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]
	An oral (gavage) dose range-finding prenatal developmental toxicity study of clomazone in Wistar Han rats. [redacted]. FMC Tracking No. 2018TOX-CLZ4336
Guidelines:	A range-finding study
Deviations:	NA
GLP:	No

Executive summary:

In a dose range-finding prenatal developmental toxicity study, clomazone was administered by oral gavage to time-mated Wistar Han rats (8/dose group) on Gestation Days 6-20. Gavage doses in 0.5% carboxymethyl cellulose (CMC, 400-800 cP) with 0.1% Tween® 80 were 0, 250, 500, and 750 mg/kg bw/day. The dose volume was 5 mL/kg. 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. Liver weight was also 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, including one female each in the 250, and 750 mg/kg bw/day groups that delivered on Gestation Day 21. Dilated pupils were noted on one to six occasions for all females in the 250, 500, and 750 mg/kg bw/day groups at approximately 2 and/or 6 hours postdosing during Gestation Days 9-20. No other remarkable clinical findings were noted at approximately 2 or 6 hours following dose administration or at the daily examinations.

Mean body weights, body weight gains, net body weights, net body weight gains, gravid uterine weights, and food consumption in the 250, 500, and 750 mg/kg bw/day groups were comparable to the control group.

No remarkable macroscopic findings were noted in the test substance-treated groups at the scheduled necropsy. Dose-responsive, higher mean liver weights (17.9%, 22.0%, and 38.7%) were noted in the 250, 500, and 750 mg/kg bw/day groups, respectively, when compared to the control group.

Intrauterine growth and survival were unaffected by test substance administration at all dosage levels. No external foetal malformations or developmental variations were noted for foetuses at all dosage levels.

Based on these results, dosage levels of 100, 250, 500, and 750 mg/kg bw/day were selected for a definitive prenatal developmental toxicity study of clomazone administered orally by gavage to time-mated Wistar Han rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Clomazone
   Lot/Batch #: PL15-0023 (JLHP212001)
   Purity: 96.3%
   Description: Yellow liquid
   CAS #: 81777-89-1
   Stability of test compound: Previous analysis confirmed that test substance was stable under conditions used in this study. 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 10-11 weeks old
   Weight at dosing: 200-224 g
   Source: [redacted]
   Acclimation period: 5 days
   Diet: PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*
   Water: Tap water, reverse osmosis-purified, *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: 20–26°C
   Humidity: 30–70%
   Air changes: At least 10 changes/hour
   Photoperiod: Alternating 12-hour light and dark cycles

B. STUDY DESIGN

1. In-life initiated/completed

   19-July-2018 to 09-August-2018

2. Animal assignment and treatment

   The dosage levels were selected based on results from an earlier prenatal developmental toxicity study in the same strains of rats. In the earlier 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 range of dosage levels in this current range-finding study aided in selection of proper dosage levels for a consequent definitive study. 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: Dose range-finding 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)	8
2	250	50	8
3	500	100	8
4	750	150	8

[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 suspensions, preparation and analysis

Suspensions of test substance in 0.5% CMC of medium viscosity and 0.1% Tween® 80 were prepared approximately weekly, separated into daily aliquots, and stored protected from light at 5°C. The homogeneity and concentration of clomazone in the dosing solutions were checked using high performance liquid chromatography using ultraviolet absorbance detection at the beginning and end of the study. The test substance was at target concentrations 95.0 to 105%, and homogeneous, RSD 1.5 to 5.6. Stability was confirmed in a concurrent study ([redacted]). Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

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 level of 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.

Mean 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 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), 3, 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.

4. Organ weights and tissue collection

The liver was weighted at necropsy for all scheduled euthanasia animals. A section of the left lobe of the liver was collected, flash frozen in liquid nitrogen, and stored in a freezer set to maintain -55°C to -85°C for possible future analysis.

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

Each viable foetus was examined externally, sexed, individually weighed, euthanized by a subcutaneous injection of sodium pentobarbital in the scapular region (if necessary), and discarded. The findings were recorded as either developmental variations or malformations.

II. RESULTS AND DISCUSSION

A. MATERNAL EFFECTS

1. Clinical observations and mortality

All females in the control, 250, 500, and 750 mg/kg bw/day groups survived to the scheduled necropsy on Gestation Day 21, including one female each in the 250, and 750 mg/kg bw/day groups that delivered on Gestation Day 21. Dilated pupils were noted on one to six occasions for all females in the 250, 500, and 750 mg/kg bw/day groups at approximately 2 and/or 6 hours postdosing during Gestation Days 9–20. Other clinical observations noted at approximately 2 or 6 hours postdosing, including cool extremities, partial closure of the eyes, and red material around the mouth or nose, occurred infrequently, at similar frequencies in the control group, and/or in a non-dose-responsive manner. There were no noteworthy clinical observations at the daily examinations.

2. Body weight and body weight gain

Mean maternal body weights, body weight gains, net body weights, net body weight gains, and gravid uterine weights in the 250, 500, and 750 mg/kg bw/day groups were comparable to that in the control group. Differences from the control group were slight and not statistically significant, with the following exceptions. A statistically significant mean body weight loss was noted in the 500 mg/kg bw/day group on Gestation Day 14–15 and a statistically significantly higher mean body weight gain was noted in the 250 mg/kg bw/day group on Gestation Day 10–11 compared to the

control group; however, these transient, non-dose-responsive differences were not of sufficient magnitude to result in statistically significant differences in mean absolute body weights for these groups.

Table 2
Dose range-finding prenatal developmental toxicity study in rats:
Maternal body weight changes (g)

Day	0 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
Day 0-3	4 ± 5.2	7	5 ± 6.2	7	3 ± 5.6	8	3 ± 6.7	8
Day 3-5	7 ± 1.0	7	6 ± 2.4	7	7 ± 2.6	8	7 ± 2.2	8
Day 5-6	7 ± 1.5	7	8 ± 3.2	7	9 ± 1.9	8	8 ± 2.3	8
Day 6-7	0 ± 2.0	7	-1 ± 3.0	7	0 ± 2.1	8	-2 ± 9.0	8
Day 7-8	3 ± 2.9	7	2 ± 3.9	7	2 ± 1.8	8	3 ± 4.1	8
Day 8-9	1 ± 3.4	7	3 ± 2.6	7	1 ± 2.9	8	1 ± 3.2	8
Day 9-10	4 ± 3.4	7	3 ± 2.5	7	5 ± 1.4	8	4 ± 3.0	8
Day 10-11	3 ± 3.5	7	6 ± 2.0[a]	7	5 ± 1.1	8	5 ± 2.2	8
Day 11-12	9 ± 3.3	7	6 ± 2.4	7	7 ± 3.2	8	7 ± 2.5	8
Day 12-13	4 ± 2.6	7	2 ± 3.1	7	3 ± 1.8	8	2 ± 2.2	8
Day 13-14	5 ± 2.1	7	4 ± 2.6	7	6 ± 2.3	8	8 ± 2.2	8
Day 14-15	3 ± 2.0	7	5 ± 3.4	7	-1 ± 3.4[a]	8	0 ± 3.2	8
Day 15-16	5 ± 3.9	7	6 ± 5.4	7	7 ± 1.7	8	5 ± 2.1	8
Day 16-17	10 ± 2.6	7	8 ± 2.6	7	9 ± 2.0	8	9 ± 3.8	8
Day 17-18	8 ± 2.6	7	12 ± 4.4	7	10 ± 4.3	8	9 ± 2.6	8
Day 18-19	11 ± 2.0	7	7 ± 5.0	7	8 ± 5.2	8	8 ± 3.5	8
Day 19-20	12 ± 4.4	7	14 ± 1.5	7	13 ± 3.2	8	11 ± 4.3	8
Day 20-21	1 ± 5.4	7	6 ± 3.6	7	5 ± 4.9	8	5 ± 3.7	8

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

3. Food consumption

Mean maternal food consumption, evaluated as g/animal/day and g/kg/day, in the 250, 500, and 750 mg/kg bw/day groups was comparable to that in the control group. Differences from the control group were slight and not statistically significant, with the following exceptions. Statistically significantly lower mean food consumption was noted in the 500 and 750 mg/kg bw/day groups on Gestation Day 7–8; however, these transient differences were not of sufficient magnitude to effect mean absolute body weights or body weight gains during this interval.

4. Gross pathology

At the scheduled necropsy on Gestation Day 21, no remarkable internal findings were observed at dosage levels of 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 and 250 mg/kg bw/day groups, all females were gravid.

5. Organ weights

Dose-responsive, statistically significantly higher mean liver weights (17.9%, 22.0%, and 38.7%) were noted in the 250, 500, and 750 mg/kg bw/day groups, respectively, when compared to the control group at the scheduled necropsy.

Table 3
Dose range-finding prenatal developmental toxicity study in rats:
Summary of liver weights (g)

	0 mg/kg bw/day	**250 mg/kg bw/day**	**500 mg/kg bw/day**	**750 mg/kg bw/day**
Mean ± SD	10.38 ± 0.561	12.24[a] ± 1.518	12.66[a] ± 1.105	14.40[a] ± 1.679
% Difference	—	17.9	22.0	38.7
N	7	7	8	8

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

6. Ovarian and uterine examinations

Intrauterine growth and survival were unaffected by test substance administration at dosage levels of 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 postimplantation 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, with the following exceptions. In the 250 mg/kg bw/day group, statistically significantly higher mean female and combined foetal weights (5.5 g and 5.6 g, respectively) were noted when compared to the control group (5.0 g and 5.1 g, respectively). These differences were considered incidental given the lack of dose response.

B. FOETAL EFFECTS

External morphological examinations

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

III. CONCLUSION

Higher mean liver weights were noted in the 250, 500, and 750 mg/kg bw/day groups in a dose-responsive manner, and dilated pupils were noted on one to six occasions for all treated females at the postdosing observations. There were no other noteworthy clinical or macroscopic observations, and no effects on survival, maternal body weight, food consumption, intrauterine growth and survival, or foetal morphology. Based on these results, dosage levels of 100, 250, 500, and 750 mg/kg bw/day were selected for a definitive prenatal developmental toxicity study of clomazone administered orally by gavage to time-mated Wistar Han rats.

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