DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.3700b [§83-3b]; Developmental Toxicity Study in Rabbits

Work Assignment No. 4-1-128 J (MRID 46808233)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 12/19/06

Secondary Reviewer
John W. Allran, M.S.
Signature: John W Allran
Date: 12/20/06

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.
Signature: Michael E Viana
Date: 12/19/06

Quality Assurance:
Mary L. Menetrez, Ph.D.
Signature: Mary L Menetrez
Date: 12/20/06

Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** [signature]
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 5/31/07
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 5/31/07

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbits; OPPTS 870.3700b [§83-3b]; 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: Zablotny, C. L., and E. W. Carney (1997) XDE-570: oral gavage teratology study in New Zealand White rabbits. The Toxicology Research Laboratories, The Dow Chemical Company, Midland, MI. Laboratory Project Study ID: 960022, August 12, 1997. MRID 46808233. Unpublished.

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

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46808233), XDE-570 (Florasulam; 99.3% a.i.; Lot No. 940714) in aqueous 0.5% methylcellulose was administered daily via oral gavage to 20 naturally mated New Zealand White rabbits/group at a dose volume of 4 mL/kg at dose levels of 0, 50, 250, or 500 mg/kg/day from gestation day (GD) 7-19. On GD 28, all surviving does were killed and a limited necropsy was performed. The liver, kidneys, and gravid uterus were removed and weighed, and the fetuses were delivered by cesarean section and examined.

One 250 mg/kg/day doe aborted on GD 22, and one 500 mg/kg/day doe aborted on GD 17. Prior to aborting, both animals displayed decreased fecal output, body weight loss, and markedly lower food consumption. At necropsy, the 500 mg/kg/day doe was found to have findings indicative of pneumonia, which was most likely due to deposition of the test substance in the lungs. One 500 mg/kg/day doe was found dead on GD 19; the cause of death was attributed to a ruptured esophagus with atelactic lungs, with thoracic adhesions and hydrothorax present.
No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, organ weights, or gross pathologic examinations in the animals that survived to scheduled termination.

The maternal LOAEL is not determined and the maternal NOAEL is 500 mg/kg/day.

There were no premature deliveries or complete litter resorptions, and no effects of treatment on the numbers of litters, live fetuses, dead fetuses, or resorptions (early), or on gestation index, fetal body weights, sex ratio, post-implantation loss, or gravid uterine weights. There were no treatment-related external, visceral, or skeletal findings.

The developmental LOAEL is not determined and the developmental NOAEL is 500 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfies the guideline requirements for a developmental toxicity study in the rabbit. Although the animals were not dosed to the limit dose, a preliminary developmental toxicity study in rabbits (MRID 46808232) was performed and indicated that a dose of 600 mg/kg/day probably would have exceeded the maximum tolerated dose and resulted in excessive maternal death. Therefore, selection of the high dose (500 mg/kg/day) used in this study was considered reasonable. Additionally, while this study did not dose the animals for the recommended interval (implantation through the day prior to cesarean section), it must be noted that this study was performed prior to the adoption of the current guidelines (OPPTS 870.3700, August, 1998).

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 rabbits. 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.

46808233.PMRA.der
.pdf

Reviewer: Tom Morris , Date May 19, 2000.

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

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

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

CITATION: Zablotny, C. L. and Carney, E. W. August 12, 1997. **XDE-570; Oral Gavage Teratology Study in New Zealand White Rabbits.** Performing Laboratory: The Toxicology Research Laboratories, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory Project Study ID: 960022. 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 20 naturally mated, adult female New Zealand White rabbits/dose at dose levels of 0, 50, 250 or 500 mg/kg bw/d by oral gavage at a dose volume of 4 mL/kg bw from days 7 through 19 of gestation. Sexually mature, virgin adult females were naturally mated with one buck (1 male: 1 female) of the same strain at HRP Inc. prior to shipment to the testing facility. Dosing volume was adjusted daily, based on dam body weight during the dosing period.

There were no treatment-related effects on mortality, clinical signs, body weight or food consumption and no treatment-related necropsy findings or changes in organ or gravid uterine weights. When corrected for gravid uterine weight, body weight was unaffected by treatment. One dam at 250 (gestation day 22) and one at 500 (gestation day 17) mg/kg bw/d aborted prior to the scheduled necropsy. Examination of the uterus indicated that the dam at 250 mg/kg bw/d had 5 normally developing fetuses and 1 unaccounted for fetus while the dam at 500 mg/kg bw/d had 5 normally developing fetuses and two aborted fetuses of which one appeared normal and the other was unaccounted for. Prior to aborting, both dams exhibited markedly lower food consumption, a body weight loss and decreased or absent faecal output. There were no gross pathological findings in the dam at 250 mg/kg bw/d. Gross pathological findings in the dam at 500 mg/kg bw/d were indicative of pneumonia. One dam at 500 mg/kg bw/d was found dead on gestation day 19. The cause of death was attributed to a ruptured esophagus with atelactic lungs. Additionally, the dam exhibited thoracic adhesions and hydrothorax. Examination of the uterus indicated 7 implantation sites (7 normally developing fetuses). The single abortion at 250 mg/kg bw/d was considered to be a spontaneous occurrence. Single abortions and total resorptions may occur spontaneously in the strain of rabbit used. The abortion at 500 mg/kg bw/d was considered to be secondary to pneumonia which was most likely due to inadvertent deposition of the test substance into the lungs.

The LOAEL for maternal toxicity was not determined. The NOAEL for maternal toxicity was >500 mg/kg bw/d based on the absence of any treatment-related findings at this dose level

There were no treatment-related effects on fetal body weight and no treatment-related external, visceral or skeletal findings observed at any dose level. There was no 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 (florasulam) was not teratogenic.

The LOAEL for developmental toxicity was not determined. The NOAEL for developmental toxicity was

>500 mg/kg bw/d based on the absence of any treatment-related effects on developmental parameters at this dose level.

The developmental toxicity study in the rabbit is classified acceptable / guideline and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

Study deficiencies: There was no treatment-related maternal or developmental toxicity at 500 mg/kg bw/d, the highest dose tested. The highest dose tested should induce some overt maternal toxicity such as slight body weight loss but not more than 10% percent maternal mortality as indicated in OECD 414. The dose levels used in this study were based on a preliminary developmental toxicity study with New Zealand White rabbits (see DACO 4.5.3 - Zablotny, C. L and Carney, E.W., August 12, 1997. XDE-570: Oral gavage teratology probe study in New Zealand White rabbits. Laboratory Project Study ID DR-0312-6565-023). In the preliminary study, treatment-related findings were observed at ≥600 mg/kg bw/d. At 1,000 mg/kg bw/d, severe maternal toxicity was manifest as increased mortality (43%; 3/7 dams) with markedly lower food consumption (up to 59% lower), severe body-weight loss and reduced faecal output prior to death. At 600 mg/kg bw/d, maternal effects included one mortality (14%, 1/7 dams). This dam exhibited markedly lower food consumption, severe body weight loss and reduced faecal output prior to death. The remaining dams at 600 mg/kg bw/d, exhibited a lower overall body-weight gain (≈16% lower; gestation days 7-19) due to a body weight loss with no change in food consumption during gestation days 7-10 and lower body-weight gain (≈56% lower) with a concomitant lower food consumption (up to 35% lower) during the remainder of gestation. No treatment-related findings were observed at 100 or 300 mg/kg bw/d. The deaths were considered to be treatment-related although the possibility of gavage error could not be eliminated as a possible cause of death since the dam at 600 mg/kg bw/d and 2 dams at 1,000 mg/kg bw/d exhibited edematous lungs. Examination of the uterus indicated that all of these dams were pregnant with normally developing fetuses. Due to the severity of the toxicity observed, all surviving dams at 1,000 mg/kg bw/d were euthanised on gestation day 17. No fetal evaluation was done since dams were sacrificed on day 20 of gestation, one day after the final dosing. Based on the findings from the preliminary developmental study, the high-dose, 500 mg/kg bw/d, was expected to result in lower body-weight gain. However, there was no evidence of maternal or developmental toxicity at any dose level up to and including 500 mg/kg bw/d; therefore, the dose levels used may not be sufficient to determine teratogenic potential of the test substance. A dose level of 600 mg/kg bw/d could have been used, although the mortality rate (14%, 1 out 7 dams) in the preliminary study at 600 mg/kg bw/d, exceeded the 10% maternal deaths as indicated in OECD 414, however, it was uncertain whether this death was treatment-related. Although the maximum tolerated dose (MTD) was not achieved in this main rabbit developmental toxicity study, the next highest dose used in the preliminary rabbit developmental toxicity study, 600 mg/kg bw/d, exceeded the MTD based on maternal deaths and body-weight gain. In the rat developmental toxicity study (see DACO 4.5.2 - Liberacki, A.B., Carney, E.W. and Kociba, R.J. June 12, 1997. Laboratory Project Study ID: DR-0312-6565-027) there was no evidence of treatment-related irreversible structural changes at any dose level up to and including 750 mg/kg bw/d (HDT); therefore under the conditions of the study, XDE-570 was not teratogenic in the rat. It is questionable if any additional information would have been attained if the high-dose level was increased to 600 mg/kg bw/d; therefore, this developmental toxicity study in rabbits is considered acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

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-difluropheny!)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulphonamide

Description: White powdery solid

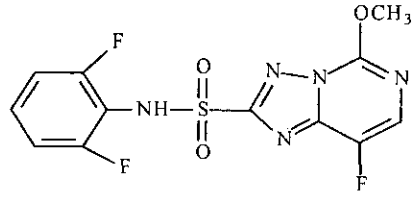
Lot/Batch #: Lot # 940714 / Test Substance # 100511

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 rabbits. Sexually mature, virgin adult females were naturally mated with one buck (1 ♂: 1 ♀) of the same strain at HRP Inc.

Strain: New Zealand White.

Age/weight at study initiation: At the time of mating the females were ≈5 to 6 months of age with a body weight range of ≈2.5 to 3.5 kg.

Source: HRP Inc., Kalamazoo, MI.

Housing: The animals were individually housed in suspended cages with flattened tube grid floors.

Diet: Animals received 2 ounces of Certified Laboratory Rabbit Chow # 5322 (Purina Mills Inc., St. Louis, MO) upon receipt and the amount of feed was increased incrementally by 2 ounces per day to a total of ≈8 ounces/day.

Water: Municipal tap water *ad libitum*

Environmental conditions:
Temperature: ≈20 °C
Humidity: 40-60%
Air changes: 12 air changes/hr
Photoperiod: 12 hrs dark/12 hrs light

Acclimation period: Approximately 6 days.

B. PROCEDURES AND STUDY DESIGN

1. **In life dates** - In-life study dates were not provided in study report. However, the observed day of coitus was considered day 0 of gestation. On day 28 of gestation, all surviving dams were euthanised and subjected to a gross pathological examination. Date of study conduct was from 22/10/96 to 12/08/97.

2. **Mating:** Adult females, approximately 5 to 6 months of age and weighing approximately 2.5 to 3.5 kg were naturally mated with one buck (1 ♂: 1 ♀) of the same strain at HRP Inc. The observed day of coitus was considered day 0 of gestation. Day 0 body weights and records of mating pairs were provided by HRP Inc., and maintained in the study records. The rabbits were shipped on day 0 or 1 of gestation.

3. **Animal Assignment:** The time-mated rabbits were randomly assigned to the dose groups, as indicated in Table 1,

using a computer generated procedure designed to achieve similar body weights across the dose groups.

TABLE 1: Animal Assignment.

Dose Level (mg/kg bw/day)	0	50	250	500
# Females	20	20	20	20

4. Dose selection rationale: The dose levels were based on an oral gavage teratology probe study in New Zealand White rabbits (see DACO 4.5.3 - Zablotny, C. L and Carney, E.W., August 12, 1997. Laboratory Project Study ID DR-0312-6565-023). Groups of seven time-mated female rabbits were administered XDE-570 suspended in aqueous 0.5 % Methocel (methylcellulose), by oral gavage, on days 7-19 of gestation at dose levels of 0, 100, 300, 600 or 1,000 mg/kg bw/d. Treatment-related findings were observed at ≥600 mg/kg bw/d. One dam at 600 mg/kg bw/d (gestation day 19) and 3 dams at 1,000 mg/kg bw/d (gestation days 10, 13 and 17) died prior to the scheduled necropsy. All of these dams exhibited markedly lower food consumption, severe body-weight loss and decreased faecal output prior to death. The deaths were considered to be treatment-related although the possibility of gavage error could not be eliminated as a possible cause of death. Examination of the uterus indicated that all of these dams were pregnant with normally developing fetuses. At 1,000 mg/kg bw/d, a significant body-weight loss was observed during gestation days 7-10 and 13-16. This was associated with concomitant lower food consumption and reduced faecal output. At 600 mg/kg bw/d, the overall body weight gain during treatment (days 7 through 19 of gestation) was lower (≈16%) compared to controls, this was attributed to a body-weight loss during gestation days 7-10 and a lower body-weight gain (≈56%) during gestation days 13-16. The body-weight loss during gestation days 7-10 was not associated with concomitant lower food consumption. The lower body-weight gain during gestation days 13-16 was associated with concomitant lower food consumption. There were no significant treatment-related effects on absolute or relative liver or kidney weights and no treatment-related gross pathological findings in the dams that survived to the scheduled necropsy on gestation day 20 at 100, 300 or 600 mg/kg bw/d. There were no treatment-related effects on caesarian section parameters examined including pregnancy rate at 100, 300 or 600 mg/kg bw/d (no data collected for dams at 1,000 mg/kg bw/d). The NOAEL for maternal toxicity was 300 mg/kg bw/d. No fetal evaluation was performed since dams were sacrificed on day 20 of gestation, one day after the final dosing; therefore, a NOAEL for developmental toxicity was not determined. In the main developmental / teratology study, 500 mg/kg bw/d was expected to result in lower body-weight gain. The lower doses were expected to provide dose-response data for any toxicity observed in the high-dose animals and to establish a NOAEL.

5. Dosage preparation and analysis The test substance was administered as a suspension in an aqueous vehicle of 0.5% Methocel (methylcellulose) such that a dose volume of 4 mL/kg bw yielded the targeted dose. The dose suspensions were prepared once prior to the start of the dosing period and once approximately midway through the dosing regimen. Analysis of all dosing suspensions from the first mix were performed to determine concentrations prior to and following the final dose of the first mix. Stability was established for the use period. In addition, the low dose and high dose suspensions from the first mix were analysed for homogeneity prior to or concurrent with the start of dosing. Reference samples of all dose suspensions including control were retained from each mix for possible analysis.

Results - **Homogeneity Analysis:** Analysis of all dose suspensions from the first mix confirmed that the test material was homogeneously suspended.

Dose Level (mixed/analysed 10/28/96)	50 mg/kg bw/day	500 mg/kg bw/day
Targeted Concentration (mg/mL)	12.5	125
Mean Observed Concentration (mg/mL)	11.6	116
Standard Deviation	0.10	1.53
% Relative Standard Deviation	0.86	1.32

Stability Analysis: Following dosing of the first mix, the concentrations of the low and high dose suspensions were 95 and 96% of their initial concentrations, respectively, indicating a stability of at least 15 days.

Date Mixed - 10/23/96		Targeted Concentration (mg/mL)	Observed Concentration (mg/mL)	% of Target	% of Initial Analysis
Day 1 Analysis	50 mg/kg bw/d	12.5	11.6	93	-
	500 mg/kg bw/d	125	116	93	-
Day 15 Analysis	50 mg/kg bw/d	12.5	11.0	88	95
	500 mg/kg bw/d	125	111	89	96

Concentration Analysis: Analyses of all dose suspensions from the first mix initially revealed mean concentrations of the test substance from 88 to 93% of targeted concentrations.

Date Mixed - 10/23/96	Targeted Concentration (mg/mL)	Observed Concentration (mg/mL)	% of Target
50 mg/kg bw/d	12.5	11.6	93
250 mg/kg bw/d	62.5	54.9	88
500 mg/kg bw/d	125	116	93

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 in aqueous 0.5% Methocel (methylcellulose) at a dosage volume of 4 mL/kg bw, on days 7 through 19 of gestation. Dosing volume was adjusted daily, based on current individual dam body weight during the dosing period.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The dams were checked daily for mortality or clinical signs. Body weight data were recorded on day 0 (by supplier), daily during the dosing period and on days 20 and 28 of gestation. Statistical analyses of body weights and body-weight gains were performed using data collected on days 0, 7, 10, 13, 16, 20 and 28 of gestation. Food consumption was recorded daily during the test period beginning on gestation day 4. On day 28 of gestation all surviving females were euthanised by an IV injection of Beuthanasia-D Special and a limited gross pathological examination (necropsy) was performed. Any obvious gross pathological alterations were recorded and the liver, kidney and gravid uterine weights were recorded. Sections of liver with gallbladder, kidneys and gross lesions were preserved in neutral, phosphate-buffered 10% formalin, but microscopic examination of tissue was not performed. In addition, the uterus, with attached ovaries was preserved in neutral phosphate-buffered 10% formalin until the reproductive data were verified and then discarded. 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

in order to determined pregnancy status. 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 that liver, kidney and gravid uterine weights were not determined. Also, the number of corpora lutea and the sex and body weight of the fetuses from these animals were not recorded. The degree to which the implantation site(s) had developed was determined to the extent possible by external examination and then discarded.

2. Fetal Evaluations - All fetuses were examined by dissection under a low power stereomicroscope for evidence of visceral alterations. This examination also included a fresh examination of the brain. All fetuses were then preserved in alcohol, eviscerated, cleared and stained with alizarin-red and examined for skeletal alterations.

D. DATA ANALYSIS

1. Statistical analyses: Maternal body weights, body-weight gains, food consumption, organ weights and mean fetal body weights per litter were evaluated by Bartlett's test for equality of variances. Based on the outcome of the Bartlett's test ($\alpha = 0.01$), a parametric or non-parametric ANOVA ($\alpha = 0.05$ for both) 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 lutes minus number of implantations), resorptions/litter and resorptions/fetal population, and fetal alterations were analysed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea, number of 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, females with resorptions only, or females found pregnant after staining their uteri were excluded from the appropriate analyses. Statistical outliers were identified using a sequential method ($\alpha = 0.05$, two-sided), and excluded only if justified by sound scientific reason. Both Dunnett's and Bonferroni's correction corrected for multiple comparisons to the control group 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:

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

$$\text{Gestation Index (\%)} = \frac{\text{\# of females with viable fetuses}}{\text{\# of pregnant females (with implants)}} \times 100$$

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

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

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: There were no treatment-related mortalities. One dam at 250 mg/kg bw/d aborted on gestation day 22 and one dam at 500 mg/kg bw/d aborted on gestation day 19. Examination of the uterus

indicated 6 implantation sites (5 normal appearing fetuses and 1 unaccounted for fetus) in the dam at 250 mg/kg bw/d and 7 implantation sites (5 normal appearing fetuses and two aborted fetuses, one of which appeared normal, the other unaccounted for) in the dam at 500 mg/kg bw/d. Prior to aborting, both dams exhibited markedly lower food consumption, a body weight loss and reduced or absent faecal output. There were no gross pathological findings in the dam at 250 mg/kg bw. Gross pathological findings in the dam at 500 mg/kg bw/d included diffuse, generalized firm lungs consistent with pneumonia. One dam at 500 mg/kg bw/d was found dead on gestation day 19. The cause of death was attributed to a ruptured esophagus with atelactic lungs. Additionally, the dam exhibited thoracic adhesions and hydrothorax. Examination of the uterus indicated 7 implantation sites (7 normal appearing fetuses). The single abortion at 250 mg/kg bw/d was considered to be a spontaneous occurrence. Single abortions and total resorptions may occur spontaneously in the strain of rabbits used. The abortion at 500 mg/kg bw/d was considered to be secondary to pneumonia which was most likely due to inadvertent deposition of the test substance into the lungs.

There were no treatment-related clinical observations.

2. Body Weight - There were no treatment-related effects on body weight or body-weight gain at any dose level up to and including 500 mg/kg bw/d, the highest dose tested (Table 2). When corrected for gravid uterine weight, body weight was unaffected by treatment. The dam at 250 mg/kg bw/d and the dam at 500 mg/kg bw/d which aborted exhibited a body-weight loss with concomitant lower food consumption prior to aborting. Compared to controls, body-weight gain was significantly lower in dams at 250 mg/kg bw/d during gestation days 10-13, however, this was not dose-related and was considered to be an incidental finding.

TABLE 2 Maternal Body Weight Gain (g ± SD) (a)

Interval		Dose Level (mg/kg bw/day)			
		0 (n = 20)	50 (n = 19)	250 (n = 20)	500 (n = 20)
Body weight (g ± SD)					
Pre-Treatment	Day 0	3101 ± 217	3102 ± 196	3112 ± 180	3143 ± 199
	Day 7	3327 ± 259	3366 ± 240	3322 ± 163	3381 ± 193
Treatment	Day 10	3353 ± 269	3368 ± 248	3350 ± 191	3365 ± 177 (n = 19) b
	Day 13	3423 ± 263	3408 ± 271	3374 ± 221	3415 ± 177 (n = 19) b
	Day 16	3504 ± 297	3473 ± 301	3435 ± 271	3497 ± 183 (n = 19) b
Post-treatment	Day 20	3543 ± 324	3546 ± 312	3463 ± 310	3575 ± 170 (n = 18) (c)
	Day 28	3679 ± 372	3683 ± 270	3663 ± 296 (n = 19) (c)	3703 ± 200 (n = 18) (c)
	Day 28 (d)	3181 ± 341	3196 ± 263	3170 ± 268 (n = 19) (c)	3246 ± 216 (n = 18) (c)
Body-weight gain (g ± SD)					
Pretreatment	Day 0-7	226.4 ± 106.2	264.5 ± 124.6	209.8 ± 107.1	238.1 ± 132.5
Treatment	Days 7-10	25.4 ± 50.5	2.2 ± 36.7	28.2 ± 61.3	2.6 ± 62.7
	Days 10-13	69.9 ± 40.9	39.3 ± 67.3	23.3 ± 54.4 *	50.6 ± 35.3
	Days 13-16	81.2 ± 59.1	65.2 ± 88	61.5 ± 89.7	81.4 ± 30.3
	Days 16-20	39.3 ± 95	72.8 ± 63	27.7 ± 96.8	58.4 ± 39.6
	Days 7-20	215.8 ± 135.1	179.5 ± 91.9	140.7 ± 225.7	196.1 ± 82.3
Post-treatment	Days 20-28	136.3 ± 110	137.6 ± 96.1	164.1 ± 73	128.0 ± 141.8
Overall	Days 0-28	578.5 ± 232.1	581.6 ± 151.8	545.1 ± 232.6	565.5 ± 207.8
	Days 0-28 (d)	79.1 ± 231.1	94.2 ± 153.0	52.4 ± 211.0	108.5 ± 218.8

(a) Data extracted from pages 30-31 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 54-57 of the study report, no statistical analysis was done. Animals which were non-pregnant or had totally resorbed litters were excluded from analysis.
(b) Change in (n) value due to values excluded from analysis due to probable gavage error.
(c) Change in (n) value due to abortion or spontaneous death.
(d) Body weight, body-weight gain corrected for gravid uterine weight.
* Statistically different from control mean by Dunnett's test, $p \leq 0.05$.

3. Food Consumption - No treatment-related effects on food consumption were observed at any dose level up to and including 500 mg/kg bw/d, the highest dose tested. The dam at 250 mg/kg bw/d and the dam at 500 mg/kg bw/d which aborted exhibited a markedly lower food consumption with a concomitant body-weight loss prior to aborting. Food consumption was significantly higher on gestation days 24-25 in dams at 500 mg/kg bw/d in comparison to controls (≈26% higher compared to controls). Due to the transient nature and the absence of any corresponding changes in body weight or body-weight gain, this was considered to be incidental.

4. Gross Pathology - There were no treatment-related effects on absolute or relative liver or kidney weights and no treatment-related gross pathological findings at any dose level up to and including 500 mg/kg bw/d, the highest dose tested. There were no gross pathological findings in the dam at 250 mg/kg bw/d aborting on gestation day 22. Gross pathological findings in the dam at 500 mg/kg bw/d aborting on gestation day 17 included diffuse, generalized firm lungs consistent with pneumonia. The death of the dam at 500 mg/kg bw/d found dead on gestation day 19 was attributed to a ruptured esophagus with atelactic lungs. Additionally, the dam exhibited thoracic adhesions and hydrothorax.

5. Caesarean Section Data - The pregnancy rate was 100, 95, 100 and 100% at 0, 50, 250 and 500 mg/kg bw/d, respectively. The gestation index was 100, 100, 95 and 90% at 0, 50, 250 and 500 mg/kg bw/d, respectively. At 250 mg/kg bw/d one dam aborted on day 22 of gestation. Examination of the uterus revealed 6 implantation sites (5 normal appearing fetuses and 1 unaccounted for fetus). At 500 mg/kg bw/d, one dam aborted on day 17 of gestation. Examination of the uterus indicated 7 implantation sites (5 normal appearing fetuses and two aborted fetuses, one appeared normal, the other was unaccounted for). Examination of the uterus of the dam at 500 mg/kg bw/d found dead on gestation day 19 indicated 7 implantations sites (7 normal appearing fetuses). The single abortion at 250 mg/kg bw/d was considered to be a spontaneous occurrence. Single abortions and total resorptions may occur spontaneously in the strain of rabbits used. The abortion at 500 mg/kg bw/d was considered to be secondary to pneumonia. No significant differences were seen in the number of corpora lutea or implantation sites. There were no significant effects on resorptions or implantation loss (pre- or post-implantation loss), between animals of the control and treatment groups. No significant differences in the litter size, the number of live fetuses/litter, or the ratio of male/female fetuses were observed between the control and treatment groups. There were no dead fetuses in the control or treatment groups. Mean fetal body weight and gravid uterine weight were not significantly affected by treatment at any dose level. Caesarean section data are summarized in Table 3.

TABLE 3 Caesarean Section Observations (a)

Observation		Dose Level (mg/kg bw/day)			
		0	50	250	500
# Animals Assigned (Mated)		20	20	20	20
# Animals Pregnant		20	19	20	20
Pregnancy Rate (%)		100	95	100	100
# Nonpregnant		0	1	0	0
Maternal Wastage	# Died	0	0	0	1
	# Died Pregnant	0	0	0	1

Observation	Dose Level (mg/kg bw/day)			
	0	50	250	500
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	1	1
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	193 9.7 ± 2.2	190 10.0 ± 1.8	187 9.8 ± 2.1	179 9.9 ± 1.4
Total # Implantations (Implantations/Dam)	171 8.6 ± 2.0	168 8.8 ± 1.5	164 8.6 ± 1.6	147 8.2 ± 2.2
Total # Pre-Implantation Loss	22	22	23	32
Pre-implantation Loss (%)	10.8 ± 11.0	10.6 ± 11.2	11.1 ± 13.2	17.1 ± 20.6
Total # Viable Litters	20	19	19	18
Total # Live Fetuses (litters) (Live Fetuses/Dam)	166 8.3 ± 1.9	172 (c) 8.5 ± 1.5	160 8.4 ± 1.7	140 7.8 ± 2.3
Gestation Index (%)	100	100	95	90
Total # Dead Fetuses (Dead Fetuses/Dam)	0 0	0 0	0 0	0 0
Total # Resorptions (Early)	5	6	4	7
Total # Resorptions/Dam	0.3 ± 0.6	0.3 ± 0.5	0.2 ± 0.4	0.4 ± 0.6
% Implantations Resorbed	2.9 (5/171)	3.6 (6/168)	2.4 (4/164)	4.8 (7/147)
% Litters with Resorptions	20 (4/20)	31.6 (6/19)	21.1 (4.19)	33.3 (6/18)
Resorptions/Litters with Resorption	1.3 (5/4)	1.0 (6/6)	1.0 (4/4)	1.2 (7/6)
Litters with Total Resorptions	0	0	0	0
Post-implantation Loss (%) (b)	2.92	3.57	2.44	4.76
Mean Fetal Weight (g ± SD) Sexes Combined	38.99 ± 4.0	37.33 ± 3.36	38.94 ± 3.72	38.87 ± 4.51
Gravid Uterine Weight (g ± SD)	501.0 ± 92.6	487.5 ± 69.1	492.7 ± 80.0	457.0 ± 102.1
Average # Males/Litter (total # of males)	4.3 ± 1.8 (86)	4.7 ± 1.5 (90)	4.5 ± 1.8 (85)	3.7 ± 1.9 (66)
Average # Females/Litter (total # of females)	4.0 ± 1.3 (80)	3.7 ± 1.6 (71)	3.9 ± 1.4 (75)	4.1 ± 1.7 (74)
Sex Ratio (% Male/% Female)	52/48	56/44	55/45	47/53

(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 66-69 of study report, no statistical analysis done.
(c) The sex of one fetus was not determined.

B. DEVELOPMENTAL TOXICITY

1. External Examination - There were no treatment-related 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. One fetus at 500 mg/kg bw/d exhibited a rudimentary tail. This was considered to be a malformation. The observed external findings occurred at a low rate of incidence, were not dose-related and / or were within the normal range for animals of this age and strain from this laboratory. External examination findings are summarized in Table 4a.

TABLE 4a. External Examinations [expressed as fetal (litter) incidence]. (a)

Observations+	Dose Level (mg/kg bw/day)			
	0	50	250	500
#Fetuses(litters) examined	166 (20)	162 (19)	160 (19)	140 (18)
#Fetuses(litters) affected	0 (0)	1 (1)	0 (0)	1 (1)
Total # fetuses (litters) with external malformations (b)	0 (0)	1 (1)	0 (0)	0 (0)
Petechial haemorrhage	0 (0)	1 (1)	0 (0)	0 (0)
Edema	0 (0)	1 (1)	0 (0)	0 (0)
Rudimentary tail (c)	0 (0)	0 (0)	0 (0)	1 (1)

+ Some observations may be grouped together
(a) Data extracted from pages 34-35 (group data) and 141-247 (individual data) of the study report.
(b) Total number of fetuses (litters) with external malformations as indicated in the study report
(c) Considered to be a malformation as indicated in the study report.

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 findings in one fetus at 50 mg/kg bw/d (ventricular septal defect) and one fetus at 500 mg/kg bw/d (retroesophogeal right subclavian artery) were considered to be malformations. The observed visceral findings occurred at a low rate of incidence, were not dose-related and / or were within the normal range for animals of this age and strain from this laboratory. Visceral examination findings are summarized in Table 4b.

TABLE 4b. Visceral Examinations [expressed as fetal (litter) incidence]. (a)

Observations+	Dose Level (mg/kg bw/day)			
	0	50	250	500
#Fetuses(litters) examined	166 (20)	162 (19)	160 (19)	140 (18)
#Fetuses (litters) affected	19 (11)	22 (9)	5 (4)	22 (8)
Total # fetuses (litters) visceral malformations (b)	0 (0)	1 (1)	0 (0)	1 (1)
Pericardial - excessive fluid	1 (1)	0 (0)	0 (0)	0 (0)
Ventricular septal defect (c)	0 (0)	1 (1)	0 (0)	0 (0)
Retroesophageal right subclavian artery (c)	0 (0)	0 (0)	0 (0)	1 (1)
Missing lung lobe/ caudal	13 (8)	14 (5)	3 (2)	18 (6)
Retrocaval ureter	5 (3)	7 (6)	2 (2)	3 (3)

+ Some observations may be grouped together
(a) Data extracted from pages 34-35 (group data) and 141-247 (individual data) of the study report.
(b) Total number of fetuses (litters) with visceral malformations as indicated in the study report.
(c) Considered to be a malformation as indicated in the study report.

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. Skeletal findings in one fetus at 250 mg/kg bw/d (centra - fused/thoracic and vertebrae - fused/thoracic) were considered to be malformations. The observed skeletal findings occurred at a low rate of incidence, were not dose-related and / or were within the normal range for animals of this age and strain from this laboratory. Skeletal examination findings are summarized in Table 4c.

TABLE 4c. Skeletal Examinations [expressed as fetal (litter) incidence]. (a)

Observations+		Dose Level (mg/kg bw/day)			
		0	50	250	500
#Fetuses(litters) examined		166 (20)	162 (19)	160 (19)	140 (18)
#Fetuses(litters) affected		91 (20)	91 (19)	87 (18)	71 (17)
Total # fetuses (litters) with skeletal malformations (b)		0 (0)	0 (0)	1 (1)	0 (0)
Skull	- delayed ossification	0 (0)	1 (1)	0 (0)	0 (0)
Hyoid	- crooked - delayed ossification	3 (2) 49 (18)	0 (0) 52 (14)	6 (4) 46 (13)	4 (4) 40 (13)
Centra	- extra site of ossification/ thoracic - fused /thoracic (c)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 1 (1)	1 (1) 0 (0)
Vertebrae	- fused/thoracic (c) - misaligned/caudal	0 (0) 0 (0)	0 (0) 0 (0)	1 (1) 6 (1)	0 (0) 0 (0)
Sternebrae	- delayed ossification - extra site of ossification - fused - irregular pattern of ossification	53 (17) 0 (0) 1 (1) 2 (2)	57 (16) 0 (0) 1 (1) 2 (2)	58 (18) 1 (1) 2 (2) 0 (0)	46 (15) 0 (0) 1 (1) 2 (2)

+ Some observations may be grouped together
(a) Data extracted from pages 34-35 (group data) and 141-247 (individual 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 as indicated in the study report.

III. DISCUSSION

A. Investigators' conclusions (extracted from page 18 of the study report): "In conclusion, oral administration of XDE-570 to time mated female rabbits resulted in no maternal or developmental effects. Though no evidence of maternal effects was seen in the present study, a previous probe study in New Zealand White rabbits used to select dose levels for the present study produced maternal toxicity at a slightly higher dose level. In this probe study, a dose of 600 mg/kg/day resulted in the death of 1 of 7 rabbits, a decrease of approximately 12% in feed consumption over the dosing period, and a transient decrease in body weight gain. A dose level of 1000 mg/kg/day in this same probe study produced severe maternal toxicity including mortality and significant weight loss (Zablotny and Quast, 1996). Based on the results of the present study, the no-observed-effect-level (NOEL) for maternal and embryonal /fetal toxicity for this study was 500 mg/kg/day. There was no evidence of teratogenicity at dose levels up to and including 500 mg/kg/day."

B. Reviewer's discussion:

1. Maternal toxicity: There were no treatment-related effects on mortality, clinical signs, body weight or food consumption and no treatment-related necropsy findings or changes in organ or gravid uterine weights. When corrected for gravid uterine weight, body weight was unaffected by treatment. One dam at 250 (gestation day 22) and one at 500 (gestation day 17) mg/kg bw/d aborted prior to the scheduled necropsy. Examination of the uterus indicated that the dam at 250 mg/kg bw/d had 5 normally developing fetuses and 1 unaccounted for fetus while the dam at 500 mg/kg bw/d had 5 normally developing fetuses and two aborted fetuses of which one appeared normal and the other unaccounted for. Prior to aborting, both dams exhibited markedly lower food consumption, a body weight loss and decreased or absent faecal output. There were no gross pathological findings in the dam at 250

mg/kg bw/d. Gross pathological findings in the dam at 500 mg/kg bw/d were indicative of pneumonia. One dam at 500 mg/kg bw/d was found dead on gestation day 19. The cause of death was attributed to a ruptured esophagus with atelactic lungs. Additionally, the dam exhibited thoracic adhesions and hydrothorax. Examination of the uterus indicated 7 implantation sites (7 normally developing fetuses). The single abortion at 250 mg/kg bw/d was considered to be a spontaneous occurrence. Single abortions and total resorptions may occur spontaneously in the strain of rabbit used. The abortion at 500 mg/kg bw/d was considered to be secondary to pneumonia which was most likely due to inadvertent deposition of the test substance into the lungs.

2. Caesarian section: No adverse effects on any caesarian section parameter examined including pregnancy rate and gestation index. Gravid uterine weight was not significantly affected by treatment.

3. Developmental toxicity: Mean fetal body weight was unaffected by treatment up to and including 500 mg/kg bw/d, the highest dose tested. There were no treatment-related external, visceral or skeletal findings observed at any dose level. There was no 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 (florasulam) was not teratogenic.

The LOAEL for maternal toxicity was not determined. The NOAEL for maternal toxicity was >500 mg/kg bw/d based on the absence of any treatment-related findings at this dose level

The LOAEL for developmental toxicity was not determined. The NOAEL for developmental toxicity was >500 mg/kg bw/d based on the absence of any treatment-related effects on developmental parameters at this dose level.

C. Study deficiencies There was no treatment-related maternal or developmental toxicity at 500 mg/kg bw/d, the highest dose tested. The highest dose tested should induce some overt maternal toxicity such as slight body weight loss but not more than 10% percent maternal mortality as indicated in OECD 414. The dose levels used in this study were based on a preliminary developmental toxicity study with New Zealand White rabbits (see DACO 4.5.3 - Zablotny, C. L and Carney, E.W., August 12, 1997. XDE-570: Oral gavage teratology probe study in New Zealand White rabbits. Laboratory Project Study ID DR-0312-6565-023). In the preliminary study, treatment-related findings were observed at ≥600 mg/kg bw/d. At 1,000 mg/kg bw/d, severe maternal toxicity was manifest as increased mortality (43%; 3/7 dams) with markedly lower food consumption (up to 59% lower), severe body-weight loss and reduced faecal output prior to death. At 600 mg/kg bw/d, maternal effects included one mortality (14%, 1/7 dams). This dam exhibited markedly lower food consumption, severe body weight loss and reduced faecal output prior to death. The remaining dams at 600 mg/kg bw/d, exhibited a lower overall body-weight gain (≈16% lower; gestation days 7-19) due to a body weight loss with no change in food consumption during gestation days 7-10 and lower body-weight gain (≈56% lower) with a concomitant lower food consumption (up to 35% lower) during the remainder of gestation. No treatment-related findings were observed at 100 or 300 mg/kg bw/d. The deaths were considered to be treatment-related although the possibility of gavage error could not be eliminated as a possible cause of death since the dam at 600 mg/kg bw/d and 2 dams at 1,000 mg/kg bw/d exhibited edematous lungs. Examination of the uterus indicated that all of these dams were pregnant with normally developing fetuses. Due to the severity of the toxicity observed, all surviving dams at 1,000 mg/kg bw/d were euthanised on gestation day 17. No fetal evaluation was done since dams were sacrificed on day 20 of gestation, one day after the final dosing. Based on the findings from the preliminary developmental study, the high-dose, 500 mg/kg bw/d, was expected to result in lower body-weight gain. However, there was no evidence of maternal or developmental toxicity at any dose level up to and including 500 mg/kg bw/d; therefore, the dose levels used may not be sufficient to determine teratogenic potential of the test substance. A dose level of 600 mg/kg bw/d could have been used, although the mortality rate (14%, 1 out 7 dams) in the preliminary study at 600 mg/kg bw/d, exceeded the 10% maternal deaths as indicated in OECD 414, however, it was uncertain whether this death was treatment-related. Although the maximum tolerated dose (MTD) was not achieved in this main rabbit developmental toxicity study, the next highest dose used in the preliminary rabbit developmental toxicity study, 600 mg/kg bw/d, exceeded the MTD based on maternal deaths and body-weight gain. In the rat developmental toxicity study (see DACO 4.5.2 - Liberacki, A.B., Carney, E.W. and Kociba, R,J. June 12, 1997. Laboratory Project Study ID: DR-0312-6565-027) there was no evidence of treatment-related irreversible structural changes at any dose level up to and including 750 mg/kg bw/d

(HDT); therefore under the conditions of the study, XDE-570 was not teratogenic in the rat. It is questionable if any additional information would have been attained if the high-dose level was increased to 600 mg/kg bw/d; therefore, this developmental toxicity study in rabbits is considered acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.