IIA 5.6.11 Teratogenicity test by the oral route in the rat

IIA 5.6.11/XX

Previous evaluation:	No
Reference:	X
Report:	[redacted] (2019) An oral (gavage) prenatal developmental toxicity study of clomazone in Wistar Han rats. [redacted] Laboratory Project ID 00105205. FMC Tracking No. 2018TOX-CLZ4337
Guidelines:	OECD Test Guideline 414; OPPTS 870.3700
Deviations:	No
GLP:	Yes

Executive summary:

The objectives of this study were to determine the potential of the test substance to induce developmental toxicity in Wistar rats after maternal exposure from implantation to 1 day prior (through Day 20 of gestation) to expected parturition, to characterize maternal toxicity at the exposure levels tested, and to determine a no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity. The study was undertaken to specifically characterize the potential of the test substance to elicit limb flexures that were alleged to have been observed in the developing foetuses of Wistar rats.

Clomazone was administered by oral gavage to time-mated Wistar Han rats (21/dose group) on Gestation Days 6-20. Gavage doses in 0.5% carboxymethyl cellulose (CMC, 400-800 cps) with 0.1% Tween® 80 were 0, 100, 250, 500, and 750 mg/kg bw/day. The dose volume was 5 mL/kg bw. Clinical observations, body weights, and food consumption were recorded at appropriate intervals. On Gestation Day 21, a laparohysterectomy was performed on each surviving female. The uteri, placentae, and ovaries were examined, and the numbers of foetuses, early and late resorptions, total implantations, and *corpora lutea* were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The foetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations.

All females survived to the scheduled necropsy. Primarily during the first three days of dose administration, dilated pupils were noted at 2-hours post-dosing in 4 and 12 females in the 500 and 750 mg/kg bw/day groups, respectively. The dilation persisted to 6-hours post-dosing in 2 and 4 females in the 500 and 750 mg/kg bw/day groups. Red material around the mouth was also noted sporadically in the 750 mg/kg bw/day group at 2-hours post-dosing throughout the dosing period. These findings were considered test substance-related but not adverse due to their transient nature.

Statistically significant lower maternal mean body weight gains were noted during Gestation Days 6–8 and 12-15 in the 750 mg/kg bw/day group compared to the control group. Statistically significant lower food consumption was noted during Gestation Days 6–10 and 15-21 in the 750 mg/kg bw/day group compared to the control group. Overall mean body weight gain (Gestation Days 6–21), mean net body weight, and net body weight gain in the 750 mg/kg bw/day group were also lower than the control group and statistically significant. The reductions in overall mean body weight gain (12.9%) and mean net body weight gain (25.2%) were considered test substance-related and adverse.

Statistically significant lower mean maternal body weight gains and food consumption were noted in the 250 and 500 mg/kg bw/day groups following the first 1 or 2 days of dosing (Gestation Days 6–7 and/or 7–8) compared to the control group. Thereafter, mean body weight gains and food consumption in the 250 and 500 mg/kg bw/day groups were comparable to the control group. The magnitude of the body weight and food consumption decrements at 250 and 500 mg/kg bw/day were not sufficient to result in lower mean absolute body weights, and therefore were considered test substance-related but not adverse. Mean body weights, body weight gains, and food consumption in the 100 mg/kg bw/day group, mean net body weights and net body

weight gains in the 100, 250, and 500 mg/kg bw/day groups, and mean gravid uterine weights at all dosage levels were comparable to the control group.

No test substance-related maternal macroscopic findings were noted. Statistically significant increases in mean absolute liver weights were 10.6%, 25.4%, and 23.3% relative to the control group in the 250, 500, and 750 mg/kg bw/day groups, respectively. This finding was considered potentially adverse at 500 and 750 mg/kg bw/day due to the magnitude of the changes.

Foetal intrauterine growth and survival and foetal morphology were not affected by maternal test substance administration at all dosage levels. There were no incidences of limb flexure observed in any test substance-treated group on either external or skeletal examination.

Based on the increased decreases in mean body weight gain and net body weight gain at 750 mg/kg bw/day and increased liver weights at 500 and 750 mg/kg bw/day, a dosage level of 250 mg/kg bw/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. Based on the lack of adverse findings or test substance-related effects on embryo/foetal development, a dosage level of 750 mg/kg bw/day (the highest dosage level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for embryo/foetal development when the test substance was administered orally by gavage to time-mated Wistar rats.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Clomazone
	Lot/Batch #:	PL15-0023 ([redacted])
	Purity:	96.3%
	Description:	Yellow liquid
	CAS #:	81777-89-1
	Stability of test compound:	Analyses confirmed that test substance was stable for up to 20 hours at room temperature and 11 days of refrigerated storage at a concentration range of 5–150 mg/mL. Homogeneity and concentration assessments of the test substance formulations were conducted for each sampling time point. Test-substance formulations were prepared weekly.
2.	Vehicle and/or positive control:	0.5% CMC with 0.1% Tween® 80
3.	Test animals	
	Species:	Rat
	Strain:	Wistar Han Crl:WI(Han) female rats
	Age at dosing:	Young adult, approximately 11-12 weeks old
	Weight at dosing:	177-216 g
	Source:	[redacted]
	Acclimation period:	2-5 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	All rats were housed individually solid-bottom cages containing appropriate bedding equipped with an automatic watering valve. Cages were arranged on the racks in group order. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).
4.	Environmental conditions	
	Temperature:	23 ± 3°C
	Humidity:	50 ± 20%
	Air changes:	At least 10 changes/hour
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

27-August-2018 to 14-September-2018

2. Animal assignment and treatment

The dosage levels were selected based on results of a range finding study conducted in the current laboratory and on the basis of reported prenatal developmental toxicity study in the same strain of rats. The selected dosage levels for this definitive study were expected to cause maternal toxicity at high dosage levels and to determine NOAELs for maternal and developmental toxicity at low dosage levels. In the range-finding study, Wistar rats were dosed with 250, 500, and 750 mg/kg bw/day by gavage on Gestation Days 6 through 20. Treatment caused approximately 18%, 22%, and 39% increases in absolute liver weights, respectively. In the earlier prenatal developmental toxicity study, Wistar rats were dosed with 250, 500, and 750 mg/kg bw/day by gavage on Gestation Days 6–19. Reduced body weight gain was observed at the mid- and high-dosage levels and reduced food intake was observed in all test substance-treated groups. At the high-dosage level, one dam died after clinical signs of lethargy and slight salivation were observed. Symptoms were seen in the high-dosage level dams on single occasions (5 of 28 dams had lethargy, each on a single day; 22 dams had slight salivation with a duration of 1-5 days). Dams with total resorptions at the high-dosage level were also significantly higher than control (4 dams/17%). The test substance was administered in 0.5% carboxymethyl cellulose with 0.1% Tween® 80 at a volume of 5 mL/kg bw based on the most recent body weight. A negative control group received 0.5% CMC with 0.1% Tween® 80 alone. Animal housing and husbandry were in accordance with the provisions of the *Guide to the Care and Use of Laboratory Animals* (National Research Council, 2011).

Table 1
Study design: Prenatal developmental toxicity test by the oral route in the rat

Group No.	Dosage level (mg/kg bw/day)[a]	Test substance concentration (mg/mL)	Number of time-mated females
1	0[b]	0 (control)	20
2	100	20	21
3	250	50	21
4	500	100	21
5	750	150	21

[a] Formulations of test substance in 0.5% CMC with 0.1% Tween® 80 were administered once daily by oral gavage on Gestation Days 6-20 at a dosing volume of 5 mL/kg.

[b] The control group received 0.5% CMC with 0.1% Tween® 80 only at 5 mL/kg.

3. Dosing, preparation and analysis

Homogeneity and concentration assessments of the test substance formulations were conducted for each sampling time point. Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% of target concentration. Homogeneity results were considered acceptable if the relative standard deviation of the mean value at each sampling location was ≤ 10% and if mean sample concentration results were within or equal to ± 15% of target concentration. Stability of the test substance in the vehicle was previously performed in conjunction with Charles River Study No. 00105203 and demonstrated that the test substance was stable in the vehicle for up to 20 hours at room temperature and 11 days of refrigerated storage at a concentration range of 5–150 mg/mL.

4. Statistics

All statistical tests were performed using WTDMS™ unless otherwise noted. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean.

Data obtained from nongravid animals were excluded from statistical analyses. Due to the use of significant figures and the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ slightly. Therefore, the use of reported individual values to calculate subsequent parameters or means will, in some instances, yield minor variations from those listed in the report data tables. Where applicable, the litter was used as the experimental unit.

Maternal body weights, body weight changes (absolute and net), food consumption, gravid uterine weights, numbers of *corpora lutea*, implantation sites, and viable foetuses, foetal body weights (separately by sex and combined), and liver weights were subjected to a parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test substance-treated groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable foetuses, early and late resorptions, total resorptions, pre- and postimplantation loss, and foetal sex distribution), total foetal malformations and developmental variations (external, visceral, skeletal, and combined), and each particular external, visceral, and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the nonparametric ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test substance-treated groups to the control group.

C. METHODS

1. Observations

All rats were observed twice daily, once in the morning and once in the afternoon, for morbidity and mortality. Individual clinical observations were recorded from the day of receipt through euthanasia. During the dosing period, these observations were performed prior to dosing. On dosing days, clinical observations were also recorded approximately two and six hours following dose administration.

2. Body weights and gravid uterine weights

Individual maternal body weights were recorded on Gestation Day 0 (by the supplier) and 5-21 (daily).

Gravid uterine weight was collected and net body weight (the Gestation Day 21 body weight exclusive of the weight of the uterus and contents) and net body weight change (the Gestation Day 0-21 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

3. Food consumption

Food consumption was measured on Gestation Days 5-21.

4. Euthanasia and necropsy

At termination, animals were sacrificed by carbon dioxide anaesthesia. Animals were subjected to a complete necropsy examination, which included evaluation of the thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

5. Ovarian and uterine examination

The uterus was weighed, and the ovaries and uterus were examined for number and distribution of *corpora lutea*, implantation sites, live and dead foetuses, and early and late resorptions. The placentae were also examined. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.

6. Foetal morphological examination

Foetal examinations were conducted without knowledge of treatment group. Foetal specimens were handled and processed in compliance with the testing facilities SOPs in such a way as to minimize foetal artefacts or mechanically induced alterations. External, internal, and skeletal foetal findings were recorded as developmental variations or malformations. All malformations were verified by a second evaluator.

Each viable foetus was examined externally, individually weighed, euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region. Limbs were examined for size, shape and position; feet were examined for carpal/tarsal flexures, and digits were counted.

All foetuses were examined for visceral anomalies by dissection in the fresh (non-fixed) state. The thoracic and abdominal cavities were opened and dissected. This examination included the heart and major vessels. Foetal kidneys were examined and graded for renal papillae development. The sex of all foetuses was confirmed by internal examination. The heads were removed from approximately one-half of the foetuses in each litter and placed in Harrison's fixative for subsequent processing and soft-tissue examination. The heads from the remaining one-half of the foetuses in each litter were examined by a mid-coronal slice. All carcasses, including the carcasses without heads, were skinned (including the nose, paws, and tail), eviscerated, and fixed in 100% ethyl alcohol for subsequent examination of skeletons. Each eviscerated foetus, following fixation in alcohol, was stained with Alizarin Red S and Alcian Blue. Skeletal examinations were subsequently carried out.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical observations and mortality

All females in the control, 100, 250, 500, and 750 mg/kg bw/day groups survived to the scheduled necropsy on Gestation Day 21. No test substance-related clinical observations were noted at the daily examinations at any dosage level. At the 2-hour post-dosing observation, dilated pupils in one or both eyes were noted in the 500 and 750 mg/kg bw/day groups in a dose-related manner. This finding was noted once in four females on Gestation Day 6 or 8 in the 500 mg/kg bw/day group. One to three occurrences were noted in 12 females in the 750 mg/kg bw/day group primarily on Gestation Day 6 or 7 with sporadic occurrences as late as Gestation Day 13. Dilated pupils were also noted in two and four females in the 500 and 750 mg/kg bw/day groups, respectively, approximately 6 hours post-dosing during Gestation Days 6–10. Of these, the finding had persisted from the 2-hour observation in one and three females in the 500 and 750 mg/kg bw/day groups, respectively. One to four occurrences of red material around the mouth were also noted sporadically in six females in the 750 mg/kg bw/day group at 2-hours post-dosing during Gestation Days 7–20. These findings were considered test substance-related but not adverse due to the transient, sporadic nature. Other findings noted in the test substance-treated groups, including rales, occurred infrequently and/or in a manner that was not dose-related.

2. Body weight and body weight gain

Lower mean maternal body weight gains were noted in the 750 mg/kg bw/day group during Gestation Days 6–8 and 13–14, resulting in lower mean body weight gains for the cumulative Gestation Days 6–9 and 12–15 intervals and for the entire treatment period (Gestation Days 6-21). These differences were generally statistically significant. Mean net body weight and net body weight gain in the 750 mg/kg bw/day group were also statistically significantly lower than the control group. The reductions in overall mean body weight gain (12.9%) and mean net body weight gain (25.2%) were considered test substance-related and adverse. Mean gravid uterine weight in the 750 mg/kg bw/day group was comparable to the control group.

Mean body weight gains in the 250 and 500 mg/kg bw/day group were statistically significantly lower than the control group at the onset of dose administration (Gestation Day 6–7). Mean body weight gains in these groups were comparable to the control thereafter. The only statistically significant difference was a higher mean body weight gain in the 500 mg/kg bw/day group for Gestation Day 8–9. Mean absolute body weights, net body weights, net body weight gains, and gravid uterine weights in the 250 and 500 mg/kg bw/day groups were comparable to the control group. Therefore, the initial effect on mean body weight gain in these groups was considered test substance-related but not adverse.

Mean body weights, body weight gains, net body weight, net body weight gain, and gravid uterine weight in the 100 mg/kg bw/day group were unaffected by test substance administration. Differences from the control group were slight and not statistically significant.

Table 2
Prenatal developmental toxicity study in rats: Maternal body weight changes (g)

Day	0 mg/kg bw/day		100 mg/kg bw/day		250 mg/kg bw/day		500 mg/kg bw/day		750 mg/kg bw/day	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Day 0-5	15 ± 7.1	19	13 ± 6.6	20	17 ± 6.3	20	16 ± 6.2	21	18 ± 7.8	21
Day 5-6	5 ± 3.4	19	4 ± 3.3	20	5 ± 3.3	20	5 ± 4.7	21	4 ± 4.3	21
Day 6-7	3 ± 2.5	19	2 ± 2.5	20	0 ± 4.1[a]	20	0 ± 4.2[a]	21	0 ± 3.6	21
Day 7-8	3 ± 2.7	19	3 ± 4.0	20	4 ± 2.6	20	2 ± 3.5	21	1 ± 2.5[a]	21
Day 8-9	4 ± 2.8	19	5 ± 3.0	20	6 ± 2.9	20	7 ± 3.0[a]	21	3 ± 4.1	21
Day 9-10	4 ± 1.8	19	5 ± 3.2	20	5 ± 3.7	20	6 ± 3.0	21	6 ± 6.7	21
Day 10-11	5 ± 3.3	19	4 ± 4.4	20	4 ± 2.9	20	5 ± 3.6	21	6 ± 4.5	21
Day 11-12	4 ± 4.6	19	4 ± 5.1	20	2 ± 4.5	20	4 ± 3.2	21	3 ± 3.2	21
Day 12-13	4 ± 3.8	19	3 ± 3.9	20	4 ± 3.3	20	4 ± 4.7	21	4 ± 4.9	21
Day 13-14	5 ± 3.6	19	6 ± 4.7	20	4 ± 3.0	20	3 ± 4.3	21	1 ± 6.1[a]	21
Day 14-15	6 ± 3.8	19	6 ± 3.2	20	7 ± 4.0	20	6 ± 4.4	21	6 ± 3.0	21
Day 15-16	7 ± 4.0	19	6 ± 3.9	20	7 ± 3.2	20	5 ± 3.9	21	6 ± 4.8	21
Day 16-17	9 ± 3.3	19	8 ± 3.4	20	9 ± 3.8	20	9 ± 3.8	21	8 ± 3.9	21
Day 17-18	9 ± 5.3	19	10 ± 4.3	20	8 ± 4.5	20	9 ± 3.2	21	11 ± 5.3	21
Day 18-19	8 ± 3.7	19	10 ± 2.5	20	8 ± 2.7	20	10 ± 2.8	21	8 ± 4.7	21
Day 19-20	13 ± 5.1	19	12 ± 4.2	20	11 ± 4.8	20	12 ± 3.5	21	11 ± 3.6	21
Day 20-21	8 ± 3.8	19	9 ± 4.3	20	7 ± 4.7	20	8 ± 3.1	21	8 ± 5.9	21
Day 6-21	93±19.7	19	93±10.9	20	85±13.4	20	90±11.3	21	81±14.3[a]	21
Net body weight	243.9 ±13.6	18	238.5 ±12.3	20	241.5 ±13.2	20	241.9 ±10.9	20	231.8 ±16.0[a]	21
Net body weight change	46.1 ±11.3	18	40.5 ±12.4	20	44.4 ±11.9	20	43.6 ±11.0	20	34.5 ±13.1[a]	21

[a] Significantly different from control by the Dunnett's criteria, $p < 0.05$.

3. Food consumption

Mean maternal food consumption in the 750 mg/kg bw/day group was lower than the control group during Gestation Days 6–10, resulting in lower mean food consumption for the cumulative Gestation Days 6–9 interval. These differences were generally statistically significant. Mean food consumption in this group was comparable to the control group during Gestation Days 10-15, but statistically significantly lower than the control group for the cumulative Gestation Days 15-21 interval. As a result, mean food consumption in the 750 mg/kg bw/day group was statistically significantly lower than the control group for the entire treatment period (Gestation Days 6-21). The effects on mean food consumption in the 750 mg/kg bw/day group corresponded to lower mean body weight gains and were considered test substance-related.

Mean food consumption in the 250 and 500 mg/kg bw/day groups was lower than the control group during Gestation Days 6–7 and 6–8, respectively, resulting in lower mean food consumption when the cumulative Gestation Day 6–9 interval was evaluated at 500 mg/kg bw/day. The differences were statistically significant. The lower mean food consumption in these groups corresponded to lower mean body weight gains at the initiation of dosing and were considered test substance-related. Mean food consumption at 250 and 500 mg/kg bw/day was comparable to the control group following the initial decrements on the first one or two days of dosing.

Mean maternal food consumption in the 100 mg/kg bw/day group was unaffected by test substance administration during the treatment period. Differences from the control group were slight and not statistically significant.

Table 3
Prenatal developmental toxicity study in rats: Food consumption during gestation (g/animal/day)

Day	0 mg/kg bw/day		100 mg/kg bw/day		250 mg/kg bw/day		500 mg/kg bw/day		750 mg/kg bw/day	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Day 5-6	17 ± 3.3	19	14 ± 2.4[b]	20	17 ± 2.3	20	16 ± 2.0	21	16 ± 1.8	21
Day 6-7	16 ± 1.6	19	14 ± 2.1	20	12 ± 3.3[b]	20	12 ± 3.4[b]	21	11 ± 3.1[b]	21
Day 7-8	16 ± 2.1	19	15 ± 2.9	20	15 ± 3.9	20	12 ± 3.2[a]	21	12 ± 4.1[b]	21
Day 8-9	16 ± 2.0	19	15 ± 3.1	20	16 ± 2.3	20	16 ± 2.3	21	13 ± 3.7[b]	21
Day 9-10	17 ± 2.0	19	15 ± 3.1	20	16 ± 2.4	20	18 ± 3.4	21	14 ± 4.7	21
Day 10-11	17 ± 2.6	19	16 ± 2.0	20	16 ± 2.3	20	18 ± 2.1	21	18 ± 4.6	21
Day 11-12	18 ± 2.8	19	18 ± 3.8	20	18 ± 3.3	20	18 ± 2.7	21	17 ± 3.2	21
Day 12-13	19 ± 5.4	19	16 ± 3.8	20	19 ± 7.0	20	20 ± 5.4	21	16 ± 2.9	21
Day 13-14	18 ± 7.1	19	18 ± 6.5	20	18 ± 2.9	20	19 ± 5.3	21	18 ± 5.9	21
Day 14-15	19 ± 1.7	19	21 ± 8.4	20	19 ± 5.6	20	18 ± 4.2	21	18 ± 3.7	21
Day 15-16	18 ± 4.6	19	17 ± 4.5	20	19 ± 4.6	20	18 ± 3.8	21	16 ± 4.5	21
Day 16-17	20 ± 2.4	19	17 ± 4.1	20	19 ± 2.8	20	19 ± 3.4	21	16 ± 5.5[b]	21
Day 17-18	19 ± 3.7	19	17 ± 3.1	20	18 ± 2.2	20	19 ± 3.0	21	18 ± 2.8	21
Day 18-19	19 ± 4.1	19	17 ± 4.2	20	20 ± 5.3	20	18 ± 3.9	21	17 ± 3.7	21
Day 19-20	19 ± 2.5	19	19 ± 5.1	20	17 ± 4.0	20	21 ± 6.3	21	17 ± 3.3	21
Day 20-21	18 ± 5.6	19	17 ± 4.3	20	16 ± 2.8	20	18 ± 2.5	21	16 ± 4.6	21
Day 6-21	18 ± 1.3	19	17 ± 41.4	20	17 ± 1.2	20	18 ± 1.5	21	16 ± 2.2[b]	21
Day 6-21 (g/kg bw/day)	70 ± 4.0	19	67 ± 4.6	20	68 ± 4.6	20	70 ± 4.8	21	63 ± 5.5[b]	21

[a] Significantly different from control by the Dunnett's criteria, $p < 0.05$.
[b] Significantly different from control by the Dunnett's criteria, $p < 0.01$.

4. Gross pathology

At the scheduled necropsy on Gestation Day 21, no test substance-related internal findings were observed at dosage levels of 100, 250, 500, and 750 mg/kg bw/day. Macroscopic findings observed in the test substance-treated groups occurred infrequently and/or in a manner that was not dose-responsive. With the exception of one female each in the control, 100, and 250 mg/kg bw/day groups, all females were gravid.

5. Organ weights

Dose-responsive, statistically significantly higher mean liver weights (10.6%, 25.4%, and 23.3%) were noted in the 250, 500, and 750 mg/kg bw/day groups, respectively, when compared to the control group at the scheduled necropsy. These differences were considered to be test substance-related and potentially adverse at 500 and 750 mg/kg bw/day due to the magnitude of the changes. Mean liver weight in the 100 mg/kg bw/day group was comparable to the control group.

Table 4
Prenatal developmental toxicity study in rats: Summary of liver weights (g)

	0 mg/kg bw/day	100 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day	750 mg/kg bw/day
Mean ± SD	11.14 ± 1.035	11.89 ± 1.123	12.32 ± 0.913[a]	13.97 ± 1.387[b]	13.74 ± 1.491[b]
% Difference	---	6.7	10.6	25.4	23.3
N	19	20	20	21	21

[a] Significantly different from control by the Dunnett's criteria, p <0.05.
[b] Significantly different from control by the Dunnett's criteria, p <0.01.

6. Ovarian and uterine examinations

Intrauterine growth and survival were unaffected by test substance administration at dosage levels of 100, 250, 500, and 750 mg/kg bw/day. Parameters evaluated included mean numbers of *corpora lutea* and implantation sites, mean litter proportions of early and late resorptions, pre- and post-implantation loss, mean number and percentage of viable foetuses, mean foetal body weights, and foetal sex ratios. There were no treatment-related effects across the dose groups and no statistically significant changes.

B. FOETAL EFFECTS

External morphological examinations

No external developmental variations were observed in foetuses in this study.

The only external malformation observed in this study was omphalocele (several loops of intestine protruded through an opening in the umbilicus with remnants of a membranous sac) noted for one foetus in the 750 mg/kg bw/day group. This finding was not considered test substance-related because it occurred in a single foetus, the mean litter proportion was not statistically significantly different from the control group and the value was within the laboratory historical control range.

Visceral morphological examinations

No test substance-related visceral developmental variations were noted.

The only visceral malformation observed in this study was lobular dysgenesis of the lungs (one lobe present on the right) noted for one foetus in the 750 mg/kg bw/day group. This finding was not considered test substance-related because it occurred in a single foetus and the mean litter proportion was not statistically significantly different from the control group. The finding had not been observed in the laboratory historical control data.

Skeletal morphological examinations

No test substance-related skeletal developmental variations were noted.

Skeletal malformations were observed in 3(3), 0(0), 2(2), 2(2), and 0(0) foetuses (litters) in the control, 100, 250, 500, and 750 mg/kg bw/day groups, respectively. Costal cartilage anomalies were noted in one foetus each in the control, 250, and 500 mg/kg bw/day groups. This finding consisted of malpositioned costal cartilage in one control foetus and costal cartilage arising from a seventh cervical rib fused to costal cartilage Number 1 (associating with sternum in normal position) for foetuses in the 250 and 500 mg/kg bw/day groups, respectively. A bifurcated costal cartilage was also noted in the same 500 mg/kg bw/day foetus. Single foetuses in the 250 and 500 mg/kg bw/day groups had vertebral centra anomalies, both with bipartite thoracic centra and the one 500 mg/kg bw/day foetus with fused and malpositioned thoracic centra. These findings were not considered test substance-related because they were noted in single foetuses in the affected groups, occurred similarly in the control group, there was no dose-response relationship, and/or the mean litter proportions were not statistically significantly different from the control

group. Remaining skeletal malformations (8 cervical vertebrae, bent limb bones [humerus, radius, and ulna, bilateral], and vertebral anomaly [extra arch and half of centrum located in lumbar region, with extra arch fused to adjacent arches]) were noted in control group. There were no incidences of limb flexure observed in any test substance-treated group at either external or skeletal examination.

Table 5
Prenatal developmental toxicity study in rats: Summary of foetal malformations

Finding	**0 mg/kg bw/day**	**100mg/kg bw/day**	**250 mg/kg bw/day**	**500 mg/kg bw/day**	**750 mg/kg bw/day**
Number examined	180	199	174	197	205
External examination					
Omphalocele	0	0	0	0	1
Visceral examination					
Lungs-lobular dysgenesis	0	0	0	0	1
Skeletal examination					
Costal cartilage anomaly	1	0	1	1	0
Vertebral centra anomaly	0	0	1	1	0
8 cervical vertebrae	1	0	0	0	0
Bent limb bone(s)	1	0	0	0	0
Vertebral anomaly with or without associated rib anomaly	1	0	0	0	0
Combined	3(3)[a]	0 (0)	2 (2)	2 (2)	2 (2)

[a] expressed as foetal (litter) incidence

III. CONCLUSION

Based on the increased decreases in mean body weight gain and net body weight gain at 750 mg/kg bw/day and increased liver weights at 500 and 750 mg/kg bw/day, a dosage level of 250 mg/kg bw/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal toxicity. Based on the lack of adverse findings or test substance-related effects on embryo/foetal development, a dosage level of 750 mg/kg bw/day (the highest dosage level tested) was considered to be the no-observed-adverse-effect level (NOAEL) for embryo/foetal development when the test substance was administered orally by gavage to time-mated Wistar rats.

([redacted] 2019)