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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

014031

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

EPA ID Nos.:

PC Code: 129122
DP Barcode: D249626; D258376
Submission No.: S526363
Case No.: 288998

Subject: **Diclosulam (XDE-564)**: Completed Toxicology DERs

To: Tobi Colvin-Snyder
Herbicide Branch, Registration Division (7505C)

From: Ghazi A. Dannan, Ph.D. Ghazi A. Dannan 01/20/00
Registration Action Branch 3, Health Effects Division (7509C)

Through: Stephen C. Dapson, Ph.D., Senior Scientist Stephen C. Dapson 01/25/2000
Registration Action Branch 3, Health Effects Division (7509C)

Registrant: The Dow Chemical Co.

Test Material: Diclosulam (XDE-564), BF-309

Action Required: Review the studies with the attached petition to use Diclosulam on peanuts.

The toxicology studies listed in the table below have been reviewed and the Data Evaluation Records are attached. All studies used technical Diclosulam (XDE-564) except for one of the two 21-day dermal toxicity studies (MRID 44103514) in which BF-309 (83.1% a.i.) was tested. Two of the studies are found unacceptable and one of these three studies is nonguideline. The acute neurotoxicity study in rats (MRID 44192601) is considered unacceptable/guideline pending receipt of requested information. The rat chronic neurotoxicity study (MRID 44103526) is considered unacceptable/nonguideline and may be upgradable; however, it does not satisfy the guideline for a subchronic neurotoxicity study because effects were not assessed at the 4 and 8 week time points.

Guideline	Study	MRID No.	Acceptable
81-8	Acute Neurotoxicity in Rats	44192601	No
82-2	21-Day Dermal Toxicity in Rabbits	44103523	Yes
82-2	21-Day Dermal toxicity in Rabbits (BF-309)	44103514	Yes
83-5	Combined Chronic/Oncogenicity Oral Study in Rats	44103525	Yes
NonGuideline	Chronic Oral Neurotoxicity in Rats	44103526	No
83-1	1-Year Feeding Study in Dogs	44207401	Yes
83-2	2-Year Carcinogenicity Study in Mice	44192602	Yes
83-3	Developmental Toxicity Study in Rats	43441032	Yes
83-3	Developmental Toxicity Study in Rabbits	44103524	Yes
83-4	2-Generation Reproduction Study in Rats	44207402	Yes
85-1	General Metabolism in Rats	44103527	Yes

The following are the Executive Summaries for the reviews:

I. Acute Neurotoxicity Screen in Rats; OPPTS: 870.6200 [§81-8]

Mattsson, J., P. Spencer, and J. Quast. 1996. XDE-564: Acute Neurotoxicity Study in Fischer 344 Rats. The toxicology Research Laboratory, The Dow Chemical Company, Midland, MI. Laboratory report # DR-0313-5691-026. July, 1996. MRID # 44192601. Unpublished.

In an acute neurotoxicity study (MRID # 44192601), rats (10/sex/group) received a single dose of XDE-564 (97.6% a.i.) by gavage (in methyl cellulose). Doses were 0, 200, 1000, or 2000 (a limit dose) mg/kg for both sexes. Clinical observations were recorded twice daily. Evaluation during the two-week study period included body weights, functional observational battery (FOB), motor activity and neuropathology. The FOB consisted of hand-held and open-field observations, grip performance, rectal temperature and landing foot splay testing. Animals were evaluated by FOB and motor activity assay once prior to exposure, on day 1 (beginning approximately 5 hours after dosing), and on days 8 and 15 of the study period. Body weights were determined on days -7,1,2,8 and 15 relative to the day of dosing (day 1). Cholinesterase inhibition was not evaluated.

At study termination on day 16, 5 rats/sex/group were perfused intracardially with glutaraldehyde/paraformaldhyde, and histopathological evaluation of peripheral and central nervous system tissue was performed on animals from the control and high dose groups only.

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 12/4/99
Branch Senior Scientist, Registration Action Branch 3/HED (7509C)

Secondary Review by: William B. Greear, M.P.H., D.A.B.T. William B. Greear 12/4/99
Registration Action Branch 3/HED (7509C)

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DATA EVALUATION RECORD

Study Type: Multigeneration Reproductive Toxicity
Species: Rat; Guideline: OPPTS 870.3800; OPP §83-4

EPA ID No.s: EPA MRID No. 44207402
EPA Pesticide Chemical Code 129122
EPA DP Barcode D249626
EPA Submission No. S526363

Test Material: XDE-564
Synonyms: Diclosulam, XR-564, XRD-564
Chemical Name: N-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro-1,2,4-triazolo-[1,5c]-pyrimidine-2-sulfonamide

Citation: Zablotny, C.L., Kociba, R.J., Quast, J.F., Redmond, J.M., Breslin, W.J. (1996): XDE-564: TWO-GENERATION DIETARY REPRODUCTION STUDY IN CD RATS, FIFRA 83-4, OECD Guideline No 416, EEC, MAFF, The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company for DowElanco, Laboratory Project Study ID: DR-0313-5691-027P1, DR-0313-5691-027P2, DR-0313-5691-027F1, DR-0313-5691-027F2, DR-0313-5691-027W1 and DR-0313-5691-027W3, 1 July 1996. (Unpublished Study); EPA MRID Number 44207402.

Executive Summary: In a multigeneration reproduction study (MRID# 44207402), groups of CD rats (30 per sex, per dose) from Charles River Breeding Laboratory, Kingston, NY, received 0, 50, 500, 750 or 1000 mg/kg/day XDE-564 (Diclosulam, XR-564, XRD-564; Purity: 97.6%; Lot No.: TSN100168) in the diet for two successive generations, due to the lack of toxicity noted with this compound the 750 mg/kg/day dose group was dropped. Each rat on study was observed twice daily for mortality, morbidity and moribundity, once daily for changes in behavior or demeanor or overt signs of toxicity. Weekly thorough clinical physical examinations were conducted on each P1 and P2 animal. All P1 animals had body weights and feed consumption recorded weekly during the 10-week pre-breeding treatment period with body weights for males recorded weekly throughout the course of the study. Sperm positive females were weighed on Days 0, 7, 14 and 21 of gestation. Females that delivered litters were weighed on Days 1,

4, 7, 14, and 21 of lactation. Feed consumption was not measured in males or females during the breeding period, but following this period, weekly feed consumption was measured in males and in sperm positive females during gestation. After parturition, feed consumption was measured on days 1, 4, 7, 11, 14, 17, 19 and 21 of lactation. Females were observed for evidence of parturition. The date of delivery was recorded as the first day the litter was observed and was designated as lactation day 0. All litters were examined as soon as possible after delivery. The following data were recorded on each litter: litter size on the day of parturition (lactation day 0), the number of live and dead pups on days 0, 1, 4, 7, 14, and 21 postpartum, and the sex and weight of each pup on days 1, 4 (before and after culling), 7, 14, and 21 of lactation. Any visible physical abnormalities or demeanor changes in the neonates were recorded during the lactation period. The F1 and F2 litters were culled to 8 pups on day 4 postpartum. All litters were weaned on day 21 postpartum. A complete necropsy of all P1 and P2 adults was performed. The eyes were examined. Data from previous studies with this compound in Fischer 344 rats showed possible effects in the liver and kidney, therefore terminal body weights and liver and kidney weights were recorded in the P2 adults for comparison of possible liver and kidney effects in the CD (Sprague-Dawley derived) strain of rat used in this study. The organ-to-body weight ratios were calculated for the P2 adults. Histologic examination of potential target organs and reproductive tissues, and all gross lesions was performed on the control and high dose groups. Prior to weaning, 10 pups/sex/dose level from the F1 and F2 litters were randomly selected for a complete necropsy.

No systemic toxicity to the parental animals was noted at the dose levels tested up to the limit dose. There were no treatment related findings in the reproductive system of animals of either sex. **The Parental (Paternal/Maternal) Systemic Toxicity NOAEL is equal to or greater than 1000 mg/kg/day and the Parental (Paternal/Maternal) Systemic Toxicity LOAEL is greater than 1000 mg/kg/day.**

No systemic or developmental toxicity was noted in the offspring of either generation. **The Offspring Systemic/Developmental Toxicity NOAEL is equal to or greater than 1000 mg/kg/day and the Offspring Systemic/Developmental Toxicity LOAEL is greater than 1000 mg/kg/day.**

No effects were noted on reproductive parameters. **The Reproductive Toxicity NOAEL is equal to or greater than 1000**

mg/kg/day and the Reproductive Toxicity LOAEL is greater than 1000 mg/kg/day.

This study is classified as Acceptable-Guideline and satisfies the requirements (OPPTS 870.3800, OPP §83-4) for a multigeneration reproduction study in rats.

Compliance: A signed and dated STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS, a statement of COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS, a QUALITY ASSURANCE STATEMENT, and a FLAGGING STATEMENT AS PER CFR 40 158.34 (the study neither met nor exceeded applicable criteria) were provided.

From pages 13-14 of the study report:
This study was conducted to meet the requirements of the Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals (EPA. 1984), the Organisation for Economic Co- Operation and Development (OECD), Guidelines for Testing of Chemicals, Section 4: Health Effects, Protocol Number 416, Two-Generation Reproductive Toxicity Studies (OECD, 1981), the European Economic Community (EEC), Methods for the Determination of Toxicity (EEC, 1988) and the Japan Ministry of Agriculture, Forestry and Fisheries (MAFF), Toxicity Test Guidelines (MAFF, 1985).

Statement of GLP Practice.
This study was conducted in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations for NonClinical Studies (FDA, 1988), the Environmental Protection Agency (EPA); FIFRA Good Laboratory Practice procedures (EPA, 1990), OECD Principles of Good Laboratory Practice, (OECD, 1982), the Japanese Ministry of Agriculture, Forestry and Fisheries Good Laboratory Practice Procedures (MAFF, 1984) and the Standard Operating Procedures of The Toxicology Research Laboratory of The Dow Chemical Company.

In addition, in response to the Final Rules amending the U.S. Animal Welfare Act that were promulgated by the U.S. Department of Agriculture effective October 30, 1989, the Animal Care and Use Activity (ACUA) that was required for the conduct of this study has been reviewed and given full approval by the Institutional Animal Care and Use Committee (IACUC). The IACUC has determined that the proposed Activity was in full accordance with these Final Rules.

THIS REVIEW CONTAINS TEXT INFORMATION SCANNED FROM THE STUDY REPORT BY THE REVIEWER INTO ELECTRONIC FORMAT (USED IN MATERIALS AND METHODS, STUDY DESIGN AND CONCLUSIONS-INVESTIGATORS SUMMARY SECTIONS).

A. Materials and Methods

Test Compound: XDE-564 (Diclosulam, XR-564, XRD-564)
Purity: 97.6%
Description: Solid, white powder
Lot No.: TSN100168
The sample of XDE-564 used in this study was obtained from DowElanco, Indianapolis, IN. Results from the initial characterization revealed a purity of 97.6% by high performance liquid chromatography (HPLC) (Russell, 1993). The identity of the major component of TSN 100168 was confirmed as XDE-564 by the combination of mass spectrometry and infrared spectroscopy. Results of the test material reassay revealed a purity of 97.6% (Russell, 1994). In addition, stability of the test material was confirmed by checking for purity at various intervals throughout the study (Table 1 of the study report).

Test Animal(s): Species: Rat
Strain: CD
Source: Charles River Breeding Laboratory, Kingston, NY
Age: 4 weeks at receipt
Body Weight: Means: 186.3-188.8 g for males, 151.2-153.9 g for females on study day -2.

Husbandry (scanned from pages 15-17 of the study report)

Male and female CD rats (Charles River Breeding Laboratory, Kingston, NY) approximately four weeks of age upon receipt were used for this study. Extra animals were ordered to ensure that a sufficient number of animals of acceptable health and weight were available to conduct the study as designed. This strain of rat was selected because of its general acceptance and suitability for toxicity testing, and the availability of a reliable commercial source. Upon arrival at the laboratory, all rats were examined for health status by a veterinarian and acclimated to the laboratory environment for approximately two weeks, according to the Standard Operating Procedures of the Reproductive Toxicology Group. For the randomization procedure, the rats were weighed and ranked according to body weight and those from the extremes of the distribution were identified and removed from the population until only the number of animals required for the study remained. These rats were randomly assigned by weight to the treatment groups to increase the probability of uniform group mean weights and standard deviations at the initiation of the study. Rats not placed on test were removed from the test room and the disposition of these animals was documented in the study file.

Identification of all rats on test was accomplished by subcutaneously implanting a uniquely coded transponder (Biomedic Data Systems, Maywood, NJ) between the shoulder blades of each rat. In the event that a transponder became dislodged during the course of the study, it was replaced with one mapped to the same alphanumeric code (i.e., a new alphanumeric code was not assigned) and noted in the study file.

Rats were housed singly in wire mesh, stainless steel cages in racks provided with deotized cage board to minimize odor and aid in maintaining a clean environment. From approximately day 19 of gestation and throughout the lactation phase of the study, females were housed in plastic cages provided with ground corn cob nesting material. The animal rooms of the facility are designed to maintain humidity at approximately 40-60%, temperature at approximately 22°C, photoperiod at 12 hrs light:12 hrs dark and air flow at 12-15 changes/hour. The room temperature and relative humidity were recorded a minimum of once daily. A feed crock and a pressure-activated stainless steel water nipple were components of all cages. A basal diet of Purina Certified Rodent Chow No. 5002 (Purina Mills Inc., St. Louis, MO) and municipal drinking water were available *ad libitum* throughout the prestudy and study periods. Analysis of the chow was performed by Purina Mills Inc. to confirm that the diet provided adequate nutrition, and to quantify the levels of selected contaminants associated with the formulation process. Drinking water obtained from the City of Midland, MI was analyzed for chemical parameters and biological contaminants by the City of Midland Water Department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals as stated in the Standard Operating Procedures of The Toxicology Research Laboratory, The Dow Chemical Company. The results of the feed and water analyses indicated no contaminants that would interfere with the conduct of the study or interpretation of the results.

B. Study Design

According to the investigators (scanned from pages 13, 18-19 of the study report):

The objective of the two-generation dietary reproduction study reported herein was to evaluate the effects of XDE-564 on reproductive function and neonatal growth and survival in rats.

Experimental Design.

Groups of 30 male and 30 female P1 rats were maintained on diets containing sufficient XDE-564 to provide dose levels of 0, 50, 500, 750, or 1000 mg XDE - 564/kg/day. These dose levels were selected based on the results of various studies discussed previously. A previously conducted 90-day dietary toxicity study in Sprague-Dawley rats indicated no systemic toxicity at a high dose level of 500 mg/kg/day. Therefore, dose levels of 750 and 1000 mg/kg/day were selected as the high dose levels. Low dose levels of 50 and 500 mg/kg/day

were added to establish a dose response and to provide data to define a no-observed-effect level. In addition, the high dose level of 1000 mg/kg/day represents a limit dose as defined by the EPA (EPA, 1984), Japan (MAFF, 1985) and OECD (OECD, 1981). The overall chronology of events for this study is depicted in Table 2 [of the study report]. Treatment of the P1 rats began at approximately six weeks of age. Prior to breeding of the P1 rats, a decision was made to terminate the 750 mg/kg/day dose level. This decision was based on the fact that after 10 weeks the premating clinical observations, body weight and feed consumption were unaffected by ingesting XDE-564 at the highest dose of 1000 mg/kg/day. The P1 rats from the 750 mg/kg/day dose level were euthanized by CO_2 inhalation and no further data were collected. The remaining P1 rats were mated (one male to one female of the respective treatment group) to produce the F1 litters. Prior to postpartum day 21 of the F1 litters, 30 males and 30 females from each treatment group were randomly selected using a computer-generated randomization procedure and assigned to the respective treatment group to become the parents for the next generation. At least one pup/sex, if available, was selected from each litter. To help assure a mating population of 30 P2 rats/sex/group, at least five additional replacement pups/sex/dose were selected randomly from litters using a computer-generated table of random numbers. These additional pups were selected to replace any P2 pups that died prior to scheduled necropsy or the first week of the P2 premating period, respectively. After approximately 12 weeks of treatment following weaning of the last F1 litter, the P2 adults were bred to produce the F2 litters.

Diet preparation: (scanned from pages 17-18 of the study report)

Preparation, Sampling and Delivery of Test Material.

The probable route of human exposure to the test material would be via ingestion of foodstuffs that may contain low levels of residues or accidental ingestion of the test substances during its manufacture, transportation, storage or use. Thus, formulation with feed represented the desired method of delivery to assess the potential reproductive and neonatal toxicity following oral administration of the test material in rats.

The stability of XDE-564 in basal rodent feed has been determined to be at least 41 days (Vedula. et al., 1994). Diets were prepared by serially diluting a test material/feed concentrate (premix) according to the Standard Operating Procedures of the Oral/Dermal Toxicology Group. The homogeneity of the diets using current mixing procedures was determined during the course of the study. Reference samples (1/sex/dose/mixing plus premix) were retained and stored at ambient temperature in a manner consistent with the sample retention policy of the laboratory and FDA and EPA Good Laboratory Practice Procedures. Analyses of the test diets to determine the concentration of the test material were performed at least three times per generation.

The concentration of the test material in the diets was calculated from weekly body weights and feed consumption data in order to maintain the targeted dose levels on a mg/kg/day basis. Test animals were maintained on the appropriate test diets for approximately 10 and 12 weeks of treatment prior to breeding of the first parental (P1) and second parental (P2) generations, respectively. To avoid potential overdosing during the breeding period, animals co-housed were provided with the female concentration for that dose group (low, middle or high). During gestation, females from each group were provided with the appropriate dietary concentration of XDE-564 given during the three weeks of breeding. Dietary concentrations supplied during lactation were adjusted using historical control feed consumption data for lactating females. Until all litters were weaned, weanlings chosen for the P2 generation received a diet containing the same concentration of XDE-564 that was given to the P1 rats during the third week of lactation. Dietary concentrations for the P2 generation were calculated as described for the P1 animals.

Test Material Analysis (scanned from page 25 of the study report)

Purity data for the test material is presented in Table 1 [of the study report]. As indicated in Table 1 [of the study report], XDE-564 was found to be stable over the duration of the dosing period. Analyses of the test diets from both the P1 and P2 generations are presented in Table 4 [of the study report]. The concentrations of XDE-564 in the individual diets measured during the study period ranged from 96% to 104% of target with average concentrations ranging from 98-102% of target. The results indicated an acceptable agreement between targeted and actual values. Analyses also indicated that the distribution of XDE- 564 in the rodent chow was homogeneous (Table 5) [of the study report]. The stability of XDE-564 in basal rodent chow was at least 41 days at a concentration of approximately 0.028%, (which is approximately 38% of the concentration of the low dose female diet at the beginning of the study (Table 6) [of the study report] had previously been established.

From Table 1 of the study report the purity of the test material was found to be 97.6%. From Table 4 of the study report the mean % of target concentration for the males was 100%±3%, 100%±2%, 100%±1% and 99%±1%, 99%±2%, 101%±1% and 100%±2% for the 50, 500, 750, and 1000 mg/kg/day dose levels, respectively. From Table 5 of the study report, the homogeneity analysis (w/v) mean for the 50 mg/kg/day female test diet was 0.0511%±0.0022, 0.0736%±0.0005, and 0.0376%±0.0006 and for the 1000 mg/kg/day male test diet was 1.11±0.014, 1.34%±0.01 and 0.715%±0.008. From Table 6 of the study report the stability of the test compound in the rat chow was 98% by day 42.

Mating and Mating Schedule: (scanned from page 19 of the study report)

Breeding of the P1 and P2 adults commenced after approximately 10 and 12 weeks of treatment, respectively. Each breeding program consisted of three 7-day cohabitation periods with one female and one male of the respective treatment group. For the P2 mating, cohabitation of male and female litter mates was avoided. Dams were observed once daily during cohabitation for positive signs of copulation. Daily vaginal lavage samples were evaluated for the presence of sperm as an indication of mating. The day on which sperm was detected or a vaginal copulatory plug was observed in situ was considered day 0 of gestation. The sperm or plug positive (presumed pregnant) females were then separated and returned to their individual cages. On the first day 19 of gestation, all presumed pregnant females were placed in nesting cages. Females which failed to mate during the first 7-day mating period were placed with an alternate male from the same treatment group for the second 7-day period; the same procedure was followed for the third 7-day mating period. Females that failed to mate were separated and transferred into nesting cages at the end of the 3-week mating period.

Dose Selection: (scanned from pages 12-13 of the study report)

Prior Toxicity Data.

XDE-564 (N-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro-1,2,4-triazolo-[1,5c]pyrimidine-2-sulfonamide) is an experimental herbicide currently undergoing toxicological testing.

In a two-week dietary toxicity study, XDE-564 was fed to male and female Fischer 344 rats (5/sex/dose) at dose levels of 0, 100, 500 or 1000 mg/kg/day (Stewart et al. 1992). No treatment-related changes were observed with respect to clinical appearance, body weights, feed consumption, hematological parameters, clinical chemistry or urinalysis parameters. Cecal weight increases (male rats given 1000 mg/kg/day) unassociated with histopathologic alterations were judged to represent a physiological adaptation to increased amount of ingesta retained in this organ. Relative liver weights of males ingesting 500 or 1000 mg/kg/day were also statistically identified as increased relative to controls; however, there were no accompanying histopathologic changes. The no-observed-effect level (NOEL) for this two-week dietary study was 100 mg/kg/day for male and 1000 mg/kg/day for female Fischer 344 rats. The-no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day for male Fischer 344 rats.

In a 13-week dietary toxicity study Fischer 344 rats were administered XDE-564 in their feed at targeted concentrations of 0 (control), 50, 100, 500, or 1000 mg/kg/day (Szabo and Davis, 1993). Additional groups of ten male and ten female rats were administered 0 (control) and 1000 mg/kg/day for 13 weeks and then maintained on control rodent chow for a 4-week recovery phase. Thirteen weeks of dietary administration of XDE-564 to male and female rats at both

500 and 1000 mg/kg/day resulted in lower in-life and terminal body weights, elevated absolute and relative kidney weights and relative liver weights and lower urinary specific gravity (males only). Minimal, treatment-associated alterations in hematologic parameters and serum creatinine were observed in male and female rats given 50,100, 500 or 1000 mg/kg/day.

Histopathologic alterations consisting mainly of slight to moderate panlobular hypertrophy with increased basophilia of hepatocytes occurred in the livers of both male (100, 500 or 1000 mg/kg/day) and female rats (1000 mg/kg/day).

The high dose male and female recovery group rats continued to have lower in-life and terminal fasted body weights and their absolute and relative kidney weights remained elevated. Other parameters affected during the 13-week portion of this study were comparable to controls after the 4-week recovery period.

In light of the minimal changes in hematological parameters and serum creatinine values, an EPA review of this study indicated the no-observable-effect-level (NOEL) for XDE-564 administration was 50 mg/kg/day in male and 100 mg/kg/day in female rats.

Most of the toxicity data available for XDE-564 were generated using the Fischer 344 strain of rat. Recently, a 13-week dietary toxicity test was conducted in Sprague-Dawley rats at dose levels of 0 (control), 25, 250 or 500 mg/kg/day to provide data for dose selection for the two-generation reproduction study herein reported (Vedula et al., 1994). Following 13 weeks of exposure, no treatment-related signs of toxicity were observed in male or female rats at 500 mg/kg/day, the highest dose-level tested. Thus, the no-observed effect level (NOEL) in this study was 500 mg/kg/day.

Observation Schedule

Parental animals: (scanned from pages 19-20 of the study report)

Physical Observations.

Throughout the test period, each rat on study was observed twice daily (a.m. and p.m.) for mortality, morbidity and moribundity. In addition, each rat on study was observed daily during the a.m. or p.m. examination for changes in behavior or demeanor or overt signs of toxicity. Also, thorough clinical physical examinations were conducted weekly on each P1 and P2 animal. All adult rats found dead or in a moribund condition were submitted for a gross pathologic examination. Adult rats found dead after normal working hours were refrigerated until a necropsy could be performed. All pups found dead were examined for possible defects and/or cause of death and discarded.

Body Weights and Feed Consumption.

All P1 animals had body weights and feed consumption recorded weekly during the 10-week pre-breeding treatment period, beginning on or before the first week of the study. Feed consumption (g/day) was equated as (initial weight of feed crock-final weight of feed crock)/(# days in measurement cycle). Body weights for males were recorded weekly throughout the course of the study. Sperm positive females were weighed on Days 0, 7, 14 and 21 of gestation. Body weights of non-confirmed mated females were not recorded. Females that delivered litters were weighed on Days 1, 4, 7, 14, and 21 of lactation. Females that failed to deliver were not weighed after their presumed day 21 gestation body weight. During breeding, feed consumption was not measured in males or females due to co-housing. Following completion of the breeding periods, weekly feed consumption again was measured in males, and dietary concentrations adjusted accordingly. During gestation, feed consumption was measured at weekly intervals in sperm positive females. Feed consumption was not recorded for non-confirmed mated females. After parturition, feed consumption was measured on days 1, 4, 7, 11, 14, 17, 19 and 21 of lactation. Feed consumption was not measured in females that failed to deliver. The same schedule was followed for the P2 generation.

Offspring: (scanned from pages 20-21 of the study report)

Litter Data.

Females were observed for signs of parturition. In so far as possible, parturition was observed for signs of difficulty or unusual duration. Litters born overnight were considered to have been delivered on the morning they were found. The date of delivery was recorded as the first day the presence of the litter was noted and was designated as lactation day 0. All litters were examined as soon as possible after delivery. The following data were recorded on each litter: litter size on the day of parturition (day 0), the number of live and dead pups on days 0, 1, 4, 7, 14, and 21 postpartum, and the sex and weight of each pup on days 1, 4 (before and after culling), 7, 14, and 21 of lactation. Any visible physical abnormalities or demeanor changes in the neonates were recorded as they were observed during the lactation period.

Culling and Weaning.

To reduce the variation in the growth of the pups, the F1 and F2 litters with a total number of pups exceeding eight were culled on day 4 postpartum. Culled litters were reduced to a total of eight pups, four males and four females, if possible. Culled pups were selected using a computer generated randomization procedure. Litters with eight or fewer pups were not culled. Preferential culling of runts was not performed. All pups which were culled were examined grossly and euthanized with Socumb euthanasia solution, (Veterinary Laboratories, Inc. Lenexa, Kansas) and discarded. All litters were weaned on day 21 postpartum. Any weanlings not held for the next

generation adult animals or selected for necropsy were examined grossly and euthanized by CO_2 inhalation and discarded. Runts were not excluded from assignment at the time of weaning.

Pathological examinations: (scanned from pages 21-23 of the study report)

Pathology - Adult Rats.

A complete necropsy of all P1 and P2 adults was performed by a board certified veterinary pathologist (ACVP) assisted by a team of trained individuals. The scheduled necropsy was performed after the majority of the respective generation had been weaned. The adults were fasted overnight, weighed, anesthetized with methoxyflurane and euthanized. The eyes were examined in situ by gently pressing a glass slide against the Cornea and observing the eyes under fluorescent light. Due to previous toxicity studies with this compound in the Fischer 344 rat showing possible effects in the liver and kidney, it was considered relevant to collect terminal body weights and liver and kidney weights in the P2 adults to aid in the assessment of possible liver and kidney effects in the CD (Sprague-Dawley derived) strain of rat. In addition, the organ-to-body weight ratios were calculated for the P2 adults. Tissues routinely collected (Table 3 (of the study report)) were saved from these rats and preserved in neutral, phosphate-buffered 10% formalin, with the exception of the testes and epididymides which were preserved in Bouin's fixative. The lungs were infused with formalin to their approximate normal inspiratory volume and the nasal cavity was flushed with formalin via the pharyngeal duct. This procedure for lungs and nasal cavity ensures rapid fixation of the tissue. Any of the adult rats that died, or were euthanized due to moribund condition prior to completion of the study were necropsied in a similar manner, however, body and organ weights were not obtained.

Histological Examination - Adult Rats.

Histologic examination of potential target organs and reproductive tissues (Table 3 (see below)), and all observed gross lesions was performed on the control and high dose groups. Examination of tissues from the low and middle groups was limited to the observed gross lesions. Gross lesions processed for histopathologic examination excluded those types of gross lesions for which a histopathologic correlate would not be present or relevant (i.e. - overgrown incisor teeth, decreased body fat reserves). A complete set of tissues, encompassing all organs listed in Table 3 (of the study report) (with the exception of auditory sebaceous gland and joint), were prepared from all rats found dead or euthanized in a moribund condition and examined in an attempt to determine the cause of death. Tissues were prepared for light microscopic evaluation by standard histologic procedures, sectioned at approximately 6 μm thickness, and stained with hematoxylin and eosin.

From Table 3, page 41 of the study report: TISSUES COLLECTED AND PRESERVED AT NECROPSY: ADRENALS, AORTA, AUDITORY SEBACEOUS GLANDS, BONE (INCLUDING JOINT), BONE MARROW, BRAIN (CEREBRUM, BRAINSTEM, CEREBELLUM), CECUM, CERVIX*, COAGULATING GLANDS*, COLON, DUODENUM, EPIDIDYMIDES*, ESOPHAGUS, EYES, GROSS LESIONS*, HEART, ILEUM, JEJUNUM, KIDNEYS*, LACRIMAL/HARDERIAN GLANDS, LARYNX, LIVER*, LUNGS, MAMMARY GLAND*, MEDIASTINAL LYMPH NODE, MEDIASTINAL TISSUES, MESENTERIC LYMPH NODE, MESENTERIC TISSUES, NASAL TISSUES, ORAL TISSUES, OVARIES*, OVIDUCTS*, PANCREAS, PARATHYROID GLANDS, PERIPHERAL NERVE, PITUITARY*, PROSTATE*, RECTUM, SALIVARY GLANDS, SEMINAL VESICLES*, SKELETAL MUSCLE, SKIN AND SUBCUTIS, SPINAL CORD (CERVICAL, THORACIC, LUMBAR), SPLEEN, STOMACH, TESTES*, THYMUS, THYROID GLAND, TONGUE, TRACHEA, URINARY BLADDER, UTERUS*, VAGINA* (*TISSUES SELECTED FOR HISTOPATHOLOGIC EVALUATION.)

Grading of certain histopathologic findings was done to reflect the severity of specific lesions when considered necessary to evaluate the contribution of a specific lesion to the health status of the animal. Very slight or slight grades were used for conditions that were present outside of the boundaries of the normal textbook appearance of an organ/tissue, but were of low severity and involved a relatively small portion of the organ or tissue. These types of changes would not be expected to significantly affect the function of the specific organ/tissue involved nor have a significant effect on the overall health of the animal. A moderate grade was used for conditions that were of sufficient severity and/or extent that the function of the organ/tissue may have been adversely affected, but not to the point of organ failure. A severe grade was used for conditions that were extensive enough to result in organ failure, and may have been life-threatening. The distribution of certain lesions was also categorized as to whether the lesion was focal, multifocal or diffuse in distribution.

Pathology - Weanling Rats.

Prior to weaning, 10 pups/sex/dose level from the F1 and F2 litters were randomly selected for a complete necropsy under the supervision of a veterinary pathologist. The pups were anesthetized with methoxyflurane and euthanized. Gross pathologic examination and preservation of tissue samples (Table 3 (of the study report)) were performed as described above for adults, except that terminal body weights and organ weights were not recorded and the testes and epididymides were preserved in formalin rather than Bouin's fixative. Histopathologic examination was not performed on the weanlings.

Statistical Analyses: (scanned from pages 23-25 of the study report)

Descriptive statistics (means and standard deviations) were reported for feed consumption. Body weights, gestation/lactation body weight gains and organ weights were first evaluated by Bartlett's test for equality of variances. Based upon the outcome of Bartlett's test, either a parametric or

nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, a Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed.

Gestation length, average time-to-mating and litter size were analyzed using a nonparametric ANOVA. If the ANOVA was significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Statistical outliers were identified by the method of Grubbs (1969) and were routinely excluded from analysis for feed consumption only. Outliers for body weight, gestation length, litter size and average time to mating were only excluded from analysis for documented, scientifically sound reasons. The fertility indices were analyzed by the Fisher exact probability test and Bonferroni's correction was used for multiple testing of groups in comparison to a single control. Evaluation of the neonatal sex ratio was performed by the binomial distribution test. Survival indices and other incidence data among neonates were analyzed using the litter as the experimental unit by the Wilcoxon test as modified by Haseman and Hoel (1974).

The nominal alpha levels used were as follows:

Bartlett's Test (Winer, 1971)	α=0.01
Parametric ANOVA (Steel and Torrie, 1960)	α=0.10
Nonparametric ANOVA (Hollander and Wolfe, 1973)	α=0.10
Dunnett's Test (Winer, 1971)	α=0.05, two-sided
Wilcoxon Rank-Sum Test (Hollander and Wolfe, 1973)	α=0.05, two-sided with Bonferroni correction (Miller, 1966)
Fisher's Test (Siegel, 1956)	α=0.05, two-sided
Censored Wilcoxon Test (Haseman and Hoel, 1974)	α=0.05, two-sided
Outlier Test (Grubbs, 1969)	α=0.02, two-sided
Binomial Distribution Test (Steel and Torrie, 1960)	α=0.05, two-sided

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was much greater than the cited alpha levels suggested. Thus, the final interpretation of numerical data considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in the light of other biologic and pathologic findings.

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NOTE FROM THE REVIEWER: THE PROTOCOL DESCRIBED ABOVE IN THE MATERIALS AND METHODS SECTION IS ACCEPTABLE TO FULFILL THE INFORMATION SUGGESTED BY THE GUIDELINE OPPTS 870.3800; OPP §83-4.

C. REPORTED RESULTS

Parental animals

Mortality and clinical signs:

One control P1 female died during the first month of the study, the investigators report that no clinical signs were observed and that urinary tract inflammation was the probable cause of death. One 50 mg/kg/day P1 female was found dead at study termination (only the carcass was located), the death was considered accidental. One control P2 female was sacrificed in moribund condition, there was evidence of a nasal fracture, clinical evidence of dyspnea and a distended digestive tract. One 500 mg/kg/day P2 male was found dead, no clinical signs were reported and necropsy revealed a mass on the pituitary gland. One 1000 mg/kg/day P2 male was also found dead with no untoward clinical signs reported, necropsy showed calculi in the urinary bladder along with distension of the urinary bladder, prostate, seminal vesicles and kidneys. No other clinical signs related to treatment were noted in P1 or P2 animals.

Body weight and Food consumption:

The following Table I (from Tables 8 and 12; pages 48-49 and 53-54 of the study report) presents selected body weight and food consumption data for P1 males (all values means ± standard deviation if available):

Table I: Body Weights, Body Weight Gains and Food Consumption P1 Males

Dose (mg/kg/day): Control	50	500	750	1000
Body Weights (g)				
Day				
-2	188.8±11.4	188.4±10.3	187.5±10.4	186.3±11.8	188.7±12.5
27	392.5±25.3	387.9±22.7	382.8±25.6	374.5*±31.2	377.1±30.5(96)[1]
48	470.8±33.6	465.8±32.1	463.8±30.4	451.4±44.8	448.5±42.9(95)
90	553.9±42.8	553.3±43.8	548.7±45.1	[3]	539.1±59.3(97)
118	592.3±50.1	594.9±47.9	587.9±49.7	[3]	576.7±64.9(97)
Body Weight Gains (g)				
Days				
-2-27[2]	203.7	199.5(98)	195.3(96)	188.2(92)	188.4(93)
-1-48[2]	282.0	277.4(98)	276.3(98)	265.1(94)	259.8(92)
-2-90[2]	365.1	364.9(100)	361.2(99)	[3]	350.4(96)
-2-118[2]	403.5	406.5	400.4(99)	[3]	388.0(96)

continued

Table I: Body Weights, Body Weight Gains and Food Consumption continued

	Food Consumption (g/animal/day)				
	Control	50	500	750	1000
Days					
1-8	25.6±1.7	26.3±1.5	27.4±2.1	26.4±1.9	27.4±2.0
22-29	28.2±2.1	28.0±1.8	29.2±2.1	28.6±2.4	28.9±2.5
43-50	28.8±2.8	28.7±2.2	29.8±2.5	29.4±3.0	29.1±2.9
85-92	27.4±2.4	27.5±2.7	28.9±2.8	[3]	29.3±2.6
120-123	28.8±3.7	28.8±3.3	30.2±3.6	[3]	30.7±3.8

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values; [3] = dose group terminated; * = $p < 0.05$ by Dunnett-test.

No treatment related effects were noted in the P1 males during the premating period. The 750 mg/kg/day dose group was dropped, according to the investigators: Prior to the P1 breeding, a decision was made to terminate the 750 mg/kg/day dose level. This decision was based on the fact that after 10 weeks the premating clinical observations, body weight and feed consumption were unaffected by ingesting XDE-564 at the highest dose of 1000 mg/kg/day.

The following Table II (from Tables 30 and 34; pages 95-96 and 100-101 of the study report) presents selected body weight and food consumption data for P2 males (all values means ± standard deviation if available):

Table II: Body Weights, Body Weight Gains and Food Consumption P2 Males

Dose (mg/kg/day):	Control	50	500	1000
		Body Weights (g)		
Day -2	99.3±13.5	96.6±16.7	96.7±14.3	94.8±13.1(96)[1]
27	353.6±31.9	345.4±33.2	353.4±37.8	343.7±36.1(97)
48	466.7±40.7	464.9±43.9	468.7±51.0	455.8±41.8(98)
90	566.5±55.9	566.6±51.0	570.4±65.9	562.3±46.8(99)
139	643.6±71.6	643.5±67.5	640.5±73.2	636.3±52.6(99)
		Body Weight Gains (g)		
Days				
-2-27[2]	254.3	248.8(98)	256.7	248.9(98)
-2-48[2]	367.4	368.3	372.0	361.0(98)
-2-90[2]	467.2	470.0	473.7	467.5
-2-139[2]	544.3	546.9	543.8	541.5(99)
		Food Consumption (g/animal/day)		
Days				
1-8	20.4±2.1	20.6±2.3	20.9±2.2	21.6±2.6
22-29	30.0±2.8	30.0±2.9	31.8±3.4	31.2±2.9
43-50	29.9±2.9	30.3±2.7	31.3±3.3	31.5±2.4
106-113	29.3±2.8	29.3±2.9	29.0±3.1	30.1±2.0
134-140	28.9±2.8	28.9±3.0	29.3±3.4	29.8±2.3

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values.

No treatment related effects were noted in the P2 males during the premating period.

The following Table III (from Tables 9 and 13; pages 50 and 55 of the study report) presents selected body weight and food consumption data for P1 females for the premating period (all values means ± standard deviation if available):

Table III: Body Weights, Body Weight Gains and Food Consumption P1 Females

Dose (mg/kg/day):	Control	50	500	750	1000
Body Weights (g)					
Day					
-2	151.2±11.2	151.7±10.6	151.2±9.4	153.9±10.4	153.3±11.0
13	195.1±16.9	197.4±14.0	195.0±14.2	200.4±16.1	199.8±15.3
34	233.5±22.5	242.3±20.6	233.8±19.5	239.9±25.0	238.9±19.5
55	253.9±26.1	265.7±23.3	256.9±22.4	264.2±28.9	262.0±23.9
69	270.0±26.3	277.6±27.8	269.8±21.6	276.3±31.6	274.6±23.6
Body Weight Gains (g)					
Days					
-2-13[1]	44.0	45.7	43.8	46.5	46.5
-2-34[1]	82.3	90.6	82.6	86.0	85.6
-2-55[1]	102.7	114.0	105.7	110.3	108.7
-2-69[1]	118.8	125.9	118.6	122.4	121.3
Food Consumption (g/animal/day)					
Days					
1-8	17.3±1.6	18.7±1.9	18.6±1.4	18.8±1.6	19.5±1.4
8-15	17.6±2.0	18.9±2.0	18.2±1.6	18.7±1.7	19.4±1.4
29-36	18.9±2.2	19.9±1.8	19.8±2.0	20.4±2.2	20.4±1.9
50-57	17.6±1.6	18.6±1.6	19.1±1.9	19.4±1.5	19.7±2.2
64-71	18.1±1.7	20.1±2.8	19.3±1.9	19.3±1.8	19.8±2.3

[1] = calculated by the reviewer from body weight mean values.

No treatment related effect was noted in the P1 females during the premating period.

The following Table IV (from Tables 10, 14 and 15; pages 51, 56 and 57 of the study report) presents selected body weight and food consumption data for P1 females for the gestation period (all values means ± standard deviation if available):

Table IV: Body Weights, Body Weight Gains and Food Consumption P1 Females

Dose (mg/kg/day):	Control	50	500	1000
		Body Weights (g)		
Day 0	277.2±22.2	286.0±26.9	275.3±24.1	281.5±25.2
Day 7	314.2±23.6	322.7±27.1	314.9±26.7	318.2±26.8
Day 14	347.7±24.8	355.8±28.6	343.9±27.9	345.6±26.1(99)[1]
Day 21	424.4±33.9	433.2±28.7	414.8±38.4	417.9±35.4(99)
		Body Weight Gains (g)		
Days				
0-7	36.9±9.1	36.8±8.8	38.0±10.0	36.7±10.1(100)
0-14[2]	70.5	69.8(99)	68.6(97)	64.1(91)
0-21	147.2±21.6	147.2±14.2	139.5±25.5(95)	136.4±19.3(93)
		Food Consumption (g/animal/day)		
Days				
0-7	23.4±1.8	23.9±2.6	24.4±2.5	24.9±2.9
7-14	25.3±1.6	26.2±2.9	26.0±2.7	26.2±1.8
14-21	24.3±2.4	24.9±2.8	26.0±3.9	24.9±2.9

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values.

No treatment related effects were noted in the P1 females during the gestation period.

The following Table V (from Tables 11, 16 and 17; pages 52, 58 and 59 of the study report) presents selected body weight and food consumption data for P1 females for the lactation period (all values means ± standard deviation if available):

Table V: Body Weights, Body Weight Gains and Food Consumption P1 Females

Dose (mg/kg/day):	Control	50	500	1000
		Body Weights (g)		
Day 1	315.4±24.6	321.1±25.9	312.7±30.2	312.7±23.8(99)[1]
4	320.2±23.6	322.1±22.0	317.5±28.1	317.2±23.4(99)
7	327.0±22.1	327.7±20.6	323.8±27.7	321.9±22.6(98)
14	350.1±24.3	351.4±26.1	348.3±25.7	341.6±26.1(98)
21	332.5±21.2	335.9±24.1	336.6±22.4	337.4±24.2

continued

Table V: Body Weights, Body Weight Gains and Food Consumption continued

P1 Females

Dose (mg/kg/day): Control	50	500	1000
Body Weight Gains (g)			
Days			
1-4	4.9±9.9	1.0±10.8(20)	4.5±8.8(93)	4.6±10.8(94)
1-7[2]	11.6	6.6(57)	11.1(96)	9.2(79)
1-14[2]	34.7	30.3(87)	35.6	28.9(83)
1-21	17.2±14.0	14.8±17.6	23.6±15.3	24.8±13.4
Food Consumption (g/animal/day)				
Days				
1-4	26.0±5.6	28.6±5.4	28.9±5.0	29.3±5.1
4-7	36.7±2.7	36.2±5.5	38.0±4.8	37.5±4.1
7-11	47.6±7.2	47.3±6.1	48.8±7.2	47.5±6.4
11-14	58.8±5.7	56.4±6.0	57.9±6.7	54.7±8.2
14-17	61.1±5.2	62.9±6.6	64.3±6.5	61.9±8.4
17-19	64.1±6.8	64.6±7.7	64.9±7.6	64.2±6.9
19-21	74.5±8.0	71.1±8.2	76.0±7.3	71.4±10.8

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values.

No treatment related effects were noted in the P1 females during the lactation period. The variation seen in the body weight gains is normal during lactation.

The following Table VI (from Tables 31 and 35; pages 97 and 102 of the study report) presents selected body weight and food consumption data for P2 females for the premating period (all values means ± standard deviation if available):

Table VI: Body Weights, Body Weight Gains and Food Consumption

P2 Females

Dose (mg/kg/day): Control	50	500	1000
Body Weights (g)			
Day -2	88.3±13.0	88.3±13.3	88.1±10.9	84.7±14.0
20	188.0±23.0	191.6±19.1	191.4±16.4	187.2±19.8(100)[1]
41	234.5±23.8	247.0±21.2	243.8±24.5	243.2±24.5
62	263.3±26.3	277.7±25.9	274.2±27.8	274.9±26.7
83	282.0±28.8	296.5±28.7	292.7±33.1	296.0±30.7
Body Weight Gains (g)				
Days				
-2-20[2]	99.7	103.3	103.3	102.5
-2-41[2]	146.2	158.7	155.7	158.5
-2-62[2]	175.0	189.4	186.1	190.2
-2-83[2]	193.7	208.2	204.6	211.3

continued

Table VI: Body Weights, Body Weight Gains and Food Consumption continued

P2 Females

Dose (mg/kg/day):	Control	50	500	1000
		Food Consumption (g/animal/day)		
Days				
1-8	17.1±1.9	17.6±1.2	17.9±1.7	17.4±2.0
15-22	19.2±2.0	20.4±1.6	20.5±2.9	20.1±1.8
36-43	19.5±1.9	20.5±2.0	20.3±2.6	21.0±2.7
57-64	19.5±2.2	20.6±2.4	20.3±2.4	21.5±2.3
78-85	18.8±2.4	19.6±1.8	19.9±2.5	20.6±2.2

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values.

No treatment related effects were noted in the P2 females during the premating period.

The following Table VII (from Tables 32, 36 and 37; pages 98, 103 and 104 of the study report) presents selected body weight and food consumption data for P2 females for the gestation period (all values means ± standard deviation if available):

Table VII: Body Weights, Body Weight Gains and Food Consumption

P2 Females

Dose (mg/kg/day):		Control	50	500	1000
			Body Weights (g)		
Day	0	284.1±30.1	300.5±33.0	289.9±36.6	296.8±33.5
	7	317.0±31.9	331.8±32.7	331.3±37.0	328.7±14.5
	14	350.4±31.3	362.1±34.2	362.9±39.3	359.1±36.5
	21	425.5±29.4	424.3±41.8	432.1±47.7	431.1±44.8
			Body Weight Gains (g)		
Days					
0-7		32.9±9.4	31.4±6.5(95)	32.4±10.1(99)	31.9±10.1(97)
0-14[1]		66.3	61.6(93)	73	62.3(94)
0-21		141.4±22.1	123.8±30.0(88)	133.2±26.9(94)	134.2±21.0(95)
			Food Consumption (g/animal/day)		
Days					
0-7		23.0±3.1	23.5±1.9	23.3±2.3	23.8±3.2
7-14		24.6±2.5	25.2±2.5	25.6±2.9	25.4±2.9
14-21		23.2±2.8	23.9±2.2	24.2±3.3	23.6±2.7

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values.

No treatment related effects were noted in the P2 females during the gestation period.

The following Table VIII (from Tables 33, 38 and 39; pages 99, 105 and 106 of the study report) presents selected body weight and food consumption data for P2 females for the lactation period (all values means ± standard deviation if available):

Table VIII: Body Weights, Body Weight Gains and Food Consumption P2 Females

Dose (mg/kg/day):	Control	50	500	1000
		Body Weights (g)		
Day 1	319.2±28.1	340.1±32.0	332.9±38.7	323.8±31.0
4	327.2±32.4	344.2±31.8	337.4±33.7	325.7±30.3
7	331.1±30.0	345.2±30.3	336.5±38.7	329.5±32.0
14	354.4±34.7	354.8±32.4	358.0±35.3	352.7±30.3
21	343.4±33.5	338.6±24.6	345.0±28.1	340.6±25.0
		Body Weight Gains (g)		
Days				
1-4	8.0±15.4	4.2±10.3(53)[1]	4.6±9.9(58)	1.8±11.1(23)
1-7[2]	11.9	5.1(43)	3.6(30)	5.7(48)
1-14[2]	35.2	14.7(42)	25.1(71)	28.9(82)
1-21	24.2±19.2	-3.0*±15.8	12.1±23.9(50)	16.7±12.3(69)
		Food Consumption (g/animal/day)		
Days				
1-4	28.9±8.8	28.0±6.6	27.9±5.2	31.3±6.9
4-7	38.8±5.5	38.1±5.9	40.2±5.9	39.7±8.6
7-11	51.6±8.4	47.7±9.3	49.0±8.0	52.2±7.6
11-14	52.7±10.2	54.1±12.9	49.2±14.3	54.1±11.6
14-17	62.5±6.2	57.8±8.6	64.0±6.9	62.5±9.9
17-19	62.9±11.1	64.0±8.7	69.8±7.0	67.1±9.6
19-21	74.0±8.4	71.6±12.3	73.8±11.4	77.7±11.9

[1] = percent of control; [2] = calculated by the reviewer from body weight mean values; * = p < 0.05 by Dunnett-test.

No treatment related effects were noted in the P1 females during the lactation period. The variation seen in the body weight gains is normal during lactation.

Parental animals and offspring

Reproductive performance:

Results for the mating of P1 parental animals and the F1 pups are summarized from the report on Table IX (from Tables 24-26, pages 87-91) as follows:

Table IX: Reproductive Performance and Lactation Observations

Dose (mg/kg/day):	Control	50	500	1000
		F0 Generation		
Observation				
Mean precoital interval (days)	3.1±2.9	2.5±1.2	2.8±1.5	2.5±1.2
		Males		
Mated	27	30	30	30
Mating index(%)	90.0	100	100	100
Fertile	24	25	27	26
Fertility index(%)	80.0	83.3	90.0	86.7
		Females		
Mated	29	30	29	29
Mating index(%)	100	100	100	100
Fertile	26	25	27	26
Fertility index(%)	89.7	83.3	90.0	86.7
Intercurrent deaths	1	1	0	0
Nonpregnant	2	4	2	3
Number of litters	26	25	27	26
Gestation index(%)	100	100	100	100
Parturition index(%)	100	100	100	100
Mean gestation interval (days)	21.8±0.4	21.9±0.3	22.0±0.4	21.9±0.3
		F1 litters		
Total pups	349	359	337	351
Live Litter size	13.0±2.8	14.0±2.1	12.3±3.4	13.2±2.6
Pups liveborn	337	351	331	342
Live birth index(%)	96.6	97.8	98.2	97.4
Pups stillborn	12	8	6	9
Pup survival				
days 0-4	329	340	328	334
Viability index(%)	97.6	96.9	99.1	97.7
days 4-21	202	197	205	204
Lactation index(%)	100	98.5	100	100

continued

Table IX: Reproductive Performance and Lactation Observations continued

Dose (mg/kg/day):	Control	50	500	1000
	F0 Generation - F1 litters			
Observation				
Live pups per litter				
day 1	12.8±2.8	13.8±2.1	12.2±3.5	13.0±2.5
day 4 precull	12.7±2.8	13.6±2.2	12.1±3.5	12.8±2.5
day 4 postcull	7.8±1.2	8.0±0.0	7.6±1.6	7.8±0.8
day 7	7.8±1.2	8.0±0.0	7.6±1.6	7.8±0.8
day 14	7.8±1.2	8.0±0.2	7.6±1.6	7.8±0.8
day 21	7.8±1.2	8.0±0.2	7.6±1.6	7.8±0.8
Sex ratio (males:female)				
day 1	50:50	53:47	47:53	50:50
Pup weights				
day 0 female	6.5±0.7	6.5±0.7	6.8±0.7	6.4±0.6(99)[1]
day 0 male	6.9±0.6	6.9±0.6	7.2±0.7	6.8±0.5(99)
day 4 precull F	9.2±1.2	9.0±1.2	9.5±1.2	8.8±1.2(96)
day 4 precull M	9.8±1.0	9.4±1.2	10.1±1.3	9.3±1.0(95)
day 4 postcull F	9.2±1.2	9.1±1.3	9.5±1.2	8.8±1.3(96)
day 4 postcull M	9.7±1.1	9.4±1.2	10.1±1.4	9.3±1.0(96)
day 7 female	15.0±1.8	14.6±1.9	15.2±1.7	14.1±2.2(94)
day 7 male	15.9±1.7	15.2±1.6	16.2±1.7	14.9±1.9(94)
day 14 female	32.1±3.2	30.6±4.5	31.6±2.6	30.3±3.6(94)
day 14 male	33.9±3.1	31.6*±4.3	33.3±2.5	31.6*±3.3(93)
day 21 female	51.4±5.5	50.2±6.2	51.6±4.0	48.5±5.5(94)
day 21 male	54.7±5.4	51.9±6.0	54.0±4.2	50.9*±5.7(93)

[1] = percent of control; * = $p < 0.05$ by Dunnett's test.

There were no treatment related effects noted on reproductive parameters or in pup survival and pup body weights. There was a slight reduction in pup body weights on days 14 and 21 but the differences were not biologically relevant and the was not seen in the subsequent generation (see below).

Results for the P2 parental animals and the F2 pups are summarized from the report on Table X (from Tables 48-50, pages 138-142) as follows:

Table X: Reproductive Performance and Lactation Observations

Dose (mg/kg/day):	Control	50	500	1000
		P2 Generation		
Observation				
Mean precoital interval (days)	3.2±1.5	2.4±1.7	2.6±1.9	4.1±4.3
		Males		
Mated	29	30	30	30
Mating index(%)	96.6	93.3	93.3	90.0
Fertile	23	20	24	20
Fertility index(%)	79.3	66.7	80.0	66.7
		Females		
Mated	29	30	30	30
Mating index(%)	100	96.7	96.7	100
Fertile	24	20	25	23
Fertility index(%)	82.8	66.7	83.3	76.7
Intercurrent deaths	1	0	0	0
Nonpregnant	4	10	5	7
Number of litters	24	20	25	23
Gestation index(%)	100	100	100	100
Parturition index(%)	100	100	100	100
Mean gestation interval (days)	21.7±0.5	22.0±0.0	21.8±0.4	21.8±0.4
		F2 litters		
Total pups	329	227	305	315
Live Litter size	13.3±3.5	11.0±4.0	11.9±3.6	13.6±3.3
Pups liveborn	320	220	298	313
Live birth index(%)	97.3	96.9	97.7	99.4
Pups stillborn	9	7	7	2
Pup survival				
days 0-4	308	219	289	307
Viability index(%)	96.2	99.5	97.0	97.7
days 4-21	182	138	189	175
Lactation index(%)	98.4	100	100	98.9
Live pups per litter				
day 1	13.0±3.6	11.0±4.0	11.6±3.5	13.5±3.2
day 4 precull	12.8±3.5	11.0±4.0	11.6±3.4	13.3±3.2
day 4 postcull	7.7±1.1	7.3±1.3	7.6±1.4	7.7±1.1
day 7	7.6±1.1	7.3±1.3	7.6±1.4	7.6±1.1
day 14	7.6±1.1	7.3±1.3	7.6±1.4	7.6±1.1
day 21	7.6±1.1	7.3±1.3	7.6±1.4	7.6±1.1

continued

Table X: Reproductive Performance and Lactation Observations continued

Dose (mg/kg/day):	Control	50	500	1000
	P2 Generation - F2 litters			
Observation				
Sex ratio (males:female)				
day 1	48:52	46:54	51:49	49:51
Pup weights				
day 0 female	6.4±0.9	6.8±0.8	6.7±0.9	6.4±0.6
day 0 male	6.8±0.8	7.2±0.7	7.1±1.0	6.8±0.6
day 4 precull F	9.1±1.4	10.1±1.4	10.0±1.6	9.5±1.0
day 4 precull M	9.6±1.5	10.5±1.3	10.5±1.6	9.9±0.9
day 4 postcull F	9.1±1.4	10.1±1.3	10.0±1.6	9.5±1.0
day 4 postcull M	9.7±1.4	10.5±1.3	10.6±1.6	9.9±1.0
day 7 female	14.9±2.3	16.3*±1.5	16.0±2.1	15.7±1.7
day 7 male	15.7±2.5	17.1±1.3	16.8±2.0	16.3±1.7
day 14 female	31.0±4.1	33.7*±2.7	32.9±4.3	32.6±2.9
day 14 male	32.4±4.5	34.7±2.5	34.3±4.4	33.5±3.6
day 21 female	49.4±5.8	52.9±3.2	52.5±5.8	51.7±4.1
day 21 male	52.0±6.4	54.6±3.2	54.8±5.4	53.3±5.2

* = $p < 0.05$ by Dunnett's test.

There were no treatment related effects noted on reproductive parameters or in pup survival and pup body weights. The Fertility Index was 82.8, 66.7, 83.3, 76.7% for the control 50, 500, and 1000 mg/kg/day dose groups, respectively; however, there was no effect on gestation or parturition indices or pup litter size.

Clinical observations

Clinical signs of toxicity:

No treatment related effects were noted in the supplied data for either generation and litters.

Necropsy results

Necropsy observations (macroscopic):

No treatment related effects were noted in gross (macroscopic) observation data provided for the P1 and P2 adults and the F1 and F2 pups.

Organ weights:

No treatment related effects were noted in the organs weights for the P2 adults.

Pathology

Microscopic examination:

From the data provided there were no treatment related macroscopical changes observed in the P1 and P2 parental animals.

There were no treatment related findings in the reproductive system of animals of either sex.

III. DISCUSSION

A. Investigators' Conclusions: (scanned from page 11 of the study report)

The objective of this two-generation dietary reproduction study was to evaluate the effects of XDE-564 on the reproductive capability and neonatal growth and survival of rats. Groups of 30 male and 30 female CD (Sprague-Dawley derived) rats, approximately six weeks of age, were fed diets that provided 0, 50, 500, 750 or 1000 mg XDE-564/kg/day for approximately 10 weeks prior to breeding. Prior to the P1 breeding, a decision was made to terminate the 750 mg/kg/day dose level. This decision was based on the fact that after 10 weeks the premating clinical observations, body weight and feed consumption were unaffected by ingesting XDE-564 at the highest dose of 1000 mg/kg/day. The remaining P1 adults were bred to produce the F1 litters. After weaning., groups of 30 male and 30 female F1 pups were randomly selected to become the second parental (P2) generation. Following approximately 12 weeks of dietary exposure, the P2 adults were bred to produce the F2 litters.

Dietary exposure of adult male and female rats to as high as the limit dose of 1000 mg/kg/day XDE- 564 resulted in no parental or neonatal toxicity. No effects were observed on the fertility indices, gestation length, time-to-mating or litter size at any dose level. In addition, tissues and organs were unaffected at necropsy, as were histopathologic evaluations of male and female reproductive organs, mammary gland, pituitary, liver, kidneys and gross lesions among either P1 or P2 adults. Similarly, no treatment-related effects on neonatal survival, body weight, sex ratio or gross pathologic alterations were observed at any dose level tested in either the F1 or F2 generation.

In conclusion, under conditions of this study, the parental no-observed - effect level (NOEL) was 1000 mg/kg/day for both males and females; likewise, the NOEL for fertility and neonatal development was 1000 mg/kg/day.

B. Reviewer's conclusions:

No systemic toxicity to the parental animals was noted at the dose levels tested up to the limit dose. There were no treatment related findings in the reproductive system of animals of either sex. For the pups, no systemic or developmental toxicity was noted in either generation. No effects were noted on reproductive parameters.

Parental (Paternal/Maternal) Systemic Toxicity NOAEL ≥ 1000 mg/kg/day
Parental (Paternal/Maternal) Systemic Toxicity LOAEL > 1000 mg/kg/day

Offspring Systemic/Developmental Toxicity NOAEL ≥ 1000 mg/kg/day
Offspring Systemic/Developmental Toxicity LOAEL > 1000 mg/kg/day

Reproductive Toxicity NOAEL ≥ 1000 mg/kg/day
Reproductive Toxicity LOAEL > 1000 mg/kg/day