CASWELL

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

MAY 30 1997

012238

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Comments and an 18-Month Feeding Study in Mice with Spinosad (XDE-104)

PC Code:	110003
DP Barcode:	D230566, D230456
Submission #:	S512485
Registration #:	6F04735

TO: Barbara Madden
Risk Characterization & Analysis Branch
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and

Adam Heyward, PM Team 13
Insecticides/Rodenticides Branch
Registration Division (7505C)

FROM: Roger Gardner, Toxicologist Roger Gardner 5/20/97
Review Section 1, Toxicology Branch 1
Health Effects Division (7509C)

THRU: Karl Baetckke, Ph.D., Chief KB 5/22/97
Toxicology Branch 1
Health Effects Division (7509C)

Actions Requested: Review of comments from the registrant and a mouse carcinogenicity study (MRID 44123601; DER attached).

Recommendations and Conclusions

1. Based on comments submitted, the chronic toxicity phase (83-1a) of the study in rats (83-5, MRID 43701507) is acceptable.

2. The LOEL for systemic toxicity is 24.1 and 30.3 mg/kg/day (for males and females, respectively), based on vacuolation of thyroid follicular cells in both sexes,

inflammation of the thyroid in females, and increased absolute and relative weights of the thyroid in females. The NOEL is 9.5 mg/kg/day for males and 12.0 mg/kg/day for females.

3. The combined incidences of hemangiomas/hemangiosarcomas in female mice from two long-term feeding studies (MRID 43701505 and 44123601) were 4/50 and 6/50 in the control groups and 6, 9 and 5 of 50 at dose levels of 4.2, 13.8, and 41.5 mg/kg/day, respectively). These results do not indicate a dose-response relationship for the incidence of hemangiomas/hemangiosarcomas in female mice, and there is no indication that spinosad is carcinogenic in mice.

I. Background

Initial review of the Toxicology database for spinosad indicated that:

- histopathology data were missing on rats in some groups from the chronic feeding study (MRID 43701507) and

- additional details on the incidence of hemangiomas in female mice in the oncogenicity study (MRID 43701505) as well as a report on a second mouse oncogenicity study were needed to clarify the oncogenic potential of spinosad.

The registrant responded by letter (dated September 27, 1996; MRID 44123600) and submitted the other long-term mouse feeding study (MRIDs 44123600 and -01). However, the missing data from the chronic feeding study in rats was not submitted. Also, results from the new mouse study are considered below and were considered by the Health Effects Division's (HED) Reference Dose (RfD)/Peer Review Committee on April 30, 1997.

II. Review of New Information

A. The long-term feeding study in rats

As noted above, previous reviews indicated that insufficient reasons were provided in the original report as to why the animals in the highest dose groups (0.05% or 0.1% XDE-105 in the diet) that died during the second year of the study were not microscopically examined. Although the report stated that there was excessive mortality at the highest dose tested (0.10%), deaths in that group were not observed to increase over the other groups until after week 88 in males and after week 68 in females. Even then mortality did not approach a level sufficient to terminate the study ahead of schedule (50%) until just before week 84 in females.

The September letter indicated, "...(based on advice offered in conversations with Agency toxicologists), the surviving male and female rats given 0.10% XDE-105 were removed from study on test days 714 and 611, respectively, due to the excessive mortality and body weight

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decrement..." This protocol adjustment changed the status of the high-mid dose group (0.05%) to that of a high dose group. According to Subdivision F guidelines, all animals in that group, as well as all animals in the control groups, should be examined for histopathology. At the very least, histopathology is missing on 14 of 50 control males, 12 of 50 0.05% dose level males, 15 of 50 control females and 8 of 50 0.05% dose level females from the main portion of the study.

Microscopic observations of animals that died during the study could support an interpretation of the significance of the changes noted in the report for the 12-month and terminal sacrifices. For example, the letter states, "The inflammation and/or necrosis in the thyroid gland (of female rats) were interpreted to have the potential of being life threatening conditions." However, there are no histological observations available for animals that died during the study to support the conclusion drawn in the letter. Also, the survival rate at the end of the study for female rats given the 0.05% dose level was greater than that for the control group (84% and 70%, respectively), which casts doubt on such an interpretation of the microscopic observations in the thyroid.

The letter further stated:

> **...the results from the microscopic examination of these tissues would not be relevant for the extrapolation of human risk. The data from the remaining dose levels provided information to adequately evaluate the chronic toxicity...of XDE-105. This belief is based on the significant toxicity which occurred in male and female rats given 0.05% XDE-105,...These effects occurred at a dose level that was half the dose that resulted in excessive mortality and the humane sacrifice of remaining rats in the 0.10% dose level. In addition, the intermediate dose level of 0.02% XDE-105 had toxicity consisting of vacuolization of the epithelial cells of the thyroid gland, which would aid in evaluating the dose response curve.**
>
> **These data appear sufficient to characterize the chronic toxicity of XDE-105 and indicate that a steep dose response curve exists for XDE-105, with excessive toxicity occurring at the 0.10% dose level, significant toxicity occurring at the 0.05% dose level, minor toxicity occurring at the 0.02% dose level and an NOEL at 0.005%.**
>
> **Furthermore, we believe that a chronic toxicity study in rodents requires sufficient toxicity at the highest dose, but not necessarily as much toxicity as that required for a maximum tolerated dose in a carcinogenicity study. The major emphasis in a chronic rodent study for risk assessment purposes is the definition of an NOEL or a close approximation thereof, in order to determine the RfD for the chemical. Chasing the toxicity of animals all the way to mortality was never the intention of the chronic toxicity study and appears to have no value for the toxicity dose-response curve, even if an ED10 is to be calculated rather than using an NOEL for risk assessment purposes.**

The RfD Committee agreed that the chronic toxicity phase (83-1a) of the study in rats (83-5, MRID 43701507) is acceptable. However, the Committee concluded that the LOEL for systemic toxicity is 24.1 and 30.3 mg/kg/day (for males and females, respectively), based on vacuolation of thyroid follicular cells in both sexes, inflammation of the thyroid in females, and increased absolute and relative weights of the thyroid in females. The Committee also

concluded that the NOEL is 9.5 mg/kg/day for males and 12.0 mg/kg/day for females. Under the conditions of the study, the RfD Committee also concluded that there was no oncogenic response.

It should also be noted that the Reference Dose is based on a feeding study in dogs which showed the lowest NOEL of 2.68 mg/kg/day with an LOEL of 8.22 mg/kg/day based on vacuolation in glandular cells (parathyroid) and lymphatic tissues, arteritis and increases in serum enzymes such as alanine aminotransferase, and aspartate aminotransferase, and triglyceride levels. The RfD Committee indicated that similar changes were reported in other species tested at higher dose levels. Therefore, the registrant's comments quoted above support upgrading the rat study to acceptable.

B. The mouse carcinogenicity studies

In a mouse oncogenicity study (MRID 44123601), Spinosad (76.1% Factor A + 11.9% Factor D) was administered to CD-1 mice (50/sex/group) for up to 18 months at dietary concentrations of 0, 0.0008 or 0.024% (equivalent to 0, 1.1 or 32.7 mg/kg/day in males and 0, 1.3 or 41.5 mg/kg/day in females). Satellite groups of 10 mice/sex/group were included for sacrifice at 12 months. This report was submitted to upgrade a previous study (MRID 43701505) in which the high dose (67 mg/kg/day) caused excessive toxicity (lower body weights and feed consumption with increased mortality) resulting in the early termination of the high-dose female mice. This second study (MRID 44123601) was started in order to comply with test guidelines which require at least three dose levels for a full evaluation of spinosad. This study was begun during the time of the previous study, and its design included both males and females, but most of the data included in the report related to the female mice in the highest dose tested (41.5 mg/kg/day).

Male mice given the 32.7 mg/kg/day dose level had lower mean body weights (e.g., at 90 days, 37.4 and 35.7 g for control and high-dose groups, respectively; $p \leq 0.05$) and body-weight gains (during study days -9 to 90, 9.8 and 8.2 g/animal for control and high-dose males, respectively; $p \leq 0.05$). There was no significant effect on mortality in the study. The incidence of thickened glandular mucosa of the stomach was increased at 12 months in both males and females (0/10 and 2/10 for control and high-dose males, respectively; and 0/10 and 5/10 for control and high-dose group females, respectively) and at 18 months (0/50 and 17/50 for control and high dose group males, respectively; and 2/50 and 27/50 for females, respectively).

Histopathologic effects were noted in females given the 41.5 mg/kg/day dose level, and the following differences were statistically significant ($p \leq 0.05$) at the 18-month observation: aggregates of alveolar macrophages in the lungs (0/10 controls and 2/10 treated females at 12 months and 8/50 and 39/50 in the control and treated females, respectively); sinus histiocytosis of the lymphnodes (0/10 controls and 2/10 treated females at 12 months and 2/49 and 20/50 in control and treated females, respectively); vacuolation of parathyroid glands (1/10 control and 3/10 treated females at 12 months and at 18 months 2/50 and 25/50

for control and treated females, respectively); myopathy of skeletal muscle (of the face and tongue) (0/50 controls and 22/50 treated females at 18 months; none were noted at 12 months); and hyperplasia of the glandular mucosa of the stomach (1/10 control and 9/10 treated at 12 months and 18/50 and 48/50 control and treated females, respectively at 18 months); and inflammation of the stomach (at the 12 month observation, 2/10 in the controls and 9/10 in the high dose group females, respectively; at 18 months, 14/50 and 29/50 in the control and high dose group females, respectively, $p \leq 0.05$). The incidence of the treatment-related histopathologic alterations increased with time.

The incidence of tumors in female mice administered 0.024% spinosad for up to 18 months was not increased relative to controls. The combined incidences of hemangiomas/hemangiosarcomas in female mice from two long-term feeding studies (MRID 43701505 and 44123601) were reported to be 4/50 and 6/50 in the control groups and 6, 9 and 5 of 50 at dose levels of 4.2, 13.8, and 41.5 mg/kg/day, respectively). These results do not indicate a dose-response relationship for the incidence of hemangiomas/hemangiosarcomas in female mice, and when considered with additional information submitted on the results from the first study (MRID 43701505), there is no indication that the test material is carcinogenic in mice.

References

43701505 Bond, D.; Stebbins, K.; McGuirk, R. (1995) XDE-105: 18-Month Dietary Oncogenicity Study in CD-1 Mice. Dow Chemical Co., Midland, Michigan: Lab Project Number: DR-0323-1194-006, April 7, 1995. Unpublished.

43701507 and 43701503 Bond, D.M., B.L. Yano, and K.E. Stebbins. (1995) XDE-105: Two year chronic toxicity, neurotoxicity, and oncogenicity study in Fischer rats. The Toxicology Research Laboratory, Dow Chemical Company, Midland, MI. Laboratory Study ID No. DR-0323-1194-005 and DR-0323-1194-005N. April 7, 1995. Unpublished.

44123601 Bond, D.M., K.E. Stebins, and R.J. McGuirk (March 4, 1996) XDE-105: 18-Month oncogenicity study in CD-1 Mice. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan. Report no. DR-0323-1194-006. Unpublished.

EPA R[illegible]er: Roger Gardner, Date 4/8/97
Review Section 1, Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: K. Dodd, Date 5/12/97
Review Section _, Toxicology Branch _ (7509C)

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DATA EVALUATION RECORD

STUDY TYPE: 83-2 (B); Oncogenicity Study in Mice (supplemental report)

DP BARCODE:D230566 SUBMISSION CODE: S477588
P.C. CODE: 110003 MRID NO.: 44123601

TEST MATERIAL (PURITY): XDE-105 (Factor D) (Spinosad)

SYNONYMS: Spinosad

CITATION: Bond, D.M., K.E. Stebins, and R.J. McGuirk (March 4, 1996) XDE-105: 18-Month oncogenicity study in CD-1 Mice. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan. Report no. DR-0323-1194-006A, MRID 44123601.

SPONSOR: DowElanco

EXECUTIVE SUMMARY: In a mouse oncogenicity study (MRID 44123601), XDE-105 (76.1% Factor A + 11.9% Factor D) was administered to CD-1 mice (50/sex/group) for up to 18 months at dietary concentrations of 0, 0.0008 or 0.024% (equivalent to 0, 1.1 or 32.7 mg/kg/day in males and 0, 1.3 or 41.5 mg/kg/day in females). Satellite groups of 10 mice/sex/group were included for sacrifice at 12 months. This report was submitted to upgrade a previous study (MRID 43701505) in which the high dose (67 mg/kg/day) caused excessive toxicity (lower body weights, feed consumption and mortality) resulting in the early termination of the high-dose female mice. This second study (MRID 44123601) was started in order to comply with test guidelines which require at least three dose levels for a full evaluation of XDE-105. This study was begun during the time of the previous study, and its design included both males and females, but most of the data included in the report related to the female mice in the highest dose tested (41.5 mg/kg/day).

Male mice given the 32.7 mg/kg/day dose level had lower mean body weights (e.g., at 90 days, 37.4 and 35.7 g for control and high-dose groups, respectively; $p \leq 0.05$) and body-weight gains (during study days -9 to 90, 9.8 and 8.2 g/animal for control and high-dose males, respectively; $p \leq 0.05$). There was no significant effect on mortality in the study. The incidence of thickened glandular mucosa of the stomach was increased at 12 months in both males and females (0/10 and 2/10 for control and high-dose males, respectively; and 0/10 and 5/10 for control and high-dose group females, respectively) and at 18 months (0/50 and 17/50 for control and high dose group males, respectively; and 2/50 and 27/50 for females, respectively).

Histopathologic effects were noted in females given the 41.5 mg/kg/day dose level, and the following differences were statistically significant ($p \leq 0.05$) at the 18-month observation: aggregates of alveolar macrophages in the lungs (0/10 controls and 2/10 treated females at 12 months and 8/50 and 39/50 in the control and treated females, respectively); sinus histiocytosis of the lymphnodes (0/10 controls and 2/10 treated females at 12 months and 2/49 and 20/50 in control and treated females, respectively); vacuolation of parathyroid glands (1/10 control and 3/10 treated females at 12 months and at 18 months 2/50 and 25/50 for control and treated females, respectively); myopathy of skeletal muscle (of the face and tongue) (0/50 controls and 22/50 treated females at 18 months; none were noted at 12 months); and hyperplasia of the glandular mucosa of the stomach (1/10 control and 9/10 treated at 12 months and 18/50 and 48/50 control and treated females, respectively at 18 months); and inflammation of the stomach (at the 12 month observation, 2/10 in the controls and 9/10 in the high dose group females,

respectively; at 18 months, 14/50 and 29/50 in the control and high dose group females, respectively, $p \leq 0.05$). The incidence of the treatment-related histopathologic alterations increased with time.

The incidence of tumors in female mice administered 0.024% XDE-105 for up to 18 months was not increased relative to controls. The combined incidences of hemangiomas/hamangiosarcomas in female mice from two long-term feeding studies (MRID 43701505 and 44123601) were reported to be 4/50 and 6/50 in the control groups and 6, 9 and 5 of 50 at dose levels of 4.2, 13.8, and 41.5 mg/kg/day, respectively). These results do not indicate a dose-response relationship for the incidence of hemangiomas/hemangiosarcomas in female mice, and when considered with additional information submitted on the results from the first study (MRID 43701505), there is no indication that the test material is carcinogenic in mice.

STUDY CLASSIFICATION:

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

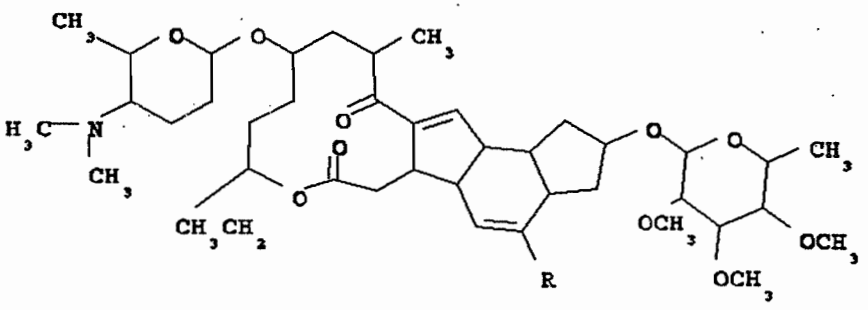
I. MATERIALS AND METHODS

A. Materials:

1. Test Material: The test material was described in the report as follows:

> The test material, XDE-105 (Lot #ACD 13651; TSN AGR 293707), ...was determined to be 88.0% active ingredient (76.1% and 11.9% Factor A and Factor D, respectively). In addition, stability of the test material was determined periodically throughout the study.

The physical state was described as a white powder, and the chemical structure was provided as follows:



Factor A: R = H
Factor D: R = CH_3

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2. Test animals: The report described the test animals as follows:

> CD-1 mice, approximately six weeks of age, were purchased from Charles River Laboratories, Portage, Michigan for use in the study...
>
> Upon arrival at the laboratory, the health of the mice was evaluated...During an acclimation period of 16 days, the mice were assigned one/cage to three testgroups per sex using a computer-based randomization program based on body weights, to ensure a homogeneous weight distribution between groups...
>
> Test animals were housed in suspended, stainless steel cages with wire-mesh floors and catch pans lined with cageboards...Animal cages were washed and cageboards changed regularly...All cages contained a feed crock and a pressure-activated stainless steel water nozzle. The animal rooms of the facility were designed to maintain adequate temperature, humidity, and photocycle for the species being used. A basal diet of Purina Certified Chow #5002, vehicle for the test material, and tap water were provided ad libitum during the prestudy and dosing periods.

B. METHODS:

1. Experimental Design: This aspect of the study was described in the report as follows:

> Sixty male and sixty female CD-1 mice/dose level were administered test diets formulated to deliver 0, 0.0008, or 0.024% XDE-105. Ten mice/sex/dose level were randomly designated at the beginning of the study for interim sacrifice after approximately 12 months. Fifty mice/sex/dose level received the test material for up to 18 months....

2. Dosing: The rationale for dosing was discussed in the report as follows:

> In a previously conducted dietary oncogenicity study, the high dose (0.036%) female mice were terminated early due to markedly lower body weights, feed consumption and excessive mortality indicative of exceeding a maximum-tolerated dose (MRID 43701505). As a result, the ONcogenicity Test Guidelines of the Japan MAFF were not fully met in regards to the number of female dose levels fully evaluated. The study reported herein was undertaken to fill this potential data gap. Since this study began before termination and final evaluation of all data from the previous study, the study design included both males and females at treatment levels of 0, 0.0008 and 0.024% XDE-105. The high dosage (0.024%) was expected to produce toxicity in multiple organs based upon the results of previously conducted subchronic and chronic toxicity studies. There were body weight differences noted at a treatment level of 0.045% following 90 days of exposure and at a treatment level of 0.036%in the oncogenicity study cited above. In addition, multiple organ systems were shown to be affectedupon histopathologic examination and numerous clinical chemistry and hematologic parameters were altered. The remaining dose level (0.0008%) was placed on study to ensure definition of a NOEL. However, the low dose level was not examined histopathologically because a NOEL was established at a higher dose level in the previous study.

Dosing preparations and analyses were discussed in the report as follows:

> ...Premixes and test diets were prepared every three to four weeks during the course of the study. Reference samples (1/dose/mix plus premix and control feed) were retained and stored

at room temperature...Test material was administered as a constant fixed percent in the diet. Test material intake (mg/kg/day) was calculated based on mean feed consumption and mean body weight data collected throughout the study.

Stability of the test compound in basal rodent chow was established in a previous study. Diet homogeneity was initiated concurrent with the start of the study and validated analytically. Analyses to verify the concentration of the test material in the diets were conducted at the start of the study and quarterly thereafter.

Results: Homogeneity Analysis: The report stated:

A diet preparation, 0.0008% (w/w), was submitted for analysis to determine homogeneity prior to the start of the study. Three core samples were taken from the diet and divided into three layers each. An approximately 5 gram aliquot was analyzed from each of the nine resulting sam[ples. The relative standard deviation over the nine samples was 6.28% for the 0.0008% diet. Based on the fact that the relative standard deviationwas lower than 10%, thefeed mixture was considered homogeneousand the mixing method was valid.

Stability Analysis: The report discussed results as follows:

Concentrations of the acive ingredients varied from 87.9 to 88.7%over 39 months. This variation was within the standard error of the analytical method, therefore, XDE-105 was considered to be stable over the duration of the dosing period.

XDE-105 was stable in rodent chow for at least 40 days at a concnetration of approximately 0.003% (w/w)...Percent of Day zero concentrations varied from 92 to 97%over the 40-day period. Variation was cnosidered within the standard error of the anlytical method...

Concentration Analysis: These results were describe in the report as follows:

...samples of all diets and the premix were assayed for concentrations of XDE-105 once prior to study start and during weeks 16, 24, 36, 39, 67, and 77..., mean percent of target for all diets and premixes ranging from 95 to 99%.

The results of daily intake of test material were described in the report as follows:

The following table correlates percent XDE-105 provided in the diet and the time-weighted average XDE-105 intake:

Percent XDE-105 in diet	Males mg/kg/day Consumed	Females mg/kg/day Consumed
0.0008	1.1	1.3
0.024	32.7	41.5

3. Observations: The parameters observed in the study were described in the report as follows:

Daily Observations. All animals were observed cageside at least twice daily for morbidity, moribundity, mortality, availability of feed and water, and clinical signs. To the extent possible, these observations included an evaluation of the skin, fur and mucous membranes, respiration, central nervous functions, and behavior pattern. Moribund animals that were not expected to survive until the next observation period, any animals found dead, were necropsied. Animals found dead after routine working hours, on weekends or holidays were refrigerated until the next scheduled workday and necropsied.

Clinical Evaluation. Clinical evaluations were conducted on all animals prior to the start of the study and weekly thereafter. Examinations included a thorough evaluation of the skin and fur, mucous membbranes respiration, nervous system and behavior pattern of each animal. Examination of nervous system function included looking for tremors, convulsions, salivation and diarrhea. In addition, the dat of initial observation, and progression or disappearance of each palpable mass was also recorded during the weekly clinical examination. The final interpretation for inlife palpable masses and significant clinical signs werebased upon the gross and histopathological examinations.

Body weights and Body Weight Gains. Body weights were recorded for all animals prestudy, weekly for the first 13 weeks fo the study, and at approximately monthly intervals thereafter. Body weight gains were subsequently calculated using these data.

Feed Consumption. The amounts of feed consumed were calculated prestudy, weekly for the first 13 weeks of the study, and for approximately a 1-week period each month thereafter, by weighing feed crocks before and after the feeding cycle allowing access to the feed suing the following equation:

$$\text{Feed consumption (mg/kg/day)} = \frac{\text{(initial wt. of feed cock - final wt. of feed cock)}}{\text{(\# days in measurement cycle)(\# animals/cage)}}$$

Feed efficienccy was calculated using mean body weight gain (bwg) data and mean feed consumption data from the first thirteen weeks of the study using the following equation:

$$\text{Feed Efficiency (g feed/day/g bwg/day)} = \frac{\text{(feed consumption/day)}}{\text{(body weight gain/day)}}$$

The time weighted average test material intake was calculated suing mean feed consumption, mean body weight , and the targeted concentration in the respective diet.

Ophthalmology. Ophthalmological examinations were conducted on all animals prior to the start of the study and at the scheduled necropsies using penlight illumination and a moistened slide with fluorescent light illumination, respectively.

Clinical Pathology.

Hematology. Blood samples for hematologic determinations were obtained from surviving mice preselected for the 12-month interim sacrifice(up to 10 mice/sex/dose level) and from the first 20 surviving mice/sex/group at the terminal sacrifice (18 months).The last five samplesfrom the female animals in all dose groups at the 18-month sample period were excluded from statistical analysis as a result of instrument malfunction encountered during analysis. Samples were collected from the orbital sinus, into EDTA tubes, from animals lightly anesthetized with with methoxyflurane. The determinations of hematocrit (HCT), hemoglobin (HGB), red blood cell(RBC), white blood cell (WBC) and platelet (PLT), differential leukocytecounts, and and an assessment of erythrocyte, leukocyte and platelet morphology conducted on all samples at 12- and 18-months ...Blood smears were prepared, stained with Wright's stain, and manually examined as necessary.

Clinical Chemistry. Blood samples for clinical chemistry determinations were obtained from surviving mice preselected for the 12-month interim sacrifice (up to 10 mice/sex/dose level) and from the first 20 surviving mice/sex/group at the terminal sacrifice 18 months). Samples were collected from the orbital sinus from animals lightly anesthetized with methoxyflurane. The following parameters were assayed...alkaline phosphatase (AP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, as well as urea nitrogen (UN), creatinine (CREAT), total protein (TP), albumin (ALB), globulin (GLOB-calculated), glucose (GLUC)cholesterol (CHOL), triglycerides (TRG), total bilirubin (TBIL), and electrolyte (NA, K, PHOS, CL, CALC)concentrations.

Anatomic Pathology. ...Terminal body weights were recorded, the animals were anesthetized with methoxyflurane, and their tracheas were exposed and clampedprior to decapitation and exsanguination. The necropsy of each mouse consisted of an examination of all external tissues and orifices. The head was removed, the cranial cavity was opened, and the brain, pituitaryand adjacent cervical tissues examined. The eyes were examined by an *in situ* technique by application of a moistened microscopic slide to each cornea while utilizing fluorescent light illumination. The nasal cavity was flushedwith neutral, phosphate-buffered 10% formalin.The skin was reflected from the carcass, the thoracic and abdominal cavities wereexposed and the viscera was examined *in situ*. All visceral tissues were dissected from the carcass and re-examined, including cut surfaces. Lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin solution by tracheal instillation using a hand-operated syringe. The nasal cavity was flushed by a similar method via the pharyngeal duct.

Representative samples of tissues...were collsecte and preserved in neutral, phosphate-buffered 10% formalin. Any masses or lesions were also preserved in formalin. Weights of the brain, heart, liver,kidneys, testes (males), and spleen were recorded and the organ weight to body weight ratios calculated for all animals.

A similar procedure was followed for animals which died or were sacrificed in a moribund in a moribund conditionwith the exception that no terminal body weight data, organ weight data, or blood/serum samples were collected.

Histopathology. All tissues...from the female control and female 0.024% groups from the 12-month and 18-month sacrifices were processed by standard procedures for light microscopic evaluation. Tissues were sectioned approxiamtely six μm thick, stained with hematoxylin-eosin, and examined by a veterinary pathologist...Histopathologic evaluation was not performed on the additional female group administered 0.008% XDE-105, because previously tested higher dose levels of 0.0025% and 0.008% had no treatment-related effects. Histopathologic evaluation was not performed on any of the male mice from the control, 0.0008% or 0.024% dose groups, because the previously tested levels of 0.0025%, 0.008%, and 0.036% XDE-105 resulted in establishing a no-observed-effect level (NOEL), of 0.008%, and and demonstrated treatment-related effects at 0.036%.

Histopathologic findings were subjectively graded to reflect the severity of specific lesions to evaluate 1) the contribution of a specific lesion to the health status of an animal, 2) exacerbation of common naturally occurring lesions as a result of the test material, and 3) dose-response relationships for tor treatment related effects. Very slight and slight grades were used for conditions that were present in excess of the normal textbook appearance of an organ/tissue, but were of minimal severity and usually with less than 10% involvement of the parenchyma. This type of change would not be expected to specifically affect the function of a 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 (up to 50%of the parenchyma) that the function of organ/tissue may may have been adversely affected, but not to the point of organ failure. The health status of the animal may not have been affected, depending on the organ/tissue involved, but generally lesions graded as moderate would

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not be life-threatening . A severe grade was used for conditions that were extensive enough to cause significant organ/tissue dysfunction or failure. This degree of change in a critical organ/tissue may have been life-threatening.

The list of tissues to be examined was presented in the report as follows:

TABLE 2

XDE-105: 18-MONTH DIETARY ONCOGENICITY STUDY IN CD-1 MICE

TISSUES COLLECTED AND PRESERVED AT NECROPSY

ADRENALS	JEJUNUM	PITUITARY
AORTA	KIDNEYS	PROSTATE
BONE (INCLUDING JOINT)	LACRIMAL/HARDERIAN GLANDS	RECTUM
BONE MARROW	LARYNX	SALIVARY GLANDS
BRAIN ((CEREBRUM, BRAINSTEM, CEREBELLUM)	LIVER	SEMINAL VESICLES
CECUM	LUNGS	SKELETAL MUSCLE
CERVIX	MAMMARY GLAND	SKIN AND SUBCUTIS
COAGULATING GLANDS	MEDIASTINAL LYMPH NODES	SPINAL CORD (CERVICLE, THORACIC, LUMBAR)
COLON	MEDIASTINAL TISSUES	SPLEEN
DUODENUM	MESENTERIC LYMPH NODES	SPLEEN
EPIDIDYMIDES	MESENTERIC TISSUES	STOMACH
ESOPHAGUS	NASAL TISSUES	TESTES
EYES	ORAL TISSUES	THYMUS
GALLBLADDER	OVARIES OVIDUCTS	THYROID GLAND
GROSS LESIONS	PNACREAS	TONGUE
HEART	PARATHYROID GLANDS	TRACHEA
ILEUM	PERIPHERAL NERVE	URINARY BLADDER
		UTERUS
		VAGINA

Statistics. Descriptive statistics only (means and standard deviations) were reported for feed consumption and leukocyte differential counts. Body weights, body weight gains, organ weights ((absolute and relative), and appropriate hematologic and clinical chemistry data were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA), followed respectively by Dunnett's test or the Wilcoxon Rank -Sum test with a Bonferoni correction for multiple comparisons. Statistical outliers were identified by a sequential test, but routinely excluded from feed consumption statistics. Outliers were excluded from other analyses only for documented, scientifically sound reasons, unrelated to treatment.

The nominal alpha levels used...were as follows:

Bartlett's	$\alpha = 0.01$
Parametric ANOVA	$\alpha = 0.10$
Nonparametric ANOVA	$\alpha = 0.10$
Dunnett's test	$\alpha = 0.05$ two-sided
Wilcoxon Rank-Sum test	$\alpha = 0.05$ two-sided
Outlier test	$\alpha = 0.02$ two-sided

Gross pathologic observations were tabulated and considered in the interpretation of final histopathologic data, but were not evaluated statistically. The cumulative incidence of appropriate histopathologic observations on all females from the control and 0.024% groups scheduled for terminal sacrifice were used in statistical analysis. Statistical analysis consisted of the pairwise comparisons of the female control

and 0.024% groups using the pairwise chi-square test with Yate's continuity correction ($\alpha = 0.10$, two-sided).

Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure ($\alpha = 0.05$) for all animals scheduled for terminal sacrifice.

II. REPORTED RESULTS

A. Mortality: There were no treatment or dose related effects on mortality observed in the study. These results are summarized from the report as follows:

Cumulative mortality at study day	Sex and dose group (% of diet)					
	Males			Females		
	0	0.008%	0.024%	0	0.0008%	0.024%
364	3/5-	2/50	4/50	2/50	1/50	7/50
552	10/50	7/50	10/50	11/50	8/50	11/50

Data presented (as)...number over total number of animals in group scheduled for the oncogenicity evaluation.

B. In-life observations: The report noted no treatment-related clinical observations that could be associated with the administration of the test material in the diets of the test animals. In addition, there were no effects observed during ophthalmologicalobservations according to the report.

C. Body weight and feed consumption:The report described these results as follows:

The mean body weights of high-dose male mice were statistically identified as lower than those of controls, typically 2.8 to 8.4%, starting at day 6 and continuing for the majority of the dosing period. There were decreases also noted in male mice of the 0.0008% dose group, however, they were normal by day 41. These transient effects were attributed to acclimation to the test dietand were considered not to be of toxicological significance.

The mean body weights of high-dose females were statistically identified as lower on test days 27 and 34, and were also attributed to the acclimation to the test diet and not to be of toxicological significance. There were no significant differences in body weights of female mice from the 0.0008% group.

Consistent with the decreased body weights of the high-dose male mice, the mean body weight gains of these animals were also depressed. Mean body weight gains were as much as 11.9 to 28.0% lower relative to cojntrols. Initially, there were decreases in the body weight gain of male mice in the 0.0008% dose group, however, these differences resolved by day 49, and were considered not to be a treatment-related effect.

Consistent with the decreased body weights, the mean body weight gains of high-dose femal mice were also depressed on day 27 through 49 and were 14 to 23% lower relative to controls and considered not toxicologically significant. There were no statistically identified differences in the body weight gains of 0.0008% female mice.

There were no meaningful differences in feed consumption of the 0.0008% and 0.024% dose group males and females.

There were also no meaningful differences noted in feed efficiencies for treated groups when compared with control groups according to the report.

Selected body weight, weight gain, feed consumption, and feed efficiency data are summarized from the report as follows:

Day of observation	Sex and dose group (% of diet)					
	Males			Females		
	0	0.008%	0.024%	0	0.0008%	0.024%
	Body weight (grams)					
-2	27.6	27.6	27.6	23.6	23.8	23.7
90	37.4	37.0	35.7*	28.9	28.9	28.7
373	41.0	40.1	38.6*	33.4	33.8	33.2
541	40.8	39.5	38.4*	34.7	36.0	33.6
	Cumulative weight gain (g/animal)					
-9 to 90	9.8	9.4	8.2*	6.6	6.6	6.4
-9 to 373	13.4	12.5	11.0*	11.1	11.5	10.8
-9 to 541	13.2	11.8	11.0	12.5	13.7	11.3
	Feed consumption (grams)					
1 to 8	5.6	5.3	5.2	5.2	5.4	5.1
85 to 92	5.1	5.1	5.2	5.5	5.4	5.6
359 to 366	4.9	5.0	5.1	5.1	5.1	5.2
527 to 534	5.1	4.9	5.3	5.2	5.2	5.3
	Feed efficiency (g feed/g body weight gain)					
1 to 8	0.23	0.21	0.21	0.26	0.27	0.25
85 to 92	0.16	0.16	0.17	0.22	0.22	0.23

* Statistically significant difference from control (Dunnett's test, $p \leq 0.05$)

D. Hematology and Clinical Chemistry: The report described the results for hematological observations as follows:

Mean white blood cell (WBC) counts of 0.024% female mice were higher than controls at 12- and 18-months. These were statistically identified only at the 12-month interval. In addition, the differential WBC count of female high-dose animals at the 12- and 18-month intervals had a shift in WBC types, specifically an increase in percent neutraphils and a decrease in percent lymphocytes. The absolute number of neutrophils and lymphocytes was also higher than control

levels. A treatment-related alteration that could have contributed to the elevated WBC count and differential count was chronic inflammation of the glandular mucosa of the stomach.

The mean WBC countof 0.0008% and 0.024% female mice was statistically identified as higher than controls at 12 months. This was considered unrelated to treatment due to the lack of repeatability at 18-months, and lack of a histopathologic correlate. The mean percent eiosinophils was increased in the female 0.024% dose group at 12-months. This was also considered unrelated to treatment due to the lack of repeatability at 18-months.

There were no treatment-related abnormalities in erythrocyte, leukocyte or platelet morphology.

The hematology results pertaining to this discussion are summarized from the report as follows:

	Sex and dose group (% of diet)					
	Males			Females		
Observation	0	0.008%	0.024%	0	0.0008%	0.024%
	12-month interval					
WBC x $10^{-3}/mm^3$	4.98	3.93	5.26	2.64	5.41**	5.19**
Differential WBC						
% NEUT	24.5	18.9	27.9	21.9	20.8	29.4
% LYMP	67.9	74.3	62.8	70.2	73.0	64.5
% LUC	0.6	0.4	0.6	0.5	0.6	0.3
	18-month interval					
WBC x $10^{-3}/mm^3$	6.21	7.30	7.98	5.43	4.19	7.08
Differential WBC						
% NEUT	28.5	27.1	29.8	24.3	27.3	33.3
% LYMP	64.2	66.8	63.7	68.0	66.8	59.6
% LUC	1.2	1.4	1.3	3.2	1.5	1.3

WBC = white blood cell
NEU= neutrophils, LYMP = lymphocytes
** Statistically different from control mean, Wilcoxon's test, $p \leq 0.05$)

Clinical chemistry results were discussed in the report as follows:

Mean sodium (NA) and chloride (CL) levels were statistically identified as higher in the 0.0008% and 0.024% females at 12-months. The mean potassium (K) was also statistically identified as higher in the 0.024% females at 12-months. These alterations in NA, K and CL levels were not repeated at 18 months and therefore were considered not toxicologically significant.

Mean calcium (CALC) levels were statistically identified as higher in 0.0008% and 0.024% females at 18-months. However, in a previously conducted bioassay, therewere no alterations in CALC levels in female mice fed diets as high as 0.036% for 12-months (this dose level was not evaluated at 180months in female mice) and 0.0008% for 18-months) (MRID 4370150?). tHEREFORE, THESE ALTERATIONS IN calc levels wer considered not to be treatment related.

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Because these results were not consistently observed during the course of the study, a summary table is not presented here.

E. Necropsy Results: Terminal body and organ weight data were discussed in the report as follows:

> The mean terminal body weights were lower than controls in male high-dose animals at the 12- and 18-month sacrifices. This weight difference was attributed to acclimation to the test diet early in the study. There were no alterations in mean terminal body weights in 0.0008% and 0.024% female mice.
>
> Relative mean liver weights were statistically identified as higher in high-dose females at the 18-month sacrifices. Mean relative liver weights were statistically identified as higher in high-dose males at 18 months. There was no histopathologic correlate to the elevated liver weights in the females (male livers not examined).
>
> Relative mean brain weights were statistically identified as higher in the high-dose males at 12-months. These higher mean relative brain weights were attributable to the lower body weights of these animals. The mean absolute heart weight of high-dose males at 12-months was statistically identified as lower than controls. This finding was also attributed to the lower mean body weight s. The relative mean heart weight of low-dose females at 18-months was statistically identified as lower than controls. This was considered not to be a treatment-related effect due to the lack of a dose response.

Gross pathological observations were discussed in the report as follows:

> The only treatment related gross pathologic effect consisted of thickening of the glandular portion of the stomach in numerous males and females from the 0.024% group. This was considered to be a primary treatment-related effect. However, at the 18-month sacrifice, a few mice from the contro and 0.0008% groups also had thickening of the glandular portion of the stomach. The low incidence of thickened stomachs in control and 0.0008% group mice was interpreted to represent the infrequent, spontaneous occurrence of this alteration, rather than a treatment-related effect.

The gross pathology results mentioned above are summarized from the report as follows:

Observation	Sex and dose group (% of diet)					
	Males			Females		
	0	0.008%	0.024%	0	0.0008%	0.024%
	12-month interval					
Stomach						
Within normal limits	10	10	8	10	10	5
Thickened glandular mucosa	0	0	2	0	0	5
	18-month interval					
Stomach						
Within normal limits	46	46	32	47	45	20
Thickened, glandular mucosa	0	1	17	2	3	27
Thickened, glandular mucosa, diffuse:	0	0	0	0	0	3
Thickened, glandular mucosa, diffuse:[1]	0	1	17	2	3	30
Hemolyzed blood:	1	0	0	0	0	0
Hemolyzed blood, lumen:	2	2	0	0	1	0
Erosion(s), &/or ulcer(s), glandular mucosa, multifocal:	1	1	1	1	1	0

[1] This duplicate entry is as it was presented in the original report.

Histopathology results were described in the report as follows:

Treatment-related histopathologic effects were observed in the lungs, lymph nodes, parathyroid glands, skeletal muscle (of the face and tongue), and stomach of female mice given 0.024% XDE-105.

Female mice from the 0.024% group had an increased incidence of slight aggregates of alveolar macrophages in the lungs, as compared to the control group, at 12 and 18 months. A grade of very slight was used for aggregates of alveolar macrophages that were interpreted to represent the normal, spontaneous occurrence of this alteration. In general, the aggregates of alveolar macrophages were most commonly present in subpleural sites, but sometimes were present deeper in the lung parenchyma. The incidence of female mice with slight aggregates of alveolar macrophages from the 0.024% group increased from 2 of 10 (20%) affected at 12 months, to 30 of 50 (60%) affected at 18 months.

Female mice from the 0.024% group had an increased incidence os sinus histocytosis in the mesenteric lymph nodes, as compared to the control group, at the 12-month and 18-month sacrifices. The incidence of female mice with sinus histocytosis from the 0.024% group increased from 2 of 10 (20%) affected at 12 mojnths, to 20 of 50 (40%) affected at 18 months.

Female mice from the 0.024% group had an increased incidence of vacuolation of the parathyroid glands, as compared to the control group. Affected parenchymal cells had single or multiple cytoplasmic vacuoles. The treatment-related vacuolation was graded as slight in all affected mice, and occurred at the 10 months and 18 months sacrifices. The incidence of female mice with vacuolation of the parathyroid glands from the from the 0.024% group increased from 3 of 10 (30%) affected at 12 months, to 25 of 50 (50%) affected at 18 months.

A treatment-related myopathy of skeletal muscle of the face and tongue was observed in females fom the 0.024% group, at 12 and 18 months. The myopathy was characterized by degeneratiove myofibers that exhibited fragmentation, swelling, atrophy, and loss of striations. Macrophages

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were at the perifery of some affected myofibers. The myopathy was graded as slight or moderate in all affected mice. The incidence of female mice with slight myopathy of the tongue from the 0.024% group increased from 1 of 10 (10%) affected at 12 months, to 17 of 50 (34%) affected at 18 months. The incidence of female mice with slight or moderate myopathy of the facial musclesfrom the 0.024% group decreased from 6 of 10 (60%) affected at 12 months, to 22 of 50 (44%) affected at 18 mnonths.

Female mice from the 0.024% group had an increased incidence and increased severity of hyperplasia of the glandular mucosa of the stomach, as compared to the control group. This treatment-related alteration was present at the 12 and 18 months, and the incidence of diffuese, moderate hyperplasia increased as the study progressed. In some mice from the control group, there was very slight to slight, multifocal hyperplasia of the stomach, concisting of dilated gastric glands in the middle to basilar region of the mucosa. In these dilated glands the cells were smaller, lessdifferentiated, and and increased in number in comparison to adjacent glands. These cystic hyperplastic foci were most common in the fundus, particularly in the region close to the forestomach. Such lesions in control mice were observed in the glandular mucosa, increasing in incidence and severity with age. They were considered a spontaneous age-associated (or background) lesion, and are consistent with hyperplasia of the glandular mucosa as described in the stomach of rats. In three frmale mice from the control group, a more severe (moderate) and diffuese hyperplasia, comparable to the treatment-related hyperplasia of mice from the 0.024% group, was observed at 18 months.

In female mice from the 0.024% group, there was a spectrum of lesions ranging from proliferative lesions similar to controls to moderate, diffuse hyperplasia involving the entire thickness of the glandular mucosa. As with controls, the lesions were most prominent in the fundic region close to the forestomach and decreased in severity towards the pyloric antrum. The hyperplastic response at 12 and 18 months appeared to involve all of the various cell types of the glandular mucosa, with with surface mucus cells and neck mucus cells representing the majority of proliferative cell types in most affected mice. In addition, there were increased numbers of undifferentiated cells. These cells did not appear dysplastic or anaplastic but rather to be immature cells which had not differentiated. The entire area of mucosa imvovled showed a similar response. In more severly affected mice and primarily later in the study, there was evagination of the proliferative response into the submucosa. In these mice, there were submucosal cystic structures lined by the same cell types evident throughout the hyperplastic gastric mucosa. The marked hyperplastic reaction of the gastric mucosa appeared to result in extension of the proliferative mucosa into the proliferative mucosa into the submucosa by thinning of the muscle layers and/or following along planes of least resistance between muscle layers, i.e., following fascial planes or along the course of blood vessels.

Although florid in nature, these proliferative lesions were hyperplastic and and not neoplastic. The proliferative response was diffuse, involving a large portion of the stomach and the entire thickness of the mucosa. even in the submucosal scystic structures, the the cells were often well-differentiated, and were the same cell types as thos observed in the mucosa. Multiple cell types including parietal cells were present in the cystic structures in some mice. They cystic structures could be demonstrated to occur by evagination of proliferating mucosal gastric glands rather than by invasion. Many cystic structures appeared to be surrounded by a thin layer of smooth muscle as might be expected if the cysts were the result of evagination. In addition, such florid hyperplastic gastric mucosal lesions similar to those seen in this study, including submucosal cysts, have been reported in mice under a variety of circumstances including autoimmune factors; nutritional changes; environmental changes; administration of chemicals; and as a spontaneous genetic lesion (citations were provided).

Additional treatment-related alterations of the stomach in female mice from the 0.024% group consisted of increased incidences of: slight or moderate chronic inflammation of the glandular mucosa; slight hyperplasia of the nonglandular mucosa; and hyperkeratosis of the nonglandular mucosa; as compared to mice from the control group.

Statistical analysis of histopathologic observations from the 18-month sacrifice was performed on female mice from the control and 0.024% groups. All of the treatment-related histopathologic observations in 0.024% group females from the 18-month sacrifice were statistically identified as different from controls.

The remainder of statistically identified histopathologic findings consisted of the following:

Females 0.024% - ovaries, decreased incidence of amyloid, moderate
Females 0.024% - ovaries, decreased incidence of cystic follicles, focal
Females 0.024% - stomach, decreased incidence of hyperplasia of the glandular mucosa, multifocal, very slight

These statistically identified ovarian alterations were not considered to be toxicologically significant or treatment-related, since the incidences of these observations were decreased relative to controls. The statistically identified decrease in the incidence of very slight, multifocal hyperplasia of the glandular mucosa of the stomach in mice from the 0.024% group was reflective of the concomitant treatment-related increased incidence of this alteration at greater severity than controls.

The incidence of tumors in mice administered 0.024% XDE-105 for up to 18 months was not statisitcally increased relative to controls. Three frmales from the 0.024% group had bronchioloalveolar adenomas at the 12-month sacrifice compared to no controls at this time point. This apparent increase in bronchioalveolar adenomas was not treatment-related, because 1) the incidence of this tumor was comaprable to controls at the 18-month sacrifice, and 2) the incidence of this tumor was not increased in mice previously tested with the higher dose of 0.036% for 12 months (males and females) and 18 months (only histopathologic evaluation at 18 months) (MRID 43701505).

A variety of palpable tumors occurred with low incidence in control and treatment groups. All were considered to be spontaneous findings unrelated to administration of XDE-105.

There were no statistically identified differences in overall moribundity and mortality pattern in male and female mice from the 0.0008% or 0.024% groups.

The microscopic lesions described were tabulated in the report as follows:

Observation	Dose (% in the diet) 0	0.008	0.024
12-month sacrifice			
Lungs (Number of tissues examined)	10	0	10
Within normal limits	6	-	3
Inflammation - subacute to chronic alveoli/septa,			
focal: - slight	2	-	0
multifocal: - slight	2	-	0
Aggregates of alveolar macrophages: - very slight	4	-	0
- slight	0	-	4
- any severity	0	-	2
Lymph nodes - mediastinal (Number of tissues examined)	10	0	10
Within normal limits	9	-	10
Hyperplasia - lymphoid:	1	-	0
Lymph node - mesenteric (Number of tissues examined)	10	0	10
Within normal limits	8	-	6
Amyloid: - very slight	1	-	1
- slight	0	-	1
- any severity	1	-	2
Hyperplasia - lymphoid:	1	-	0
Sinus histiocytosis	0	-	2
Lymph node - miscellaneous (Number of tissues examined)	1	0	0
Hyperplasia - reactive	1	-	-
Parathyroid glands (Number of tissues examined)	10	0	10
Within normal limits	9	-	7
Amyloid, interstitium: - very slight	1	-	0
Vacuolation: - slight	1	-	3
Skeletal muscle (Number of tissues examined)	10	0	10
Within normal limits	9	-	9
Inflammation - acute, multifocal: - slight	1	-	1
- subacute to chronic, focal: - slight	1	-	0
Myopathy: - slight	0	-	0
Stomach (Number of tissues examined)	10	0	10
Within normal limits	8	-	1
Hyperkeratosis - nonglandular mucosa:	0	-	4
Hyperplasia, glandular mucosa, focal: - very slight	1	-	0
- slight	0	-	5
diffuse: - moderate	0	-	4
multifocal or diffuse: - any grade	1	-	9
Hyperplasia, nonglandular mucosa: - slight	1	-	4
Inflammation - chronic, glandular mucosa: - slight	2	-	9

Observation	Dose (% in the diet) 0	0.008	0.024
18-month sacrifice			
Lungs (Number of tissues examined)	50	0	50
Within normal limits	32	-	8
Aggregate(s) of mononuclear (predominately lymphoid) cells, interstitium, multifocal: - slight	1	-	1
Alveolar histiocytosis:	0	-	1
Fibrosis, pleura, multifocal:	0	-	1
Hyperplasia, alveolar cell, focal: - slight	2	-	5
multifocal: - slight	0	-	1
focal or multifocal: - slight	2	-	6
Inflammation - subacute to chronic alveoli/septa, focal: - slight	2	-	0
multifocal: - slight	4	-	4
multifocal: - moderate	0	-	1
focal or multifocal: - any severity	6	-	5
Aggregates of alveolar macrophages: - very slight	6	-	9
- slight	2	-	30*
- any severity	8	-	39*
Lymph nodes - mediastinal (Number of tissues examined)	50	0	50
Within normal limits	49	-	47
Hyperplasia - lymphoid:	0	-	1
Hyperplasia - reactive:	1	-	1
Sinus histiocytosis:	0	-	1
Lymph node - mesenteric (Number of tissues examined)	49	-	50
Missing:	1	-	0
Within normal limits	38	-	21
Amyloid: - very slight	2	-	0
- slight	4	-	4
- any severity	6	-	4
Congestion:	1	-	3
Extramedullary hematopoiesis:	2	-	2
Hyperplasia - reactive:	1	-	1
Inflammation - chronic active, focal: - slight	1	-	0
multifocal: - moderate	0	-	1
focal or multifocal: - any severity	1	-	1
Necrosis, lymphoid tissue: - slight	1	-	0
Sinus histiocytosis	2	-	20*
Lymph node - miscellaneous (Number of tissues examined)	2	-	4
Hyperplasia - lymphoid:	1	-	1
Hyperplasia - reactive:	1	-	3

Observation	Dose (% in the diet) 0	0.008	0.024
Parathyroid glands (Number of tissues examined)	46	0	49
Missing:	4	-	1
Within normal limits	41	-	24
Amyloid, interstitium: - very slight	2	-	0
- slight	1	-	2
- any severity	3	-	2
Vacuolation: - slight	2	-	25*
Skeletal muscle (Number of tissues examined)	50	0	50
Within normal limits	50	-	28
Myopathy: - slight	0	-	21*
- moderate	0	-	1
- any severity	0	-	22*
Stomach (Number of tissues examined)	50	0	50
Within normal limits	24	-	1
Amyloid: - very slight	5	-	1
Erosion(s), glandular mucosa, multifocal:	1	-	0
Hyperkeratosis - nonglandular mucosa:	7	-	19*
Hyperplasia, glandular mucosa, focal: - very slight	13	-	1*
- slight	2	-	12*
- moderate	0	-	5*
diffuse:	3	-	30*
multifocal or diffuse: - any severity	18	-	48*
Hyperplasia, nonglandular mucosa: - slight	3	-	22*
Inflammation - chronic, glandular mucosa: - slight	14	-	34*
- moderate	0	-	5*
- any severity	14	-	29*
Inflammation - chronic active, glandular mucosa: - slight	1	-	0

* Statistically significantly different from control ($p \leq 0.05$).

The incidence of neoplastic lesions was reported in the study as follows:

Observation	Dose (% in the diet) 0	0.008	0.024
12-month sacrifice			
Lungs: Number of tissues examined	10	0	10
Adenoma, bronchioalveolar, benign, primary:	0	1	3
Oral tissues: Number of tissues examined	10	0	10
Squamous cell carcinoma, hard palate, malignant, primary, no metastasis:	1	-	0
Uterus: Number of tissues examined	10	0	10
Endometrial stromal polyp, benign, primary:	1	-	0

Observation	Dose (% in the diet) 0	0.008	0.024
18-Month Sacrifice			
Adrenals: Number of tissues examined	50	0	50
Pheochromocytoma, benign, primary:	0	-	1
Bone: Number of tissues examined	50	0	50
Chondroma, cranium, benign, primary:	1	-	0
Cecum: Number of tissues examined	50	0	50
Leiomyoma, benign, primary:	0	-	1
Cervix: Number of tissues examined	50	0	50
Leiomyoma, malignant, primary, no metastasis:	2	-	0
Lacrimal/Hardarian Gland: No. tissues examined	50	0	50
Adenoma, benign, primary:	1	-	0
Liver: Number of tissues examined	50	0	50
Adenoma, hepatocellular, benign, primary:	1	-	1
Hemangioma, benign, primary:	0	-	1
Hemangiosarcoma, malignant, primary, no metastasis:	2	-	2
Lungs: Number of tissues examined	50	0	50
Adenoma, bronchioalveolar, benign, primary:	7	-	9
Multiple organs: Number of tissues examined	7	0	6
Hemangiosarcoma (spleen), malignant, secondary:	1	-	0
Rhabdomyosarcoma, (back), malignant, secondary:	1	-	0
Lymphosarcoma, malignant, primary:	3	-	3
Ovaries: Number of tissues examined	5-	0	50
Adenoma, benign, primary:	1	-	2
Hemangiosarcoma, malignant, primary, no metastasis	1	-	0
Leiomyosarcoma, malignant, primary, no metastasis:	1	-	0
Luteoma, benign, primary:	1	-	0
Thecoma, benign, primary:	1	-	0
Pituitary: Number of tissues examined	49	0	50
Adenoma, anterior (pars distalis), benign, primary:	2	-	2
Skin and subcutis: Number of tissues examined	50	0	50
Squamous cell carcinoma, inguinal, malignant, primary, no metastasis:	0	-	1
Rhabdomyosarcoma, back, malignant, primary, metastasis:	1	-	0
Spleen: Number of tissues examined	50	0	50
Hemangiosarcoma, malignant, primary, metastasis:	1	-	0
Thyroid: Number of tissues examined	50	0	50
Adenoma, follicle(s), benign, primary:	0	-	1

Observation	Dose (% in the diet) 0	0.008	0.024
Uterus: Number of tissues examined	50	0	50
Endometrial stromal polyp, benign, primary:Hemangioma,	1	-	2
benign, primary:	1	-	2
Hemangiosarcoma, malignant, primary, no metastasis:	1	-	0
Leiomyoma, benign, primary:	3	-	0
Stromal cell sarcoma, endometrium, malignant, primary, no metastasis:	1	-	1
Deciduoma, benign, primary:	0	-	1
Vagina: Number of tissues examined	50	0	50
Basal cell adenoma, benign, primary:	1	-	0

No statistically significant differences were noted between treated and control groups.

III. DISCUSSION

A. Authors' Conclusions: The investigators described their conclusions in the report as follows:

> XDE-105 was administered to male and female CD-1mice via their diet for up to 18 months. The time weighted average dosages ingested, based upon mean feed consumption and mean body weightdata were 0, 1.1, and 32.7 mg/kg/day for males, and 0, 1.3 and 41.5 mg/kg/day for females provided diets containing 0, 0.0008%, and 0.024% XDE-105, respectively.
>
> Lower mean body weights and body-weight gains in high-dose male mice were considered related to treatment. There was no statistically identified effect on mortality in mice ingesting diets containing 0, 0.0008%, and 0.024% XDE-105.
>
> Treatment-related gross pathologic effects consisted of thickening of the glandular portion of the stomach in a significant number of high-dose males and females. Histopathologic effects consisted of aggregates of alveolar macrophages in the lungs, sinus histiocytosis of the lymphnodes, vacuolation of parathyroid glands, myopathy of skeletal muscle (of the face and tongue), and hyperplasia and inflammation of the stomach of mice given 0.024% XDE-105. The incidence of the treatment-related histopathologic alterations increasedwith time. The incidence of tumors in female mice administered 0.024% XDE-105 for up to 18 months was not increased relative to controls.

B. Reviewer's discussion and conclusions: In a previous mouse oncogenicity study (MRID 43701505), XDE-105 was given to CD-1 mice for up to 18 months at 0, 0.0025, 0.008, or 0.036% in the diet (equivalent to 0, 3.4, 11.4, or 50.9 mg/kg/day in males and 0, 4.2, 13.8, or 67.0 mg/kg/day in females). The high-dose females (67.0 mg XDE-105/kg/day) were terminated on day 455 (approximately 15 months) of the study because of excessive body weight loss and mortality. At that time of the study the mortality for female mice from that dose group 30/50 (60%) compared to 10% for controls. In high dose males at 80 weeks the mortality was 21/48 (40%) compared to 24% in controls. At 50 weeks, mean body weights in both sexes were about 10% lower than controls at the highest dose

level, and mean cumulative weight gains were 37% lower. Decreased amounts of body fat were also noted in both sexes at the 0.036% level. Based on these results, hematologic effects, increased incidences of thickening of the gastric mucosa in females, and histologic changes in the stomach of males, the systemic LOEL was established as 50.9 mg/kg/day in male mice and 67.0 mg/kg/day in female mice. The NOEL is 11.4 mg/kg/day for males and 13.8 mg/kg/day for females.

Dosing was considered adequate in males based on an increased incidence and severity of hyperplasia and inflammation of the stomach mucosa at 50.9 mg/kg/day (highest dose tested). In female mice, 67 mg/kg/day (highest dose tested) was excessive and is, therefore, inappropriate to assess the carcinogenic potential of XDE-105. Microscopic examinations of the high dose female mice were not conducted on those animals that died or were sacrificed prior to termination of the study. The DER for the previous study (MRID 43701505) concluded, "Such an examination is critical because of the incidence of total hemangiomas and/or hemangiosacromas at any site in female mice from the control, low, and mid dose groups (4, 6, and 9, respectively)." The previous DER did not accept the study as satisfying the requirement for a carcinogenicity study [§83-2 (b)] in mice. The previous review further stated, "The study may be upgraded when histopathology data from the high dose females and data from an ongoing Supplemental Study (MRID 44123601 reviewed here) are provided."

Subsequently, additional information on the incidence of hemangiomas/hemangiosarcomas has been submitted along with the study reviewed here (MRID 44123600 and -01). The additional informtion was described in a letter from the sponsor of this study as follows:

2. Mouse Carcinogenicity Study (MRID #437015-05)

The preliminary review by the Agency of the XDE-105 CD-1 mouse carcinogenicity study (Study #1, ...) has stated that: "This study does not satisfy the guideline requirements for a carcinogenecity (study)in mice. No microscopic examinations of the highdose females that either died prior to terminal sacrifice or were sacrificed early were conducted and/or submitted. Such an examination is critical because of the incidence of the total hemangiomas and/or hemangiosarcomas at any site from females in the control, low and mid dose groups(4, 6 and 9, respectively)." These data are tabulated below from the above study (MRID 43701505).

Study #1

Dose (%)	0	0.0025	0.008	0.036
Number of mice examined	50	50	50	0
Total mice with a hemangioma or hemangiosarcoma, any site	4/50	6/50	9/50	-

In addition, the individual tissues from which the combined data were derived from this study are presented below:

Study #1

Dose (%)	0	0.0025	0.008	0.036
Number of mice examined	50	50	50	0
Cervix - hemangioma, benign, primary	1/50	1/50	0/50	-
Liver - hemangioma, benign, primary	0/50	1/50	2/50	-
Liver - hemangiosarcoma, malignant, primary, metastasis	1/50	0/50	0/50	-
Lymph node - mesenteric - hemangioma, benign, primary	0/50	2/50	1/50	-
Ovaries - hemangioma, benign, primary	1/50	0/50	0/50	-
Skin and subcutis - hemangioma, back, benign, primary	0/50	1/50	0/50	-
Spleen - hemangiosarcoma, malignant, primary, no metastasis	1/50	0/50	3/50	-
Uterus - hemangioma, benign, primary	1/50	1/50	4/50	-
Uterus - hemangiosarcoma, malignant, primary, no metastasis	0/50	1/50	0/50	-
Total mice with a hemangioma or hemangiosarcoma, any site	4/50	6/50	9/50	-

In order to reduce concern over the incidence of hemangiomas and/or hemangiosarcomas in female mice given 0.008% XDE-105, DowElanco is submitting the results from a second CD-1 mouse oncogenicity study (Study #2). In this study, groups of 50 mice/sex/dose were given feed containing 0, 0.0008% or 0.024% XDE-105 for up to 18 months. A complete set of tissues was examined from all female mice given 0 or 0.024% XDE-105. There were no significant differences in the incidences of hemangiomasand/or hemangiosarcomasin the specific tissues in which these tumor types were identified in female mice. Complete data on the primary tumors can be found in the accompanying report entitled XDE-105: 18-Month Dietary Oncogenicity Study in CD-1 Mice. DECO HET DR-0323-1194-006A. The tumor incidence summary is in Table 50, on pages 164, 165, and 166of this report (). The combined incidenceof hemangiomas and/or hemangiosarcomas noted at any site was not specifically tabulated in this accompanying report due to the low number of mice with these tumor types. However, when tabulated in this manner, there were six mice with a primary hemangioma/hemangiosarcomatype of tumor in 50 control female mice and five mice with a primary hemangioma/hemangiosarcoma type of tumor in 50 female mice given 0.024% XDE-105 as listed below:

Study #2

Dose (%)	0	0.0008	0.024
Number of mice	50	50*	50
Liver - hemangioma, benign, primary	0/50	-	1/50
Liver - hemangiosarcoma, malignant, primary, no metastasis	2/50	-	2/50
Ovaries - hemangiosarcoma, malignant, primary, no metastasis	1/50	-	0/50
Spleen - hemangiosarcoma, malignant, primary, no metastasis	1/50	-	0/50
Uterus - hemangioma, benign, primary	1/50	-	2/50
Uterus - hemangiosarcoma, malignant, primary, no metastasis	1/50	-	0/50
Total mice with a hemangioma or hemangiosarcoma, any site	6/50	-	5/50

* Tissues from these mice were not examined histologically.

The animal identification of the individual female mice with hemangioma and/or hemangiosarcoma is as follows:

Study #2

Dose (%)	0	0.0008	0.024
Liver - hemangioma, benign, primary	-	-	93A4940
Liver - hemangiosarcoma, malignant, primary, no metastasis	93A4830 93A4856	-	93A4934 93A4969
Ovaries - hemangiosarcoma, malignant, primary, no metastasis	93A4857	-	-
Spleen - hemangiosarcoma, malignant, primary, no metastasis	93A4812	-	-
Uterus - hemangioma, benign, primary	93A4815	-	93A4936 93A4939
Uterus - hemangiosarcoma, malignant, primary, no metastasis	93A4850	-	-

These data indicate that there was no increase in the incidence of hemangiomas and/or hemangiosarcomasin female mice given 0.024% XDE-105 (Study #2) within any individual tissue or or when all sites are combined.

Therefore, the incidence of hemangiomas and/or hemangiosarcomas in females given 0.008% XDE-105 in the first oncogenic study in CD-1 mice should be interpreted as being the result of random variability in the background incidence of these neoplasms. Based on the combined data from both of these studies standard convention wouild indicate that sufficient information is available to support the conclusion that XDE-105 does not cause a treatment-related increase in the incidence of hemangiomas and/or hemangiosarcomas at any site.

Combined data are summarized as follows:

Dose level (mg/kg/day)	Incidence (No. with tumor/no. examined)
Controls	
Study #1	4/50
Study #2	6/50
Studies 1 & 2	10/100
1.3	- *
4.2	6/50
13.8	9/50
41.5	5/50
67.0	- **

* No animals were examined at this dose level.
** This dose level was considered excessive.

These results do not indicate a dose-response relationship with respect to the incidence of hemangioma/hemangiosarcomas at any site in CD-1 mice treated with XDE-105 for 18 months.