.3
Day 6	265.4±11.8	266.9±13.2	270.9±15.3	269.4±10.8
Day 12	293.1±16.0	295.6±18.4	297.5±18.0	271.2±31.0*
Day 19	367.6±23.6	365.9±23.7	361.9±30.4	327.6±46.6**

a Data taken from Table 3, pages 43-44 of the report. N = 24, 25, 24, and 22 for the control, low, mid, and high dose groups, respectively.
*, ** $p < 0.05$ (*) or $p < 0.01$ (**) vs. control.

As noted in the above table, maternal absolute body weight was significantly decreased at the 200 mg/kg/day dose level beginning on gestation day 11 and continuing until study termination. At day 11, the decrease in body weight was 7% from control; by day 19 of gestation, group mean maternal absolute bodyweight was decreased 11%. Gravid uterine weights were reported as 83.20±15.01g, 86.64±10.78g, 83.99±11.06g, and 66.58±23.56g*, respectively. Using these data, "corrected" maternal body weight on day 20 was calculated as 308.9g, 300.8g, 292.7g, and 270.7g, respectively, with statistical significance at the 200 mg/kg/day dose level.

Changes in maternal body weight gain over the course of this study are summarized in the following table (Table 3):

Table 3. Mean Maternal Bodyweight Gain (g) in animals dosed with Amine Oxide on days 6 through 19 of gestation.[a]

Interval	Dosage (mg/kg/day)			
	0	25	100	200
Days 0 -6	31.2±8.7	31.6±7.6	33.2±8.6	33.7±7.4
Days 6-9	11.7±7.9	14.1±6.5	10.9±4.6	2.6±19.8**
Days 15-18	40.0±9.6	39.9±7.4	36.2±12.3	21.1±21.2**
Days 6-20	126.7±16.1	120.5±15.5	105.8±24.4**	67.0±43.8**
Days 0-20	157.9±20.8	152.2±18.8	139.0±30.2	100.7±48.6**

a Data taken from Table 4, page 45 of the report. N = 24, 25, 24, and 22 for the control, low, mid,

and high dose groups, respectively.
*, ** $p < 0.05$ (*) or $p < 0.01$ (**) vs. control.

As shown in the above table, weight gain for maternal rats at the 200 mg/kg dose level was significantly decreased from control for periods of the study covering dosing and post-dosing, as well as the study period as a whole. At the 200 mg/kg dose level, weight gain in maternal rats was decreased by 78%, 48%, 48%, and 37% , respectively, for the study periods days 6-9, 15-18, 6-20, and 0-20, in comparison to control weight gains for these same time frames.

At the 100 mg/kg/day dose level, group mean maternal body weight gain was decreased as well for the time periods days 18-20 and 6-20 by 31% and 17% respectively.

3. Food Consumption - Food consumption for maternal animals is presented in the following Table (Table 4):

Table 4. Mean Maternal Food Consumption (g/kg/day) in animals dosed with Amine Oxide on days 6 through 19 of gestation.[a]

Interval	Dosage (mg/kg/day)			
	0	25	100	200
Days 0 -6	97.2±7.5	94.4±8.4	97.8±7.5	96.3±9.9
Days 6-9	91.6±9.7	92.3±7.9	89.4±5.4	72.1±21.0**
Days 15-18	85.5±8.8	77.3±8.5*	76.5±10.4**	67.2±15.0**
Days 6-20	85.1±5.8	81.0±5.1	79.1±5.4**	67.5±9.7**
Days 0-20	84.4±4.7	80.8±4.8	80.6±4.4	73.5±7.8**

a Data taken from Table 4, page 45 of the report. N = 24, 25, 24, and 23 for the control, low, mid, and high dose groups, respectively.
*, ** $p < 0.05$ (*) or $p < 0.01$ (**) vs. control.

As noted in the above table, reduced food consumption was noted at both the 100 and 200 mg/kg/day dose levels. At the 200 mg/kg/day dose level, food consumption (on a mg/kg/ basis) was reduced by 21%, 22%, 21%, and 13% for the 6-9, 15-18, 6-20, and 0-20 day time frames, respectively. At 100 mg/kg, statistical differences in food consumption were observed for days 15-18 (11% reduction) and days 6-20 (8%) but not for other times. Of interest is a 10% reduction in food consumption for the 25 mg/kg/day maternal dosed group on days 15-18.

While not calculated in the report, the reviewer made food efficiency calculations for the control, 100, and 200 mg/kg dose levels covering the period of dosing (days 6-19). Using individual food consumption data and body weight gain data, food efficiency (grams b.w. / grams food consumed x 100) was calculated. **Thus, control food efficiency was determined to be 39%, and high dose food efficiency 28%.**
These calculations, showing an 11% decrease in food efficiency, support a treatment-related toxicity at 200 mg/kg/day.

At 100 mg/kg/day, food efficiency was calculated as 35%. Thus, although body weight and food consumption were decreased, food efficiency for the dosing period was not significantly affected in relation to control food efficiency.

4. Gross Pathology - There were no treatment-related gross pathologic findings noted in any of the dams.

5. Cesarean Section Data - Cesarean section observations are presented in Table 5. As the data suggest, the effects of treatment with amine oxide were confined to the 200 mg/kg dose level. These effects consisted of an increase in resorptions (mainly early resorptions), and decreased mean litter weight as well as decreased mean weight of male and female pups at this dose.

Table 5. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	25	100	200
Animals Assigned (Mated)	25	25	25	25
Animals Pregnant	24	25	24	24
Pregnancy Rate (%)	(96)	(100)	(96)	(96)
Nonpregnant	1	0	1	1
Maternal Wastage				
Died	0	0	0	2
Died Pregnant	0	0	0	2
Died Nonpregnant	0	0	0	0
Aborted	0	0	0	0
Premature Delivery	0	0	0	0
Total Corpora Lutea(FTG)	406	442	397	414
Corpora Lutea/Dam	16.9	17.7	16.5	18.8
Total Implantations(FTG)	359	385	362	338
Implantations/Dam	15.0	15.4	15.1	15.4
Total Litters	24	25	24	24
Total Live Fetuses	339	375	348	297
Live Fetuses/Dam	14.1	15.0	14.5	13.5
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam				
Total Resorptions	20	10	14	**41**
Early	18	9	13	**40**
Late	2	1	1	1
Embryonic Deaths/Dam	0.8	0.4	0.6	**1.9**
Early	0.8	0.4	0.5	**1.8**
Late	0.1	0	0	0
Litters with Total Embryonic Deaths	0	0	0	1
Pre-implantation loss (%)	11.5	12.8	8.8	18.3
Post-implantation loss (%)	5.8	2.5	3.8	**12.1**
Mean Litter Weight (g)	3.55	3.47	3.49	**2.99****
*Mean Fetal Weight (males)	3.65	3.54	3.60	**3.07****
Mean Fetal Weight (females)	3.46	3.38	3.40	**2.91****
Sex Ratio (% Male/Female)[b]	45.9/54.1	53.6/46.4	44.6/55.4	49.1/50.9

a Data extracted from the study report, page 48, and from pages81-84.
b Total Corpora Lutea and Total Implantations were calculated by the reviewer.
• Significantly different from controls at $p < 0.05$.
** Significantly different from controls at $p<0.01$.
FTG - Full term gestating females

B. DEVELOPMENTAL TOXICITY Fetal alterations were described as malformations (irreversible changes that occur at low incidences), or variations (common findings in this species or strain and reversible delays or accelerations in development). Fetal evaluations were based on examination of 339, 375, 348, and 297 Cesarean delivered live fetuses in 24, 25, 24, and 21 litters from the 0, 25, 100, and 200 mg/kg/day dose groups.
External, visceral, and skeletal fetal findings are presented in Tables 4a, 4b, and 4c below.

Table 4a. External examinations[a]

Observations [b]	Dose (mg/kg/day)			
	0	25	100	200
#Fetuses(litters) Examined	339(24)	375(25)	348(24)	297(21)
Jaw: micrognathia	0(0)	0(0)	0(0)	1(1)
Tail:kinked	0(0)	0(0)	0(0)	1(1)
Hindlimbs: digits fused	0(0)	0(0)	0(0)	1(1)

a Data extracted from the study report Table 10, page 51.
b Values for individual observations are as follows: fetal(litter).

As shown, one fetus at 200 mg/kg/day had micrognathia, listed as a malformation. Other alterations were considered variations.

Table 4b. Visceral (soft tissue) examinations[a]

Observations [b]	Dose (mg/kg/day)			
	0	25	100	200
#Fetuses(litters) examined	176(24)	194(25)	180(24)	152(21)
Tongue: small	0(0)	0(0)	0(0)	1(1)
Vessels: innominate absent	1(1)	1(1)	0(0)	0(0)
Vessels: umbilical artery descended to left of urinary bladder	1(1)	1(1)	0(0)	0(0)

a Data extracted from the study report Table 11, page 52.
b Values for individual observations are in fetal(litter) format.

As noted in the above table, one fetus at 200 mg/kg/day was identified with a small tongue. One fetus in the control group and one fetus at the 25 mg/kg/day dose level had an absent innominate vessel. The 25 mg/kg/day dose group fetus also had the umbilical artery descending to the left of the urinary bladder.

Table 4c. Skeletal variations[a]

Observations	Dose (mg/kg/day)			
	0	25	100	200
#Fetuses (#litters) examined	176(24)	194(25)	180(24)	152(21)
Thoracic vertebrae: centrum, bifid	1(1)	6(3)	5(5)*	9(8)**
Lumbar vertebrae: arch, incompletely ossified	0(0)	0(0)	1(1)	0(0)
Lumbar vertebrae: centrum, bifid	0(0)	0(0)	1(1)	0(0)
Saacral vertebrae: arches, fused	0(0)	1(1)	0(0)	0(0)
Caudal vertebrae: none present	0(0)	1(1)	0(0)	0(0)
Sternal centra: 1st, incompletely ossified	2(2)	7(5)	3(2)	9(7)**
Sternal centra: 2nd, incompletely ossified	0(0)	4(2)	2(1)	1(1)
Pelvis: pubis, incompletely ossified	7(5)	12(4)	7(3)	16(6)**
Pelvis: ischium, incompletely ossified	2(2)	6(2)	3(1)	0(0)

a Data extracted from the study report Tables 12 and 13, pages 53 and 54.
b Values for individual observations
• Significantly different from controls at $p < 0.05$.
** Significantly different from controls at $p<0.01$.

As noted above, for skeletal examinations, malformations were identified as: one control fetus with fused arches of the 4th cervical vertebra; one 25 mg/kg/day fetus with fused arches of the 3rd sacral vertebra. In the control fetus mentioned, additional variations in ossification were present (unossified 1st sternal vertebra; incompletely ossified pubes). In the 25 mg/kg/day fetus mentioned, no caudal

vertebrae were observed. This was the fetus noted with a thread-like tail at gross external examination.

Variations noted from skeletal examination of fetuses in this study included bifid centrum (which was significantly increased in fetal and litter incidence at 100 and 200 mg/kg), incompletely ossified sternal centra (significantly increased at 200 mg/kg), and incomplete ossification of the pubes and/or ischia, significantly increased at 200 mg/kg.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS The authors of this study concluded that the Maternal NOAEL is 25 mg/kg/day, based on adverse clinical signs, reduced body weight gain, and reduced food consumption at the 100 mg/kg/day dose level. The developmental toxicity NOAEL was determined to be 25 mg/kg/day, based on delays in skeletal ossification observed at 100 mg/kg/day.

B. REVIEWER'S DISCUSSION

In this study, amine oxide (32% w/v) was administered by oral gavage to 25 Crl:CD ®BR VAF/Pplus® female rats/dose at dose levels of 0, 25, 100, and 200 mg/kg/day from days 6 through 19 of gestation. On day gestation day 20, all surviving dams were sacrificed and necropsied. Maternal toxicity was manifest at the 100 and 200 mg/kg/day dose levels, as shown by increased incidence of clinical toxicity, decreased body weight gain, and decreased food consumption. Cesarean section results showed, at the 200 mg/kg/day dose, increased early resorptions, and decreased fetal weight for male and female pups. Developmental toxicity in this study was shown at the 100 and 200 mg/kg/day dose levels primarily by increases in the incidence of delayed skeletal ossification at several sites (bifid thoracic vertebrae centra, incompletely ossified pubes, and decreases in the number of ossified caudal vertebrae, sternal centers, and metacarpals. Based on the results of this study, both the Maternal and Developmental NOAEL are determined to be 25 mg/kg/day. The Maternal LOAEL and Developmental LOAEL are both determined to be 100 mg/kg/day, based on (for maternal toxicity) decreased body weight gain, clinical signs, and decreased food consumption. For developmental toxicity, the LOAEL is based on the increase in incomplete ossification observed at several anatomical sites in male and female fetuses. There was no significant dose-related increase in incidence of developmental malformations in this study.

This study is classified as **acceptable** (guideline) and satisfies the requirement (OPPTS 870.3700) for a developmental toxicity study in the rat.