DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.3700a [§83-3a]; Prenatal Developmental Toxicity Study in Rats

Work Assignment No. 4-1-128 K (MRID 46808234)

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Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rats; OPPTS 870.3700a [§83-3a]; OECD 414

PC CODE: 129108 **DP BARCODE:** D331116
TXR#: 0054348

TEST MATERIAL (PURITY): XDE-570 (99.3% a.i.)

SYNONYMS: Florasulam; *N*-(2,6-Difluorophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo (1,5-*c*)pyrimidine-2-sulfonamide; XR-570; XRD-570; DE-570

CITATION: Liberacki, A. B., Carney, E. W., and R. J. Kociba (1997) XDE-570: oral gavage teratology study in CD rats. The Toxicology Research Laboratories, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: DR-0312-6565-027, June 12, 1997. MRID 46808234. Unpublished.

SPONSOR: Dow AgroSciences Canada, Inc., 2100-450 1 St. SW, Calgary, AB, Canada

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46808234), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) in aqueous 0.5% methylcellulose was administered daily via oral gavage to 25-27 time-mated CD (Sprague Dawley) rats/group at a dose volume of 4 mL/kg at dose levels of 0, 50, 250, or 750 mg/kg/day from gestation day (GD) 6-15. On GD 21, all surviving dams were killed and a limited necropsy was performed. The kidneys and uterus were removed and weighed, and the fetuses were delivered by cesarean section.

No adverse treatment-related effects were observed on mortality, clinical signs, or gross pathology.

Four 750 mg/kg/day dams died on study. One female was found dead on GD 9; one female was killed for humane reasons on GD 10; and two females were found dead on GD 13. These animals did not display clinical signs of toxicity prior to death. In three of the dams, necropsy revealed dark or firm lungs, with gavage error noted as the probable cause of death.

At 750 mg/kg/day, body weights were decreased ($p<=0.05$) by 4-6% during GD 6-19, resulting in decreased ($p<=0.05$) body weight gains during treatment (GD 6-16; decr. 16%). Food consumption was also decreased (not statistically analyzed) by 6-13% during the treatment period. Additionally at this dose, absolute and relative (to body weight) kidney weights were increased ($p<=0.05$) by 8 and 12%, respectively. The kidney findings were considered treatment-

related since adverse kidney effects (increased kidney weights, hypertrophy, and histopathology) were observed in several rat studies at ≥ 250 mg/kg/day.

The maternal LOAEL is 750 mg/kg/day, based on decreased body weights (4-6%), body weight gains (16%), food consumption (6-13%), and increased kidney weights. The maternal NOAEL is 250 mg/kg/day.

There were no effects of treatment on the numbers of implantations, live or dead fetuses, litters, or resorptions, or post-implantation loss.

There were no treatment-related external, visceral, or skeletal malformations.

At 750 mg/kg/day, a slight decrease ($p<=0.05$) in fetal body weight (4%) was observed; however, this finding was attributed to the decrease in maternal body weight observed in this dose group. The delayed ossification (not significant) of the skull, ribs, and sternebrae, also seen at 750 mg/kg/day, was within normal range of the historical control data.

The developmental LOAEL is not determined and the developmental NOAEL is 750 mg/kg/day.

This study is classified **acceptable/ guideline** and satisfies the guideline requirements (OPPTS 870.3700a; OECD 414) for a developmental toxicity study in rats.

COMPLIANCE: Signed and dated GLP Compliance, Quality Assurance, and Data Confidentiality statements were provided.

NOTE: This DER summarizes EPA conclusions regarding effects observed in the developmental toxicity study in rats. A detailed DER completed by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

COMMENTS: EPA concurs with the PMRA toxicology evaluation, no conclusions have been changed.

46808234.PMRA.der
.pdf

Reviewer: Tom Morris , **Date** May 10, 2000.

STUDY TYPE: Prenatal Developmental Study - Rat; OPPTS 870.3700; OECD 414.

TEST MATERIAL (PURITY): XDE-570 (Purity - 99.3%)

SYNONYMS: XR-570, XRD-570, DE-570, florasulam.

CITATION: Liberacki, A. B., Carney, E. W. and Kociba, R. J. . June 12, 1997. **XDE-570; Oral Gavage Teratology Study in CD Rats.** Performing Laboratory: The Toxicology Research Laboratories, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: DR-0312-6565-027. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a developmental toxicity study, XDE-570 (Purity - 99.3%), prepared as a suspension in aqueous 0.5% Methocell (methylcellulose), was administered to 25-27 naturally mated adult female CD (Sprague-Dawley) rats/dose at dose levels of 0, 50, 250 or 750 mg/kg bw/d by oral gavage at a dose volume of 4 mL/kg bw from days 6 through 15 of gestation. Sexually mature, virgin adult females were naturally mated with one buck (1 male: 1 female) of the same strain at Charles River Breeding Laboratories prior to shipment to the testing facility. Dosing volume was adjusted daily, based on dam body weight during the dosing period.

Four high-dose dams died prior to the scheduled necropsy. Gross pathological examination revealed dark or firm lungs in three out of four dams, with gavage error noted as the probable cause of death. One dam also exhibited haemolysed blood in the digestive tract. The fourth dam exhibited decreased body fat and a dilated renal pelvis. The cause of death for this dam was not determined, however, a treatment-related cause was not excluded. There were no adverse treatment-related clinical signs. Body weight was significantly lower in the high-dose dams from gestation days 9 through 19. During treatment (gestation days 6-15), the overall body-weight gain was significantly lower in the high-dose dams. This was attributed to significantly lower body-weight gain during the first week of treatment (≈55 and 35% during gestation days 6-9 and 9-12, respectively) and was associated with concomitant lower food consumption (≈11 and 13% at gestation days 6-9 and 9-12, respectively). Overall (gestation days 0-21) body-weight gain was significantly lower (≈10%) in the high-dose dams. When corrected for gravid uterine weight, the overall body-weight gain remained lower (≈11%). Absolute and relative kidney weights were significantly increased (≈8 and 12%, respectively) in the high-dose dams. This was considered to be treatment-related, however, there were no corroborating treatment-related gross pathological findings and in the absence of a histopathological examination the toxicological significance is uncertain. Treatment-related increased kidney weights and histopathological findings in the kidney (hypertrophy of epithelial cells in the collecting ducts) were observed at similar or lower dose levels in other rat studies (90-day and 2-year dietary studies and 2-generation reproduction study). There were no treatment-related gross pathological findings. There were no treatment-related effects on any caesarian section parameter examined including pregnancy rate. All dams terminated on gestation day 21 delivered viable fetuses and there were no dead fetuses at any dose level, including the controls. Gross pathological examination of the four high-dose dams dying prior to the scheduled termination indicated that these dams were pregnant with normally developing embryos respective to gestational age. Gravid uterine weight was not significantly affected by treatment at any dose level.

The LOAEL for maternal toxicity was 750 mg/kg bw/d based on lower body weight, body-weight gain and food consumption and increased kidney weights. The NOAEL for maternal toxicity was 250 mg/kg bw/d.

Mean fetal body weight was slightly lower at 250 and 750 mg/kg bw/d compared to controls (≈3-4% for both). Although this was statistically significant, it was not considered to be biologically or toxicologically significant since

the differences appeared to be minor, there was no clear dose-response between the doses and the values were within the normal range for animals of this strain and age from this laboratory. There were no treatment-related external, visceral or skeletal findings observed at any dose level. There was no evidence of treatment-related irreversible structural changes; therefore, under the conditions of this study, XDE-570 was not teratogenic.

The LOAEL for developmental toxicity was not determined. The NOAEL for developmental toxicity was 750 mg/kg bw/d based on the absence of any significant treatment-related effects on developmental parameters at this dose level.

The developmental toxicity study in the rat is classified acceptable / guideline and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-570 as named in the study. Chemical Name (CA nomenclature): N-(2,6-difluorophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulphonamide

Description: White powdery solid

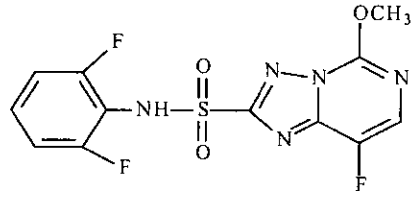
Lot/Batch #: Test Substance # 100511 / Lot # 940714

Purity: 99.3 % a.i. (determined by HPLC).

Compound Stability: The test substance was re-assayed after study determination and was confirmed at 99.3% (Knowles, et al., 1997, Lab Report Code GHE-P-6448)

CAS #: 145701-23-1

Structure



2. **Vehicle and/or positive control:** The test substance was administered as a suspension in an aqueous solution of 0.5% Methocell (methylcellulose) such that a dose volume of 4 mL/kg bw yielded the appropriate dose.

3. **Test animals:**

Species:	Adult female time-mated rats. Sexually mature, virgin adult females were naturally mated with one male of the same strain at Charles River Breeding Laboratories.	
Strain:	CD (Sprague-Dawley)	
Age/weight at study initiation:	At breeding, the animals were ≈9 weeks of age with a body weight range of 200-250 g.	
Source:	Charles River Breeding Laboratories, Portage, MI.	
Housing:	Animals were housed individually in wire bottom cages.	
Diet:	Certified Rodent Chow #5002 (Purina Mills Inc., St. Louis, MO) *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	22 °C
	Humidity:	40-70%
	Air changes:	12-15 changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 4 days.	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - Start: May 13-22, 1996 (day 0 -day vaginal plug detected).
End: June 3 - 12, 1996 (gestation day 21, caesarian section)

2. **Mating:** Sexually mature, adult virgin females, were naturally mated with one male of the same strain at the Charles River Breeding Laboratories in Portage, MI. Females were checked for *in situ* copulation plugs the following morning and those found with such plug were removed and caged separately. The day on which the vaginal plug was detected was considered day 0 of gestation. Day 0 body weights were provided by Charles River Breeding Laboratories. Rats were shipped on either day 0 or 1 of gestation and arrived at the test facilities on either day 1 or 2 of gestation.

3. **Animal Assignment:** - Time-mated dams were randomly assigned to the study as summarized in Table 1 using a computer generated procedure designed to increase the probability of uniform group weights and standard deviations

at the start of the study.

TABLE 1: Animal Assignment.

Dose Level (mg/kg bw/day)	0	50	250	750
# Females	25	25	25	27

4. Dose selection rationale: The dose levels were based on an oral gavage teratology probe study in rats (Liberacki, A. B., *et al, 1996, Dow Chemical Laboratory Project ID - DR-0312-6565-024, submitted but a complete review of* the data was not done). In the oral gavage teratology probe study, groups of 10 female CD rats were administered XDE-570 as a suspension in an aqueous solution of 0.5% Methocel (methylcellulose) on days 6 through 15 of gestation at dose levels of 0, 100, 500, 750 or 1,000 mg/kg bw/d. Severe maternal toxicity was observed at 1,000 mg/kg bw/d as evidenced by significant decreases in maternal body weights and body-weight gain early in the dosing period as well as significant maternal mortality (5/10 animals - 50%) by gestation day 12. The remaining dams at this dose level were sacrificed on gestation day 13 due to severe maternal toxicity observed without any further data collection. At 750 mg/kg bw/d treatment-related findings included significant decreases in body-weight gain (33%) on gestation days 6 through 9 and significant increases in absolute and relative kidney weights (12-33%) compared to controls. No significant findings were observed on any maternal parameters at dose levels of 100 or 500 mg/kg bw/d and no embryonal/fetal effects were observed at doses up to and including 750 mg/kg bw/d. The high dose of 750 mg/kg bw/d was expected to result in decreased body-weight gains early in the dosing period and kidney weight increases compared to controls. The lower dose levels of 250 and 500 mg/kg bw/d were selected to provide dose-response data for any toxicity observed in the high-dose animals and a no-observed-effect level (NOEL).

5. Dosage preparation and analysis - The test substance was prepared as a suspension in an aqueous solution of 0.5% Methocel (methylcellulose) such that a dose volume of 4 mL/kg bw yielded the appropriate dose. The dose suspensions were mixed once prior to initiation of dosing and once midway through the dosing period. Samples of the initial mix were analysed to determine concentrations prior to dosing. Dose concentrations were also determined midway through the dosing period in order to establish stability of the test compound for the use period. In addition the low and high dose suspensions were analysed for homogeneity of mixing. Samples were analysed by HPLC with UV detection and external standard quantification. Reference samples of all dose suspensions including control were retained and stored at room temperature in a manner consistent with accepted policy (FDA and EPA GLP).

Results

Homogeneity Analysis: - Analysis of the low- and high-dose concentrations indicated that the test material was homogeneously suspended.

Date Mixed - 5/15/96	Low Dose	High Dose
Location	Observed Concentration (mg/mL)	
Top	11.7	195
Middle	12.3	195
Bottom	12.3	193
Mean	12.1	194
Standard Deviation	0.35	1.2
% Relative Standard Deviation	2.89	0.62

* Table extracted from page 23 of the study report.

Stability Analysis: Re-analysis of the dosing suspensions, from the first mix, following the first half of the dosing regime (midway through dosing period) revealed mean dose concentrations ranging from 99 to 109%, indicating a stability of at least 13 days.

Concentration Analysis: Initial analysis (prior to dosing) of all dosing suspensions from the first mix revealed mean concentrations of the test substance ranging from 97 to 103% of targeted concentrations.

	% of Target Concentration		
Date Mixed - 5/15/96	ACL Report # 96-116 (prior to initiation of dosing)	ACL Report # 96-125 (midway through the dosing period)	Mean
Control	ND	ND	ND
Low-dose	97	99	98
Mid-dose	101	106	104
High-dose	103	109	106

* Table extracted from page 22 of the study report.
ND: Not detected with estimated limit of detection of 0.01 ppm.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: Doses were administered once daily by oral gavage, the suggested route of administration for studies of this type, in aqueous 0.5% Methocel (methylcellulose) at a dose volume of 4 mL/kg bw, on days 6 through 15 of gestation. Dosing volume was adjusted daily, based on current dam body weight during the dosing period.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - All dams were observed daily for mortality or clinical signs. Body weight data were recorded on day 0 (by supplier), daily during gestation days 6 through 16 and on gestation days 19 and 21. Statistical analyses of body weights and body-weight gains were performed using data collected on gestation days 0, 6, 9, 12, 16, 19 and 21. Food consumption was determined at 3-4 day intervals beginning on gestation day 3. On gestation day 21, all dams which survived the dosing regimen were weighed, euthanised by CO_2 inhalation after which a limited necropsy was performed. Any obvious structural or pathologic changes observed were noted and the gravid uterine and kidney weights recorded. Gross lesions and sections of the kidneys were preserved in neutral phosphate buffered 10% formalin. Microscopic examination of tissues was not conducted. The uterine horns were exteriorized through an abdominal incision and the following data recorded: 1) the number and position of the fetuses *in utero*; 2) the number of live and dead fetuses, 3) the number and position of resorption sites, 4) the number of corpora lutea, 5) the sex and body weight of each fetus and 6) any gross alterations. The uteri of apparently non-pregnant animals were stained with a 10% aqueous solution of sodium sulfide and examined

for evidence of early resorptions. The number of corpora lutea were not recorded for females which were not visibly pregnant at caesarian section. Dams which died prior to scheduled termination were submitted for a complete necropsy examination. The necropsy was similar to dams sacrificed at the scheduled necropsy except kidney weights were not recorded. In addition, the number of corpora lutea and the sex and body weight of each fetus were not recorded. The degree to which the implantation site(s) had developed was determined to the extent possible and the fetuses discarded.

2. Fetal Evaluations - At least one half of the fetuses in each litter, selected using a computerized random program, were examined immediately by dissection under a low power stereo-microscope for evidence of visceral alterations. The heads of the fetuses examined by dissection were removed and placed in Bouin's fixative and examined by serial sectioning technique of Wilson (1965). All fetuses were preserved in alcohol, eviscerated and stained with alizarin red-S. Skeletal examinations were conducted on all fetuses that were not selected for visceral examinations. Visceral and skeletal examinations of the fetuses from dams which died prior to the scheduled termination were not performed.

D. DATA ANALYSIS

1. Statistical analyses: Food consumption data were summarized by mean and standard deviation without further statistical analysis. Maternal body weights, body-weight gains, organ weights and fetal body weights were evaluated by Bartlett's test for equality of variances ($\alpha = 0.01$). Based on the outcome of the Bartlett's test ($\alpha = 0.05$ for both), a parametric or nonparametric ANOVA was performed. If the ANOVA was significant, analysis by Dunnett's test ($\alpha = 0.05$, two-sided) or Wilcoxon Rank-Sum test ($\alpha = 0.05$, two-sided) with Bonferroni's correction was performed, respectively. Frequency of pre-implantation loss (number of corpora lutea minus number of implantations), resorptions/litter and resorptions/fetal population, fetal alterations among litters and the fetal population were analysed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implantations, and litter size were evaluated using a non-parametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analysed using the Fischer exact probability test ($\alpha = 0.05$, two-sided) with Bonferroni's correction. Fetal sex ratios were analysed using a binomial distribution test ($\alpha = 0.05$, two-sided). Non-pregnant females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from the appropriate analyses. Statistical outliers were identified using a sequential method ($\alpha = 0.05$, two-sided), and excluded if justified by sound scientific reasons unrelated to treatment. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control groups to keep the experiment-wise alpha at 0.05. Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was expected to be much greater than the cited alpha level would suggest. Therefore, the final interpretation of the numerical data took into consideration the statistical analyses along with other factors such as dose-response relationships and whether the results were significant in light of other biologic and pathologic findings.

2. Indices: The following indices were calculated from caesarean section records of animals in the study:

$$\text{Pregnancy Rate (\%)} = \frac{\text{\# of females with visible implantations}}{\text{Total \# bred}} \times 100$$

$$\text{Pre-implantation loss} = \frac{\text{\# of corpora lutea - \# implants}}{\text{\# of corpora lutea}} \times 100$$

$$\text{Post-implantation loss} = \frac{\text{\# of implantations - \# viable progeny}}{} \times 100$$

# of implantations

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls (reproductive indices, external, visceral and skeletal alterations).

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: Four high-dose dams died prior to the scheduled necropsy. One female was found dead on gestation day 9, one was sacrificed in moribund condition on gestation day 10 and two died on gestation day 13. Of these four dams, only one exhibited clinical observations prior to death. One dam was noted as having scabbed abrasions on the muzzle on gestation day 1 through 4. This abrasion was noted upon arrival of the animal to the laboratory and was most likely inflicted during mating. Gross pathological examination revealed dark or firm lungs in three out of four dams, with gavage error noted as the probable cause of death. One dam also exhibited haemolysed blood in the digestive tract. The fourth female exhibited decreased body fat and a dilated renal pelvis. The cause of death for the fourth female was not determined, however, a treatment-related cause was not excluded. Gross pathological examination also indicated that all four animals were pregnant with normally developing embryos respective to gestational age.

There were no adverse treatment-related clinical observations. An increased incidence of salivation and perineal soiling were observed in the high-dose dams (Table 2). In all animals, the salivation observed occurred immediately following dosing and was resolved within 10 minutes. Due to the immediate onset of salivation and its short duration it was considered most likely to be a local effect resulting from deposition of residual test material into the oral cavity during the gavage procedure and not toxicologically relevant. Perineal soiling was interpreted by the study author to be a treatment-related effect. However, it is not an adverse effect and could be considered a secondary or indirect effect, possibly due to lack of grooming resulting from urine acidification or to the presence of excretory products of the test substance in the urine.

TABLE 2. Clinical Observations (values expressed as incidence/total number examined) (a)

Observation	0 mg/kg bw/d	50 mg/kg bw/d	250 mg/kg bw/d	750 mg/kg bw/d
Salivation post dosing	0/25	0/25	0/25	14/27
Perineal soiling	0/25	0/25	0/25	3/27

(a) Data obtained from page 24 of the study report.

2. Body Weight - Body weight was significantly lower (≈4-6%) in the high-dose dams from gestation days 9 through 19 (Table 3). During treatment (gestation days 6-16), the overall body-weight gain was significantly lower in the high-dose dams (≈16% lower). This was attributed to significantly lower body-weight gain during the first week of treatment (≈55 and 35% during gestation days 6-9 and 9-12, respectively) and was associated with concomitant lower food consumption (≈11 and 13% at gestation days 6-9 and 9-12, respectively). During the second week of treatment (gestation days 12-16) and during the post-treatment interval (gestation days 16-19 and 19-21) body-weight gain in the high-dose dams was not significantly different from controls. The overall (gestation days 0-21) body-weight gain was significantly lower (≈10%) in the high-dose dams. When corrected for gravid uterine weight, the overall body-weight gain remained lower (≈11%; no statistical analysis done). No treatment-related effects were observed on body weight or body-weight gains in dams at 50 or 250 mg/kg bw/d.

TABLE 3: Maternal body weight and body-weight gain. (a)

Interval		Dose Level (mg/kg bw/day)			
		0 (n = 25)	50 (n = 24)	250 (n = 24)	750 (n = 27)
Body weight (g ± SD)					
Pre-Treatment	Day 0	226.5 ± 11.0	225.1 ± 10.6	227.1 ± 11.3	227.8 ± 11.6
	Day 6	262.1 ± 12.1	259.7 ± 13.7	260.2 ± 13.7	259.9 ± 12.9
Treatment	Day 9	279.0 ± 12.7	276.0 ± 15.0	274.7 ± 14.5	268.6 ± 13.7 * (n = 26)
	Day 12	298.2 ± 12.2	295.4 ± 15.6	294.9 ± 1.37	281.6 ± 18.8 * (n = 23)
	Day 16	328.3 ± 15.0	325.8 ± 19.5	324.7 ± 16.5	314.6 ± 18.2 *
Post-treatment	Day 19	372.9 ± 18.1	369.7 ± 23.7	368.1 ± 19.8	356.6 ± 24.2 *
	Day 21	409.9 ± 23.4	406.3 ± 28.9	405.2 ± 21.0	392.7 ± 28.5
	Day 21 (b)	308.6 ± 19.2	307.4 ± 20.1	307.6 ± 15.1	299.8 ± 20.7
Body-weight gain (g ± SD)					
Pretreatment	Day 0-6	35.6 ± 7.2	34.6 ± 6.4	33.1 ± 6.6	32.1 ± 6.5
Treatment	Days 6-9	16.9 ± 6.0	16.3 ± 5.7	14.6 ± 7.5	7.6 ± 10.8 #
	Days 9-12	19.3 ± 4.1	19.3 ± 5.8	20.2 ± 6.8	12.6 ± 11.8 #
	Days 12-16	30.0 ± 7.9	30.4 ± 7.6	29.7 ± 7.2	33.1 ± 8.0
	Days 6-16	66.2 ± 13.3	66.1 ± 14.1	64.5 ± 14.7	55.6 ± 13.6 *
Post-treatment	Days 16-19	44.7 ± 6.4	43.9 ± 6.0	43.5 ± 6.4	41.9 ± 8.6
	Days 19-21	36.9 ± 8.5	36.5 ± 8.2	37.1 ± 5.8	36.1 ± 8.1
Overall	Days 0-21	183.4 ± 24.8	181.1 ± 24.7	178.1 ± 21.5	166.1 ± 25.1 *
	Days 0-21 (b)	82.1 ± 19.1	82.3 ± 18.1	80.5 ± 14.6	73.3 ± 17.5

(a) Data extracted from pages 27-28 of the study report. Overall body-weight gain corrected for gravid uterine weight was calculated by reviewer from individual body weight data obtained from pages 53-56 of the study report, no statistical analysis was done. Animals which were non-pregnant or had totally resorbed litters were excluded from analysis.
(b) Body weight, body-weight gain corrected for gravid uterine weight.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$
\# Statistically different from control mean by Wilcoxon test, $p \leq 0.05$

3. Food Consumption - Compared to control dams, food consumption was lower in the high-dose dams throughout treatment (gestation days 6-16). The lower food consumption was most notable during the first week of treatment (≈11 and 13% during gestation days 6-9 and 9-12, respectively) and was associated with significantly lower body-weight gain (≈55 and 35% during gestation days 6-9 and 9-12, respectively). During the post-treatment interval, food consumption was comparable to controls. Food consumption of dams at 50 or 250 mg/kg bw/d was unaffected by treatment. Food consumption data are summarized in Table 4.

TABLE 4: Maternal food consumption (g/animal/day ± SD) (a)

Interval		Dose Level (mg/kg bw/day)			
		0 (n = 25)	50 (n = 24)	250 (n = 24)	750 (n = 27)
Pre-Treatment	Days 3-6	22.8 ± 1.6	22.3 ± 2.0	22.6 ± 2.0	22.9 ± 2.0 (n = 25)

Interval		Dose Level (mg/kg bw/day)			
		0 (n = 25)	50 (n = 24)	250 (n = 24)	750 (n = 27)
Treatment	Days 6-9	23.3 ± 1.5	23.0 ± 2.2	22.1 ± 2.8	20.8 ± 2.8
	Days 9-12	24.8 ± 1.5	25.0 ± 1.9	24.6 ± 2.4	21.5 ± 3.1 (n = 22) (b)
	Days 12-16	25.8 ± 1.9	26.2 ± 2.4	25.2 ± 2.6	24.2 ± 2.1 (n = 23) (b)
Post-treatment	Days 16-19	28.4 ± 2.7	28.5 ± 3.2	27.8 ± 3.0	29.2 ± 2.9
	Days 19-21	27.7 ± 2.7	27.8 ± 3.1	27.4 ± 2.6	28.3 ± 3.2

(a) Data extracted from pages 27-28 of the study report. Animals which were non-pregnant were excluded. Food consumption data were summarized by mean and standard deviation without further statistical analysis.
(b) Varying n values due to early deaths or exclusion of values for animals which spilled excessive amounts of food.

4. Gross Pathology - Absolute and relative kidney weights were significantly higher (≈8 and 12%, respectively) in the high-dose dams compared to controls (Table 5). This was considered to be treatment-related, however, there were no corroborating treatment-related gross pathological findings and in the absence of a histopathological examination the toxicological significance is uncertain. Treatment-related increased kidney weights and histopathological findings in the kidney (hypertrophy of epithelial cells in the collecting ducts) were observed at similar or lower dose levels in other rat studies (90-day and 2-year dietary and 2-generation reproduction study). There were no treatment-related gross pathological findings in the dams sacrificed at scheduled termination of the study. Gross pathological examination revealed dark or firm lungs in three out of four dams, with gavage error noted as the probable cause of death by the attending pathologist. One dam also exhibited haemolysed blood in the digestive tract. The fourth dam exhibited decreased body fat and a dilated renal pelvis. The cause of death for the fourth dam was not determined, however, a treatment-related cause was not excluded. All other tissues were within normal limits. Gross pathological examination also indicated that all four dams were pregnant with normally developing embryos respective to gestational age.

TABLE 5. Maternal absolute (g ± SD) and relative (g/100 g bw ± SD) kidney weights. (a)

Dose Level (mg/kg bw/day)		0 (n = 25)	50 (n = 24)	250 (n = 24)	750 (n = 23)
Kidney	Absolute	2.037 ± 0.203	2.056 ± 0.170	2.036 ± 0.158	**2.190 ± 0.198 ***
	Relative	0.497 ± 0.042	0.508 ± 0.046	0.503 ± 0.030	**0.559 ± 0.045 ***

(a) Data extracted from pages 29 of the study report.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.

5. Caesarean Section Data - The pregnancy rate was 100, 96, 96 and 100% at 0, 50, 250 and 750 mg/kg bw/d, respectively (Table 6). All dams terminated on gestation day 21 delivered viable fetuses and there were no dead fetuses at any dose level, including the controls. Gross pathological examination of the four high-dose dams dying prior to the scheduled termination indicated that all were pregnant with normally developing embryos respective to gestational age. No dams aborted or delivered pre-maturely. The number of corpora lutea, implantation sites, resorptions and implantation losses (pre- or post-implantation) were unaffected by treatment. No significant differences in litter size, number of live fetuses/litter, or ratio of male/female fetuses were observed between the control and treatment groups. Gravid uterine weight was not significantly affected by treatment at any dose level. Mean fetal body weight was slightly lower at 250 and 750 mg/kg bw/d compared to controls (≈3-4% for both). Although these differences were statistically significant, they were not considered to be biologically or toxicologically significant since the differences appeared to be minor, there was no clear dose-response between the mid- and high-dose dams and the values were within the normal range (historical control range - 5.03 to 5.6 g) for animals of this strain and age from this laboratory.

TABLE 3: Caesarean section observations. (a)

Observation		Dose Level (mg/kg bw/day)			
		0	50	250	750
# Animals Assigned (Mated)		25	25	25	27
# Animals Pregnant		25	24	24	27
Pregnancy Rate (%)		100	96	96	100
# Nonpregnant		0	1	1	0
Maternal Wastage	# Died	0	0	0	4
	# Died Pregnant	0	0	0	4
	# Died Nonpregnant	0	0	0	0
	# Aborted	0	0	0	0
	# Premature Delivery	0	0	0	0
Total # Litters		25	24	24	23
Total # Corpora Lutea Corpora Lutea/Dam		418 16.7 ± 2.3	396 16.5 ± 2.9	392 16.3 ± 2.1	369 16.0 ± 3.2
Total # Implantations Implantations/Dam		349 14.0 ± 1.5	328 13.7 ± 1.5	336 14.0 ± 2.0	311 13.5 ± 2.0
Total # Pre-Implantation Loss		69	68	56	58
Pre-implantation Loss (%)		15.4 ± 11.9	15.8 ± 11.0	13.8 ± 10.7	14.2 ± 12.4
Total # Live Fetuses Live Fetuses/Litter		333 13.3 ± 1.7	308 12.8 ± 1.9	319 13.3 ± 1.8	286 12.4 ± 2.3
Total # Dead Fetuses Dead Fetuses/Litter		0 0	0 0	0 0	0 0
Total # Resorptions		16	20	17	25
Total # Resorptions/Dam		0.6 ± 0.8	0.8 ± 1.0	0.7 ± 1.0	1.1 ± 1.4
% Implantations Resorbed		4.6 (16/349)	6.1 (20/238)	5.1 (17/336)	8.0 (25/311)
% Litters with Resorptions		44.0 (11/25)	58.3 (14/24)	50.0 (12/24)	56.5 (13/23)
Resorptions/Litters with Resorption		1.5 (16/11)	1.4 (20/14)	1.4 (17/12)	1.9 (25/13)
Litters with Total Resorptions		0	0	0	0
Post-implantation Loss (%) (b)		4.71 ± 6.19	6.24 ± 7.59	4.76 ± 6.07	8.03 ± 10.05
Mean Fetal Weight (g ± SD)	Sexes Combined	5.50 ± 0.28	5.49 ± 0.25	**5.30 ± 0.23** *	**5.27 ± 0.24** *
Gravid Uterine Weight (g ± SD)		101.3 ± 11.4	98.9 ± 14.3	97.7 ± 12.3	92.8 ± 14.8
Average # Males/Litter (total # of males)		7.3 ± 2.2 (182)	7.3 ± 1.9 (176)	6.6 ± 2.0 (159)	6.2 ± 1.9 (143)
Average # Females/Litter (total # of females)		6.0 ± 2.0 (151)	5.5 ± 1.8 (132)	6.7 ± 1.4 (160)	6.2 ± 2.3 (143)
Sex Ratio (% Male/% Female)		55/45	57/43	50/50	50/50

(a) *Data extracted from page 30 and pages 61-64 of the study report.*
(b) Post-implantation loss calculated by reviewer from data obtained on pages 61-64 of study report, no statistical analysis done.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.
Historical control range for fetal body weight (sexes combined): 5.03 - 5.6 g.

B. DEVELOPMENTAL TOXICITY

1. External Examination - There were no external findings observed at any dose level. There was no significant

treatment-related effect on the total number of fetuses or litters with external findings. External examination findings are summarized in Table 4a.

TABLE 4a. External examinations (a)

Observations+	Dose Level (mg/kg bw/day)			
	0	50	250	750
#Fetuses(litters) examined	333 (25)	308 (24)	319 (24)	286 (23)
#Fetuses(litters) affected	0 (0)	0 (0)	0 (0)	0 (0)

+ Some observations may be grouped together
(a) Data extracted from pages 31-33 (group data) and 75-258 (individual litter data) of the study report.
Expressed as fetal (litter) incidence

2. Visceral Examination - There were no treatment-related visceral findings observed at any dose level. There was no significant treatment-related effect on the total number of fetuses or litters with visceral findings. Visceral examination findings are summarized in Table 4b.

TABLE 4b. Visceral examinations (a)

Observations+		Dose Level (mg/kg bw/day)			
		0	50	250	750
#Fetuses(litters) examined		173 (25)	161 (24)	165 (24)	148 (23)
#Fetuses (litters) affected (b)		0 (0)	1 (1)	0 (0)	0 (0)
Total # fetuses (litters) malformed visceral		0 (0)	1 (1)	0 (0)	0 (0)
Kidneys	- hydronephrosis (c)	0 (0)	1 (1)	0 (0)	0 (0)
	- hydroureter	0 (0)	1 (1)	0 (0)	0 (0)

+ Some observations may be grouped together
(a) Data extracted from pages 31-33 (group data) and 75-258 (individual litter data) of the study report.
(b) Total number of fetuses (litters) with visceral malformations as indicated in study report.
(c) Considered to be a malformation.
Expressed as fetal (litter) incidence

3. Skeletal Examination - There were no treatment-related skeletal findings observed at any dose level. There was no significant treatment-related effect on the total number of fetuses or litters with skeletal findings. However, there may be a slight increased incidence of delayed development at 750 mg/kg bw/d compared to controls as indicated by a slight increased incidence of delayed ossification of the ribs and sternebrae and lower fetal body weight. However the incidences of delayed ossification were not statistically significant, and all findings were within the normal range of the CD (Sprague-Dawley) rat historical control data for this laboratory and showed no clear indication of a dose-response relationship. All other alterations occurred at low frequencies, were not dose-related or were within the normal range of the CD (Sprague-Dawley) rat historical control data for this laboratory. Skeletal examination findings are summarized in Table 4c.

TABLE 4c. Skeletal examinations (a)

Observations+		Dose Level (mg/kg bw/day)			
		0	50	250	500
#Fetuses(litters) examined		160 (25)	147 (24)	154 (24)	138 (23)
#Fetuses(litters) affected		71 (22)	77 (24)	78 (21)	83 (23)
Total # fetuses (litters) malformed skeletal (b)		1 (1)	0 (0)	5 (4)	1 (1)
Skull	- delayed ossification - extra ossification site - misplaced suture line	6 (5) 1 (1) 0 (0)	4 (4) 0 (0) 0 (0)	15 (7) 0 (0) 0 (0)	11 (7) 0 (0) 1 (1)
Centra	- delayed ossification cervical - delayed ossification thoracic	50 (19) 6 (5)	66 (19) 6 (5)	61 (17) 2 (2)	62 (23) 0 (0)
Ribs	- delayed ossification - Class I wavy - Class II wavy (c) - extra - calloused (c)	1 (1) 3 (2) 0 (0) 0 (0) 1 (1)	1 (1) 0 (0) 0 (0) 0 (0) 0 (0)	7 (5) 6 (4) 5 (4) 1 (1) 0 (0)	8 (4) 1 (1) 0 (0) 0 (0) 1 (1)
Limbs	- delayed ossification metacarpals	0 (0)	1 (1)	0 (0)	0 (0)
Sternebrae	- delayed ossification (d) - irregular pattern of ossification	17 (10) 5 (5)	14 (12) 2 (2)	12 (9) 0 (0)	27 (12) 3 (3)
Bouin's Observations					
Eyes and forebrain	- microphthalmia (d)	1 (1)	1 (1)	1 (1)	0 (0)

+ Some observations may be grouped together
(a) Data extracted from pages 31-33 (group data) and 75-258 (individual litter data) of the study report.
(b) Total number of fetuses (litters) with skeletal malformations as indicated in the study report.
(c) Considered to be a malformation.
(d) Base number of fetuses adjusted to account for damage to sternebrae in positions #5 or #6 during skeletal processing (could not be evaluated). Expressed as fetal (litter) incidence
Historical control values:
- delayed ossification of sternebrae - 4 to 29% (observed incidence at 750 mg/kg bw/d was approximately 19%)
- delayed ossification of ribs - 0 to 9% (observed incidence at 750 mg/kg bw/d was approximately 6%)

III. DISCUSSION

A. Investigators' conclusions (extracted from page 19 of the study report): "In conclusion, oral administration of XDE-570 to time-mated CD rats resulted in maternal toxicity in dams administered 750 mg/kg/day as evidenced by decreased feed consumption, body weight and body-weight gain. In addition, an increased incidence of perineal soiling was also interpreted to represent a treatment-related effect. Three of four early deaths which occurred at 750 mg/kg/day were attributed to gavage error based upon gross pathologic findings in the lungs of these rats. Findings in the fourth female were limited to a decreased amount of body fat and a dilated renal pelvis. The cause of death for this female was not determined and, therefore may have been treatment-related. No significant maternal effects were observed in rats administered 50 or 250 mg/kg/day and no adverse embryonal/fetal effects were observed at any dose level tested. Therefore, the no-observed-effect-level (NOEL) for maternal toxicity was 250 mg/kg/day; the embryonal/fetal NOEL was 750 mg/kg/day."

B. Reviewer's discussion:

1. **Maternal toxicity**: Four high-dose dams died prior to the scheduled necropsy. Gross pathological examination

revealed dark or firm lungs in three out of four dams, with gavage error noted as the probable cause of death. One dam also exhibited haemolysed blood in the digestive tract. The fourth dam exhibited decreased body fat and a dilated renal pelvis. The cause of death for this dam was not determined, however, a treatment-related cause was not excluded. There were no adverse treatment-related clinical signs. Body weight was significantly lower in the high-dose dams from gestation days 9 through 19. During treatment (gestation days 6-16), the overall body-weight gain was significantly lower in the high-dose dams. This was attributed to significantly lower body-weight gain during the first week of treatment (≈55 and 35% during gestation days 6-9 and 9-12, respectively) and was associated with concomitant lower food consumption (≈11 and 13% at gestation days 6-9 and 9-12, respectively). Overall (gestation days 0-21) body-weight gain was significantly lower (≈10%) in the high-dose dams. When corrected for gravid uterine weight, the overall body-weight gain remained lower (≈11%). Absolute and relative kidney weights were significantly increased (≈8 and 12%, respectively) in the high-dose dams. This was considered to be treatment-related, however, there were no corroborating treatment-related gross pathological findings and in the absence of a histopathological examination the toxicological significance is uncertain. Treatment-related increased kidney weights and histopathological findings in the kidney (hypertrophy of epithelial cells in the collecting ducts) were observed at similar or lower dose levels in other rat studies (90-day and 2-year dietary studies and 2-generation reproduction study). There were no treatment-related gross pathological findings.

2. Caesarian section: There were no treatment-related effects on any caesarian section parameter examined including pregnancy rate. All dams terminated on gestation day 21 delivered viable fetuses and there were no dead fetuses at any dose level, including the controls. Gross pathological examination of the four high-dose dams dying prior to the scheduled termination indicated that these dams were pregnant with normally developing embryos respective to gestational age. Gravid uterine weight was not significantly affected by treatment at any dose level.

3. Developmental toxicity: Mean fetal body weight was slightly lower at 250 and 750 mg/kg bw/d compared to controls (≈3-4% for both). These differences were statistically significant, however, they were not considered to be biologically or toxicologically significant since the differences appeared to be minor, there was no clear dose-response between the doses and the values were within the normal range for animals of this strain and age from this laboratory. There were no treatment-related external, visceral or skeletal findings observed at any dose level. There was no significant treatment-related effect on the total number of fetuses or litters with external, visceral or skeletal findings. There was no evidence of treatment-related irreversible structural changes; therefore, under the conditions of this study, XDE-570 was not teratogenic.

The LOAEL for maternal toxicity was 750 mg/kg bw/d based on lower body weight, body-weight gain and food consumption and increased kidney weights. The NOAEL for maternal toxicity was 250 mg/kg bw/d.

The LOAEL for developmental toxicity was not determined. The NOAEL for developmental toxicity was 750 mg/kg bw/d based on the absence of any significant treatment-related effects on developmental parameters at this dose level.

C. Study deficiencies There are no deficiencies which would impact on the outcome of the study. The developmental toxicity study in the rat is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.