EPA Reviewer: John E. Whalan   _John E. Whalan_ Date: 6-5-02
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M.   _[signature]_ Date: 7/9/02
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit [OPPTS 870.3700 (§83-3)]

DP BARCODE: D268553   SUBMISSION CODE: none
P.C. CODE: 082583   TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): XRD-537 BE (Cyhalofop butyl, 97.1% a.i.)

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propanoate;
             XDE-537 BE; XDE-537; XRD-537; XRD-537 nBu; XDE-537 nBu;
             XRD-537 n Butyl Ester; DEH-112

CITATIONS: Aoyama, H. (1994) A teratogenicity study in rabbits with XRD 537 BE. The
             Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo
             187, Japan. Laboratory Study ID. GHF-P-1391, March 8, 1994. MRID
             45014710. Unpublished.

             E.W. Carney, _et al._ (2001) XDE-537 Teratogenicity Study in Rabbits: Reanalysis
             of Fetal anomaly Data. Toxicology & Environmental Research and Consulting,
             The Dow Chemical Company, Midland, Michigan. Report ID DR-0287-1467-
             012, May 21, 2001, MRID 45413901

SPONSOR: Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan;
             Dow Chemical Japan Ltd., Seavans North 2-1, Shibaura 1-chome, Minato-ku,
             Tokyo 105, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45014710 and
45413901), 18 inseminated specific pathogen free Japanese White (Kbl:JW) rabbits per group
were administered XRD-537 BE (Lot No.: AGR 295713; purity: 97.1%) by gavage in 1%
aqueous carboxymethylcellulose at doses of 0, 40, 200, or 1000 mg/kg/day on gestation days
(GDs) 6-18, inclusive, with the day following the day of artificial insemination designated as GD
0. On GD 27, all surviving does were sacrificed and necropsied, and all fetuses were weighed
and subjected to external examination, then sexed internally and examined viscerally by fresh
dissection, followed by evisceration and processing for skeletal examination.

The evaluation of this study is complicated by a high incidence of maternal mortality. In the
1000 mg/kg/day group, seven deaths occurred on GDs 15-19 following hematuria on the day of
death and, in most cases, one and/or two days prior to death; one dam was killed _in extremis_ on

GD 13 due to exhibiting "ventral posture;" and an additional dam died on GD 25 without exhibiting any abnormal clinical signs. In the 200 mg/kg/day group, one dam died on GD 21 after exhibiting loose stool and/or soiled fur in the lower abdominal/external genital region, hematuria, and feces with yellowish white, viscous material; and another dam aborted and was sacrificed on GD 25, following observations of hematuria and loose stool. Hematuria was also observed in one dam each in the control and 200 mg/kg/day groups during the pre-dosing interval, in another control dam on GD 24, and in one dam in the 200 mg/kg/day group on GD 18. The number of animals exhibiting hematuria during the dosing interval was significantly increased (p<0.01) at the 1000 mg/kg/day dose level. Considering the pattern of occurrence, what was reported as hematuria was probably concentrated, blood-colored urine which can be attributed to dehydration, rather than to disease or the test article.

Absolute body weights and body weight gains were similar in all groups through GD 18, but increased in a dose-related manner after dosing ceased (GDs 18-27) except for the controls which lost body weight. Adjusted body weight also increased in a dose-related manner. Food consumption mirrored body weight gain, with the most notable anomaly being a significant decrease in mean food consumption in the control dams due to anorexia in 5 of 18 dams and reduced food consumption in several other dams.

The predominant necropsy findings from the animals that died after exhibiting hematuria included "cloudy" or dark colored kidneys, whitish membranous material on the gastric mucosa or white spots in the stomach, urinary bladder distended with red or brown urine, and scanty gastrointestinal contents. Necropsy findings from the surviving animals also included "dark" or cloudy colored kidneys in three high-dose animals, and "cloudy" colored kidneys in one mid-dose animal. The significance of these findings is unknown.

The maternal LOAEL is 200 mg/kg/day based on maternal death. The maternal NOAEL is 40 mg/kg/day.

There were no treatment-related effects on intrauterine parameters. The overall incidence rates for litters containing fetuses with external, visceral, and/or skeletal malformations in the 0, 40, 200, and 1000 mg/kg/day groups were 2/17 (11.8%), 5/18 (27.8%), 7/16 (43.8%), and 0/9, respectively.

At the 200 mg/kg/day dose level, but not at the 1000 mg/kg/day dose level, the litter incidence of total malformations was significantly greater than controls (p<0.05) and exceeded the historical control range (11.8-31.3%), and the litter incidence of skeletal malformations was also increased (6/16 vs. 1/17 for controls; p<0.05) although there were no increased incidences of any individual malformations. These increases were most likely due to maternal stress rather than test article toxicity.

The developmental NOAEL is ≥1000 mg/kg/day (limit dose).

This study is classified as **Acceptable/Guideline** and does satisfy the requirements for a developmental toxicity study [OPPTS: 870.3700 (83-3b)] in rabbits.

COMPLIANCE: Signed and dated Data Confidentiality, Good Laboratory Practice, Quality Assurance, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE
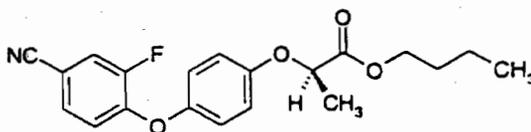
    Description: Off-white powder
    Lot No.: AGR 295713
    Purity: 97.1%
    Stability of compound: "Stable in a dark and cold environment"
    CAS No.: 122008-85-9
    Structure:

2. Vehicle and/or positive control

    The vehicle control article was a 1 % aqueous solution of carboxymethylcellulose in purified water (CMC, Kanto Chemical Co., Inc., Lot no. 207N1441). No positive control was used in this study.

3. Test animals

    Species: Rabbit
    Strain: Specific pathogen free Japanese White (Kbl:JW)
    Age and weight at study initiation: approximately 18 weeks; 3484-4402 g. on GD 0
    Source: Ina Research Laboratory, KITAYAMA LABES Co., Ltd.
    Housing: individually in suspended aluminum cages with wire-mesh floors, 350 mm x 480 mm x 330 mm
    Diet: Solid feed (GC4, Oriental Yeast Co., Ltd.) was available *ad libitum*.
    Water: Well water passed through precipitating and sedimentary procedures and sterilized with hypochlorous acid and ultraviolet light exposure was available *ad libitum*.
    Environmental conditions:
        Temperature: 22±2°C
        Humidity: 55±10%
        Air changes: approximately 15/hour
        Photoperiod: 12 hr light/12 hr dark
    Acclimation period: 17 days prior to study initiation

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the maternal and developmental toxicity potential of XRD-537 BE when administered by gavage to rabbits on GD 6-18, inclusive.

1. In life dates

Start: November 9, 1992; end: December 11, 1992

2. Artificial insemination

Females were inseminated using pooled semen collected from 3 resident males of the same strain as the females. Diluted semen was used to inseminate twelve or sixteen does. Each doe received an intravenous injection of human chorionic gonadotropin immediately following insemination. The day after the day of artificial insemination was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were randomized and assigned to groups in such a way as to equalize group means and standard deviations of body weights.

TABLE 1: Animal assignment		
Test Group	Dosage Level (mg/kg/day)	Number Assigned
1. Control	0	18
2. Low-Dose	40	18
3. Mid-Dose	200	18
4. High-Dose	1000	18

Data taken from text, p. 6, MRID 45014710.

4. Dose selection rationale

Doses were selected based on the results of preliminary studies in the rabbit. The report stated that there were no overt toxic effects on maternal rabbits or their fetuses at dose levels up to 1000 mg/kg/day. No other data from the preliminary studies were provided.

5. Dosing

All doses were administered in a volume of 5 mL/kg of body weight, based on the most recently recorded body weight.

6. Dose solution preparation and analysis

Test article formulations were prepared weekly during the study. The report did not give details of the method of dose solution preparation other than stating that "the test

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substance was suspended in purified water with the aid of 1% CMC." The report also did not mention the storage conditions of the dosing formulations. Duplicate samples of all dosing solutions from all three mixes were collected for concentration analysis prior to use. The analytical method used was high performance liquid chromatography. Stability analysis was conducted at an earlier date, in conjunction with a preliminary study (IET 90-0174), and these results were summarized in the report, but no data were included. Homogeneity analysis of dosing solutions of the test article in 1% CMC was conducted in conjunction with a developmental toxicity study in rats (MRID 45014709); however, the method of dose solution preparation was not described for this study either and may have been different from that used in the current study. A separate stability and homogeneity study (MRID 45000528) was provided in which suspensions of radiolabelled and/or non-radiolabelled XRD-537 BE in 0.5% CMC either alone or with 2% Tween 80 were analyzed for homogeneity of radioactivity and/or stability.

Results –
Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the low-, mid-, and high-dose formulations were 96-100%, 98-101%, and 97-100%, respectively, of nominal. All of the individual measured concentrations were within 5% of nominal.

Homogeneity analysis: Homogeneity was confirmed in MRID Nos. 45014709 and 45000528. However, in MRID 45000528 it was noted that the solutions became non-homogenous when allowed to stand for 5-30 minutes after preparation.

Stability analysis: The study author stated that results of the preliminary study (IET 90-0174) indicated that the test substance was stable in a 1% CMC solution for at least 10 days. The storage conditions used in the preliminary study (IET 90-0174) were not described, and none of the data from the preliminary study were provided. Stability of solutions of the test material in 0.5% CMC with 2% Tween 80 for up to 21 days of storage at 4°C was confirmed in MRID 45000528.

The analytical results from the concentration analyses of the dosing formulations were satisfactory. However, the study did not include adequate information about the storage conditions of the dosing formulations, and there was no mention made of stirring the dosing formulations during dosing to maintain homogeneity. Nevertheless, the rabbits likely received the expected dosages.

C. <u>OBSERVATIONS</u>

1. <u>Maternal observations and evaluations</u>

The animals were observed at least once a day from GD 0 through GD 27 for clinical signs and mortality. Body weights were recorded on GDs 0, 3, 6-18, 21, 24, and 27, and food consumption was recorded on alternate days during GDs 0-26 and on GD 27. Dams were sacrificed on GD 27 by intravenous injection of sodium pento-

barbital and subjected to gross necropsy. The reproductive tract was excised, and the number of corpora lutea on each ovary was recorded. The gravid uterine weight as well as numbers of viable and nonviable fetuses, resorptions, and implantations were recorded. Resorptions or dead fetuses were classified as implantation sites, placental remnants, or macerated fetuses. While these categories were not further defined in the study report, the reviewer is interpreting them as roughly corresponding to early resorptions, late resorptions, and late fetal deaths, respectively, although "macerated fetuses" could also include some late resorptions, and "placental remnants" could also include some early resorptions. Uteri without gross evidence of implantation were checked for evidence of early implantation loss by the Salewski method. In addition, the liver, spleen, kidneys, and ovaries were retained in 10% neutral buffered formalin. All animals that aborted, died, or were sacrificed moribund were subjected to gross necropsy.

2. Fetal evaluations

Fetal and placental weights were recorded, then fetuses were euthanized and subjected to external examination, including examination of the eyes by removing the palpebral skin. Fetuses were sexed internally and examined for visceral alterations by the Stuckhardt and Poppe fresh dissection technique. Fetal thoracic and abdominal organs were retained in 10% neutral buffered formalin, along with the placentas. All fetal skeletons were stained with Alizarin Red S and cleared in 70% glycerin, than subjected to skeletal examination.

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weights and body weight changes, maternal food consumption, gravid uterine weights, fetal and placental weights, and numbers of corpora lutea, total implantations, and viable fetuses were subjected to statistical analysis as follows. Bartlett's test was used to evaluate the homogeneity of variances. Data with homogeneous variances were subjected to a one-way parametric analysis of variance followed by Dunnett's t-test or Scheffe's multiple comparison test if significant. Data with heterogeneous variances were subjected to the Kruskal-Wallis test followed by a Dunnett-type mean rank test or a Scheffe-type mean rank test if significant. Incidence data such as clinical signs, gross necropsy findings, fetal sex ratios, and fetal and litter incidences of malformations and variations were analyzed using Fisher's exact probability test. Litter proportions of resorptions and fetal deaths were analyzed using the Mann-Whitney U test. A minimum significance level of $p<0.05$ was used for all analyses.

The sponsor, the Dow Chemical Company, submitted a supplemental study report (MRID 45413901; May 21, 2001) which revealed the statistical methods used to evaluate incidences of lumbar ribs were not entirely appropriate. In the initial study report, it states that a Fisher's exact probability test was used for data on the

incidences of maternal rabbits having fetuses with malformations and variations (i.e., a litter based statistic), which is an acceptable method. However, the investigators also ran an additional Fisher's test to analyze the <u>percentage of fetuses</u> with specific malformations and variations, which is <u>not</u> appropriate. That the investigators ran separate Fisher's tests for both the percentage of affected litters and the percentage of affected fetuses was corroborated by the appearance of Table 10 in the report, in which statistical significance for fetal and litter incidences are denoted separately.

The lumbar rib data were reanalyzed using the Censored Wilcoxon test, which is a litter-based analysis, but which uses the fetal incidence data as a secondary ranking factor when the litter is totally unaffected, and combined the probabilities from both analyses. Using an appropriate litter-based statistical method that is ideally suited for developmental toxicity incidence data, no evidence of an increase in the incidence of lumbar ribs was found. The results of this reanalysis are reflected in this DER.

2. <u>Historical control data</u> were not provided although the historical control ranges for preimplantation loss, overall litter incidence of malformations, and the fetal incidence of the skeletal variation lumbar ribs were mentioned in the results or discussion sections of the study report. Information regarding the number of studies included, dates of the studies, source of the animals, and the vehicle(s) and route(s) of administration was not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

There were seven deaths in the 1000 mg/kg/day group on GDs 15-19 following the observation of hematuria on the day of death and, in most cases, one and/or two days prior to death. The animal that died on GD 15 also exhibited hypoactivity on GD 14. An additional animal from the 1000 mg/kg/day group died on GD 25 without exhibiting any abnormal clinical signs, and one animal was killed *in extremis* on GD 13 due to exhibiting "ventral posture." In the 200 mg/kg/day group, one animal died on GD 21 after exhibiting loose stool and/or soiled fur in the lower abdominal/external genital region on GDs 14-21, hematuria on GDs 19-21, and feces with yellowish white, viscous material on GD 19. One animal from the 200 mg/kg/day group aborted and was sacrificed on GD 25, following observations of hematuria on GDs 18 and 22 and loose stool on GDs 14-19 and 21-24.

Clinical signs observed from animals that survived to termination included the following: one female in each of the control and 200 mg/kg/day groups exhibited hematuria during the pre-dosing interval, on GDs 1 and 5, respectively; and hematuria was also observed from a different control group animal on GD 24 and from one animal from the 200 mg/kg/day group on GD 18.

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At the 1000 mg/kg/day dose level, the number of animals exhibiting hematuria during the dosing interval (GD 7-19) was significantly greater than controls (0/17, 0/18, 2/18, and 7/18 animals from the 0, 40, 200, and 1000 mg/kg/day groups, respectively; p<0.01). The number of animals exhibiting hematuria at any time during the study was 2/17, 0/18, 4/18, and 7/18 (n.s.) animals from the 0, 40, 200, and 1000 mg/kg/day groups, respectively.

2. Body weight

Selected maternal body weight data are given in Table 2. Absolute body weights and body weight gains were similar in all groups through GD 18, but increased in a dose-related manner after dosing ceased (GDs 18-27) except for the controls which lost body weight. Adjusted body weight also increased in a dose-related manner.

TABLE 2: Selected mean maternal body weights and body weight changes during gestation (g)				
GD	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Absolute body weights				
0	3850	3868	3868	3865
6	3966	3979	3986	3969
12	4016	4030	4049	3991
18	4054	4102	4067	4068
24	4087	4208	4146	4269
27	4041	4209	4228	4279
Adjusted body weight[a]	3591	3778	3822	3929
Body weight changes				
0-6	116	111	118	104
0-18	204	234	199	203
18-27	-13	107	161	211
0-27	191	341	360	414

Data taken from Tables 3 and 4, pp. 24-25, and 26, respectively, MRID 45014710.
[a]Adjusted body weight = terminal body weight minus gravid uterine weight.

3. Food consumption

Selected maternal food consumption data are given in Table 3. There were non-statistical decreases in mean food consumption of the 1000 mg/kg/day group for all measuring intervals between GDs 8 and 18 (71-91% of controls). These decreases do not corresponded to the mean body weight gains which were similar in all groups during the dosing interval. Mean food consumption by the 1000 mg/kg/day group was statistically increased at all measuring intervals between GDs 20 and 27 (150-246% of controls; p<0.05 or 0.01); mean food consumption by the 200 mg/kg/day

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group was statistically increased during GDs 24-27 (198-202% of controls; p<0.05); and mean food consumption by the 40 mg/kg/day group was statistically increased at the GD 22-24 interval (170% of controls; p<0.05). Thus, food consumption mirrored body weight gain, with the most notable anomaly being a significant decrease in mean food consumption in the control dams due to anorexia in 5 of 18 dams and reduced food consumption in several other dams.

TABLE 3: Selected mean maternal food consumption during gestation (g/animal/day)				
GD	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1.000 mg/kg/day
0-2	181	172	177	175
2-4	183	176	183	182
4-6	177	166	178	178
6-8	164	152	169	152
8-10	170	153	161	145 (85)[a]
10-12	145	147	142	125 (86)
12-14	128	134	125	117 (91)
14-16	136	125	127	97 (71)
16-18	144	143	136	113 (78)
18-20	129	158	136	158 (122)
20-22	105	141	133	158* (150)
22-24	71	121* (170)	106	130* (183)
24-26	54	101	107* (198)	133** (246)
26-27	47	87	95* (202)	112* (238)

Data taken from Table 5, p. 27, MRID 45014710.
[a] Number in parentheses is per cent of control; calculated by reviewer.
Significantly different from control; *p<0.05, **p<0.01.

4. Gross pathology

Necropsy findings from the seven high-dose animals that were found dead on GDs 15-19 after exhibiting hematuria included the following: "cloudy" colored kidneys in four animals, "dark" colored kidneys in two animals, and enlarged kidneys with red spots in one animal; whitish membranous material on the gastric mucosa in three animals; urinary bladder distended with red or brown urine in two animals; trichobezoars (hairballs) in two animals, one of which also had white spots in the stomach; scanty gastrointestinal contents in three animals, one of which also had red contents in the small intestine; and ascites in the animal that had enlarged kidneys with red spots. Necropsy findings from the high-dose animal that was killed *in extremis* on GD 13 included "cloudy" colored kidneys and black, watery stomach contents. Necropsy findings from the high-dose animal that was found dead on GD 25, without

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any prior abnormal clinical signs included watery stomach contents and an umbilical hernia of small intestine. Necropsy findings from the mid-dose animal that was found dead on GD 21 after exhibiting hematuria and loose stool included "cloudy" colored kidneys and a trichobezoar in the stomach with whitish membranous material on the gastric mucosa. Necropsy findings from the mid-dose animal that was sacrificed on GD 25 following loose stool, hematuria, and abortion included a trichobezoar in the stomach and gaseous distension of the large intestine.

Necropsy findings from the nine surviving high-dose animals included "dark" colored kidneys in one animal and "cloudy" colored kidneys in two animals. Necropsy findings from the 16 surviving mid-dose animals included "cloudy" colored kidneys in one animal, trichobezoars in three animals, watery and scanty large intestinal contents in one animal, and gaseous distension of the large intestine in one animal. One low-dose animals had a trichobezoar. Necropsy findings from control group animals included trichobezoars in six animals, one of which also had gaseous distension of the large intestines, and one of which also had a pale liver.

5. Cesarean section data

Data collected at the scheduled cesarean section are summarized in Table 4. There were no total litter resorptions. There were no significant differences between the treated and control groups in the mean numbers of corpora lutea/dam, late fetal deaths (macerated fetuses), late resorptions (placental remnants), early resorptions (implantation sites), post-implantation losses, or fetal sex ratios. The mean number of implantations per dam in the 1000 mg/kg/day dose group was significantly decreased as compared to controls (6.0 vs. 9.5 for controls; $p<0.05$), and corresponded to an increase in the mean pre-implantation loss (34.6 vs. 12.0% for controls). Since dosing was initiated after implantation was believed to have occurred, the decreased mean number of implantations and increased pre-implantation loss are probably not an adverse effect of treatment and may just be an artifact of the small sample size.

The mean number of viable fetuses per litter was significantly decreased at the 1000 mg/kg/day dose level (5.6 vs. 9.1 for controls; $p<0.01$); however, this was probably due to the increased pre-implantation loss and decreased number of implantations rather than being an adverse effect of treatment. The significantly ($p<0.05$) increased mean fetal weights for both male and female fetuses at the 1000 mg/kg/day dose level were most likely due to the smaller liter size of this group.

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TABLE 4: Cesarean section observations				
Observation	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
No. Animals Assigned	18	18	18	18
No. Animals Pregnant	17	18	18	18
Pregnancy Rate[a] (%)	94	100	100	100
Maternal Mortality	0	0	1	9[b]
Delivered Early/Aborted	0	0	1	0
Pregnant at scheduled necropsy	17	18	16	9
Total Corpora Lutea[c]	183	190	185	82
Corpora Lutea/dam	10.8	10.6	11.6	9.1
Total Implantations[c]	161	150	153	54
Implantations/Dam	9.5	8.3	9.6	6.0*
Preimplantation Loss[d] (mean %)	12.0	21.1	17.5	34.6
Postimplantation Loss (mean %)	3.5	6.9	13.0	9.9
Total Live Fetuses	155	138	132	50
Viable Fetuses/Litter	9.1	7.7	8.3	5.6**
Mean Fetal Weight (g)				
Males	35.4	40.1	37.4	44.2*
Females	34.6	37.6	35.4	43.2*
Sex Ratio (% Male)	51.0	53.6	54.5	50.0
Total fetal resorptions and deaths per litter	0.35	0.67	1.31	0.44
"Macerated fetuses[e]" per litter	0.06	0.44	0.75	0.33
"Placental remnants[e]" per litter	0.06	0.00	0.31	0.00
"Implantation sites[e]" per litter	0.24	0.22	0.25	0.11
Dams with all resorptions	0	0	0	0

Data taken from Tables 1 and 7 and Appendices 21-24, pp 20, 29, and 59-62, respectively, MRID 45014710.
[a] Calculated by reviewer as Pregnancy rate = (number of animals pregnant/number of animals mated) x 100.
[b] Includes one animal killed *in extremis*.
[c] Calculated by reviewer using individual data.
[d] Calculated by reviewer from individual data as group means for the following:
Preimplantation Loss = [(Corpora Lutea-Implantations)/Corpora Lutea] x 100.
[e] These terms were not further defined in the study report. The reviewer is interpreting them as follows: "macerated fetuses" roughly corresponds to late fetal deaths, although some late resorptions could also be included; "placental remnants" roughly corresponds to late resorptions, although some early resorptions could also be included; and "implantation sites" roughly corresponds to early resorptions, excluding any that are included as "placental remnants." Significantly different from control; *p<0.05, **p<0.01.

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B. DEVELOPMENTAL TOXICITY

The numbers of fetuses (litters) examined in the 0, 40, 200, and 1000 mg/kg/day groups were 155 (17), 138 (18), 132 (16), and 50 (9), respectively. The overall incidence rates for litters containing fetuses with external, visceral, and/or skeletal malformations in the 0, 40, 200, and 1000 mg/kg/day groups were 2/17 (11.8%), 5/18 (27.8%), 7/16 (43.8%), and 0/9, respectively. Selected fetal morphological data are given in Table 5.

1. External examination

   One fetus from the 200 mg/kg/day group had multiple external malformations, including craniorachischisis, microphthalmia, open eyelids, cleft palate, and club hand. No external malformations were observed in the 0, 40, or 1000 mg/kg/day groups, and no external developmental variations were observed in any group.

2. Visceral examination

   Dilatation of the lateral ventricles was observed in the fetus from the 200 mg/kg/day group that had multiple external malformations. An undescended testis was observed in one fetus from each of the 0, 40, and 200 mg/kg/day groups. There were no visceral malformations observed in the 1000 mg/kg/day group. The only observed visceral variation was thymic remnant in the neck, which occurred in 10 (6), 16 (9), 14 (8), and 5 (2) fetuses (litters) of the 0, 40, 200, and 1000 mg/kg/day groups, respectively.

3. Skeletal examination

   The incidence rates of litters containing fetuses with skeletal malformation in the control, low-, mid-, and high-dose groups was 1/17, 4/18, 6/16 (p<0.05), and 0/9, respectively. A variety of different malformations were observed with no consistent pattern. No more than two fetuses were affected in any litter of any treatment group. The most commonly observed skeletal variation was lumbar ribs, which were observed in all treated and control groups, though with no statistically significant differences in the fetal or litter incidences. Lumbar rib incidence is within historical control levels, and is a normal background occurrence in the Japanese White rabbit and other strains of rabbits. The skeletal variation 27 presacral vertebrae with 13th ribs was observed at similar fetal and litter incidences in all treated and control groups. Other skeletal variations which occurred at single or low incidences included asymmetry or splitting of the sternebrae, shortening of the 12th ribs, 27 presacral vertebrae without 13th ribs, and 25 presacral vertebrae. The overall fetal incidences of skeletal variations were increased at the 200 and 1000 mg/kg/day treatment levels (41/155, 50/132, and 30/50 fetuses from the 0, 200, and 1000 mg/kg/day groups, respectively; p<0.05 and 0.001); however, there were no differences between the overall litter incidences of skeletal variations between the treated and control groups.

TABLE 5: Fetal malformations and variations [Number of litters (fetuses)]				
Observation	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Malformations				
Multiple external malformations [a][b]	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Dilatation of the lateral ventricles [b]	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Undescended testis	1/17 (1/155)	1/18 (1/138)	1/16 (1/132)	0/9 (0/50)
Absent skull bones	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Fusion of parietal bones	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	0/9 (0/50)
Splitting of the parietal bones	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	0/9 (0/50)
Splitting of the cervical vertebral arches	0/17 (0/155)	1/18 (1/138)	1/16 (1/132)	0/9 (0/50)
Splitting of the thoracic vertebral arches	1/17 (1/155)	0/18 (0/138)	0/16 (0/132)	0/9 (0/50)
Splitting of ossification centers of the thoracic vertebral bodies	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Fusion of the caudal vertebral bodies	0/17 (0/155)	0/18 (0/138)	2/16 (2/132)	0/9 (0/50)
Fusion of the sternebrae	1/17 (1/155)	2/18 (2/138)	2/16 (2/132)	0/9 (0/50)
Bifurcation of the ribs	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Shortening of the femur	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Total with skeletal malformations	1/17 (2/155)	4/18 (4/138)	6/16* (6/132)	0/9 (0/50)
Variations				
Thymic remnant in the neck	6/17 (10/155)	9/18 (16/138)	8/16 (14/132)	2/9 (5/50)
Lumbar ribs	12/17 (32/155)	14/18 (27/138)	14/16 (42/132)	6/9 (23/50)
27 presacral vertebrae with 13th ribs	5/17 (6/155)	5/18 (10/138)	5/16 (6/132)	4/9 (4/50)
27 presacral vertebrae	2/17 (2/155)	0/18 (0/138)	1/16 (1/132)	1/9 (1/50)
Asymmetry of the sternebrae	0/17 (0/155)	0/18 (0/138)	0/16 (0/132)	1/9 (1/50)
Splitting of the sternebrae	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	1/9 (2/50)
Shortening of the 12th ribs	1/17 (1/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
25 presacral vertebrae	1/17 (1/155)	0/18 (0/138)	0/16 (0/132)	0/9 (0/50)
Total with skeletal variations	15/17 (41/155)	15/18 (38/138)	14/16 (50/132)	7/9 (30/50)

Data taken from text, pp 15-16 and Tables 8, 9, and 10, pp 30, 31, and 32, respectively, MRID 45014710.
[a]Multiple external malformations included craniorachischisis, microphthalmia, open eyelids, cleft palate, and club hand.
[b]Observed in the same fetus.
[c]These two skeletal malformations were observed in two fetuses from the same litter.
Significantly different from control; * $p<0.05$, **$p<0.01$, or ***$p<0.001$.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that XRD-537 BE resulted in maternal toxicity at 200 and 1000 mg/kg/day as evidenced by maternal mortality, hematuria, body weight losses, and decreased food consumption. Similar findings were not observed in the probe study, in which there was no maternal toxicity at doses up to 1000 mg/kg/day. The study author stated that "although the reason for this inconsistency is not clear at present, it might be caused by a difference in susceptibility between batches of rabbits." The decreased mean numbers of implants and live fetuses at 1000 mg/kg/day were due to increased preimplantation losses. Because treatment began after implantation was believed to have occurred, the lower implantation rate of the 1000 mg/kg/day group was probably not treatment related. The study author stated that the significantly increased litter incidence of total malformations was probably incidental "since 1) statistical significance seemed to be due to a relatively low control value..., 2) type of malformations was not consistent among groups or within a group, and 3) no malformations were found in the highest dose level of 1000 mg/kg." The study author reported that a statistically significant increase in fetal incidence of lumbar ribs was a treatment-related effect based upon a Fisher's exact probability test. The Dow Chemical Company later determined that this was an inappropriate statistical method and performed a renalaysis using the Censored Wilcoxon test (MRID 45413901). Using an appropriate litter-based statistical method that is ideally suited for developmental toxicity incidence data, no evidence of an increased incidence of lumbar ribs was found. The high incidence of lumbar ribs in the concurrent controls (20.6% of fetuses; 70.6% of litters) and among historical controls (8.1-34.6% of fetuses; no litter historical data presented) indicates that lumbar rib is a normal background occurrence in the Japanese White rabbit and in other rabbit strains such as the New Zealand White. The study author concluded that the maternal and developmental toxicity NOAEL was 40 mg/kg/day, and the maternal and developmental toxicity LOAEL was 200 mg/kg/day. The study author also concluded that 1000 mg/kg/day represented an LD_{50} for the maternal rabbits in this study.

B. REVIEWER'S DISCUSSION (in consultation with Stephen Dapson, RAB3, and after HIARC discussion)

1. MATERNAL TOXICITY

It is uncertain whether necropsy findings of "cloudy" or "dark" kidney discoloration and whitish membranous material on the gastric mucosa could have been test article related. There was a higher incidence of hematuria in the 1000 mg/kg/day group during the treatment interval with corresponding decreases in body weight gain and food consumption. It is unlikely that hematuria could have been caused by the test article because it was observed in two animals (one control and one from the 200 mg/kg/day group) prior to the initiation of dosing, and in a second control group animal during the post-dosing interval. If there had been blood in the urine, this would be a serious finding and it should have been identified via urinalysis, but it was

not. It is more likely that hematuria was a misdiagnosis.

According to Harkness and Wagner,[1] normal rabbit urine can be pale yellow to red-orange to brown, and vary from clear to opaque and resemble pus or blood. The color of rabbit urine, caused by porphyrin and bilirubin derivatives, is intensified during dehydration or on certain pigmented or high calcium diets. Bloody urine is very rare in rabbits and rodents. If the urine is indeed bloody, then cystitis (inflammation of the urinary bladder), urolithiasis (calculi), and leptospirosis should be considered. Most cases of "bloody" urine turn out to be a porphyrin-pigmented basic urine or a sanguineous vaginal discharge from a tumor or abortion. Considering the pattern of occurrence, what was reported as hematuria was probably concentrated, blood-colored urine which can be attributed to dehydration, rather than to disease or the test article.

The maternal LOAEL is 200 mg/kg/day based on maternal death. The maternal NOAEL is 40 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

There were no increases in mean numbers of fetal deaths or resorptions per litter.

b. Altered growth

No evidence of altered growth of the fetuses was seen in this study.

c. Developmental variations

The most commonly observed skeletal variation was lumbar ribs, which were observed in all treated and control groups, though with no statistically significant differences in the fetal or litter incidences. Lumbar rib incidence is close to historical control levels, and is a normal background occurrence in the Japanese White rabbit and other strains of rabbits.

d. Malformations

The increased litter incidences of skeletal malformations and total malformations at the 200 mg/kg/day dose level were most likely due to maternal stress rather than test article toxicity.

The developmental NOAEL is ≥1000 mg/kg/day (limit dose).

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[1]John E. Harkness and Joseph E. Wagner. **The Biology and Medicine of Rabbits and Rodents.** 2nd Edition. Lea & Febiger, Philadelphia. 1983.

C. STUDY DEFICIENCIES

The study report did not describe dose formulation preparation or storage conditions, and there was no mention made of stirring the dosing solutions during dosing to maintain homogeneity. Nevertheless, non-homogeneity was not a problem in any other toxicity studies, and the dosing measurements suggest that the formulations were homogeneous. Ideally, more rabbits should have been used. Since this study was performed prior to implementation of the OPPTS Harmonized Test Guidelines, only 12 rabbits per group are required. Randomization procedures and sacrifice order were not provided. The study report did not include a copy of the study protocol and any amendments.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and does satisfy the requirements for a developmental toxicity study [OPPTS: 870.3700 (§83-3)] in rabbits.