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

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MAY 30 1997

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Toxicology Data Submitted by DowElanco to Support Registration of Spinosad for Use on Cotton as an Insecticide.

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TO: Barbara Madden
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and

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FROM: Roger Gardner, Toxicologist Roger Gardner 5/22/97
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THRU: Karl Baetckke, Ph.D., Chief KB 5/29/97
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Actions Requested: DowElanco requested an Experimental Use Permit (EUP) for spinosad on cotton. Some of the data supporting this EUP request were previously submitted and reviewed elsewhere (D210443, D210471, D210473 and D212834). Data reviewed as part of this memorandum include:

| MRID | Study |
|---|---|
| 43701502 | 21-day dermal toxicity |
| 43701503 | chronic feeding study in rats |
| 43701504 | chronic feeding study in dogs |
| 43701505 | mouse oncogenicity |
| 43701506 | multigeneration reproduction in rats |
| 43701507 | rat oncogenicity |

Also considered in this memorandum are reports on an acute oral toxicity study and range finding studies for the rat and rabbit developmental toxicity studies (MRIDs 43770701 through -03). The metabolism studies (MRIDs 43701508 through -11), also submitted with this package, have been reviewed elsewhere (D221461). This memorandum is confined to review of the submitted toxicology data, and Toxicity Profiles based on the full data base are included in other reviews (D227006 and 232207).

Recommendations and Conclusions

1. The acute oral toxicity category for the technical grade active ingredient (spinosad) is III based on results from two study reports (MRID 43770701 and 43414515).

2. In a chronic dog feeding study, the LOEL is 8.22 mg/kg/day (300/360 ppm), based on increases in serum alanine aminotransferase, aspartate aminotransferase, and triglycerides levels, and the presence of tissue abnormalities including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid). The NOEL is 2.68 mg/kg/day (100/120 ppm).

3. In the chronic feeding/carcinogenicity/neurotoxicity study with rats, 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. Under the conditions of the study, there was no oncogenic response.

4. In the mouse oncogenicity study, the systemic LOEL is 0.036% or 360 ppm (50.9 and 67.0 mg/kg/day in male and female mice, respectively)based on decreased weight gains, increased mortality, hematologic effects, and gross finding of increased thickening of the gastric mucosa in females and the histologic changes in the stomach of males. The NOEL is 0.008% (80 ppm; 11.4 and 13.8 mg/kg/day for males and females, respectively).

5. The NOEL for maternal toxicity in the rat developmental toxicity study is $\geq$200 mg/kg/day (highest dose tested), and there were no developmental effects that could be attributed to administration of spinosad. The NOEL for developmental toxicity is also $\geq$200 mg/kg/day.

6. In the rabbit developmental toxicity study, the highest dose tested (50 mg/kg/day) approached an adequate level as indicated by the range-finding study. However, results from the two studies suggested that the NOEL for maternal toxicity is $\geq$50 mg/kg/day. There were also no developmental effects noted in the definitive study, and the NOEL for developmental toxicity is $\geq$50 mg/kg/day.

7. For the reproduction study in rats, the LOEL for systemic toxicity is 100 mg/kg/day based on increases in heart, kidney, liver, spleen, and thyroid weights (both sexes), corroborative histopathology in the spleen and thyroid (both sexes), heart and kidney (males only), and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. The NOEL for systemic toxicity is 10 mg/kg/day. The LOEL for reproductive toxicity is 100 mg/kg/day based on decreases in litter size, survival ($F_2$ litters only), and body weights in the offspring and increased incidence of dystocia and/or vaginal bleeding after parturition with associated increases in mortality in the dams. The NOEL for reproductive toxicity is 10 mg/kg/day.

8. In a 21-day dermal toxicity study with a formulation of spinosad, the LOEL is 300 mg/kg/day for female rabbits and 1000 mg/kg/day for male rabbits, based on very slight to slight hyperplasia of the gastric mucosa in both sexes. The NOEL is 200 mg/kg/day for females and 500 mg/kg/day for males.

9. The dog study (see point 2., above) was used as the basis for a reference dose (RfD) since it showed the lowest NOEL of 2.68 mg/kg/day with an LOEl of 8.22 mg/kg per day. Similar changes were reported in other species tested at higher dose levels. Spinosad is not a reproductive or developmental toxicant. Therefore, only a 100-fold uncertainty factor (UF) was applied to the NOEL of 2.68 mg/kg/day in the dog study. The resulting RfD is calculated to be 0.0268 mg/kg/day.

10. Additional information on positive controls included with the rat chronic feeding study (MRID 43701503 and -07) was sufficient to upgrade two previously reviewed neurotoxicity studies (MRIDs 43557501 and 43557504). The two developmental toxicity range-finding studies (MRIDs 43770702 and -03) also supported upgrading of the previously reviewed developmental toxicity studies (MRIDs 43557505 and 43414521). Upgraded Data Evaluation Records for these studies are attached along with DERs on the new studies.

## I. Background

In a letter dated November 30, 1994, DowElanco requested consideration of submitted toxicology data to support new registrations for technical grade Spinosad (XDE-105) and for use of its formulation, NAF-85 (44% a.i.) on cotton as an insecticide. The active ingredient

is a fermentation product of *Saccharopolyspora spinosa* and is extracted and purified for formulation as a combination of two factors (Factors A and D).

## II. Summary of New Information

A. Technical grade active ingredient

1. Acute Oral Toxicity (MRID 43770701)

The previously submitted results (MRID 43414515) indicated an $LD_{50}$ value for male Fischer 344 rats of 3738 mg/kg/day. That value was apparently based on additional data which was not included, but cited in the original report. The report containing the additional data was cited in the original report as Wright, et al., 1992b and currently has been assigned MRID 43770701. Based on the review of MRIDs 43414515 and 43770701 together (DER attached), there is adequate information available to support the determination that the acute oral $LD_{50}$ = 3738 mg/kg for male rats. **These results place spinosad into Toxicity Category III.**

2. Chronic feeding studies

i. 12-Month feeding study in dogs (MRID 43701504)

In a chronic toxicity study, Spinosad (87.2% ai) was administered to four beagle dogs/sex/dose in the diet at dose levels of 50/60, 100/120, or 300/360 ppm (1.44, 2.68, or 8.46 mg/kg/day, respectively, for males; 1.33, 2.72, or 8.22 mg/kg/day, respectively, for females) for 52 weeks.

Male beagles in the 300/360 ppm treatment group had serum levels of alanine aminotransferase 257 and 207% higher at 26 and 52 weeks, respectively; and serum levels of aspartate aminotransferase and triglycerides 147 and 132% higher, respectively, at 26 weeks than beagles in the control group; no comparable differences were observed in females in the 300/360 ppm group. Male and female beagles in the 300/360 ppm treatment groups were found to have slight vacuolated cell aggregations in lymphoid tissues (4/4 males, 2/4 females), slight to moderate inflammation of arteries in the epididymis (1/4 males) or cerebral meninges (1/4 females), and slight glandular cell vacuolation of the parathyroid (2/4 males). Although female beagles in the 300/360 ppm treatment group had absolute and relative thyroid weights that were approximately 160% higher than beagles in the control and lower dose treatment groups, no treatment-related microscopic changes were observed in these tissues. No dogs died during the study. No treatment-related differences were observed between the clinical appearance, body weights, food consumption, ophthalmology, hematology, or urine of the treated and control animals. No definitive treatment-related differences in organ weights were observed between the treated and control animals. No gross pathological differences were observed between beagles in the treatment and control groups. All microscopic tissue abnormalities, other than those mentioned, occurred

randomly and sporadically in all study groups. No neoplastic tissue was observed in beagles in the treatment or control groups. **The LOEL is 8.22 mg/kg/day (300/360 ppm), based on increases in serum alanine aminotransferase, aspartate aminotransferase, and triglycerides levels, and the presence of tissue abnormalities, including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid). The NOEL is 2.68 mg/kg/day (100/120 ppm).**

This chronic toxicity study in dogs is **acceptable** and satisfies the guideline requirement for a chronic oral toxicity study (§83-1b) in dogs.

ii. Chronic feeding studies in rats (MRID 43701507 and 43710503)

In a rat oncogenicity/chronic toxicity/neurotoxicity study (MRID 43701507 and 43701503), Spinosad (88% ai) was administered to Fischer 344 rats (65/sex/group) for up to 2 years at dietary levels of 0, 0.005, 0.02, 0.05, or 0.1% w:w (equivalent to 0, 2.4, 9.5, 24.1, or 49.4 mg/kg/day in males, and 0, 3.0, 12.0 30.3, or 62.8 mg/kg/day in females). A group of 15/sex/group, randomly designated as a satellite group were scheduled for sacrifice at 12 months. Ten satellites/sex/group underwent neurobehavioral testing at pre-test, 3, 6, 9, and 12 months, and a subset of 5/sex in the control and high dose group were assessed for neuropathology. The remaining satellite rats were evaluated for chronic toxicity at 12 months. The dosage equivalents in the satellites were 0, 4.6, 9.2, 23.0, or 46.0 mg/kg/day for males, and 0, 5.7, 11.4, 28.5, or 57.0 mg/kg/day for females. In the highest dosage group (0.1% XDE-105), there was excessive mortality and males were terminated at 102 weeks and females at 87.3 weeks. Since the MTD was exceeded at this dose, the 0.05% groups were evaluated as the high-dose for histologic findings and organ weights.

At 0.02% spinosad, slight vacuolation of the follicular epithelial cells of the thyroid was observed in males (7/49) and females (34/50) scheduled for terminal sacrifice ($p < 0.05$). In the 0.05% group, vacuolation of epithelial cells of the thyroid was seen at 12 months in all males (slight) and all females (slight to moderate); at terminal sacrifice, slight vacuolation of epithelial cells of the thyroid was observed in the majority of males and females and inflammation of the thyroid was seen in 3/50 males and 32/50 females. Absolute and relative weights of the thyroid were significantly increased (2-fold) in females at 24 months. The incidence of very slight inflammation of the lungs was increased in both sexes at 12 and 24 months; the increase in females was significant at 24 months (37/50 at 0.05% compared to 8/50 in controls; $p < 0.05$). In the 0.1% group, there was no effect on survival or weight gain in the first year of the study. By week 77, weight gain was depressed 14% (males) and 23% (females) compared to controls. At the 12-month sacrifice the following histopathologic changes were seen, slight degeneration of the heart in 3/10 males and 4/10 females, aggregates of reticuloendothelial (RE) cells in the larynx of 7/10 and spleen of 9/10 females, degeneration/regeneration of the glandular mucosa in the stomach of 9/10 females and inflammation of the lungs of 6/10 males and all females. Slight vacuolation of the kidney tubules was observed in 9/10 females. Vacuolation of follicular epithelia and moderate inflammation of the thyroid were present in the majority of rats at 0.1%. Absolute or

relative weights of the heart, kidney, liver, spleen, and thyroid were significantly increased at 12 months. Gross findings in the main group (24 months)included decreased body fat, degenerative and inflammatory lesions of the heart, lungs, the glandular mucosa of the stomach, and the skeletal muscle, hydrothorax, and enlargement of the thyroid. Histologic examination of tissues of rats receiving 0.1% were not conducted at 24 months since the MTD had been exceeded. **The LOEL for systemic toxicity is 9.5 mg/kg/day, based on vacuolation of the epithelial follicular cells of the thyroid in both sexes. The NOEL is 3.0 mg/kg/day. Under the conditions of the study, there was no oncogenic response.**

In the chronic neurotoxicity portion of this study (MRID 43710503), no effects on the Functional Observation Battery or on motor activity were observed after 3, 6, 9, or 12 months of dosing at a dietary level of 0.1% XDE-105 (equivalent to 46.0 mg/kg/day in males or 57.0 mg/kg/day in females). Histopathologic observations of the central and peripheral nervous system of the control and high dose groups revealed a number of lesions which were considered spontaneous and unrelated to dosing. These lesions were generally very slight and the incidences and severity in the high dose group were similar to that in the control group (both sexes). The positive control data provided appropriate positive responses. **A LOEL for neurobehavioral effects and neuropathic effects was not established. The NOEL for neurotoxicity is 0.1% XDE-105, the highest dose tested (46.0 mg/kg/day in males and 57.0 mg/kg/day in females).**

The chronic toxicity/oncogenicity study is **acceptable** and the chronic neurotoxicity study is **acceptable**. The classification of the chronic toxicity phase of the study has been upgraded based on information discussed elsewhere (D230566 and D230456). The chronic neurotoxicity study satisfies the guideline requirements for a chronic neurotoxicity study (§83-7) in rodents. Also, the positive control data for the neurotoxicity portion of the study has been accepted as the basis for upgrading the classification of other neurotoxicity studies reviewed previously (MRIDs 43557501 and 43557504).

iii. Mouse oncogenicity study (MRID 43701505)

In a mouse oncogenicity study, spinosad (76.1% Factor A + 11.9% Factor D) was administered to CD-1 mice (50/sex/group) for up to 18 months at 0, 0.0025, 0.008, or 0.036% in the diet (0, 25, 80, or 360 ppm which is 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). Two satellite groups of 10 mice/sex/group were included for sacrifice at 3 and 12 months. The high-dose females (0.036% XDE) were terminated on day 455 (approximately 15 months) of the study because of marked body weight loss and excessive mortality.

At the highest dietary level (0.036%/360 ppm XDE-150), the mortality rate for females at 65 weeks was 30/50 (60%) compared to 10% for controls and in males at 80 weeks was 21/48 (40%) compared to 24% in controls. At 50 weeks, mean body weights in both sexes were about 10% lower than controls in both sexes and mean cumulative weight gains were 37% lower. Decreased amounts of body fat were clinically observed in both sexes at 0.036%. At

3 and 12 months, hemoglobin and hematocrit values were significantly decreased in high-dose males and significantly decreased in high-dose females at 3 months. An increased incidence of thickened glandular mucosa of the stomach was seen in both sexes and in males this correlated histologically with an increased incidence of mucosal inflammation and increased severity and incidence of hyperplasia of the glandular gastric mucosa.

No important effects of dosing were observed in the low- and mid-dose groups.

**Based on the decreased weight gains, increased mortality, the hematologic effects, and the gross finding of increased thickening of the gastric mucosa in females and the histologic changes in the stomach of males, the systemic LOEL was established as 0.036% or 360 ppm which is equivalent to 50.9 mg/kg/day in male mice and at 67.0 mg/kg/day in females. The NOEL is 0.008% (80 ppm), which is equivalent to 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 the highest dose level. In females the highest dose was excessive and, therefore, inappropriate to assess the carcinogenic potential and the mid-dose (0.008% or 80 ppm) showed no toxicity. The study is classified as **acceptable** based on additional information discussed elsewhere (D230566 and D230456).

iii. Developmental Toxicity

Rats (MRID 43770702)

Spinosad was administered in 0.5% aqueous Methocel A4M to groups of 10 mated Sprague-Dawley strain rats by gavage at dose levels of 0, 10, 50, or 150 mg/kg/day (Part 1) or 0, 200, 250 or 300 mg/kg/day (Part 2) from gestation day 6 through 16 (gestation day 0 was the day mating occurred) (MRID 43770702). This study was conducted to define a dose range for the main rat study reviewed previously (see MRID 43557505). Females were observed for changes in appearance or behavior, and body weight and food consumption were determined at intervals during gestation. Animals were sacrificed on gestation day 16, and at necropsy, gross observations of organs and organ weights of the kidneys, spleen, heart and liver were obtained, and reproductive observations were made. Histological observations of the liver, kidney, spleen, thyroid gland, trachea, ovaries, oviducts, uterus, cervix and vagina from two pregnant rats given 0 mg/kg/day and five pregnant rats given 200 mg/kg/day were recorded.

Maternal toxicity was reported at doses of 200, 250 and 300 mg/kg/day and was characterized by decreased body weight at Days 9, 12 and 16 of gestation (5-8% less than controls for all treated groups), and decreased body weight gain for Days 6-9, 6-16, and 0-16 of gestation which were not consistent with increased dose or the decreased mean body weights for treated groups in comparison with controls. Along with these marginal weight and weight gain decreases, there were no clinical signs reported, and organ weights were not

affected by administration of spinosad. There were also no increases in the incidence of gross observations that could be related to administration of the test substance, and histological observations were limited to two control group animals and 5 animals from the 200 mg/kg/day dose group.

The limited results of this study suggest that the 200 mg/kg/day dose level, which was the highest dose tested in a definitive developmental toxicity study (MRID 43557505), was not high enough to cause maternal toxicity in pregnant Sprague-Dawley rats. But based on results from a rabbit developmental toxicity study (MRID 43414521), the insufficient dose range evaluated in the definitive study should not be considered a critical deficiency. The highest dose in the rabbit study of 50 mg/kg/day approached a toxic level, which suggests that the rabbit is more sensitive to spinosad toxicity, and repeating the rat developmental toxicity study (MRID 43557505) would not add significantly to the data required for developmental toxicity testing.

This study does not, by itself, satisfy §83-3 guideline requirements for a rat developmental toxicity study but should be classified as acceptable supplementary data. The study was intended to define the dose range to be evaluated in a definitive developmental toxicity study, and it is adequate support for upgrading the main developmental toxicity study in rats to acceptable.

Rabbits (MRID 43770703)

Spinosad was administered in 0.5% aqueous Methocel A4M to groups of 7 mated New Zealand White strain rabbits by gavage at dose levels of 0, 50, 100, 200 or 400 mg/kg/day from gestation day 7 through 19 (gestation day 0 was the day mating occurred). This study was conducted to define a dose range for the main rabbit study reviewed previously (MRID 43557521). Females were observed for changes in appearance or behavior, and body weight and food consumption were determined at intervals during gestation. Animals in the 100, 200, and 400 mg/kg/day groups were sacrificed on gestation day 13, while those given 0 and 50 mg/kg/day were sacrificed on gestation day 20. At necropsy, gross observations of organs and organ weights of the kidneys and liver were obtained, and reproductive observations were made. Histological observations of the stomach of one control group and three from the 50 mg/kg/day dose group animals were recorded.

Maternal toxicity was reported at doses of 100, 200 and 400 mg/kg/day and was characterized by decreased defecation (1/7, 4/7, 7/7, 7/7, and 7/7 in the 0, 50, 100, 200 and 400 mg/kg/day dose groups, respectively), decreased body weight (at day 13 group mean body weights were 99, 91, 87 and 86% of the control mean values for the 50, 100, 200 and 400 mg/kg/day dose groups, respectively), decreased body weight gain for Days 10-13 (the 50 mg/kg/day dose group's weight gain was 98% of control value and the 100, 200 and 400 mg/kg/day lost 5-6 times the amount gained by the control and 50 mg/kg/day dose groups), and reduced feed consumption (78, 3, 4 and 0.7% of the control values for the 50, 100, 200, and 400 mg/kg/day dose groups, respectively). Liver weights and liver-to-body-weight ratios

were decreased in the 50 mg/kg/day dose group (76% and 81% of the control values, respectively).

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The limited results of this study suggest that the 50 mg/kg/day dose level at least approaches a maternally toxic dose based on the responses noted at 100 mg/kg/day and above. Therefore, the study reviewed previously can be upgraded to acceptable, and **the NOEL for maternal toxicity in the rabbit is 50 mg/kg/day.**

This study does not satisfy §83-3 guideline requirements for a rabbit developmental toxicity study but should be classified as acceptable supplementary data. The study was intended to define the dose range to be evaluated in a definitive developmental toxicity study and is acceptable support for upgrading the definitive study to acceptable.

iv. Reproduction Toxicity (MRID 43701506)

In a 2-generation reproduction study spinosad (88.0% a.i.) was administered to 30 Sprague Dawley rats/sex/dose in diet at target dose levels 0, 0.005, 0.02, and 0.2% w/w (equivalent to 0, 3, 10 and 100 mg/kg/day). Exposure to the $P_1$ animals began at 6 weeks of age and lasted for 10 weeks prior to the first mating to produce the $F_{1a}$ pups. Exposure to the $F_{1a}$ pups (30/sex) began at weaning and lasted for at least 12 weeks prior to mating to produce the $F_2$ pups. One week after weaning the $F_{1a}$ pups, the $P_1$ animals were mated again to produce an $F_{1b}$ generation. All animals were mated on a 1:1 ratio.

Parental toxicity was characterized in the high-dose animals by treatment-related increases in dystocia (↑3 and 17%) and vaginal bleeding after parturition (↑23 and 24%) and associated increases in mortality (↑7 and 10%, $P_1$ and $F_{1a}$ dams), increases in the absolute and/or relative weights of the heart, kidney, liver, spleen, and thyroid ($P_1$ and $F_{1a}$, both sexes), and histopathology in the lungs, mesenteric lymph nodes, spleen, and thyroid ($P_1$ and $F_{1a}$, both sexes), heart ($P_1$ males only), kidney, and prostate ($P_1$ and $F_{1a}$, males only) and in the stomach ($P_1$ and $F_{1a}$, females only). Histopathology in the lungs was characterized by an increase incidence of multifocal subacute to chronic inflammation of the interalveolar septae along with multifocal aggregates of alveolar macrophages. For the spleen and mesenteric lymph nodes, the histopathology was described as sinus histiocytosis. The primary lesion in the thyroid was diffuse cytoplasmic vacuolation of the follicular epithelial cells with associated chronic active inflammation and necrosis. Treatment-related histopathologic lesions found exclusively in the high-dose males were degeneration of the myocardium with or without inflammation, tubular degeneration in the kidneys, and chronic active inflammation of the prostate. The treatment-related histopathologic lesion found exclusively in the high-dose females was characterized as dilation of the glandular crypts with cellular debris in the pyloric region of the stomach. There were no treatment-related effects noted in the reproductive function or performance of the high-dose $P_1$ or $F_{1a}$ adults. For the low or mid-dose $P_1$ or $F_{1a}$ adults, no treatment-related effects were noted in the clinical signs, mortality, food consumption, body weights, reproductive function and performance, organ weights, gross pathology, or histopathology. **The LOEL for systemic toxicity is 100**

**mg/kg/day based on increases in heart, kidney, liver, spleen, and thyroid weights (both sexes), corroborative histopathology in the spleen and thyroid (both sexes), heart and kidney (males only), and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. The NOEL for systemic toxicity is 10 mg/kg/day.**

Reproductive toxicity, which appears to be related to the systemic toxicity in the dams, was characterized in the high-dose offspring by decreases in the numbers of pups born alive (↓22-35%) and the mean litter sizes (↓23-38%) on days 1 and 4 ($F_{1a}$, $F_{1b}$), and body weight decreases (↓1-11%) throughout lactation ($F_{1a}$, $F_{1b}$, and $F_2$). There were no treatment-related gross pathologic changes noted in the offspring of any generation/treatment group. For the low- and mid-dose treatment groups, no treatment-related effects on the body weights, clinical signs, litter size, or survival indices were noted. **The LOEL for reproductive toxicity is 100 mg/kg/day based on decreases in litter size, survival ($F_2$ litters only), and body weights in the offspring and increased incidence of dystocia and/or vaginal bleeding after parturition with associated increases in mortality in the dams. The NOEL for reproductive toxicity is 10 mg/kg/day.**

The reproductive study in the rat is classified **Acceptable**, and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4) in rats.

B. Studies with the formulation (NAF-85)

Subchronic (21-Day) Dermal Study (MRID 43701502)

In a repeated dose dermal toxicity study conducted in two phases, XDE-105 (Spinosad; 43.4% ai), formulated as NAF-85, was applied to the shaved skin of New Zealand White rabbits. In Phase I, five rabbits/sex/dose received dose levels of 100, 500 or 1000 mg/kg for 6 hours/day; there were a total of 15 applications during a 21-day period. In Phase II, five females/dose received dose levels of 200, 300 or 400 mg/kg for 6 hours/day for a total of 15 applications during a 21-day period.

No rabbits died during the study. Very slight to slight hyperplasia of the gastric mucosa was observed in females treated at $\geq$300 mg/kg/day and males treated at 1000 mg/kg/day. Multiple dark foci of the gastric mucosa with associated histopathological findings occurred in females treated at $\geq$400 mg/kg/day. A majority of treated and control rabbits exhibited aggregates of reticuloendothelial cells in the dermis at the dermal site which was considered to be a background, spontaneous condition that was exacerbated by treatment. **The LOEL is 300 mg/kg/day for female rabbits and 1000 mg/kg/day for male rabbits, based on very slight to slight hyperplasia of the gastric mucosa in both sexes. The NOEL is 200 mg/kg/day for females and 500 mg/kg/day for males.**

This subchronic toxicity study is classified **acceptable**, and satisfies the guideline requirements for a subchronic dermal study (82-2) in rabbits.

## III. Discussion

The Health Effects Division RfD/Peer Review Committee met on August 1, 1996 to discuss the toxicology data in support of the registration of spinosad. The Committee met again on April 30, 1997, to consider data on the carcinogenic potential of spinosad.

The dog study 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. Similar changes were reported in other species tested at higher dose levels. Spinosad is not considered to be a reproductive or developmental toxicant. Therefore, only a 100-fold uncertainty factor (UF) was divided into the NOEL of 2.68 mg/kg/day in the dog study. The resulting reference dose was calculated to be 0.0268 mg/kg/day.

## References


43414515 Gilbert, K.S., K.A. Johnson and K.A. Stebbins XDE-105: Acute Oral Toxicity Study in Fischer 344 Rats and CD-1 Mice. Unpublished Report No(s) DR-0323-1194-017A, DR-0323-1194-017R, DR-0323-1194-017M submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414521 Vedula, U., W.J. Breslin, and B.L. Yano XDE-105: Oral Gavage Teratology Study in New Zealand White Rabbits. Unpublished Report No(s) DR-0323-1194-011 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43557501 Albee, R.R., N.M. Berdaxco, and B.L. Yano XDE-105: Acute Neurotoxicity Study in Fischer 344 Rats. Unpublished Report No(s) DR-0323-1194-009R, DR-0323-1194-009A, DR-0323-1194-009B, DR-0323-1194-009C, DR-0323-1194-009DD submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43557504 Wilmer, J.W., P.J. Spencer, B.L. Yano, and D.M. Bond XDE-105: 13-Week Neurotoxicity Study in Fischer 344 Rats. Unpublished Report No(s) DR-0323-1194-001, DR-0323-1194-001A, and DR-0323-1194-001B submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43557505 Liberacki, A.B., W.J. Breslin, and B.L. Yano XDE-105: Oral Gavage Teratology Study in Sprague-Dawley Rats. Unpublished Report No(s) DR-0323-1194-003 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43701502 Vedula, U., and B.L. Yano (August 30, 1994) NAF-85: Probe and 21-day repeated dose dermal toxicity study in New Zealand White Rabbits. Toxicology Research Laboratory; Health and Environmental Sciences; Dow Chemical Company, Midland, MI 48674. Report No. DR-0341-0784-002 and DR-0341-0784-002R.




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43701503 Spencer, P.J., and B.L. Yano. (April 28, 1995) XDE-105: Chronic neurotoxicity study in Fischer rats. The Toxicology Research Laboratory, Dow Chemical Company, Midland, MI. Report No. DR-0323-1194-005N.

43701504 Harada, T. (January 30, 1995) XDE-105: 12-Month Oral Chronic Toxicity Study in Dogs. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan. Report No. IET 91-0080.

43701505 Bond, D.; Stebbins, K.; McGuirk, R. (April 7, 1995) XDE-105: 18-Month Dietary Oncogenicity Study in CD-1 Mice. Dow Chemical Co., Midland, Michigan: Report No. DR-0323-1194-006.

43701506 Breslin, W.; Quast, J.; Vedula, U. (December 20, 1994) XDE-105: Two Generation Dietary Reproduction Study in Sprague-Dawley Rats. Dow Chemical Co., Midland, Michigan. Report Nos. DR-0323-1194-008A0: DR-0323-1194-008L0: DR-0323-1194-008W1.

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

43770701 Wright, F.L., M.J. Keton, and D.W. Grothe (November 9, 1992) XDE-105: The Acute Toxicity of XDE-105 Administered Orally to Fischer 344 Rats. Toxicology Research Laboratory, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN. Report no. R42590.

43770702 Vedula, U., B.L. Yano, and W.J. Breslin (December 18, 1992) XDE-105: Oral Gavage Teratology Probe Study in Sprague-Dawley Rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan. Report no. DR-0323-1194-002 and -002A.

43770703 Vedula, U., B.L. Yano, and W.J. Breslin (December 18, 1992) XDE-105: Oral Gavage Teratology Probe Study in New Zealand White Rabbits. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan. Report no. DR-0323-1194-007.

012237

Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

**This document supercedes the DER in HED document 011597.**

Study Type: Acute Oral Toxicity Study
Guideline 81-1
Species: Rat

EPA Identification Nos.: EPA MRID No. 434145-15
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-017A, DR-0323-1194-017R, DR-0323-1194-017M

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: Acute Oral Toxicity Study in Fischer 344 Rats and CD-1 Mice.

Author(s): Gilbert, K.S., K.A. Johnson and K.A. Stebbins

Report Issued: August 2, 1994

Executive Summary: The acute oral $LD_{50}$ for CD-1 strain male and female mice is >5000 mg/kg of body weight. The results presented in this report (MRID 43414515) and results from a second report (MRID 43770701) indicate an acute oral $LD_{50}$ value for male Fischer 344 rats of 3738 mg/kg. **These results place XDE-105 into Toxicity Category III.**

Core Classification: This study by itself does not satisfy §81-1 guideline requirements for an acute oral toxicity study in rats and is classified as Supplimentary. It is classified as acceptable when the results are combined with those from a previous study (MRID 43770701).

## Materials and Methods

A. Test Animals: Male and female Fischer 344 rats and CD-1 mice were used. They were acclimated for a period of at least 7 days. The animals were about 8 weeks of age at the start of the test, and were obtained from Charles River Laboratories, Inc., Kingston, New York.

B. Test Substance: XDE-105 (87.9% a.i.) was supplied as a solid (Reference no. AGR293707).

C. Test substance preparation: The report described preparation of the test substance for dosing as follows:

> **The test material was administered as a 50% suspension in 0.5% Methocel.**

D. Experimental design: Animals were randomly assigned to test groups as follows:

| Test Group | Dose Level (mg/kg)* | Number Assigned | |
|---|---|---|---|
| | | Males | Females |
| Rats | 5000 | 5 | 5 |
| Mice | 6000 | 5 | 5 |

* Diet was provided *ad libitum* but was withdrawn overnight prior to dosing by gavage.

E. Observations: The observations procedures were described in the report as follows:

> **Careful in-life observations were mate frequently the day of treatment and at least once each working day throughout the two-week observation period...Each surviving animal was weighed prestudy, the day of treatment, and on test days 2, 8 and 15. A necropsy was performed on all animals.**
>
> **...Following inspection of the externum and body orifices, the nasal, cranial, thoracic, oral and abdominal cavities were opened and the visceral organs were examined both *in situ* and following dissection.**

## Reported Results

A. Clinical signs and mortality: Four males and one female rats died (2 males on day 7 and the remainder on day 8), and one male and two female mice died on Day 11 and

Day 12, respectively. Clinical signs frequently observed during the observation period included lacrimation, salivation, chromorhinorrhea, chromodacryorrhea, rapid respiration, urine and fecal soiling in the perineal area, incoordination and decreased activity. Most of the effects were noted within an hour after dosing and persisted for the entire observation period in one male rat and one female mouse.

B. Body weights: Group mean body weights are summarized from the report as follows:

| | Mean body weight (g) | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| Species | Day 2 | Day 8 | Day 15 | Day 2 | Day 8 | Day 15 |
| Rats | 177.6<br>n=5 | 149.4<br>n=1 | 179.9<br>n=1 | 130.1<br>n=5 | 127.5<br>n=4 | 148.4<br>n=4 |
| Mice | 31.9<br>n=5 | 30.6<br>n=5 | 32.8<br>n=4 | 24.7<br>n=5 | 22.7<br>n=5 | 23.6<br>n=3 |

C. Gross necropsy: Necropsy results were described in the report as follows:

All rats dying spontaneously had perineal soiling. Other effects, primarily involving the gastrointestinal tract, were noted only for individual rats. These included one rat each having gastric erosions or ulcers and hemolyzed blood in the digestive tract, clear fluid in the stomach, pale liver or generalized visceral congestion. Due to the interval of several days following treatment, these lesions are considered nonspecific rather than direct effects of the test material upon the GI tract. All rats that survived to the scheduled necropsy after two weeks had decreased amounts of adipose tissue. A small subcutaneous abscess present i one female rat was considered a spontaneous lesion unrelated to treatment.

All of the surviving male mice, and one of the surviving female mice had increased size of their spleens. Two of the surviving males had increased size of their livers, and multifocal pale areas in their livers. One male mouse with these liver alterations also had decreased amount of body fat. One of the surviving females had a pale liver and decreased amount of body fat. All of the gross pathologic observzations in surviving mice were considered to be treatment related alterations, indicative of systemic toxicity. One of the females, that died during the observation period, had generalized congestion of the lungs. This alteration was considered to be a non-specific alteration, not indicative of treatment related toxicity. The other two mice that died had no other significant gross pathologic observations.

## Discussion

A. Authors' Conclusions: The authors' discussion of the results was reported as follows:

> **Under the conditions of this study the acute oral $LD_{50}$ of XDE-105 for male Fischer 344 rats was 3738 mg/kg and was greater than the limit dose of 5000 mg/kg for female Fischer 344 or male and female CD-1 mice.**

B. Reviewer's Discussion and Conclusions: See "Executive Summary" above.

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Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. KB 5/29/97
Toxicology Branch I/HED

DATA EVALUATION RECORD

**This document supercedes the DER in HED Document Number 011597.**

Study Type: Developmental Toxicity
Guideline §83-3
Species: Rabbit

EPA Identification No.s: EPA MRID No. 43414521
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-011

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: Oral Gavage Teratology Study in New Zealand White Rabbits

Author(s): Vedula, U., W.J. Breslin, and B.L. Yano

Report Issued: January 7, 1994

Executive Summary: XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 20 mated New Zealand White strain rabbits by gavage at dose levels of 0, 2.5, 10 or 50 mg/kg/day from gestation day 7 through 19 (gestation day 0 was the day mating occurred) (MRID 43414521). Females were observed for changes in appearance or behavior, and body weight and food consumption were determined at intervals during gestation. Animals were sacrificed on gestation day 21 and reproductive observations were made and uteri were weighed and examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

The report concluded that maternal toxicity was observed at the highest dose tested (50 mg/kg/day) and was indicated by decreased defecation (in 6/20 animals compared with 2/10 in the control group), decreased body weight gain (28% less than that for the control group during gestation), and reduced food consumption (the high dose group consumed an average amount that was 74% of the control group value). However, there was only a 1-2% difference in the mean body weights between the control and 50 mg/kg/day dose groups. These high dose

group results, along with results from a range-finding study (MRID 4370703), suggested that **the NOEL for maternal toxicity is ≥50 mg/kg/day.** Although the incidence of aborted pregnancies was higher in the 50 mg/kg/day dose group than historical control values, the treatment and observation periods in the range-finding study were not adequate to confirm the investigators' conclusion (the study was terminated at gestation day 20 when abortions were noted at gestation days 22 and 27 in the main study).

There were no developmental effects that could be attributed to administration of XdE-105. **The NOEL for developmental toxicity is ≥50 mg/kg/day.**

Core Classification: This study, along with the range-finding study (MRID 4370703) satisfies §83-3 guideline requirements for a rabbit developmental toxicity study and should be classified as acceptable. The highest dose tested (50 mg/kg/day) approached an adequate level as indicated by the range-finding study.

## Materials and Methods

A. Test Animals: Adult female time-mated New Zealand White strain rabbits were used. They were approximately 6 months of age on arrival at the laboratory and were acclimated until gestation Day 7. Animals selected for the study weighed 2.63-3.53 kg., and weights were obtained by the breeder on gestation day 0. The animals were from Hazleton Research Products, Inc., Kalamazoo, Michigan. Animals were shipped and received on Day 0 or Day 1 of gestation according to the report.

B. Mating Procedures: The mating procedure was described in the report as follows:

> Adult females...were naturally mated with one buck of the same strain... The observed day of breeding was considered Day 0 of gestation.

C. Test Substance: Technical grade XDE-105 (8806% a.i.; 76.1% factor A and 11.9% factor B) was supplied as a solid (lot no. ACD13651), and the dosages are expressed as the active ingredient.

D. Vehicle: 0.5% aqueous Methocel A4M.

E. Dose Solution: The test substance was suspended in the vehicle and was administered in a volume of 2 ml/kg body weight. Dose solutions were prepared before the start of the study and adjusted based on daily body weights during the study according to the report. Samples of each dosing solution were analyzed by HPLC at the sponsor's laboratory at the start of the study to verify the test substance's concentration (89-106% of nominal concentrations, from Table 1 in the report). Samples of each dose solution were also analyzed after 8 days for stability and found to be stable.

F. Study Design: Mated animals were assigned to four groups as follows:

| Test Group | Dose Level (mg/kg/day)* | Number Assigned |
|---|---|---|
| Control | 0 | 20 |
| Low Dose | 2.5 | 20 |
| Mid Dose | 10 | 20 |
| High Dose | 50 | 20 |

* Doses were administered by gavage on gestation days 7 through 19.

G. Observations: The animals were observed daily for clinical signs and mortality. They were weighed on gestation days 0, 7-19, 20 and 28 during the study. Food consumption was determined on gestation days 4-28.

On gestation day 28 surviving animals were sacrificed.

The liver, gall bladder, kidneys and gravid uterus of each animal were removed and weighed, and the ovaries and uterus were examined to determine the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses.

Fetuses were sexed and weighed individually. The report stated that each fetus was examined as follows:

> The uteri of apparently non-pregnant animals were stained with a 10% aqueous solution of sodium sulfide and examined for evidence of early resorptions. All fetuses were examined by dissection...for evidence of visceral alterations. This examination also included an examination of the brain. All fetuses were then preserved in alcohol, eviscerated, cleared and stained with alizarin red S and examined for skeletal alterations.

H. Statistical Analysis: The report described the methods used as follows:

> Descriptive statistics (means and standard deviations) were calculated for feed consumption. Maternal body weights, body weight gains, organ weights (absolute and relative), and fetal body weights were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, analysis by the Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed, respectively. Stastical evaluation of the frequency of pre-implantation loss and resorption among litters and the fetal population was performed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea and implants, and litter size were evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed suing the Fisher Exact probability test. Fetal sex ratios were analyzed by suing a binomial distribution test. Nonpreganant females,

females pregnant following staining or females having totally resorbed litters were excluded from the appropriate analyses. Statistical outliers were identified using a sequential method, but values were not excluded unless justified by sound scientific reasons unrelated to treatment.

The nominal alpha levels used were as follows:

| | |
|---|---|
| Bartlett's Test | $\alpha=0.01$ |
| 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 with Bonferroni's correction |
| Fisher's Test | $\alpha=0.05$, one-sided |
| Censored Wilcoxon Test | $\alpha=0.05$, one-sided |
| Binomial Distribution Test | $\alpha=0.05$, one-sided |
| Sequential Outliers Test | $\alpha=0.02$, two-sided |

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

I. Historical Control Data: Historical control data from 33 studies were included in the report. Nine of those were oral/gavage studies conducted from September, 1990 to January, 1993 and will be considered as appropriate in this DER (see page 11 below).

## Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report indicated that one of the high dose group does was found dead on Day 18 of gestation. The report noted, "This animal exhibited generalized, dark, mottled and firm lungs and...normal appearing fetuses *in utero*." The cause of death was attributed by the authors to gavage error.

The investigators noted a treatment related increase in the incidence of animals with decreased defecation in the high dose group. These results are summarized from the report as follows:

| Observation | Dose level (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| Number of animals | 20 | 20 | 20 | 20 |
| Decreased defecation: No. with | 2 | 1 | 1 | 6 |

The report also noted:

> Two rabbits from the high dose group showed signs of early termination of pregnancy (aborted fetuses) on gestation days 22 and 27...Decreased feed consumption, severe weight loss and decreased fecal output were noted in both of these animals prior to abortion. Gross pathologic observation (of one rabbit) revealed serosanguinous ascites of the abdominal cavity, atelectasis of the left apical lobe of the lungs, multifocal pale areas in the wall of the gall bladder, mucoid exudate in the trachea, a dark focus in the cortex of one kidney, decreased ingesta in the digestive tract and hemorrhagic vaginal wall. Aside from showing signs of the recent abortion of fetuses, (the other rabbit) appeared normal at necropsy.

2. Body Weight and Food Consumption: The report noted:

> There were no significant treatment-related effects on body weights...at any dose level. A statistically significant decrease in body weight gain was observed in the high dose dams during Days 7 through 10 of gestation. This time interval corresponds to the start of the dosing period and hence the decrease in body weight gain was interpreted to be treatment related. The body weight gains of rabbits given the 50 mg/kg/day were also decreased throughout the remainder of the dosing period, but the decreases were not statistically significant.

These results are summarized from the report as follows:

| Observation | Dose level (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| Mean body weight (g) on gestation day | | | | |
| 0 | 3039.5 | 3082.1 | 3085.2 | 3087.7 |
| 7 | 3314.4 | 3375.3 | 3393.5 | 3370.9 |
| 10 | 3365.0 | 3439.7 | 3439.2 | 3366.7 |
| 20 | 3600.0 | 3689.8 | 3666.7 | 3561.7 |
| 28 | 3740.6 | 3835.2 | 3822.7 | 3750.9 |
| 28[a] | 3325.3 | 3405.6 | 3345.6 | 3295.3 |
| Mean body weight gain gestation days | | | | |
| 0 - 7 | 274.9 | 293.2 | 308.2 | 283.2 |
| 7 - 10 | 50.6 | 64.4 | 45.8 | -4.1* |
| 7 - 20 | 285.7 | 314.5 | 273.3 | 196.6 |
| 20 - 28 | 140.5 | 145.4 | 156.0 | 193.6 |
| 0 - 28 | 701.1 | 753.1 | 736.5 | 703.9 |

[a] Body weight adjusted by subtracting gravid uterine weight.
* Significantly different from controls, Dunnett's Test ($p \leq 0.05$).

Feed consumption was also reduced at the highest dose tested. The investigators described the findings as follows:

> The feed consumption of rabbits given 50mg/kg/day was decreased throughout most of the dosing period. Severely decreased feed consumption was observed in 6 of 20 high dose rabbits during the dosing period. Decreased fecal output also was found in these rabbits, consistent with decreased feed consumption. A compensatory increase in the feed consumption of the affected rabbits was observed following the dosing period. In addition, two rabbits exhibiting a severe increase in feed consumption either died or aborted, thereby further increasing the mean feed consumption at the high dose during the post-dosing period.

Selected results are summarized from the report as follows:

| Observation | Dose level (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| Mean food consumption (g/day) days | | | | |
| 4 - 5 | 214.9 | 220.1 | 210.2 | 211.8 |
| 7 - 8 | 187.1 | 211.3 | 206.4 | 187.3 |
| 8 - 9 | 191.5 | 211.1 | 205.9 | 185.0 |
| 9 - 10 | 189.6 | 214.0 | 207.3 | 171.8 |
| 11 - 12 | 179.8 | 208.3 | 185.8 | 155.4 |
| 14 - 15 | 175.0 | 191.6 | 180.6 | 129.8 |
| 17 - 18 | 186.4 | 208.3 | 200.7 | 165.7 |
| 21 - 22 | 180.7 | 194.1 | 183.3 | 192.9 |
| 22 - 23 | 173.1 | 181.6 | 169.3 | 194.6 |
| 23 - 24 | 150.3 | 174.3 | 159.9 | 184.6 |
| 24 - 25 | 150.3 | 174.3 | 159.9 | 184.6 |
| 25 - 26 | 144.5 | 153.9 | 144.3 | 147.8 |
| 27 - 28 | 150.4 | 158.1 | 152.6 | 147.1 |

Significantly differences from controls were not noted in Table 4 of the original report.

3. Uterine Observations; The report indicated that there were no treatment-related effects on pregnancy rate, litter size, fetal sex ratio, and gravid uterine weight or on the numbers of corpora lutea, implantations, resorptions, and pre- or post-implantation losses. These results are summarized from the report as follows:

| Observation | Dose level (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| Number of animals | 20 | 20 | 20 | 20 |
| Number died: | 0 | 0 | 0 | 1 |
| Pregnant | 0 | 0 | 0 | 1 |
| Non-pregnant | 1 | 0 | 3 | 2 |
| No. aborted | 0 | 0 | 0 | 2 |
| With viable fetuses at termination | 19 | 20 | 17 | 15 |

| Observation | Dose level (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| Number of animals | 20 | 20 | 20 | 20 |
| Corpora lutea/doe | 8.8 | 9.0 | 9.7 | 9.3 |
| Implantations/doe | 7.5 | 7.4 | 8.8 | 8.2 |
| Litters with resorptions | 7 | 2 | 7 | 6 |
| Resorptions/litter | 0.5 | 0.1 | 0.5 | 0.5 |
| Resorp./litter w. resorp. | 1.4 | 1.3 | 1.0 | 1.3 |
| Dead fetuses/litter | 0.0 | 0.0 | 0.0 | 0.0 |
| Mean litter size | 7.0 | 7.3 | 8.3 | 7.7 |
| Mean gravid uterus weight (g) | 415.3 | 429.6 | 477.1 | 45.6 |
| Mean fetal body weight (g) | 39.5 | 39.5 | 38.2 | 39.8 |
| % males | 54 | 50 | 50 | 43 |

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

B. Developmental End Points: The investigators noted that malformations were seen in 14, 8, 5 and 4 fetuses overall from the control, low, mid and high dose groups, respectively. These observations were described in the report as follows:

> The malformations (in the control group) included retroeso-phageal right subclavian artery in three fetuses (3 litters), missing caudal lung lobe in eight fetuses (4 litters), umbilical hernia in one fetus, extra lumbar vertebra in one fetus and fused ribs in one fetus...Five fetuses (5 litters, in the low dose group) exhibited missing caudal lung lobes. The remaining three fetuses (sic) each had a single malformation consisting of missing left intermediate lung lobe, extra semilunar valve, missing apical lung lobe or ectopic kidney.
>
> Of the five malformed fetuses (in the mid dose group), three exhibited missing caudal lung lobes (in 3 litters) and fused ribs were noted in two rabbits (2 litters)...The malformations (in the high dose group) included forelimb flexure in one rabbit, missing caudal lung lobe in two rabbits (2 litters), and an ectopic kidney in one rabbit.

No skeletal variations were noted by the investigators in their discussion.

Selected data are summarized from the report as follows:

| Observation | Dose (mg/kg/day) 0 | 2.5 | 10 | 50 |
|---|---|---|---|---|
| No. fetuses/litters examined for alterations: | 133/19 | 145/20 | 141/17 | 115/15 |
| Retrocaval ureter | 9/6 | 8/8 | 4/4 | 7/3 |
| Hyoid: delayed ossification | 48/15 | 78/19 | 53/14 | 50/13 |
| Sternebrae: delayed ossif. | 54/15 | 64/17 | 49/13 | 52/14 |
| Lumbar spurs | 32/12 | 33/14 | 39/14 | 26/10 |

* Statistically significantly different from controls, $p \leq 0.05$, Fisher's Exact test.

The authors compared the incidences of fetal alterations with historical control data as follows:

## Discussion

A. Authors' Conclusions: The authors' conclusion was reported as follows:

> ...maternal toxicity was observed in the 50 mg/kg/day dose group rabbits as evidenced by decreased feed consumption, decreased fecal output and decreased body weight gain during the treatment period. In addition two of the high dose rabbits aborted prior to scheduled necropsy. No maternal effects were observed at 2.5 or 10 mg/kg/day and no adverse developmental effects at any dose level tested. Thus, the maternal no-observed-effect level (NOEL) for XDE-105 was determined to be 10 mg/kg/day. The NOEL for embryonal/fetal toxicity and teratogenicity was 50 mg/kg/day, the highest dose tested.

B. Reviewer's Discussion and Conclusions: Body weight gain in the high dose group was 108% less than that of the control group (-4.1 g compared to 50.6 g) for the Day 7-10 interval during the first three days of dosing (see page 6 above). Food consumption for the Day 7-10 interval was 4% less than controls in the high dose group (computed as a mean of the Days 7-8, 8-9, and 9-10 means, 181.4 g/day compared to 189.4 g/day for the interval; see page 7 above). The body weight for the control and high dose groups at Day 10 of gestation were 3365.0 and 3366.7 g, respectively. The difference of 54.7 g. body weight gain is only 1-2% of the average body weight for the control and high dose groups. Results of the body weight, weight gain, and food consumption observations suggest that the high dose group response may not be toxicologically significant as indicated in the report, particularly with respect to the reference to "severly decreased" food consumption (see page 6 above).

In addition, the compensatory effect noted by the investigators (see page 6 above) is also apparent in the other treated groups, and a dose response relationship is not evident. Using the sums of mean food consumption values for Days 21-22, 22-23, 23-24, 24-25, 25-26, 26-27 and 27-28 post-dosing, the totals for the low, mid and high dose groups are 9, 2 and 11% greater than the control group value. The maximum difference between the control group and highest dose group is seen at Day 23-24 (22% increase) and the maximum for the low dose group (16%) is also seen at Day 23-24. The mid dose group value for Day 23-24 was 6% greater than the control value. This comparison of low and high dose group food consumption post-dosing with that of the control group further suggests that the effects on food consumption may not be related to administration of the test substance.

Statistically significant differences were not consistently noted for body weight, body weight gain or food consumption results, and there were no differences noted with respect to absolute and relative organ weights (data not included here). No animals were characterized in the report as emaciated and anorexia was not noted.

The only clinical observations attributed to the test substance were decreased fecal output and increased incidence of abortions. Individual animal data on those animals with reduced fecal output in the high dose group indicated that the two animals that aborted exhibited decreased food consumption from the time dosing began (Day 7-8), but their food consumption values were not identified as statistical outliers until the Day 17-18 observation.

Decreased fecal output was also noted in two animals from the control group; one on gestation days 16-17 (animal 93A3915) and one on days 12-13 (animal no. 93A3920). By comparison, the observation appeared to occur in a similar pattern in three of the six high dose group animals (93A3973, 93A3976 and 93A3984), which may be coincidental.

Two other high dose group animals (93A3980 and 93A3981) had extended periods of decreased fecal output associated with decreased food consumption. One of those animals (93A3980) aborted on gestation Day 22, and the other (93A3981) had a successful pregnancy with 9 fetuses (compared to a group mean litter size of 7.7) with 1 resorption.

These results do not clearly suggest toxicologically significant effects, but a range-finding study, mentioned in the Introduction to the study report, described similar results as follows:

> In a recent oral gavage teratology probe study, XDE-105 was administered to groups of seven inseminated New Zealand White rabbits on gestation days 7 through 19 at doses of 0, 50, 100, 200 or 400 mg/kg/day. Marked decreases in feed consumption and significant

body weight losses during the exposure period were observed at 100, 200 and 400 mg/kg/day dose groups. As a result of the inanition and body weight loss observed in these animals, all rabbits in the 100, 200 and 400 mg/kg/day dose groups were euthanized on Day 13. Dams given 50 mg/kg/day had slightly decreased feed consumption and statistically significant decreases in body weight gain during the exposure period. The absolute and relative liver weights were also decreased in dams given 50 mg/kg/day. Three out of seven rabbits of the 50 mg/kg/day dose group had hemolyzed blood in the lumen of the stomach and had histopathologic evidence of hyperplasia and nuclear changes in the epithelial lining of the stomach. No effect of treatment on litter size, preimplantation loss or resorptions was observed at 50 mg/kg/day.

The full report on the range-finding study (MRID 4370703) is considered in a separate DER which described that study as follows:

XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 7 mated New Zealand White strain rabbits by gavage at dose levels of 0, 50, 100, 200 or 400 mg/kg/day from gestation day 7 through 19 (gestation day 0 was the day mating occurred) (MRID 43770703). This study was conducted to define a dose range for the main rabbit study reviewed previously (see MRID 43414521). Females were observed for changes in appearance or behavior, and body weight and feed consumption were determined at intervals during gestation. Animals in the 100, 200, and 400 mg/kg/day dose groups were sacrificed on gestation day 13, while those given 0 or 50 mg/kg/day were sacrificed on Day 20. At necropsy, gross observations of organs and organ weights of the kidneys and liver were obtained, and reproductive observations were made. Histological examination of the stomach were made on one and three rabbits from the 50 mg/kg/day groups, respectively.

Maternal toxicity was reported at doses of 100, 200 and 400 mg/kg/day and was characterized by decreased defecation (in 1/7, 4/7, 7/7, 7/7 and 7/7 animals from the 0, 50, 100, 200 and 400 mg/kg/day dose groups, respectively), decreased body weight (at Day 13 group mean body weights were 99, 91, 87, and 86% of the control mean value for the 50, 100, 200 and 400 mg/kg/day groups, respectively), decreased body weight gain (for Days 10-13 the 50 mg/kg/day group weight gain was 98% of the control value and the 100, 200 and 400 mg/kg/day dose groups lost 5-6 times the amount gained by the control animals), and reduced feed consumption (78, 3, 4 and 0.7% of the control feed consumption for the 50, 100, 200 and 400 mg/kg/day dose groups, respectively). Liver weight and liver-to-body-weight ratios were decreased in the 50 mg/kg/day dose group (76% and 81% of the control values, respectively).

The results of this study suggest that 50 mg/kg/day at least approached a maternally toxic dose based on the responses noted at 100 mg/kg/day and above. Therefore, the study reviewed previously can be upgraded to acceptable, and **the NOEL for maternal toxicity is 50 mg/kg/day.**

Historical control data on abortions at the testing facility is summarized as follows:

| Study Number | Number Mated | Number Pregnant | Number Aborted |
|---|---|---|---|
| 24 | 28 | 21 | 0 |
| 25 | 22 | 19 | 1 |
| 26 | 18 | 17 | 1 |
| 27 | 18 | 15 | 0 |
| 28 | 18 | 14 | 0 |
| 29 | 20 | 17 | 0 |
| 30 | 24 | 22 | 0 |
| 31 | 20 | 20 | 0 |
| 33 | 20 | 18 | 0 |
| Mean ± S.D. | 20.89±3.333 | 18.11±2.667 | --- |
| (Max. - Min.) | (18 - 28) | (14 - 22) | (0 - 1) |

These data indicate that the incidence of abortions in untreated control groups can be as high as 1/17 (6%) compared with 2/17 in the high dose group of the study reviewed here. The authors attributed the two aborted pregnancies in the high dose group to the test substance.

012237

Primary Review by: Roger Gardner Roger Gardner 5.22.97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

**This document supercedes the DER in HED Document No. 011597.**

Study Type: Acute Neurotoxicity Study
Guideline 81-8
Species: Rat

EPA Identification Nos.: EPA MRID No. 435575-01
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-009R, DR-0323-1194-009A, DR-0323-1194-009B, DR-0323-1194-009C, DR-0323-1194-009DD

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: Acute Neurotoxicity Study in Fischer 344 Rats.

Author(s): Albee, R.R., N.M. Berdaxco, and B.L. Yano

Report Issued: March 11, 1994

Executive Summary: In an acute oral neurotoxicity study, groups of 10 male and 10 female Fischer 344 strain rats were given a single dose of 0, 200, 630, or 2000 mg XDE-105/kg body weight. The highest dose is considered to be a limit dose. There were no effects of XDE-105 observed on the functional observational battery (FOB), motor activity, or histological observations of the nervous system. Therefore, **the NOEL for acute mammalian neurotoxicity in rats is ≥2000 mg/kg.**

Core Classification: This study satisfies §81-8 guideline requirements for an acute oral mammalian neurotoxicity study and is upgraded to acceptable (guideline) (see HED Document No. 011597 for previous review). There were adequate positive control data from the testing laboratory presented in the report on the chronic toxicity/carcinogenicity/ neurotoxicity study with rats (MRIDs 43701507 and 43710503).

## Materials and Methods

A. Test Animals: Male and female Fischer 344 rats were used. They were acclimated for a period of at least 7 days. The animals were about 8 weeks of age at the start of the test, and were obtained from Charles River Laboratories, Inc., Kingston, New York.

B. Test Substance: XDE-105 (87.9% a.i.) was supplied as a solid (Lot no. ACD13651).

C. Test substance preparation: The report described preparation of the test substance for dosing as follows:

> The dosage solutions, administered by gavage, were adjusted for purity and were analyzed for concentration prior to treatment. Based on the analytical data, the administered high dose was 90% of the target concentration, the middle was 95% of the target, and the low dose was 99% of the target concentration. The analysis showed also that the dosing solutions were homogeneous.
>
> The rats received the test material by single-dose gavage in aqueous methyl cellulose at a dose volume of 10 ml/kg body weight.

D. Experimental design: Animals were randomly assigned to test groups as follows:

| Test Group | Dose Level (mg/kg)* | Number Assigned | |
|---|---|---|---|
| | | Males | Females |
| Low dose | 200 | 10 | 10 |
| Mid dose | 630 | 10 | 10 |
| High dose | 2000 | 10 | 10 |

* Diet was provided *ad libitum* but was withdrawn overnight prior to dosing by gavage.

E. Observations: The observations procedures were described in the report as follows:

> Ten rats/sex/group were used for the FOB and motor activity assays, which were conducted once prior to XDE-105 administration (dosing day -1), approximately 5 to 6 hours post-dosing (day 1), and then on days 8 and 15 of the study. In addition, body weights were determined on days -1, 1, 2, 8 and 15. After study completion at 15 days, 5 rats/sex/group were fixed by perfusion and examined for gross pathologic alterations.

The number and type of observations made in the study were summarized in the report as follows:

| | Test Periods | Number of Rats | Obs or Data Points/Rat | Total Obs or Data Points | Number of Means (±SD) |
|---|---|---|---|---|---|
| Body weight | 5 | 80 | 1 | 400 | 40 |
| Hindlimb grip | 4 | 80 | 3 | 960 | 32 |
| Forelimb grip | 4 | 80 | 3 | 960 | 32 |
| Landing splay | 4 | 80 | 3 | 960 | 32 |
| Motor activity | 4 | 80 | 6 | 1920 | 224 |
| Clin obs (categories) | 3 | 80 | 6 categories | 1440 | NA |
| FOB (categories) | 4 | 80 | 6 categories | 1920 | NA |
| FOB (ranked obs) | 4 | 80 | 12 | 3840 | NA |
| Necropsy (# tissues) | 1 | 80 | 51 | 4080 | NA |
| Neuropath (# tissues) | 1 | 20 | 30 | 60 | NA |
| | | | TOTAL | 17,080 | 360 |

Functional observational battery (FOB) parameters were described in the report as follows:

| FOB Parameter | Recorded As |
|---|---|
| Measurement/Count | |
| Body weight | grams |
| Hindlimb grip performance | grams force |
| Forelimb grip performance | grams force |
| Landing foot splay | distance between hind feet (cm) |

| FOB Parameter | Recorded As |
|---|---|
| Hand-held Observations | |
| General (thin, fat, red ocular/nasal crusts, etc.) | Description |
| Palpebral closure | Rank |
| Pupil size | Normal, increased, or decreased |
| Lacrimation (clear periocular wetness) | Rank |
| Salivation (clear perioral wetness) | Rank |
| Abnormalities of skin or haircoat | Description |
| Perianal staining | Present or absent |
| Abnormal movements (e.g., muscle tone, tremors, convulsions) | Description |
| Abnormal respiration (e.g., increased, wheezing) | Description |
| Reactivity to handling | Rank |
| Open-field observations | |
| Level of activity | Rank |
| Responsiveness to sharp noise | Rank |
| Responsiveness to touch | Normal, increased, or decreased |
| Responsiveness to tail pinch | Rank |
| Abnormal behavior (e.g., stereotypies, locomotor) | Description |
| Gait abnormalities | Rank |
| Urine volume voided during FOB | Rank |
| Number of fecal pellets voided during FOB | Rank |

The report described procedures for measuring grip performance as follows:

> ...the rats were selected in a random manner and given to the observer in such a way that the observer did not know the treatment status of the animal. The observer then placed the rat's forelegs on a bench and the hind feet were set on a horizontal screen attached to a strain gauge. The observer then smoothly but firmly pulled backward on the rat's tail until the rat's grip on the screen was broken.
>
> ...The average of three trials was used for statistical analysis. Forelimb grip performance was similarly tested. In this application, a bench was not used, and the rats were placed so that the forefeet were on a screen and the hindfeet were on a smooth horizontal plastic surface. The test sequence was the same as for hindlimb testing. Because grip performance is affected by body weight, each rat's individual grip performance (grams of pull) was divided by its body weight, resulting in a grip performance measure of grams pull/gram body weight.

The report described the procedure for determining landing foot splay as follows:

> The rats were selected in a random manner and given to the observer in such a way that the observer did not know the treatment status of the animal. The tarsal joint pad of each hindfoot was marked with ink. The animal was then dropped from a height of 30 cm onto the recording sheet. This procedure was repeated three times,...The distance from center to center of the ink marks was measured and the average of the three splay values was used for statistical analysis.

Motor activity observation procedures were described in the report as follows:

> Twenty-four motor activity cages (also referred to as chambers), visually isolated from each other, were located in a quiet room (approximately 60 dBA) that had low light intensity (approximately 1.5 lux). Each motor activity cage consisted of a circular clear plastic alley...An infrared photobeam bisected the cage so that the beam crossed the alley in 2 locations.
>
> To ensure that the animals had been undisturbed prior to motor activity testing, the motor activity assay was started one hour prior to conduct of the FOB at each data collection interval. Each animal was tested individually for motor activity. All test sessions consisted of six 8-minute epochs, totalling 48 minutes of testing per animal...Total activity counts for each epoch were recorded. Each beam break that lasted more than 100 msec constituted an activity count. This minimum duration was set to discount such activities as tail-flicking, rearing, head bobbing, etc.
>
> Cages were calibrated prior to testing each day...any photocell showing a difference exceeding 4 centiseconds was readjusted to assure equivalence of devices.
>
> The experimental design...is referred as (sic) a split-plot factorial design with two between block (sex and dose)treatments and two within-block (epoch and day) treatments (also known as $SPF_{p.q}$ design).

The procedures used to perfuse and fix nervous tissue for microscopic evaluation were described in the report as follows:

> Rats (5/sex/group) were perfused intracardially with 0.05 M phosphate buffer containing 0.7% sodium nitrite, followed by a phosphate-buffered solution of 1.5% glutaraldehyde-4% formaldehyde (c. 540 mOsM). A complete gross examination was conducted on all animals...The remaining 5 rats/sex/dose were...not perfusion fixed...Tissues from these rats were fixed by immersion in neutral phosphate buffer 10% formalin.
>
> Tissues for neuropathologic evaluation were prepared from all perfusion fixed rats in the control and high dose groups. Nine transverse sections of the brain were prepared from the olfactory lobe, cerebrum (frontal, parietal, temporal, and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and medulla oblongata. The following tissues were also prepared: trigeminal ganglion, pituitary gland, eyes with

optic nerve, spinal cord (cervical and lumbar), nasal tissues with the olfactory epithelium, and skeletal muscles (gastrocnemius and interior tibial). Tissues from the central nervous system were...stained with hematoxylin & eosin...Peripheral nerves (sciatic, tibial and sural) and additional dorsal root ganglia (cervical and lumbar) were...stained with toluidine blue.

F. Statistical Analyses: Statistical methods were generally described in the report as follows:

Statistical analyses were conducted on body weights, grip performance, landing foot splay, and motor activity. Grip performance data were transformed to grams pull divided by grams body weight to minimize confounding from changes in body weight. Motor activity counts were reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment. FOB observations were evaluated by a test of proportions beginning with the greatest difference in distributions between control and high dose. Evaluation of other FOB dose levels and categories continued in a trend fashion until the first non-significant finding (i.e., differences between all further distributions were too small to be significant).

Means and standard deviations were calculated and homogeneity of variance was confirmed with the F-max test ($\alpha$ = 0.01).

The study design had two sexes and four major data collection periods; pre-exposure (day -1), day 1, day 8 and day 15. Initial statistical analyses, therefore, were factorial repeated measure analyses to account for data from both sexes at all time periods in one statistical analysis. By using sex as a factor, statistical power of the test was increased by increasing the degrees of freedom. Body weight analysis also included day 2 of the study period. In factorial repeated-measure tests, the inclusion of pre-exposure data in the analysis makes relevant only the analyses which include factors of both treatment and time. The following interactions were studied:

Treatment x Time -- Does treatment change the pre-existing differences between groups at any time interval? this interaction will not identify which time interval or which exposure level is different in case of statistical significance.
Treatment x Time x Sex -- Does treatment change the pre-existing differences between the sexes at any time interval?
Treatment x Time x Epoch (motor activity only) -- Does treatment change the pre-existing differences in distribution of motor activity across epochs at any time interval?

...To reduce the rate of false declarations, the type I error rate ($\alpha$) per comparison was set at 0.02...The corrections for multiple statistical analyses were applied to $\alpha$ only, and probability values were reported without correction.

The types and number of statistical tests were tabulated in the report as follows:

| Dependent variable | Type of test[a] | Number of primary tests |
|---|---|---|
| Body weight | Rep-ANOVA | 2 (Txd & TxdxS) |
| FOB (ranked observations) | Test of proportions | Until non-significant |
| Grip performance | | |
| Forelimb | Rep-ANOVA | 2 (Txd & TxdxS) |
| Hindlimb | Rep-ANOVA | 2 (Txd & TxdxS) |
| Landing foot splay | Rep-ANOVA | 2 (Txd & TxdxS) |
| Motor activity | | |
| Total counts | Rep-ANOVA | 2 (Txd & TxdxS) |
| Epochs (nested by day) | Rep-ANOVA | 1 (TxdxE) |

Factors: T=treatment, d=day, S=sex, E=epoch. Repeated across days. For motor activity repeated across days and across epochs.

[a] Rep-ANOVA was calculated by rep-MANOVA with one dependent variable. This format avoids the requirement of sphericity of the variance/covariance matrix of the Rep-ANOVA. Multivariate index was the Hoteling-Lawley Trace statistic.

## Reported Results

A. Clinical signs, grip performance and landing foot splay: the report described results of cageside and FOB observations as follows:

> No treatment-related effects were seen during cageside or clinical observations.
>
> No treatment-related effects were seen in hand-held and open field observations at any time during the study.

No effects were noted on grip performance or landing foot splay.

B. Body weights: Group mean body weights are summarized from the report as follows:

| | Mean body weight (g) | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| Dose (mg/kg) | Day 2 | Day 8 | Day 15 | Day 2 | Day 8 | Day 15 |
| 0 | 155.3 | 181.7 | 178.2 | 97.0 | 115.3 | 113.6 |
| 200 | 151.6 | 180.1 | 178.7 | 96.5 | 113.8 | 112.5 |
| 630 | 150.1 | 178.9 | 177.6 | 96.1 | 117.3 | 114.0 |
| 2000 | 152.5 | 179.7 | 177.2 | 93.1 | 115.6 | 114.1 |

The results of statistical analyses for body weight results were described in the report as follows:

> There was a significant treatment-day interaction in 2000 and 630 mg/kg-groups, however, there was no treatment-by-day-by-sex interaction. Close examination of the body weight data suggested a treatment-related effect for day 2 only. When day 2 was removed from the statistical model there was no significant difference among the groups (treatment-by-day p=0.8214).

C. Motor activity: Results of the statistical analyses of motor activity observations were discussed in the report as follows:

> The treatment-by-day interaction was not significant (i.e., the groups did not significantly differ overall under baseline conditions compared to under treatment conditions). However, a treatment-by-day-by-sex interaction was significant, which meant that males and females reacted differently to treatment...
>
> When the data from males and females were analyzed separately, the males showed a significant treatment-by-day interaction. The analysis next addressed the difference between days in male rats...a trend is present under baseline conditions. Specifically, day -1 high dose male activity was greater than the controls but was not greater at any of the remaining intervals. When day -1 data were removed from the model, the treatment-by-day was no longer significant. The statistically significant data reflect an idiosyncrasy of baseline data in male rats, and do not, therefore, express an effect of the test compound. Although there were no treatment-related changes in motor activity, significant sex and day main effects (both p values << 0.001)were seen in this study unrelated to treatment. These significant effects show that the absence of a treatment effect in this study is unlikely to be due to an overall lack of power of the system to detect a significant effect if there was one.

D. Neuropathology: Necropsy results were described in the report as follows:

> Gross pathologic lesions in both fixation groups (perfusion or immersion fixed) included: hemolyzed blood in the stomach, strangulated mesenteric fat, a liver hernia, testicular hemorrhage, and a preputial abscess. These alterations occurred in a maximum of one rat/sex/dose level with no predilection for the highest dose level and were not attributed to XDE-105.
>
> ...A number of spontaneous lesions were identified in the brains of control and XDE-105 exposed rats (5/sex from the control and high dose groups). These lesions were characterized as swollen axons in several regions of the brain, including the nucleus gracilus (medulla oblongata), parietal

lobes of the cerebrum, spinal cord, and pars nervosa of the pituitary gland; degeneration of individual nerve fibers in the trapezoid body (medulla oblongata), dorsal root ganglia, and trigeminal ganglia; unilateral retinal optic nerve atrophy; and mineralization of the cornea and blood vessels in proximity to the eye. The distribution of these lesions did not suggest that they were treatment related, but reflected the occurrence of spontaneous lesions.

Selected incidences of histopathologic observations are summarized from the report as follows:

| Lesion | Dose (mg/kg/day) 0 | 2000 |
|---|---|---|
| **Males** | | |
| Brain - cerebrum: No. examined | 5 | 5 |
| Swollen axons, parietal lobe - focal, very slight | 1 | 0 |
| Brain - medulla oblongata: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, trapezoid body | | |
| Multifocal - very slight | 4 | 2 |
| Focal - very slight | 0 | 2 |
| Dorsal root ganglia with roots - lumbar: No. examined | 5 | 5 |
| Degeneration - individual nerve fiber, focal - very slight | 1 | 0 |
| Eyes: No. examined | 5 | 5 |
| Atrophy - retina, unilateral - very slight | 1 | 0 |
| Mineralization - blood vessels, bilateral - very slight | 1 | 0 |
| Mineralization - cornea, unilateral - very slight | 2 | 0 |
| Mineralization - cornea, bilateral - very slight | 3 | 3 |
| Pituitary: No. examined | 5 | 5 |
| Swollen axons - posterior (pars nervosa) | | |
| multifocal - very slight | 4 | 4 |
| focal - very slight | 0 | 0 |
| Spinal cord - lumbar: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, focal - very slight | 0 | 1 |
| Trigeminal ganglia: No. examined | 5 | 5 |
| Degeneration - individual nerve fiber, focal - very slight | 0 | 1 |

| | Females | |
|---|---|---|
| Brain - medulla oblongata: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, trapezoid body | | |
| Multifocal - very slight | 3 | 2 |
| Focal - very slight | 0 | 2 |
| Swollen axons - gracile nucleus, focal - very slight | 0 | 1 |
| Cranial nerve - optic: No. examined | 5 | 5 |
| Mineralization - blood vessels, focal - very slight | 0 | 1 |
| Dorsal root ganglia with roots - cervical: No. examined | 5 | 5 |
| Degeneration - individual nerve fiber, focal - very slight | 0 | 1 |
| Dorsal root ganglia with roots - lumbar: No. examined | 5 | 5 |
| Degeneration - individual nerve fiber, | | |
| multifocal - very slight | 1 | 0 |
| Eyes: No. examined | 5 | 5 |
| Atrophy - retina, unilateral - very slight | 0 | 0 |
| Mineralization - blood vessels, bilateral - very slight | 0 | 0 |
| Mineralization - cornea, unilateral - very slight | 3 | 2 |
| Mineralization - cornea, bilateral - very slight | 2 | 2 |
| Pituitary: No. examined | 5 | 5 |
| Swollen axons - posterior (pars nervosa) | | |
| multifocal - very slight | 1 | 1 |
| focal - very slight | 1 | 1 |
| Spinal cord - lumbar: No. examined | 5 | 5 |
| Swollen axons - focal - very slight | 0 | 1 |
| Spinal cord - cervical: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, focal - very slight | 1 | 1 |
| Swollen axons - focal = very slight | 1 | 1 |

## Discussion

A. Authors' Conclusions: The authors' discussion of the results was reported as follows:

> ...A single oral gavage dose of 630 or 2000 mg XDE-105/kg body weight caused a transient dose-response decrease in body weight. Decreased body weight was apparent the day after dosing but was not present 1 week after dosing. The body weight of rats from the low dose group was unaffected by treatment...XDE-105, however, had no effect on the functional observational battery or motor activity and did not result in treatment-related neuropathology.

B. Reviewer's Discussion and Conclusions: See "Executive Summary" above.

012237

Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. KB 5/27/97
Toxicology Branch I/HED

DATA EVALUATION RECORD

**This document supercedes the DER in HED Document No. 011597.**

Study Type: Subchronic Neurotoxicity Study
Guideline 82-7
Species: Rat

EPA Identification Nos.: EPA MRID No. 435575-04
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-001, DR-0323-1194-001A, and DR-0323-1194-001B

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: 13-Week Neurotoxicity Study in Fischer 344 Rats.

Author(s): Wilmer, J.W., P.J. Spencer, B.L. Yano, and D.M. Bond

Report Issued: July 13, 1993

Executive Summary: In a 13-week feeding neurotoxicity study, groups of 10 male and 10 female Fischer 344 strain rats were given daily levels of 0, 0.003, 0.006, 0.012 or 0.06% (0, 2.2, 4.3, 8.6, and 42.7 mg XDE-105/kg body weight for males and 0, 2.6, 5.2, 10.4 and 52.1 mg/kg/day for females). There were no effects of XDE-105 observed on the functional observational battery (FOB), motor activity, or histological observations of the nervous system. Therefore, **the NOEL for acute mammalian neurotoxicity in rats is ≥42.7 or 52.1 mg/kg/day for male and female rats, respectively.**

Core Classification: This study satisfies §82-7 guideline requirements for a subchronic mammalian neurotoxicity study and is classified as acceptable (guideline) (MRID 43557504). This study was an extension of a subchronic feeding study, and there were adequate positive control data from the testing laboratory

presented in the report of the chronic toxicity/carcinogenicity/ neurotoxicity study (MRID 43701507 and 43701503).

## Materials and Methods

A. Test Animals: Male and female Fischer 344 rats were used. They were acclimated for a period of at least 7 days. The animals were about 8 weeks of age at the start of the test, and were obtained from Charles River Laboratories, Inc., Kingston, New York.

B. Test Substance: XDE-105 (87.9% a.i.) was supplied as a solid (Lot no. ACD13651).

C. Experimental design: Animals were randomly assigned to test groups as follows:

| Test Group | Dose Level (% diet)* | Number Assigned | |
|---|---|---|---|
| | | Males | Females |
| Control | 0 | 10 | 10 |
| Low dose | 0.003 | 10 | 10 |
| Low-mid dose | 0.006 | 10 | 10 |
| High-mid dose | 0.012 | 10 | 10 |
| High dose | 0.06 | 10 | 10 |

* Diets were provided *ad libitum* for 13 weeks. They were equivalent to approximately 0, 2.2, 4.3, 8.6, and 42.7 mg/kg/day for males or 0, 2.6, 5.2, 10.4 and 52.5 mg/kg/day for females.

D. Observations: The observations procedures were described in the report as follows:

> The FOB, grip performance, hindlimb landing foot splay testing and motor activity assay were conducted on 10 animals/sex/group once during the prestudy period and then monthly during the dosing period. After completion of 13 weeks of the study, a neuropathologic evaluation of central and peripheral nervous systems was conducted on 5 animals/sex/group, following whole body perfusion fixation.

The number and type of observations made in the study were summarized in the report as follows:

| | Test Periods | Number of Rats | Obs or Data Points/Rat | Total Obs or Data Points | Number of Means (±SD) |
|---|---|---|---|---|---|
| Body weight | 4 | 100 | 1 | 400 | 50 |
| Hindlimb grip | 4 | 100 | 3 | 1200 | 40 |
| Forelimb grip | 4 | 100 | 3 | 1200 | 40 |
| Landing splay | 4 | 100 | 3 | 1200 | 40 |
| Motor activity | 4 | 100 | 6 | 2400 | 280 |
| FOB (categories) | 4 | 100 | 7 categories | 2800 | NA |
| FOB (ranked obs) | 4 | 100 | 12 | 4800 | NA |
| Necropsy (# tissues) | 1 | 100 | 51 | 5100 | NA |
| Neuropath (# tissues) | 1 | 20 | 30 | 60 | NA |

Functional observational battery (FOB) parameters were described in the report as follows:

| FOB Parameter | Recorded As |
|---|---|
| <u>Measurement/Count</u> | |
| Hindlimb grip performance | grams force |
| Forelimb grip performance | grams force |
| Landing foot splay | distance between hind feet (cm) |
| <u>Hand-held Observations</u> | |
| General (thin, fat, red ocular/nasal crusts, etc.) | Description |
| Palpebral closure | Rank |
| Pupil size | Normal, increased, or decreased |
| Lacrimation (clear periocular wetness) | Rank |
| Salivation (clear perioral wetness) | Rank |
| Abnormalities of skin or haircoat | Description |
| Perianal staining | Present or absent |
| Abnormal movements (e.g., muscle tone, tremors, convulsions) | Description |
| Abnormal respiration (e.g., increased, wheezing) | Description |
| Reactivity to handling | Rank |

| FOB Parameter | Recorded As |
|---|---|
| Open-field observations | |
| Level of activity | Rank |
| Responsiveness to sharp noise | Rank |
| Responsiveness to touch | Normal, increased, or decreased |
| Responsiveness to tail pinch | Rank |
| Abnormal behavior (e.g., stereotypies, locomotor) | Description |
| Gait abnormalities | Rank |
| Urine volume voided during FOB | Rank |
| Number of fecal pellets voided during FOB | Rank |

The report described procedures for measuring grip performance as follows:

> ...the rats were selected in a random manner and given to the observer in such a way that the observer did not know the treatment status of the animal. The observer then placed the rat's forelegs on a bench and the hind feet were set on a horizontal screen attached to a strain gauge. The observer then smoothly but firmly pulled backward on the rat's tail until the rat's grip on the screen was broken...The average of three trials was used for statistical analysis. Forelimb grip performance was similarly tested. In this application, a bench was not used, and the rats were placed so that the forefeet were on a screen and the hindfeet were on a smooth horizontal plastic surface. The test sequence was the same as for hindlimb testing. Because grip performance is affected by body weight, each rat's individual grip performance (grams of pull) was divided by its body weight, resulting in a grip performance measure of grams pull/gram body weight.

The report described the procedure for determining landing foot splay as follows:

> ...the observer did not know the treatment status of the animal. The tarsal joint pad of each hindfoot was marked with ink. The animal was then dropped from a height of approximately 30 cm onto the recording sheet. This was repeated three times...The distance from center to center of the ink marks was measured and the average of the three splay values was used for statistical analysis.

Motor activity observation procedures were described in the report as follows:

> Sixteen motor activity cages (also referred to as chambers), visually isolated from each other, were located in a quiet dim room. Each motor activity cage consisted of a circular clear plastic alley...An infrared photobeam bisected the cage so that the beam crossed the alley in 2 locations.
>
> Each animal was tested individually for motor activity. All test sessions consisted of six 8-minute epochs, totalling 48 minutes of testing per animal...Total activity counts for each

epoch were recorded. Each beam break that lasted more than 100 msec constituted an activity count. This minimum duration was set to discount such activities as tail-flicking, rearing, head bobbing, etc.

Cages were calibrated prior to testing each day...any photocell showing a difference exceeding 4 centiseconds was readjusted to assure equivalence of devices.

The experimental design...is referred as (sic) a split-plot factorial design with two between block (sex and dose)treatments and two within-block (epoch and day) treatments (also known as $SPF_{pr.qr}$ design).

The procedures used to perfuse and fix nervous tissue for microscopic evaluation were described in the report as follows:

Rats (5/sex/group) were perfused intracardially with 0.05 M phosphate buffer containing 0.7% sodium nitrite, followed by a phosphate-buffered solution of 1.5% glutaraldehyde-4% formaldehyde (c. 540 mOsM). A complete gross examination was conducted on all animals...The remaining 5 rats/sex/dose were...not perfusion fixed...Tissues from these rats were fixed by immersion in neutral phosphate buffer 10% formalin.

Tissues for neuropathologic evaluation were prepared from all perfusion fixed rats in the control and high dose groups. Nine transverse sections of the brain were prepared from the olfactory lobe, cerebrum (frontal, parietal, temporal, and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and medulla oblongata. The following tissues were also prepared: trigeminal ganglion, pituitary gland, eyes with optic nerve, spinal cord (cervical and lumbar), nasal tissues with the olfactory epithelium, and skeletal muscles (gastrocnemius and interior tibial). Tissues from the central nervous system were...stained with hematoxylin & eosin...Peripheral nerves (sciatic, tibial and sural) and additional dorsal root ganglia (cervical and lumbar) were...stained with toluidine blue.

F. Statistical Analyses: Statistical methods were generally described in the report as follows:

Statistical analyses were conducted on monthly body weights, grip performance, landing foot splay, and motor activity. Grip performance data were transformed to grams pull divided by grams body weight to minimize confounding from changes in body weight. Motor activity counts were reported as their square roots to minimize problems of heterogeneity of variance and departure from normality that commonly occur from treatment. Means and standard deviations were calculated for each test period. FOB observations were evaluated by a test of proportions beginning with the greatest difference in distributions between control and high dose. Evaluation of other FOB dose levels and categories continued in a trend fashion until the first non-significant finding (i.e.,

differences between all further distributions were too small to be significant).

Because extreme departures from homogeneity of variance can affect the F ratio, the F-max test was performed ($\alpha$ = 0.01). When departure from homoscedasticity was judged too extreme by the study director, outlying data points farthest from the mean were removed (one at a time) until homoscedasticity was achieved.

Evaluation of the variance indicated a significant departure from homoscedasticity for the 13-week female hindlimb landing foot splay. This departure was considered to be due to an unusually low variance in the control group and not to a particular outlier, therefore no outlying data points were removed.

The study design had four major data collection periods; pre-exposure, weeks 4, 8 and 13. A factorial repeated measure analysis was run to account for data at all time periods. In repeated-measure tests that include a baseline data point, the inclusion of pre-exposure data in the analysis makes relevant only the analyses which include factors of both treatment and time. The following interactions were studied:

Treatment x Time -- Does treatment change the pre-existing differences between groups at any time interval? This interaction will not identify which time interval or which exposure level is different in case of statistical significance.

Treatment x Time x Sex -- Does treatment change the pre-existing differences between the sexes at any time interval?

Treatment x Time x Epoch (motor activity only) -- Does treatment change the pre-existing differences in distribution of motor activity across epochs at any time interval?

...To reduce the rate of false declarations, the type I error rate ($\alpha$) per comparison was set at 0.02...The corrections for multiple statistical analyses were applied to $\alpha$ only, and probability values were reported without correction.

The types and number of statistical tests were tabulated in the report as follows:

| Dependent variable | Type of test* | Number of primary tests |
|---|---|---|
| Body weight | Rep-ANOVA | 2 (Txd & TxdxS) |
| Grip performance | | |
| Forelimb | Rep-ANOVA | 2 (Txd & TxdxS) |
| Hindlimb | Rep-ANOVA | 2 (Txd & TxdxS) |
| Landing foot splay | Rep-ANOVA | 2 (Txd & TxdxS) |
| Motor activity | | |
| Total counts | Rep-ANOVA | 2 (Txd & TxdxS) |
| Epochs (nested by day) | Rep-ANOVA | 1 (TxdxE) |

Abbreviations: T=treatment, d=day, S=sex, E=epoch. Repeated across days. For motor activity repeated across days and across epochs.

* Rep-ANOVA (repeated-measure analysis of variance using multivariate approach and the Pillai Trace Statistic).

## Reported Results

A. Clinical signs, grip performance and landing foot splay: The report described results of FOB observations as follows:

> No treatment-related effects were seen on the monthly hand-held and open field observations. No differences were noted in gait or posture, in muscle tone, or in hindlimb extensor thrust responses. Sensory responses were judged normal, as were activity and reactivity.
>
> On week 8, spontaneous activity (Level of Activity) in the open field ranged from none to pronounced. When male and female data from week 8 were combined, moderate activity was seen in 11/20 controls, 5/20 low dose, 7/20 first mid dose, 8/20 second mid dose, and 5/20 high dose. Although no dose-response was apparent, the contrast of 11/20 for controls vs. 5/20 for high dose stimulated a *post hoc* test of proportions. When control 11/20 was compared to high dose 5/20 by a test of proportions, $z = 1.95$, $p > 0.02$. Thus, based on lack of dose-response, lack of statistical significance, and a modest expected background occurrence of reduced activity, activity in the open field was considered to be unaffected by XDE-105.

No effects were noted on grip performance or landing foot splay.

B. Body weights: According to the report, there were no statistically significant effects of XDE-105 on body weight. Group mean body weights are summarized from the report as follows:

| Dose (% diet) | Mean body weight (g) | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| | Pretest | Week 4 | Week 13 | Pretest | Week 4 | Week 13 |
| 0 | 130.8 | 242.7 | 329.2 | 91.8 | 139.4 | 177.0 |
| 0.003 | 128.4 | 227.8 | 331.8 | 91.5 | 140.0 | 176.9 |
| 0.006 | 129.4 | 237.7 | 325.1 | 95.3 | 141.6 | 163.6 |
| 0.012 | 129.7 | 238.8 | 326.0 | 92.4 | 142.0 | 184.9 |
| 0.06 | 130.4 | 239.5 | 324.8 | 91.5 | 138.7 | 179.3 |

C. Motor activity: Results of the statistical analyses of motor activity observations were discussed in the report as follows:

> The statistical data analysis of on (sic) the epoch data shows that XDE-105 did not have an effect on total motor activity counts. The genders did not react differently to treatment. The distribution of motor activity counts across epochs was not affected by treatment, i.e., there was no treatment-related change in habituation over time...
>
> Although there were no treatment-related changes in motor activity, significant gender (females were more active than males; p=0.004), epoch (activity decreased across epochs; p<0.001), and month (activity increased acrossed (sic) months; p<0.001) effects were seen unrelated to treatment (data in study file). These significant effects show that the absence of a treatment effect in this study are unlikely to be due to an overall lack of power of the system to detect a significant effect if there were one.

D. Neuropathology: Necropsy results were described in the report as follows:

> Hemorrhage involving the stomach, head, testis, and urinary bladder were noted in some of the rats. In addition, isolated occurrences of a liver hernia and a hindlimb fracture were identified. These alterations only occurred in a maximum of 1 rat/sex/dose level, with no predilection for the highest dose level and were therefore not attributed to XDE-105.

Selected incidences of histopathologic observations are summarized from the report as follows:

Spinosad (XDE-105) §82-7: Rat

| Lesion | Dose (mg/kg/day) 0 | 2000 |
|---|---|---|
| **Males** | | |
| Brain - medulla oblongata: No. examined | 5 | 5 |
| Swollen axons - gracile nucleus - very slight | 1 | 0 |
| Degeneration - individual nerve fibers, trapezoid body - very slight | 5 | 5 |
| Eyes: No. examined | 5 | 5 |
| Mineralization - blood vessels - very slight | 0 | 1 |
| Mineralization - cornea, unilateral - very slight | 4 | 2 |
| Mineralization - cornea, bilateral - very slight | 1 | 2 |
| Pituitary: No. examined | 5 | 5 |
| Swollen axons - pars nervosa - very slight | 4 | 5 |
| Cyst - anterior (pars distalis), focal | 0 | 1 |
| Spinal cord - cervical: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers - very slight | 2 | 1 |
| Spinal cord - lumbar: No. examined | 5 | 5 |
| Degeneration - individual nerve fiber - very slight | 0 | 1 |
| Spinal nerve roots: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, lumbar - very slight | 2 | 1 |
| **Females** | | |
| Brain - medulla oblongata: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers, trapezoid body - very slight | 4 | 4 |
| Eyes: No. examined | 5 | 5 |
| Atrophy - retina, unilateral - slight | 0 | 1 |
| Mineralization - cornea, unilateral - very slight | 1 | 4 |
| Mineralization - cornea, bilateral - very slight | 4 | 1 |
| Gasserian ganglia: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers - very slight | 2 | 1 |
| Peripheral nerve - optic: No. examined | 5 | 5 |
| Atrophy - unilateral | 0 | 1 |
| Pituitary: No. examined | 5 | 5 |
| Swollen axons - posterior (pars nervosa) - very slight | 2 | 2 |
| Spinal cord - cervical: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers - very slight | 3 | 1 |
| Spinal cord - lumbar: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers - very slight | 2 | 1 |
| Spinal nerve roots: No. examined | 5 | 5 |
| Degeneration - individual nerve fibers - very slight | 0 | 1 |

## Discussion

A. Authors' Conclusions: The authors' discussion of the results was reported as follows:

> XDE-105 had no effect at any time on handled and open field observations, grip performance or landing foot splay, either in males or in females. XDE-105 also did not affect any aspect of motor activity, either in males or in females. The results of the neuropathologic evaluation did not indicate that XDE-105 had any effect on the central and peripheral nervous system.
>
> The results of the present...(study) indicated...the neurotoxicological NOEL 0.06% (42.7 & 52.1 mg/kg/day for males and females, respectively).

B. Reviewer's Discussion and Conclusions: See "Executive Summary" above.

012237

Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

**This document supercedes the DER in HED Document Number 011597.**

Study Type: Developmental Toxicity
Guideline §83-3
Species: Rat

EPA Identification No.s: EPA MRID No. 43557505
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-003

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: Oral Gavage Teratology Study in Sprague-Dawley Rats

Author(s): Liberacki, A.B., W.J. Breslin, and B.L. Yano

Report Issued: February 1, 1993

Executive Summary: XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 30 mated Sprague-Dawley strain rats by gavage at dose levels of 0, 10, 50 or 200 mg/kg/day from gestation day 6 through 16 (gestation day 0 was the day sperm were found in vaginal lavage or a vaginal plug was found) (MRID 43557505). Females were observed for changes in appearance or behavior, and body weight and food consumption were determined at intervals during gestation. Animals were sacrificed on gestation day 21 and reproductive observations were made and uteri were weighed and examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

Marginal maternal toxicity was reported at the highest dose tested and was indicated by decreased body weight gain (46% less than that for the control group during gestation days 6-9 and 11% less for the day 9-12 interval), and slightly reduced body weight at Day 12 (high dose group animals weighed an average of 4% less than the control group animals). These weight differences were not noted to occur with dose-related absolute and

relative liver, kidney, heart, and spleen weight changes, and no animals were described in the report as having dose-related clinical signs.

**The NOEL for maternal toxicity is ≥200 mg/kg/day.**

There were no developmental effects that could be attributed to administration of XdE-105. **The NOEL for developmental toxicity is ≥200 mg/kg/day.**

Core Classification: This study does not, by itself, satisfy §83-3 guideline requirements for a rat developmental toxicity study, but it should be classified as acceptable. The highest dose tested (200 mg/kg/day) was not adequate, but the limited results of a range-finding study (MRID 43770702) suggested that the 200 mg/kg/day dose level used as the highest dose in this study (MRID 43557505) was not high enough to cause maternal toxicity in pregnant Sprague-Dawley rats. Based on results from a rabbit developmental toxicity study (MRID 43414521), the insufficient dose range evaluated in the definitive study should not be considered a critical deficiency. The highest dose in the rabbit study of 50 mg/kg/day approached a toxic level, which suggests that the rabbit is more sensitive to XDE-105 toxicity, and repeating the rat developmental toxicity study (MRID 43557505) would not add significantly to the data required for developmental toxicity testing.

## Materials and Methods

A. Test Animals: Adult virgin female Sprague-Dawley strain rats were used. They were approximately 9 weeks of age on arrival at the laboratory and were acclimated for at least one week. Animals selected for the study weighed 200-300 g. The animals were from Charles River Breeding Laboratories, Raleigh, NC.

B. Mating Procedures: The mating procedure was described in the report as follows:

> ...females...were bred overnight with males of the same strain (one male: one female) and vaginal lavage samples were evaluated for presence of sperm. The day on which vaginal sperm was detected or when a vaginal plug was observed *in situ* was considered Day 0 of gestation.

C. Test Substance: Technical grade XDE-105 (8806% a.i.; 76.1% factor A and 11.9% factor B) was supplied as a solid (lot no. ACD13651), and the dosages are expressed as the active ingredient.

D. Vehicle: 0.5% aqueous Methocel A4M.

E. Dose Solution: The test substance was suspended in the vehicle and was administered in a volume of 2 ml/kg body weight. Dose solutions were prepared before the start of the study and adjusted based on daily body weights during the study according to the report. Samples of each dosing solution were analyzed by HPLC at the sponsor's laboratory at the start of the study to verify the test substance's concentration (95-102% of nominal concentrations, from Tab 1 in the report). Samples of each dose solution were also analyzed after one month for stability and found to be stable.

F. Study Design: Mated animals were assigned to four groups as follows:

| Test Group | Dose Level (mg/kg/day)* | Number Assigned |
|---|---|---|
| Control | 0 | 30 |
| Low Dose | 10 | 30 |
| Mid Dose | 50 | 30 |
| High Dose | 200 | 30 |

* Doses were administered by gavage on gestation days 6 through 16.

G. Observations: The animals were observed daily for clinical signs and mortality. They were weighed on gestation days 0, 6-16, 19 and 21 during the study. Food and water consumption were determined at 3-4 day intervals beginning on gestation day 0.

On gestation day 21 surviving animals were sacrificed.

The liver, kidneys, spleen, heart and gravid uterus of each animal were removed and weighed, and the ovaries and uterus were examined to determine the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses.

Fetuses were sexed and weighed individually. The report stated that each fetus was examined as follows:

> Uteri not containing visible implantations were stained with a 10% aqueous solution of sodium sulfide and examined for evidence of early resorptions. At least one-half of the fetuses in each litter were examined immediately by dissection...for evidence of visceral alterations. The heads of rat fetuses examined by dissection were...examination by the serial technique... All fetuses were then preserved in alcohol, eviscerated, cleared and stained with alizarin red S. Skeletal examinations were conducted on all fetuses that were not given visceral examinations.

H. Statistical Analysis: The report described the methods used as follows:

> Descriptive statistics (means and standard deviations) were calculated for feed consumption. Maternal body weights, body weight gains, organ weights (absolute and relative), and fetal body weights were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, analysis by the Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed, respectively. Stastical evaluation of the frequency of pre-implantation loss and resorption among litters and the fetal population was performed using a censored Wilcoxon test with Bonferroni's

correction. The number of corpora lutea and implants, and litter size were evaluated using a nonparametric ANOVA followed by the Wilcoxon Rank-Sum test with Bonferroni's correction. Pregnancy rates were analyzed suing the Fisher Exact probability test. Fetal sex ratios were analyzed by suing a binomial distribution test. Nonpreganant females, females pregnant following staining or females having totally resorbed litters were excluded from the appropriate analyses. Statistical outliers were identified using a sequential method, but values were not excluded unless justified by sound scientific reasons unrelated to treatment.

The nominal alpha levels used were as follows:

| | |
|---|---|
| Bartlett's Test | $\alpha$=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 with Bonferroni's correction |
| Fisher's Test | $\alpha$=0.05, one-sided |
| Censored Wilcoxon Test | $\alpha$=0.05, one-sided |
| Binomial Distribution Test | $\alpha$=0.05, one-sided |
| Sequential Outliers Test | $\alpha$=0.02, two-sided |

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

I. Historical Control Data: Historical control data was compiled from 5 studies conducted over the period from September, 1988 to May, 1990. Two of those studies were done using gavage, two used the dietary route, and one used the dermal route.

## Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report indicated that none of the test animals died during the study. There were also no treatment-related signs of toxicity reported.

2. Body Weight and Food Consumption: The report noted:

> Feed consumption was not affected at any dose level tested. Water consumption of dams given 10 or 50 mg/kg/day was not affected by treatment, however, a slight increase in water consumption (60.5 g/animal/day compared with 51.1 g/animal/day in the control group, not a statistically significant difference) was noted in dams given 200 mg/kg/day after completion of the dosage regimen (Days 19-21).

Decreases in body weights were statistically identified in dams given 10 mg/kg/day on Days 6 and 9 of gestation. However, these decreases were not considered treatment related as actual treatment with XDE-105 did not begin until Day 6 of gestation, rats in this group were approximately eight grams lighter than control animals at study start, animals at higher dose levels were not affected on corresponding days, and body weight gains of dams in this dose level were not affected by treatment. No effects were observed on body weights or body weight gains of dams given 50 mg/kg/day. A significant decrease in body weight was noted on Day 12 in dams given 200 mg/kg/day. Body weight changes in dams given 200 mg/kg/day were associated with significant decreases in body weight gains on Days 6-9, 9-12, and 6-16. A significant compensatory increase was noted in dams given 200 mg/kg/day after completion of the dosing regime.

These results are summarized from the report as follows:

| | Dose level (mg/kg/day) | | | |
|---|---|---|---|---|
| Observation | 0 | 10 | 50 | 200 |
| Mean body weight (g) on gestation day | | | | |
| 0 | 264.5 | 256.6 | 261.7 | 263.6 |
| 6 | 306.9 | 296.6* | 301.6 | 304.8 |
| 9 | 323.0 | 310.8* | 315.2 | 313.4 |
| 13 | 343.2 | 311.0 | 334.9 | 329.3* |
| 16 | 374.5 | 362.2 | 366.1 | 364.8 |
| 19 | 428.9 | 413.9 | 419.9 | 414.4 |
| 21 | 469.8 | 456.6 | 465.2 | 463.8 |
| 21[a] | 350.5 | 338.1 | 345.6 | 340.9 |
| Mean body weight gain gestation days | | | | |
| 0 - 6 | 42.5 | 40.0 | 39.9 | 41.2 |
| 6 - 9 | 16.0 | 14.2 | 13.6 | 8.6* |
| 9 - 12 | 20.3 | 20.2 | 19.6 | 16.0* |
| 6 - 16 | 67.5 | 65.6 | 64.5 | 60.0 |
| 19 - 21 | 40.9 | 42.6 | 45.3 | 49.4* |
| 0 - 21 | 205.3 | 200.0 | 203.5 | 200.2 |

[a] Body weight adjusted by subtracting gravid uterine weight.
* Significantly different from controls, Dunnett's Test ($p \leq 0.05$).

3. Uterine Observations; The report indicated that there were no treatment-related effects on pregnancy rate, litter size, fetal sex ratio, and gravid uterine weight or on the

numbers of corpora lutea, implantations, resorptions, and pre- or post-implantation losses. These results are summarized from the report as follows:

| Observation | Dose level (mg/kg/day) 0 | 10 | 50 | 200 |
|---|---|---|---|---|
| Number of animals | 30 | 30 | 30 | 30 |
| Number died: | 0 | 0 | 0 | 0 |
| Non-pregnant | 3 | 2 | 1 | 3 |
| With viable fetuses at termination | 27 | 28 | 29 | 27 |
| Corpora lutea/dam | 18.3 | 18.5 | 19.1 | 19.8 |
| Implantations/dam | 17.5 | 17.4 | 17.5 | 18.0 |
| Resorptions/litter | 1.3 | 1.2 | 1.2 | 0.9 |
| Resorp./litter w. resorp. | 1.6 | 1.8 | 1.9 | 2.0 |
| Mean litter size | 16.2 | 16.1 | 16.2 | 17.1 |
| Mean gravid uterus weight (g) | 119.2 | 118.6 | 119.6 | 123.0 |
| Mean fetal body weight (g) | 5.3 | 5.4 | 5.4 | 5.2 |
| % males | 50 | 48 | 52 | 51 |

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

B. Developmental End Points: The investigators noted that malformations were seen in a total of 4 fetuses in the control (1), low (0), mid (1) and high dose (2) groups, respectively.

No skeletal variations were noted by the investigators in their discussion.

Selected skeletal data are summarized from the report as follows:

| Observation | Dose (mg/kg/day) 0 | 10 | 50 | 200 |
|---|---|---|---|---|
| No. fetuses/litters examined for alterations: | 214/27 | 220/28 | 230/29 | 223/27 |
| Skull: delayed ossification | 1/1 | 5/4 | 4/4 | 4/3 |

| Observation | Dose (mg/kg/day) 0 | 10 | 50 | 200 |
|---|---|---|---|---|
| Cervical centra: delayed ossification | 73/21 | 62/21 | 101/25 | 91/23 |
| Sternebrae: delayed ossif. | 15/9 | 15/11 | 24/13 | 25/15 |
| Thoracic centra: delayed ossification | 2/2 | 2/2 | 3/3 | 4/4 |

* Statistically significantly different from controls, $p \leq 0.05$, Fisher's Exact test.

The authors stated that the incidences of fetal effects were within historical control ranges.

## Discussion

A. Authors' Conclusions: The authors' conclusion was reported as follows:

> Administration of XDE-105 to rats via oral gavage at a dose level of 200 mg/kg/day resulted in maternal toxicity as evidenced by a statistically significant decrease in mean body weight on Day 12 and body weight gains on Days 6-16. No maternal effects were observed on dams given 10 or 50 mg/kg/day. No effects were observed on reproductive and embryonal/fetal parameters at any dose level tested.

B. Reviewer's Discussion and Conclusions: Average weight gain for the high dose group was 46% less than controls for the Day 6-9 interval and 11% less than controls for the Day 9-12 interval. For the period following dosing the weight gain for the 200 mg/kg/day animals was 4% more than the controls for the post-dosing interval (based on the mean body weights for Days 16 and 21 [uncorrected]), and the largest difference between the highest dose group and controls for post-dosing weight gain was 21% for the Day 19-21 interval (high dose group gained more than the control group animals).

Mean body weights in the 200 mg/kg/day dose group for Day 12 averaged 4% less than those in the control group animals. The mean body weights for the low and the high dose groups at Day 19 was 3% less than controls and 1% less than controls by Day 21. These results and the absence of effects on the incidence of clinical signs and organ weight effects (data not included in this DER) suggest that the weight gain results may not be toxicologically significant. Therefore, range-finding study results should be considered.

The authors noted in the introduction of the study report:

> In a pilot developmental toxicity study, XDE-105 was administered by gavage in a 10% acacia solution to mated CD female rats (5 animals per dose) at dose levels of 0, 10, 30, 100, or 300 mg/kg/day on Days 6 through 17 of gestation. Maternal toxicity as evidenced by dose-related decreases in body weight and feed consumption, was noted in animals given 100 and 300 mg/kg/day. Fetal weights were depressed at 300 mg/kg/day. However, fetal viability and morphology were not affected at any dose level tested.

The summary of the range-finding study is not sufficient for determining the adequacy of the dose range tested in the main developmental study since the effects at the highest dose tested in the main study (200 mg/kg/day) may be marginal.

# DATA EVALUATION REPORT

012237

SPINOSAD (XDE-105)

Study Type: 82-2; Probe and 21-day repeated dose dermal toxicity

Work Assignment No. 1-22A (MRID 43701502)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
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Date: 3/12/96

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Date: 3/12/96

Project Manager
William Spangler, Ph.D.

Signature: William J Spangler
Date: 3/15/96

Quality Assurance
Reto Engler, Ph.D.

Signature: Reto Engler by WJS
Date: 3/15/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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EPA Reviewer: M. Copley, DVM, DABT Roger Gardner, Date 4-2-97
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, DVM, DABT KB for, Date 5/29/97
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose Dermal Toxicity - 21-day rabbit
OPPTS Number: 870.3200 OPP Guideline Number: §82-2

DP BARCODE: D219011 SUBMISSION CODE: S492760
P.C. CODE: 110003 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-105 (Spinosad; formulated as NAF-85, 43.4% ai)

SYNONYMS: Spinosad

CITATION: Vedula, U., and B.L. Yano. 1994. NAF-85: Probe and 21-day repeated dose dermal toxicity study in New Zealand White Rabbits. Toxicology Research Laboratory; Health and Environmental Sciences; Dow Chemical Company, Midland, MI 48674. August 30, 1994. Laboratory Project No. DR-0341-0784-002 and DR-0341-0784-002R. MRID 43701502. Unpublished.

SPONSOR: DowElanco, 9330 Zionsville, Indianapolis, IN 46268.

EXECUTIVE SUMMARY:

In a repeated dose dermal toxicity study (MRID 43701502) conducted in two phases, XDE-105 (Spinosad; 43.4% ai), formulated as NAF-85, was applied to the shaved skin of New Zealand White rabbits. In Phase I, five rabbits/sex/dose received dose levels of 100, 500 or 1000 mg/kg for 6 hours/day; there were a total of 15 applications during a 21-day period. In Phase II, five females/dose received dose levels of 200, 300 or 400 mg/kg for 6 hours/day for a total of 15 applications during a 21-day period.

No rabbits died during the study. Very slight to slight hyperplasia of the gastric mucosa was observed in females treated at ≥300 mg/kg/day and males treated at 1000 mg/kg/day. Multiple dark foci of the gastric mucosa with associated histopathological findings occurred in females treated at ≥400 mg/kg/day. A majority of treated and control rabbits exhibited aggregates of reticuloendothelial cells in the dermis at the dermal site which was considered to be a background, spontaneous condition that was exacerbated by treatment. **The LOEL is 300 mg/kg/day for female rabbits and 1000 mg/kg/day for male rabbits, based on very slight to slight hyperplasia of the gastric mucosa in both sexes. The NOEL is 200 mg/kg/day for**

**females and 500 mg/kg/day for males.**

This subchronic toxicity study is classified **acceptable**, and does satisfy the guideline requirements for a subchronic oral study (82-2) in rabbits.

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





## I. MATERIALS AND METHODS

### A. MATERIALS:

1. Test Material: XDE-105
   Description: Liquid, formulated as NAF-85 (Factor A plus Factor D; 43.4% ai, [redacted] and [redacted] other ingredients)
   Lot/Batch #: B372-41
   Purity: 43.4% ai
   Stability of compound: Not provided
   CAS #: Not provided
   Structure: Not provided

2. Vehicle and/or positive control: None

3. Test animals: Species: Rabbits
   Strain: New Zealand White
   Age and weight at study initiation: "Adult" (age not reported); body weights at -1 week for Phase I males, 2128-2503 g, for Phase I females 2267-2548 g, and for Phase II females 2359-2861 g
   Source: Hazelton Research Products, Inc., Kalamazoo, MI
   Housing: Individually housed in stainless steel suspended cages with flattened tube grid floors
   Diet: 4 oz. of Purina Certified Rabbit Chow (#5322) daily
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: approximately 20 C
   Humidity: 40-60%
   Air changes: 12-15 changes/hour
   Photoperiod: 12-hour light/dark
   Acclimation period: ≥14 days

B. STUDY DESIGN:

1. In-life dates - Start: 2/21/94 End: 3/15/94

2. Animal assignment

Twenty male and 40 female rabbits were selected for use on the basis of body weight. Twenty rabbits of each sex were randomly assigned to the Phase I test groups, and 20 female rabbits were randomly assigned to the Phase II test groups, each shown in Table I, based on body weights.

TABLE 1: STUDY DESIGN.

| Test Group | Dose to Animal[a] (mg/kg) | Male | Female |
|---|---|---|---|
| Phase I | | | |
| Control | 0 | 5 | 5 |
| Low | 100 | 5 | 5 |
| Mid | 500 | 5 | 5 |
| High | 1000 | 5 | 5 |
| Phase II | | | |
| Control | 0 | --- | 5 |
| Low | 200 | --- | 5 |
| Mid | 300 | --- | 5 |
| High | 400 | --- | 5 |

[a] The high dose of 1000 mg/kg/day used in the Phase I portion of the study was selected on the basis of results from previously conducted acute studies, and the results of a probe study, included in this report. In the probe study, four male New Zealand White rabbits (2/dose) received a dermal application of undiluted NAF-85 (43.4% ai) at 500 or 1000 mg/kg for 6 hours/day for 4 consecutive days. The treated males exhibited no clinical signs of systemic toxicity or dermal irritation, consumed their entire ration of daily feed, and gained 0.05-0.25 kg body weight during the course of the study.



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3. Preparation and treatment of animal skin

The test animals were acclimated to elastic jackets, intended to hold the test material dressing in dermal contact for 6 hours/day, for 4 days prior to the first application. The study was conducted in two phases. In the Phase I portion of the study, five rabbits/sex/dose received topical applications of undiluted NAF-85 at 100, 500 or 1000 mg/kg for 6 hours/day for a total 15 applications during a 21-day period. In the Phase II portion of the study, five females/dose level received topical applications of undiluted NAF-85 at 200, 300 or 400 mg/kg 6 hours/day for a total of 15 applications during a 21-day period. In both phases, the test substance was applied to a 10- x 15-cm clipped area (approximately 10% body surface area) on the back of each rabbit and covered with a porous gauze patch that was backed by nonabsorbent cotton and held in place with an elastic jacket. The jackets and patches were removed approximately 6 hours after application. The treated area was wiped thoroughly with a water-dampened towel to remove residual test material.

Rabbits in the control group were exposed to pesticide-free distilled water in a volume similar to the amount of test material applied to the high-dose animals.

Dose amounts were adjusted weekly based on the most recent individual animal body weight. All rabbits were dosed for at least 2 days immediately prior to necropsy.

4. Statistics

The equality of means for data from the treatment groups was established using Bartlett's test of homogeneity of variances. For significant results, the data were subjected to transformations (the common log, inverse, and square root, in sequence) to obtain equality of variances, followed by a Bartlett's test after each transformation. When Bartlett's test was satisfied, no further transformation were applied, or if no transformations resulted in homogeneous variances, the transformed data or raw data with the lowest Bartlett's statistic was used. Inlife body weights were evaluated using a three-way RM-ANOVA for time (the repeated factor), sex, and dose. A Bonferroni correction was used to compensate for multiple comparisons with the control group, and was applied for the time-dose interaction. Terminal body weight, absolute and relative organ weights (excluding testes), hematological (excluding differential WBC) and clinical chemistry parameters were evaluated using a two-way ANOVA with the factors of sex and dose.

A one-way ANOVA was performed separately for each sex if the sex-dose interaction was significant. Prior to evaluation of the sex-dose interaction, Dunnett's test was conducted to compare individual dose groups to the control group if there was a statistically significant dose effect. Absolute and relative testes weights were analyzed using a one-way ANOVA; if results were significant, separate doses were compared to the controls using the Dunnett's test. Final numerical data analyses involved statistical analyses and such factors as dose-response relationships and whether the results were plausible based on other biological and pathological findings. WBC differential counts were analyzed for means and standard deviations only. Bartlett's test was conducted at the 99% confidence level; all other one- and two-way ANOVA tests were conducted at the 5 or 10%, two-sided risk levels. Three-way RM-ANOVA tests were conducted at 99, 95 or 90% confidence levels.

C. METHODS:

1. Observations

Animals were observed once daily for signs of mortality and moribundity once daily during the work week and twice daily on the weekends. A clinical examination that included evaluations of the skin, fur, mucous membranes, respiration, central nervous system function, swelling, masses, and behavior was conducted at the same time as the dermal scoring at weekly intervals and 1 day prior to necropsy. A cage-side examination of these parameters was made during daily the work week except when a clinical examination was conducted.

2. Body weight

All animals were weighed prior to the initiation of treatment and at weekly intervals during the dosing periods.

3. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on each rabbit prior to and at the termination of the study.

4. Food consumption and compound intake

Food consumption was not measured during the course of the study since rabbits "typically consume their entire ration of 4 ounces of food per day." [page 17]

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5. Blood

Blood was collected and hematology and clinical chemistry parameters were evaluated on all surviving animals at study termination. The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT)* | X | Leukocyte differential count* |
| X | Hemoglobin (HGB)* | | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC)* | | Mean corpusc. HGB conc.(MCHC) |
| X | Erythrocyte count (RBC)* | | Mean corpusc. volume (MCV) |
| X | Platelet count* | | Reticulocyte count |
| | Blood clotting measurements* | X | Cell morphology |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

b. Clinical Chemistry

| | | | |
|---|---|---|---|
| | ELECTROLYTES | | OTHER |
| X | Calcium* | X | Albumin* |
| X | Chloride* | X | Blood creatinine* |
| | Magnesium | X | Blood urea nitrogen* |
| X | Phosphorus* | | Total Cholesterol |
| X | Potassium* | X | Globulins |
| X | Sodium* | X | Glucose* |
| | | X | Total bilirubin |
| | | | Direct bilirubin |
| | ENZYMES | X | Total serum protein (TP)* |
| | | | Triglycerides |
| X | Alkaline phosphatase (ALK) | | Serum protein electrophores |
| | Cholinesterase (ChE) | | |
| | Creatine phosphokinase | | |
| | Lactic acid dehydrogenase (LDH) | | |
| X | Serum alanine aminotransferase (also ALT, SGPT)* | | |
| X | Serum aspartate aminotransferase (also AST, SGOT)* | | |
| | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.

6. Sacrifice and Pathology

All animals were sacrificed on schedule and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta | X | Brain |
| X | Salivary glands* | X | Heart | X | Periph.nerve |
| X | Esophagus* | X | Bone marrow | X | Spinal cord (3 levels) |
| X | Stomach* | X | Lymph nodes | | |
| X | Duodenum* | X | Spleen | X | Pituitary |
| X | Jejunum* | X | Thymus | X | Eyes (optic n.) |
| X | Ileum* | | | | |
| X | Cecum* | | | | |
| X | Colon* | | UROGENITAL | | GLANDULAR |
| X | Rectum* | | | | |
| XX | Liver*+a | XX | Kidneys*+a | X | Adrenal gland |
| X | Gall bladder* | X | Urinary bladder* | | Lacrimal gland |
| X | Pancreas* | XX | Testes*+ | X | Mammary gland |
| X | Appendix | X | Epididymides | X | Parathyroids |
| | | X | Prostate | X | Thyroids |
| | RESPIRATORY | | Seminal vesicle | | |
| X | | X | Ovaries | | |
| X | Trachea* | X | Uterus* | | OTHER |
| X | Lung* | X | Oviducts | | |
| X | Nose | X | Vagina | X | Bone |
| | Pharynx | X | Cervix | X | Skeletal muscle |
| X | Larynx | | | X | Skin (treated/untreated*) |
| | | | | X | All gross lesions and masses* |
| | | | | X | Mediastinal tissues |
| | | | | X | Mesenteric tissues |
| | | | | X | Oral tissues |
| | | | | X | Sacculus rotundus |

[a] Liver and kidneys were not evaluated histologically in the Phase II portion of the study due to lack of treatment-related effects in Phase I.
* Required for repeated dose dermal toxicity studies based on Subdivision F Guidelines.
+ Organ weight required in repeated dose dermal toxicity studies.

## II. RESULTS

### A. Observations

1. Toxicity - No rabbits exhibited obvious treatment-related abnormalities during the course of the study. Two 100 mg/kg females (Phase I) had scabs and/or scars at the application site after study weeks 1 or 2 due to fighting and not application of the test material. One 1000 mg/kg female (Phase II) exhibited very slight erythema at study week 2, which was attributed to removal of the dried test material and gauze patch that had adhered to the skin during application; erythema was not observed in this rabbit at study week 3.

2. Mortality - No test animals died during the course of the study.

B. Body weight and weight gain

No treatment-related effects in the final body weights and body weight gains were observed in any of the treatment groups. The decreased final body weight for the 500 mg/kg/day females on day 21, although statistically significant ($p < 0.05$), was not associated with any specific pathological alteration, and was attributed to random variability. The study authors stated that the lower body weights for the 500 mg/kg/day females "were probably secondary to the lower body weights of this dose group animals from the start of the pre-study period and not directly due to the administration of the test material." [page 24]

At the termination of the experiment, average body weights for the Phase I males were 2833 g for the control, 2926 g for the 100 mg/kg, 2982 g for the 500 mg/kg, and 2752 g for the 1000 mg/kg groups; for the Phase I females were 3109 g for the control, 3139 g for the 100 mg/kg, 2861 g for the 500 mg/kg, and 2935 g for the 1000 mg/kg groups; and for the Phase II females were 2655 g for the control, 2643 g for the 200 mg/kg, 2656 g for the 300 mg/kg, and 2635 g for the 400 mg/kg groups.

C. Ophthalmoscopic examination

No treatment-related ophthalmoscopic alterations were observed in rabbits from any of the treatment groups. One 300 mg/kg/day female (Phase II) had excessive tearing of the left eye due to conjunctivitis that was not considered to be treatment-related.

D. Blood work

1. Hematology - No treatment-related effects in hematology were observed in any of the treatment groups.

2. Clinical Chemistry - No treatment-related effects in clinical blood chemistry were observed in any of the treatment groups.

E. Sacrifice and Pathology:

1. Organ weight - No treatment-related effects in relative or absolute organ weights were observed in any of the treatment groups.

2. Gross pathology - Multiple dark foci of the gastric mucosa with associated histopathological findings were



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observed in 1/5 females from each of the 400 and 500 mg/kg/day groups and 3/5 females from the 1000 mg/kg/day group. No treatment-related alterations in gross pathology were observed in the male treatment groups or the 100, 200 and 300 mg/kg/day female treatment groups. Minor alterations observed in rabbits at various dose levels were concluded to be spontaneous lesions unrelated to treatment.

3. Microscopic pathology -

a) Non-neoplastic - Very slight to slight hyperplasia of the gastric mucosa was observed in 1/5 females from the 300 mg/kg/day group, 4 or 5/5 females from each of the 400, 500, and 1000 mg/kg/day groups, and 3/5 males from the 1000 mg/kg group (Table 2). The hyperplasia was characterized by an increased number of mitotic figures and basophilic cells in the gastric pit region and increased numbers of mucous surface cells.

TABLE 2. INCIDENCE OF HYPERPLASIA OF THE GASTRIC MUCOSA IN CONTROL AND TREATED RABBITS AT STUDY TERMINATION.[a,b]

| Treatment Rate (mg/kg/day) | Affected Animals per Total | |
|---|---|---|
| | Males | Females |
| PHASE I | | |
| 0 | 0/5 | 0/5 |
| 100 | 0/5 | 0/5 |
| 500 | 0/5 | 4/5 |
| 1000 | 3/5 | 5/5 |
| PHASE II | | |
| 0 | --- | 0/5 |
| 200 | --- | 0/5 |
| 300 | --- | 1/5 |
| 400 | --- | 5/5 |

[a] Data were obtained from Table 31, pages 86-87 and Table 33, page 89, in the study report.

[b] The severity rating was "very slight" for the 300-1000 mg/kg/day females, and "slight" for the 1000 mg/kg/day males.



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No other alterations were considered to be treatment-related. Acanthosis occurred in 4/5 females from each of the 200, 300, and 400 mg/kg/day groups, 1/5 females from the 500 mg/kg/day group, and 3/5 females from the 1000 mg/kg/day group; no other test groups exhibited acanthosis. The majority of control and treated rabbits had aggregates of reticuloendothelial cells in the dermis at the dermal site, characterized by accumulation of reticuloendothelial cells and variable numbers of heterphils immediately beneath the epidermis (Table 3). The increased incidence and severity observed in the treated rabbits compared to the controls indicated that treatment "somewhat exacerbated a background, spontaneous condition." [page 25] Similar aggregates of reticuloendothelial cells noted in untreated skin adjacent to the dermal test sites were considered to be spontaneous lesions. Aggregates of reticuloendothelial cells or degenerated muscle fibers in the muscle immediately beneath the dermal test site or the skin adjacent to the dermal test site were observed in individual Phase I test animals from the control male group, the 100 and 500 mg/kg/day male and female groups, and the 1000 mg/kg/day male group. These alterations were considered to be induced by handling of the test animals and the application of bandages that held the test material in contact with the skin, and were not observed in the Phase II test animals. [pages 25-26] Liver and kidney alterations noted in males and females from the Phase I control and 1000 mg/kg/day groups were considered to be spontaneous and not treatment-related.

TABLE 3. INCIDENCE OF AGGREGATES OF RETICULOENDOTHELIAL CELLS IN THE DERMIS AT THE DERMAL SITE OF CONTROL AND TREATED RABBITS AT STUDY TERMINATION.[a]

| Treatment Rate (mg/kg/day) | Affected Animals per Total[b] | |
|---|---|---|
| | Males | Females |
| PHASE I | | |
| 0 | 2/5 | 3/5 |
| 100 | 4/5 | 5/5 |
| 500 | 5/5 | 5/5 |
| 1000 | 5/5 | 5/5 |
| PHASE II | | |
| 0 | --- | 2/5 |
| 200 | --- | 5/5 |
| 300 | --- | 4/5 |
| 400 | --- | 4/5 |

[a] Data were obtained from Table 31, pages 86-87, and Table 33, page 89, in the study report.
[b] The severity rating was "very slight" for all affected groups, except was "slight" for 1/5 males in the 100 mg/kg/day group, 2/5 males in the 500 mg/kg/day group, and 1/5 males and 1/5 females in the 1000 mg/kg/day groups.

b) Neoplastic - No neoplastic tissue was observed in the treated or control rabbits.

## III. DISCUSSION

### A. Investigator's Conclusions

The study authors concluded that the NOEL for systemic toxicity of NAF-85 was 200 mg/kg/day for female rabbits and 500 mg/kg/day for male rabbits. Repeated dermal application at ≥100 mg/kg/day caused a minor increase in reticuloendothelial cells in the dermis of the dermal test site, and at ≥100 mg/kg/day, caused very slight thickening of the skin epidermal layer of females dosed at

≥200 mg/kg/day. Males treated at 1000 mg/kg/day and females treated at ≥300 mg/kg/day had very slight to slight hyperplasia of the stomach mucosa.

B. Reviewer's Discussion

XDE-105 (Spinosad; 43.4% ai), formulated as undiluted NAF-. 85, was applied to the shaved skin of five New Zealand White rabbits/sex/dose at dose levels of 100, 500 or 1000 mg/kg for 6 hours/day for a total of 15 application over a 21-day period. Five additional female rabbits/dose were similarly treated at 200, 300 or 400 mg/kg for 6 hours/day for a total of 15 applications over a 21-day period.

No rabbits died during the course of the study. There were no treatment-related differences in body weights or body weight gains in the treatment groups by the end of the study.

Treatment-related stomach alterations were observed in females treated at ≥300 mg/kg/day and males treated at 1000 mg/kg/day. Hyperplasia of the gastric mucosa was very slight in one 300 mg/kg/day female and four or five females from the 400, 500, and 1000 mg/kg/day females, and was slight in 3/5 of the 1000 mg/kg/day males. Multiple dark foci of the gastric mucosa with associated histopathological findings were observed in 1/5 females from each of the 400 and 500 mg/kg/day groups and 3/5 females from the 1000 mg/kg/day group. Aggregates of reticuloendothelial cells in the dermis at the dermal site occurred in the majority of treated and control rabbits; treatment exacerbated this background, spontaneous condition. Very slight acanthosis was observed at the dermal test site of a variable number of females treated at ≥200 mg/kg/day, and was not observed in any other test groups. No treatment-related effects were observed in body weights, body weight gains, hematology, blood chemistry, ophthalmological or urinalysis parameters, or organ weights or morphology.

IV. **Study deficiencies**

No significant deficiencies were noted in this study.

# DATA EVALUATION REPORT

XDE-105 (SPINOSAD)

Study Type: 83-1b; 12-Month Oral Chronic Toxicity Study in Dogs

Work Assignment No. 1-22B (MRID 43701504)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

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## Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: R.L. Gardner, ~~Ph.D.~~ Roger Gardner, Date 4-2-97
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: M. Copley, D.V.M., D.A.B.T. MC, Date 5/27/97
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity [feeding] - dogs
OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

DP BARCODE: D219011 SUBMISSION CODE: S492760
P.C. CODE: 110003 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-105 (87.2% ai)

SYNONYMS: Spinosad

CITATION: Harada, T. (1995) XDE-105: 12-Month Oral Chronic Toxicity Study in Dogs. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan. Laboratory Project Study ID IET 91-0080. January 30, 1995. MRID 43701504. Unpublished.

SPONSOR: DowElanco Division, Dow Chemical Japan Ltd, Tennoz Central Tower, 2-24, Higashi Shinagawa 2-chome, Shinagawa-ku, Tokyo 140, Japan.

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRID 43701504), XDE-105 (Spinosad, 87.2% ai) was administered to four beagle dogs/sex/dose in the diet at dose levels of 50/60, 100/120, or 300/360 ppm (1.44, 2.68, or 8.46 mg/kg/day, respectively, for males; 1.33, 2.72, or 8.22 mg/kg/day, respectively, for females) for 52 weeks.

Male beagles in the 300/360 ppm treatment group had serum levels of alanine aminotransferase 257 and 207% higher at 26 and 52 weeks, respectively; and serum levels of aspartate aminotransferase and triglycerides 147 and 132% higher, respectively, at 26 weeks than beagles in the control group; no comparable differences were observed in females in the 300/360 ppm group. Male and female beagles in the 300/360 ppm treatment groups were found to have slight vacuolated cell aggregations in lymphoid tissues (4/4 males, 2/4 females), slight to moderate inflammation of arteries in the epididymis (1/4 males) or cerebral meninges (1/4 females), and slight glandular cell vacuolation of the parathyroid (2/4 males). Although female beagles in the 300/360 ppm treatment group had absolute and relative thyroid weights that were approximately 160% higher than beagles in the control and lower dose treatment groups, no treatment-related microscopic changes were observed in these tissues. No dogs died during the study. No treatment-related

differences were observed between the clinical appearance, body weights, food consumption, ophthalmology, hematology, or urine of the treated and control animals. No definitive treatment-related differences in organ weights were observed between the treated and control animals. No gross pathological differences were observed between beagles in the treatment and control groups. All microscopic tissue abnormalities, other than those mentioned, occurred randomly and sporadically in all study groups. No neoplastic tissue was observed in beagles in the treatment or control groups. **The LOEL is 8.22 mg/kg/day (300/360 ppm), based on increases in serum alanine aminotransferase, aspartate aminotransferase, and triglycerides levels, and the presence of tissue abnormalities, including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid). The NOEL is 2.68 mg/kg/day (100/120 ppm).**

This chronic toxicity study in dogs is **acceptable** and does satisfy the guideline requirement for a chronic oral toxicity study (§83-1b) in dogs.

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

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test Material: XDE-105
   Description: Off-white to pale yellow powder
   Lot/Batch #: AGR293707
   Purity: 87.2% ai
   Stability of compound: Stable at room temperature
   CAS #: Not provided
   Structure: Not provided

2. Vehicle and/or positive control: None

3. Test animals: Species: Beagles
   Strain: Not identified
   Age and weight at study initiation: 6-7 months of age; body weights 7.5-9.2 kg for males, 8.1-10.0 kg for females
   Source: Ohito Biotec Center Inc., Shuzenji-cho, Tagata-gun, Shizuoka
   Housing: Individual stainless steel cages (835 x 900 x 800 mm) within a dog room
   Diet: powder certified DS diet, ad libitum (but restricted to 300 g/dog/day prior to 13 weeks and to 250 g/dog/day after 13 weeks)
   Water: sterilized well water, ad libitum
   Environmental conditions:
   Temperature: 24 ± 2 C

Humidity: 55 ± 10%
Air changes: 15 per hour
Photoperiod: 12-hour light/12-hour dark
Acclimation period: 3-4 weeks

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B. STUDY DESIGN

1. In life dates Start: 11/25/92 End: 12/24/93

2. Animal assignment

Dogs (16 of each sex) were assigned to the test groups in Table 1 on the basis of body weight using a computer-assisted randomization procedure.

TABLE 1. STUDY DESIGN

| Test Group | Conc. in Diet[a] (ppm) | Nominal Dose to Animal (mg/kg/day) | Animals Assigned | |
|---|---|---|---|---|
| | | | Male | Female |
| Control | 0 | 0 | 4 | 4 |
| Low | 50/60 | 1.5 | 4 | 4 |
| Mid | 100/120 | 3.0 | 4 | 4 |
| High | 300/360 | 9.0 | 4 | 4 |

[a] To prevent obesity, the amount of food provided the dogs each day was reduce at 13 weeks. To maintain the same level of treatment, the concentration of XDE-105 in the feed was increased accordingly.

3. Dose selection rationale

Dose selection was based on the results of a 13-week subchronic study with dogs (MRID 43444102; reviewed by EPA in a report dated 5/30/95) in which XDE-105 concentrations of 150, 300, and 900 (females) or 1350/900 (males) ppm were evaluated. The study author concluded that the NOEL was 150 ppm. The LOEL was 300 ppm, based on the observance of cytoplasmic vacuolation or vacuolated cell aggregation in a variety of tissues.

4. Diet preparation and analysis

The treated diet was prepared prior to the initiation of the study and every 4 weeks thereafter. Appropriate amounts of

test substance were mixed with a portion of the basal feed using a mortar, then the mixture was blended with additional feed using a mechanical mixer. The treated feed was stored in sealed plastic bags within a plastic container in the dark at 4 C. Approximately once each week, a portion of the treated feed was removed to the animal room and stored in an aluminum container at room temperature until moistened (to minimize spillage) and fed to the animals. Uneaten feed was removed and replaced daily.

To determine the stability of XDE-105 in the feed, samples were collected from the 50 ppm treatment feed that was prepared immediately prior to the initiation of feeding. The samples were stored moist at room temperature for 24 hours; dry at room temperature for 0, 5, or 8 days; or dry at 4 C in the dark for 5 weeks. To determine the homogeneity of the XDE-105/feed mixture, samples were collected from the top, middle, and bottom portions of the treated feed that was prepared immediately prior to the initiation of feeding. To confirm the concentration of XDE-105 in the treated feed, samples of the feed for each dose level were collected at each preparation interval from the middle of the mixer. The control diet was also analyzed to confirm that contamination had not occurred.

Results:

Stability Analysis (duplicate samples):
0 day (initial sample)- 48.0 ppm, 48.1 ppm
Room temperature, moistened 50 ppm
1 day - 45.4 ppm, 46.2 ppm
Room temperature, dry 50 ppm
5 days - 46.3 ppm, 46.8 ppm
8 days - 43.7 ppm, 45.8 ppm
4 C, dry 50 ppm
35 days - 46.6 ppm, 47.1 ppm
Homogeneity Analysis:
50 ppm: 46.5-49.6 ppm (average 48.0 ppm, 96% nominal)
100 ppm: 94.6-105.1 ppm (average 99.3 ppm, 99% nominal)
300 ppm: 283.5-291.7 ppm (288 ppm; 96% nominal)
Concentration Analysis (Appendix 1):
50 ppm treatment: 46.0-52.9 ppm
60 ppm treatment: 52.8-59.9 ppm
100 ppm treatment: 91.7-105.9 ppm
120 ppm treatment: 109.1-120.9 ppm
300 ppm treatment: 283.5-306.1 ppm
360 ppm treatment: 315.1-376.2 ppm

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

5. Statistics

Body weights, urine volume and specific gravity, hematology, blood biochemistry, and organ weights were analyzed using either Dunnett's or Scheffe's multiple comparison methods. Food consumption and all other urine parameters were analyzed using Mann-Whitney's U test. Clinical signs, mortality, ophthalmology, and pathology were analyzed using Fisher's exact probability test. Significance was determined at the 5 and 1% levels.

C. METHODS:

1. Observations

Animals were inspected at least once a day for signs of toxicity and mortality. Each animal was given a detailed physical examination weekly, and a detailed neurologic examination during weeks 49-50.

2. Body weight

Animals were weighed at the initiation of treatment, once each week through 13 weeks, and once every 4 weeks thereafter. Animals were also weighed before necropsy.

3. Food consumption and compound intake

Food consumption for each animal was determined daily during the dosing period. Mean daily diet consumption was calculated as g food/animal/day. Compound intake was calculated as mg food/kg body weight/day.

4. Ophthalmoscopic examination

Ophthalmoscopic exams were performed on all dogs using a direct ophthalmoscope and a portable fundus camera prior to study initiation and during weeks 26 and 52 of dosing.

5. Blood

Blood was collected prior to study initiation and during weeks 13, 26, and 52 of dosing. Samples were collected from the cephalic vein of all animals following overnight starvation. Hematology analyses were conducted on all samples; clinical chemistry analyses were conducted on all samples except those collected during week 13. The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT)* | X | Leukocyte differential count* |
| X | Hemoglobin (HGB)* | X | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC)* | X | Mean corpusc. HGB conc.(MCHC) |
| X | Erythrocyte count (RBC)* | X | Mean corpusc. volume (MCV) |
| X | Platelet count* | | Reticulocyte count |
| | Blood clotting measurements* | | |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

* Required for chronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| X | Calcium* | X | Albumin* |
| X | Chloride* | X | Blood creatinine* |
| | Magnesium | X | Blood urea nitrogen* |
| X | Phosphorus* | X | Total Cholesterol |
| X | Potassium* | X | Globulins |
| X | Sodium* | X | Glucose* |
| | | X | Total bilirubin |
| | | X | Total serum protein (TP)* |
| | ENZYMES | X | Triglycerides |
| | | | Serum protein electrophores |
| X | Alkaline phosphatase (ALK) | X | Albumin/globulin ratio |
| | Cholinesterase (ChE) | | |
| X | Creatine phosphokinase | | |
| | Lactic acid dehydrogenase (LDH) | | |
| X | Serum alanine aminotransferase (also ALT, SGPT)* | | |
| X | Serum aspartate aminotransferase (also AST, SGOT)* | | |
| X | Gamma glutamyl transferase (also GGT, GGPT) | | |
| | Glutamate dehydrogenase | | |

* Required for chronic studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from each animal (24-hour pooled sample) prior to study initiation and during weeks 13, 26, and 52 of dosing. The CHECKED (X) parameters were examined.

| | | | |
|---|---|---|---|
| X | Appearance | X | Glucose |
| X | Volume | X | Ketones |
| X | Specific gravity | X | Bilirubin |
| X | pH | X | Blood |
| X | Sediment (microscopic) | | Nitrate |
| X | Protein | X | Urobilinogen |

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta* | XX | Brain* |
| X | Buccal mucosa | XX | Heart* | X | Periph.nerve* |
| X | Tonsils | X | Bone marrow* | X | Spinal cord (3 levels)*T |
| X | Salivary glands* | X | Lymph nodes* | | |
| X | Esophagus* | XX | Spleen* | XX | Pituitary* |
| X | Stomach* | X | Thymus* | X | Eyes (optic n.)*T |
| X | Duodenum* | | | | |
| X | Jejunum* | | UROGENITAL | | GLANDULAR |
| X | Ileum* | | | | |
| X | Cecum* | XX | Kidneys*+ | XX | Adrenal gland* |
| X | Colon* | X | Urinary bladder* | X | Lacrimal gland[T] |
| X | Rectum* | XX | Testes*+ | X | Mammary gland[T] |
| XX | Liver*+ | X | Epididymides | XX | Parathyroids*++ |
| X | Gall bladder* | X | Prostate | XX | Thyroids*++ |
| XX | Pancreas* | | Seminal vesicle | | |
| | | XX | Ovaries*+ | | |
| | RESPIRATORY | X | Uterus* | | |
| | | X | Penis | | |
| X | Trachea* | | | | |
| X | Lung* | | | | |
| X | Nose (Nasal Cavity) | | | | OTHER |
| X | Pharynx | | | X | Bone* |
| X | Larynx | | | X | Skeletal muscle* |
| | | | | X | Skin* |
| | | | | X | All gross lesions and masses* |

* Required for chronic studies based on Subdivision F Guidelines
+ Organ weight required in chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

## II. RESULTS

### A. Observations

1. Mortality - No animals died during the course of the study.

2. Clinical Signs - No treatment-related differences were observed between the appearance of animals in the treatment and control groups. Epileptic convulsions were observed in two males, one in the control group and one in the 300/360 ppm group, that were littermates. One female in the control group exhibited decreased spontaneous motor activity and a bloody mucoid stool during weeks 13-14 of the study.

B. Body weight and weight gain

No treatment-related differences were observed between the body weights of the control and treated beagles during the study (Table 2). Body weights of the male beagles in the 50/60 ppm treatment group were lower than the control animals beginning about week 24, and gained statistical significance ($p < 0.05$) between 36 and 52 weeks; the weight of the four dogs in this group ranged from 10.0 to 11.2 kg (average 10.6 kg), compared to 10.3 to 12.7 kg for dogs in all other groups.

TABLE 2. AVERAGE BODY WEIGHTS AND BODY WEIGHT GAINS OF BEAGLES[a]

| Conc. in Diet (ppm) | Body Weight (kg) | | | | Total Body Weight Gain (kg) |
|---|---|---|---|---|---|
| | 0 Weeks | 13 Weeks | 28 Weeks | 52 Weeks | |
| Male | | | | | |
| 0 | 8.6 | 11.4 | 11.7 | 12.3 | 3.7 |
| 50/60 | 8.6 | 10.8 | 10.5 | 10.6* | 2.0 |
| 100/120 | 8.6 | 11.3 | 11.5 | 11.9 | 3.3 |
| 300/360 | 8.6 | 10.4 | 10.7 | 11.3 | 2.7 |
| Female | | | | | |
| 0 | 9.0 | 10.8 | 11.2 | 11.5 | 2.5 |
| 50/60 | 9.0 | 11.0 | 11.5 | 12.4 | 3.4 |
| 100/120 | 9.0 | 11.0 | 11.1 | 11.6 | 2.6 |
| 300/360 | 9.0 | 10.9 | 11.1 | 11.6 | 2.6 |

[a] Data obtained from Tables 5 and 6, pages 54-55, and Appendices 6 and 7, pages 154-157, in the study report. Body weight gains calculated by reviewer from average group weights at 0 and 52 weeks.
* Significantly different ($p < 0.05$) from the control.

C. Food consumption and compound intake

1. Food consumption - Food consumption by the treated animals was similar to pretest values and/or the control group throughout the study. In general, all animals ate all of the food that they were provided each day.

2. Compound consumption - During the study, male and female beagles ingested 89-96% of the nominal dose (Table 3).

TABLE 3: CONSUMPTION OF XDE-105[a]

| Conc. in Diet (ppm) | Nominal Dose to Animal (mg/kg/day) | Consumption of Test Substance (mg/kg/day) | |
|---|---|---|---|
| | | Male | Female |
| 50/60 | 1.5 | 1.44 | 1.33 |
| 100/120 | 3.0 | 2.68 | 2.72 |
| 300/360 | 9.0 | 8.46 | 8.22 |

[a] Data obtained from Tables 9 and 10, pages 58-59, in the study report.

D. Cphthalmoscopic examination

Nc treatment-related abnormalities of the eyes were noted during the study.

E. Blood work

1. Hematology - No treatment-related differences in hematology were observed between the treated and control beagles. At the 26-week interval, male beagles in all treatment groups had eosinophil counts of 0.1-0.2 x $10^3/mm^3$, compared to 0.5 x $10^3/mm^3$ for the controls; and female beagles in the 300/360 ppm treatment group had erythrocyte counts of 7.35 x $10^6/mm^3$, compared to counts of 6.60-6.82 x $10^6/mm^3$ for female beagles in the control and lower treatment groups. Although the 26-week differences were statistically significant, the values were within the normal ranges for these parameters.

2. Clinical chemistry - Male beagles in the 300/360 ppm treatment group had higher mean serum levels of alanine aminotransferase (glutamic pyruvic transaminase, GPT) at 26 and 52 weeks, and higher serum levels of aspartate aminotransferase (glutamic oxaloacetic transaminase, GOT) and triglycerides (TG) at 26 weeks than beagles in the control and lower dose treatment groups (Table 4). The increases were transient and were not observed at 52 weeks. No other treatment-related differences in blood chemistry were observed between the 300/360 treatment and control male beagles, and no differences were observed between the 50/60 or 100/120 treatment beagles and the control male beagles

during the study.

No treatment-related differences in blood chemistry were observed between the treated and control female beagles during the study. One female in the control group (animal 101) had an elevated level of plasma alkaline phosphatase (ALP) activity at 13 weeks only; alkaline phosphatase activity for this dog was 111, 346, 166, and 67 U/L at pretest, 13, 26, and 52 weeks, respectively.

TABLE 4. ALANINE AMINOTRANSFERASE (GPT), ASPARTATE AMINOTRANSFERASE (GOT), AND TRIGLYCERIDES IN CONTROL AND HIGH-DOSE (300/360 PPM) MALE BEAGLES.[a]

| Treatment Interval (weeks) | GPT (U/L) | GOT (U/L) | Triglycerides (mg/dL) |
|---|---|---|---|
| 0 ppm Males | | | |
| 0 | 40 | 32 | 37 |
| 26 | 44 | 34 | 41 |
| 52 | 40 | 32 | 42 |
| 300/360 ppm Males | | | |
| 0 | 47 | 32 | 39 |
| 26 | 113 | 50* | 54* |
| 52 | 83 | 35 | 39 |

[a] Data obtained from Table 17, pages 94-99, in the study report.
* Significantly different ($p < 0.05$) from the control.

F. Urinalysis

No treatment-related differences in urinalysis parameters were observed between the treated and control beagles during the study.

G. Sacrifice and Pathology

1. Organ weight - Female beagles in the 300/360 ppm treatment group had absolute and relative thyroid weights that were

higher than beagles in the control and lower dose treatment groups (Table 5). No other treatment-related differences in organ weights were observed between the 300/360 treatment and control female beagles, and no differences were observed between the 50/60 or 100/120 treatment beagles and the control female beagles during the study. No significant differences in absolute or relative organ weights were observed between male beagles in the treatment and control groups.

TABLE 5. ABSOLUTE AND RELATIVE THYROID WEIGHTS OF FEMALE BEAGLES AFTER 52 WEEKS OF TREATMENT.[a]

| Dose Level (ppm) | Thyroid Weight | |
|---|---|---|
| | Absolute (mg) | Relative |
| 0 | 1043 | 0.0092 |
| 50/60 | 1069 | 0.0088 |
| 100/120 | 1024 | 0.0088 |
| 300/360 | 1662* | 0.0143* |

[a] Data obtained from Table 22, page 110-111, in the study report.
* Significantly different ($p < 0.05$) from the control.

2. Gross pathology - No pathological differences were observed between beagles in the treatment and control groups. Lesions, tissue discoloration, and other abnormalities occurred randomly and sporadically in all study groups.

3. Microscopic pathology

a) Non-neoplastic - Tissue abnormalities, including slight vacuolated cell aggregations in lymphoid tissues, slight to moderate inflammation of arteries in the epididymis or cerebral meninges, and slight glandular cell vacuolation of the parathyroid (males only), were observed in male and female beagles in the 300/360 ppm treatment group (Table 6). Mineralization of the kidneys (papillary) was seen in most dogs of both sexes in the control and treatment groups at terminal sacrifice. All other abnormalities occurred randomly and sporadically in all study groups.

TABLE 6. TREATMENT-RELATED ABNORMALITIES OBSERVED IN MALE AND FEMALE BEAGLES RECEIVING 300/360 PPM.[a]

| Tissue Abnormalities | Number of Affected Animals/Total | |
|---|---|---|
| | Male | Female |
| Vacuolated cell aggregates: | | |
| Spleen | 1/4 | 1/4 |
| Faucial tonsil | 4/4 | 2/4 |
| Lymph node | 2/4 | 2/4 |
| Intestine | 3/4 | 0/4 |
| Arteritis: | | |
| Epididymis | 1/4 | 0/4 |
| Cerebral meninges | 0/4 | 1/4 |
| Glandular cell vacuolation: | | |
| Parathyroid | 2/4 | 0/4 |

[a] Data obtained from Tables 23 and 24, pages 112-118, and Appendices 20 and 21, pages 223-229, in the study report.

b) Neoplastic - No neoplastic tissue was observed in beagles in the treatment or control groups.

## III. DISCUSSION

### A. Investigator's Conclusions

The study author concluded that the LOEL for both sexes was 300/360 ppm, on the basis of changes in the blood chemistry indicative of hepatotoxicity and tissue abnormalities. The NOEL was 100/120 ppm.

### B. Reviewer's Discussion

Although the rationale for dose selection appears adequate, the dogs may have been able to tolerate a slightly higher dose. XDE-105 had no apparent effect on male and female beagles in the 50/60 ppm or 100/120 ppm treatment groups

(1.44 or 2.68 mg/kg/day, respectively, for males; 1.33 or 2.72 mg/kg/day, respectively, for females) and only minimal toxic effects at the highest treatment level, 300/360 ppm (8.46 and 8.22 mg/kg/day for males and females, respectively). The effect of XDE-105 on beagles at 300/360 ppm included changes in blood chemistry (GOT/GPT) that suggested hepatotoxicity, but there were no liver weight changes or correlating histologic changes. Changes in the histology of other organs, including the spleen, faucial tonsil, lymph nodes, intestine, and parathyroid were observed.

No dogs died during the study. No treatment-related differences were observed between the clinical appearance, body weights, food consumption, ophthalmology, hematology, or urine of the treated and control animals.

Male beagles in the 300/360 ppm treatment group had serum levels of alanine aminotransferase 257 and 207% higher at 26 and 52 weeks, respectively; and serum levels of aspartate aminotransferase and triglycerides 147 and 132% higher, respectively, at 26 weeks than beagles in the control group; no comparable differences were observed in females in the 300/360 ppm group. Alanine aminotransferase, aspartate aminotransferase, and triglyceride levels in female beagles in the 300/360 ppm treatment group were not significantly different than beagles in the control and other treatment groups. No other differences in blood chemistry between the treated and control animals were noted.

Three of four female beagles in the 300/360 ppm treatment group had absolute thyroid weights of 1617-2146 mg and relative thyroid weights of 0.0151-0.0170, compared to absolute weights of 791-1435 mg and relative weights of 0.0060-0.0121 for beagles in the control and lower dose treatment groups. No related microscopic changes were observed in the thyroids of the affected animals. No differences were observed between the thyroids of male beagles in the treatment and control groups. No other differences in organ weights were observed between the treated and control animals.

No gross pathological differences were observed between beagles in the treatment and control groups. Male and female beagles in the 300/360 ppm treatment groups had slight vacuolated cell aggregations in lymphoid tissues (4/4 males, 2/4 females), slight to moderate inflammation of arteries in the epididymis (1/4 males) or cerebral meninges (1/4 females), and slight glandular cell vacuolation of the parathyroid (2/4 males). All microscopic tissue abnormalities, other than those mentioned, occurred randomly and sporadically in all study groups. No neoplastic tissue

was observed in beagles in the treatment or control groups.

The histologic changes at the highest dose in the 12-month study (8.22-8.46 mg/kg/day) closely resembled the histologic changes seen at the mid-dose (9.7-10.5 mg/kg/day) in a previous subchronic study with beagles (MRID 43444102) that was discussed by the chronic study author. A review of the 13-week subchronic study was provided to the Dynamac reviewers by OPPTS. It appeared that at this treatment level (approximately 8-10 mg/kg/day), increasing the dosing period from 13 weeks to 12 months did not intensify the severity of the lesions.

It was reported that the dogs in the 12-month study were examined by a Functional Observation Battery at 48-49 weeks and found normal (data not provided in this MRID). In the 13-week study, doses that were clearly toxic (45 mg/kg/day for 5.5 weeks followed by 33 mg/kg/day for 7.5 weeks in males, and 30 mg/kg/day for 13 weeks in females) resulted in death (1/4 males); marked weight loss (both sexes); decreased spontaneous motor activity (2 males); and vacuolation of nerve cells in the cervical spinal cord (4/4 males), the thoracic and lumbar spinal cords (2/4 males and 3/4 females), and the cerebellum and pons (1/4 females).

The LOEL is 8.22 mg/kg/day (300/360 ppm), based on increases in serum alanine aminotransferase, aspartate aminotransferase, and triglycerides levels, and the presence of tissue abnormalities, including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid). The NOEL is 2.68 mg/kg/day (100/120 ppm).

## IV. Study deficiencies

No significant deficiencies were noted in this study.

012237

# DATA EVALUATION REPORT

SPINOSAD

Study Type: 83-2 (B); Oncogenicity Study in Mice

Work Assignment No. 1-22C (MRID 43701505)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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## Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Roger Gardner Roger Gardner, Date 7/22/96
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Pamela Hurley Pamela McHurley Date 6/20/96
Review Section I, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Study in Mice

OPPTS Number: 870.4200 OPP Guideline Number: §83-2 (b).
DP BARCODE: D219011 SUBMISSION CODE: S492760
P.C. CODE: 110003 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-105 (88.0% a.i.; 76.1% Factor A+11.9% Factor D)

SYNONYMS: Spinosad (Factor A + Factor D)

CITATION: 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. MRID 43701505. Unpublished.

SPONSOR: DowElanco, Indianapolis, ID

EXECUTIVE SUMMARY:

In a mouse oncogenicity study (MRID 43701505), 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 0, 0.0025, 0.008, or 0.036% in the diet (0, 25, 80, or 360 ppm which is 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). Two satellite groups of 10 mice/sex/group were included for sacrifice at 3 and 12 months. The high-dose females (0.036% XDE) were terminated on day 455 (approximately 15 months) of the study because of marked body weight loss and excessive mortality.

At the highest dietary level (0.036%/360 ppm XDE-150), the mortality rate for females at 65 weeks was 30/50 (60%) compared to 10% for controls and in males at 80 weeks was 21/48 (40%) compared to 24% in controls. At 50 weeks, mean body weights in both sexes were about 10% lower than controls in both sexes and mean cumulative weight gains were 37% lower. Decreased amounts of body fat were clinically observed in both sexes at 0.036%. At 3 and 12 months, hemoglobin and hematocrit values were significantly decreased in in high-dose males and significantly decreased in high-dose females at 3 months. An increased incidence of thickened glandular mucosa of the stomach was seen in both sexes and in males this correlated histologically with an increased incidence of mucosal inflammation and increased severity and incidence of hyperplasia of the glandular gastric

mucosa.

No important effects of dosing were observed in the low- and mid-dose groups.

**Based on the decreased weight gains, increased mortality, the hematologic effects, and the gross finding of increased thickening of the gastric mucosa in females and the histologic changes in the stomach of males, the systemic LOEL was established as 0.036% or 360 ppm which is equivalent to 50.9 mg/kg/day in male mice and tentatively at 67.0 mg/kg/day in females. The NOEL is 0.008% (80 ppm), which is equivalent to 11.4 mg/kg/day for males and 13.8 mg/kg/day for females.**

Under the conditions of this study, there was no evidence of carcinogenic potential.

Dosing was considered adequate in males based on an increased incidence and severity of hyperplasia and inflammation of the stomach mucosa at the highest dose level. In females the highest dose was excessive and, therefore, inappropriate to assess the carcinogenic potential and the mid-dose (0.008% or 80 ppm) showed no toxicity. In addition, the study authors did not conduct microscopic examinations of the high dose females that either died prior to terminal sacrifice or were sacrificed early. Therefore, the submitted study is classified as supplementary and does not satisfy the guideline requirements for a carcinogenicity study [§83-2 (b)] in mice. The study may be upgraded when data from an ongoing Supplemental Study are provided. An EPA memo (ID 282443) dated 1/5/94 (Study pages 862-866) indicated that the Sponsor has initiated a supplemental study using 60 mice/sex/dose at 0.00%, 0.0008% and 0.024% of XDE-105 in the diet.

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



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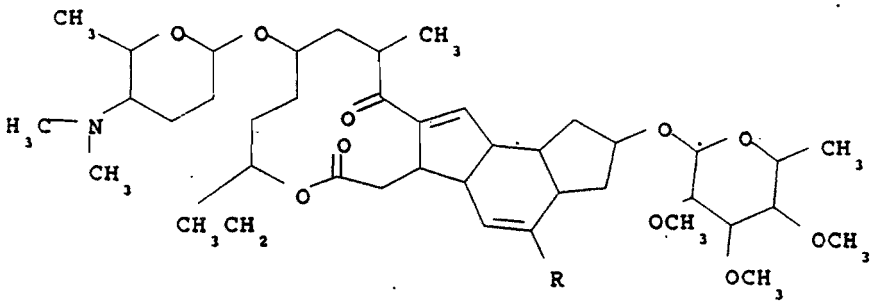
## I. MATERIALS AND METHODS

### A. MATERIALS:

1. Test Material: XDE-105 (Mixture of Factors A and D)
   Description: Technical; white solid
   Lot/Batch #: ACD 13651; TSN AGR 293707
   Purity: 88.0% a.i. (76.1% Factor A + 11.9% Factor D)
   Stability of compound: Stable in storage under unspecified conditions for up to 16 months.

   CAS #: Not provided

   FACTOR A - R = H; FACTOR D - R- $CH_3$



2. Vehicle and/or positive control: Diet

3. Test animals: Species: Mouse
   Strain: CD-1
   Age and weight at study initiation: 5-6 weeks
   Source: Charles River Laboratories, Portage, MI
   Housing: Animals were housed one/cage in suspended stainless steel cages with wire-mesh floors.
   Diet: Purina Certified Chow #5002, ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21.6 C
   Humidity: 52.7%
   Air changes: 10-12/hr
   Photoperiod: 12 hr dark/12 hr light
   Acclimation period: 14 days

B. STUDY DESIGN:

1. In life dates - Start: 9/25/92 End: 3/29/94

2. Animal assignment

Animals were assigned to treatment groups as indicated in Table 1 using a set of computer-generated random numbers based on body weights.

TABLE 1: STUDY DESIGN

| Test Group | Concentration in Diet (%) | Dose to Animals (mg/kg/day) M/F [a] | Number of Animals | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Main Study 18 Months | | Interim Sacrifice (Months) 3 | | 12 | |
| | | | M | F | M | F | M | F |
| Control | 0 | 0 | 50 | 50 | 10 | 9 | 10 | 10 |
| Low | 0.0025 (25 ppm) | 3.4/4.2 | 50 | 50 | 10 | 10 | 10 | 10 |
| Mid | 0.0080 (80 ppm) | 11.4/13.8 | 50 | 50 | 10 | 10 | 10 | 10 |
| High | 0.0360 (360 ppm) | 50.9/67.0 | 50 | 50 | 10 | 10 | 10 | 10 |

a Data were extracted from study report, Table 1, page 54.

3. Dose Selection: The rational for dose selection was based on results from a previously reviewed subchronic study on CD-1 mice (MRID 43566602). Following 90 days of exposure of mice to 0.045% XDE-105, body weight differences and many changes in clinical chemistry and hematologic parameters involving multiple organs were detected. Because the effects progressed during the 13 weeks, the potential existed that the high-dose proposed for the chronic study would have an effect on survivability.

Based upon the results of the subchronic study, 0.036% (360 ppm) was selected as a high-dose for the subsequent oncogenicity study in mice. Low- and mid-dose levels chosen were 0.0025% (25 ppm) and 0.0080% (80 ppm), respectively.



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4. Diet Preparation and Analysis: Premixes and test diets were prepared every three to four weeks and stored at room temperature. Prior to the start of the study, homogeneity was tested in samples taken from each of three levels (top, middle, and bottom) of test formulations prepared at low (0.0025%) and mid (0.008%) concentrations. During week 5 of the study, homogeneity of the 0.0025% diet was tested again. A stability assay was performed on a formulation prepared at 0.003% for a previously conducted study. Stability was determined after 14, 21, 28, and 40 days of storage in rodent chow (temperature was not reported). Concentration analyses were performed on all diets once prior to the start of the study and during weeks 8, 17, 20, 25, 37, 49, 63, and 75.

   Results: Homogeneity Analysis: The concentrations for the 0.0025% and 0.008% diets were 74-114% of the intended concentrations with relative standard deviations of 5.46-11.81%.

   Stability Analysis: The concentration of the test substance over a 40 day period was 92-97% of the initial concentration.

   Concentration Analysis: The concentrations of the analyzed diets (all dose levels) were 83-119% of the intended concentrations.

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

5. Information on how often animals received fresh diet was not provided.

6. Statistics - Bartlett's test for equality of variances was applied to body weight, organ weight, appropriate hematology, and blood chemistry data. This was followed by an analysis of variance and Dunnett's test or the Wilcoxon Rank-Sum test. Differences in mortality were tested by the Gehan-Wilcoxon procedure. Linear trends were evaluated using the Cochran-Armitage Trend test, and if found to be significant, were followed by a pairwise chi-square test with Yate's continuity correction.

C. METHODS:

1. Observations:

Animals were observed twice daily for signs of toxicity and mortality. Clinical examinations were performed once a week to observe for changes in skin, fur, mucous membranes, respiration, central nervous system function, and behavior. The appearance and progress of palpable masses were recorded during these weekly examinations.

2. Body weight

Animals were weighed at weekly intervals for the first 13 weeks and monthly thereafter.

3. Feed consumption and compound intake

Feed consumption for each animal was determined weekly for the first 13 weeks and for approximately a one week period of each month thereafter. Feed efficiency was calculated using mean body weight gain and mean feed consumption data from the first 13 weeks of the study. Compound intake values were calculated as time-weighted averages from the mean feed consumption, mean body weight, and the targeted concentration in the diet.

4. Ophthalmoscopic examination

The eyes of all the animals were examined prior to initiation of the study. Ophthalmoscopic examinations were also performed prior to the three scheduled necropsies.

5. Blood Analyses

Blood was collected for hematology and clinical analyses from all the mice prior to sacrifice at the 3- and 12-month interim sacrifices and from 20 mice/sex/group one week prior to the terminal sacrifice at 18 months. Prior to blood sampling, animals were anesthetized with methoxyflurane. The following CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT) | X | Leukocyte differential count |
| X | Hemoglobin (HGB) | | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC) | | Mean corpusc. HGB conc.(MCHC) |
| X | Erythrocyte count (RBC) | | Mean corpusc. volume (MCV) |
| X | Platelet count | | Reticulocyte count |
| | Blood clotting measurements | | |
| | (Thromboplastin time) | | |
| | (Thromboplastin time) | | |
| | (Clotting time) | | |
| | (Prothrombin time) | | |

b. Clinical Chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| X | Calcium | X | Albumin |
| X | Chloride | X | Blood creatinine |
| | Magnesium | X | Blood urea nitrogen |
| X | Phosphorus | X | Total Cholesterol |
| X | Potassium | | Globulins |
| X | Sodium | X | Glucose |
| | ENZYMES | X | Total bilirubin |
| X | Alkaline phosphatase (ALK) | X | Total serum protein (TP) |
| | Cholinesterase (ChE) | X | Triglycerides |
| | Creatine phosphokinase | | Serum protein electrophoresis |
| | Lactic acid dehydrogenase (LDH) | | |
| X | Serum alanine aminotransferase (also SGPT) | | |
| X | Serum aspartate aminotransferase (also SGOT) | | |
| | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

6. Urinalysis

Data on urinalyses were not submitted. These data are not required for carcinogenicity studies based on Subdivision F.

7. Sacrifice and Pathology

All animals that died or were sacrificed in a moribund condition and those sacrificed on schedule were subjected to gross pathological examination. Microscopic examinations were conducted on the control and high dose groups at 3 and 12 months (males and females) and at 18 months (males). For females, microscopic examinations were conducted on the control and mid-dose group at 18 months. These examinations were not conducted on any high dose females that either died or were sacrificed at approximately 15 months. For the animals that were examined above, the CHECKED

(X) tissues in the following table were collected for histological examination. Additionally, the (XX) organs were weighed. The tissues collected from the high-dose females prematurely sacrificed at approximately 15 months were preserved for possible future examination. In addition to microscopic examinations of the control and high dose animals, the following tissues and organs were microscopically examined in the low and mid-dose groups: cervix (♀), epididymides (♂), kidneys, liver, lungs, mesenteric and mediastinal lymph nodes, ovaries (♀), oviducts (♀), pancreas, parathyroids, skeletal muscle, spleen, stomach, thymus, tongue, uterus (♀), vagina (♀) and gross lesions.

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta | XX | Brain |
| X | Salivary glands | XX | Heart | X | Periph.nerve |
| X | Esophagus | X | Bone marrow | X | Spinal cord (3 levels) |
| X | Stomach | X | Lymph nodes | X | Pituitary |
| X | Duodenum | XX | Spleen | X | Eyes (optic n.) |
| X | Jejunum | X | Thymus | | |
| X | Ileum | | | | GLANDULAR |
| X | Cecum | | UROGENITAL | X | Adrenal gland |
| X | Colon | XX | Kidneys | X | Lacrimal gland |
| X | Rectum | X | Urinary bladder | X | Mammary gland |
| XX | Liver* | XX | Testes | X | Parathyroids |
| X | Gall bladder | X | Epididymides | X | Thyroids |
| X | Pancreas | X | Prostate | | |
| | | X | Seminal vesicles | | OTHER |
| | RESPIRATORY | X | Ovaries | X | Bone |
| X | Trachea | X | Uterus | X | Skeletal muscle |
| X | Lung | | | X | Skin |
| X | Nose | | | X | All gross lesions and masses |
| | Pharynx | | | | |
| X | Larynx | | | | |

* The (XX) organs were weighed.

## II. RESULTS

### A. Observations

1. Toxicity - The 0.036% females were terminated at approximately 15 months because of their increased mortality, decreased body weight gain, and general debilitated condition (roughened fur, perineal soiling, dermatitis of the ear, and lacrimation). An increased incidence of perineal soiling was observed in the 0.036% males. There were no clinical observations related to dietary levels of 0.0025 or 0.008% XDE-105.

2. Mortality - Table 2 summarizes the data on mortality and percent survival. At approximately 15 months of the study, survival rates for the high-dose females was 40% (90% for the concurrent controls). This survival rate was less than the FIFRA guideline requirement of 50% for a 15-month interval. Because of the increased mortality rate, marked body weight gain deficits, and their debilitated condition, the surviving females from this group were sacrificed on day 455 of the study.

No significant differences were observed in survival rates in male mice in any of the treated groups nor in the low- and mid-dose female groups throughout the study when compared to the respective control groups. At 18 months, survival rates ranged from 56-71% in males and 74-87% in females.

Table 2. Mortality of Mice Fed spinosad for 80 Weeks. [a]

| Cumulative Mortality | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Weeks | Males | | | | Females | | | |
| Dose (ppm) | 0 | 20 | 80 | 360 | 0 | 20 | 80 | 360 |
| 28 | 0/50 | 1/48 | 1/49 | 1/48 | 1/50 | 1/50 | 0/48 | 4/50 |
| 36 | 1/50 | 1/48 | 2/49 | 2/48 | 1/50 | 1/50 | 0/48 | 6/50 |
| 40 | 1/50 | 1/48 | 2/49 | 1/48 | 1/50 | 1/50 | 1/48 | 8/50 |
| 52 | 3/50 | 1/48 | 5/49 | 2/48 | 3/50 | 2/50 | 1/48 | 18/50 |
| 60 | 3/50 | 8/48 | 7/49 | 4/48 | 5/50 | 4/50 | 1/48 | 24/50 |
| 68 | 4/50 | 12/48 | 9/49 | 9/48 | 6/50 | 7/50 | 3/48 | 30/50 |
| 80 | 12/50 | 17/48 | 14/49 | 21/48 | 9/50[b] | 13/50 | 6/48 | -- |

a Data extracted from study report (Table 7, pp. 61-62). Values are the number dead over total number of animals. Animals dying accidentally were not included.
b Values for the control, low and mid-doses are for week 79; high-dose females were terminated on day 455.

B. Body weight-At the 3-, 12-, and 18-month intervals, the mean body weights of the high-dose males were lower than the controls (90-95% of the controls; $p < 0.05$ at the 18-month interval). At the 12-month interval, mean body weight of the high-dose females was 9% less than the control value ($p < 0.05$).

Mean body weight gain data at selected intervals are summarized in Table 3. From day 19 of dosing and throughout the dosing period, mean body weight gain of



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the high-dose males were ~21-50% lower than the concurrent controls ($p < 0.05$). From the beginning of dosing, mean body weight gain of the high-dose females was also lower than the concurrent controls, but the differences were not statistically significant until much later in the study (day 182). From day 182 of dosing until day 455, when the high-dose females were sacrificed, mean body weight gains of the high-dose females were ~20-42% lower than the controls. The decreased body weight gains in the high-dose animals was judged to be treatment related.

At 0.008 and 0.0025%, mice of both sexes did not exhibit treatment-related decreases in mean body weights compared to the concurrent controls.

Table 3. Mean body weight gain (g) at selected intervals in mice fed XDE-105 for up to 18 months. [a]

| Males | | | | |
|---|---|---|---|---|
| Days of dosing | Dietary Level (%) | | | |
| | 0 | 0.0025 | 0.0080 | 0.0360 |
| -1 | 28.3 | 28.9 | 28.9 | 29.6 |
| 7 | 29.9 (1.5) | 30.3 (1.4) | 30.1 (1.2) | 29.6 (1.0) * |
| 19 | 31.3 (3.0) | 31.8 (3.0) | 31.3 (2.4) * | 30.1 * (1.5) * |
| 33 | 33.1 (4.8) | 33.2 (4.4) | 33.0 (4.2) | 32.0 * (3.4) * |
| 89 | 36.8 (8.5) | 36.7 (7.9) | 36.7 (7.8) | 34.9 * (6.2) * |
| 347 | 39.4 (11.2) | 40.3 (11.3) | 39.5 (10.6) | 35.8 * (7.1) * |
| 403 | 39.2 (11.1) | 39.6 (10.6) | 39.8 (10.9) | 35.5 * (6.9) * |
| 543 | 38.1 (10.0) | 40.1 (11.2) | 38.8 (9.8) | 34.4 * (5.7) * |
| **Females** | | | | |
| Days of dosing | Dietary level (%) | | | |
| | 0 | 0.0025 | 0.0080 | 0.0360 |
| -1 | 22.1 | 22.5 | 22.4 | 22.5 |
| 19 | 24.8 (2.7) | 25.2 (2.7) | 25.0 (2.5) | 24.7 (2.2) |
| 33 | 25.8 (3.6) | 26.1 (3.6) | 25.9 (3.4) | 25.7 (3.2) |
| 89 | 28.5 (6.3) | 29.1 (6.6) | 29.1 (6.6) | 28.5 (6.0) |
| 182 | 30.6 (8.5) | 31.3 (8.8) | 31.0 (8.6) | 29.2 * (6.7) * |
| 347 | 32.5 (10.4) | 33.5 (11.0) | 32.9 (10.5) | 29.2 * (6.6) * |
| 403 | 32.6 (10.5) | 34.0 (11.4) | 33.7 (11.2) | 29.1 * (6.3) * |
| 431 | 32.4 (10.4) | 33.4 (10.9) | 33.3 (10.8) | 28.8 * (6.0) * |
| 543 | 33.1 (11.0) | 34.8 (12.3) | 34.4 (12.0) | --[b] |

a Numbers listed parenthetically represent the mean body weight gain calculated from -1 day of dosing. These data were extracted from study report, Tables 14 and 15, pages 77-86.
b High dose females were sacrificed on day 455 due to excessive mortality.
* Significantly different from controls ($p < 0.05$)

C. Food consumption and compound intake

1. Food consumption - Food consumption for the high-dose males was lower (89-98%) than the concurrent controls from dose day 21 to day 312; from day 333 through the remainder of the study (day 536), food consumption was comparable to the controls. Food consumption for the high-dose females was 95-132% of the concurrent controls up to day 147 of the study; thereafter, from day 168 to day 452, food consumption was lower than the controls (83-91%). Food consumption for both sexes receiving 0.0025 or 0.008% was similar to the controls. No statistical analyses were made on the food consumption data.

2. Compound consumption - Time-weighted average compound consumption in mg/kg/day are summarized in Table 1.

3. Food efficiency - Food conversion efficiency, calculated through study day 91, was similar for all groups.

D. Ophthalmoscopic examination - There were no treatment-related effects involving the eyes of any of the dosed animals.

E. Blood analyses:

1. Hematology - Group mean values for hemoglobin and hematocrit were decreased in the 0.036% males at the three scheduled sacrifices; values were significantly ($p < 0.05$) different at the 3- and 12-month intervals (82-88% of the controls). In the high-dose females, hemoglobin and hematocrit, were 91-92% of the controls ($p < 0.05$) at the 3-month interval. The decreases in hematocrit and hemoglobin at the 3-month interval were corroborated by histopathological changes in the spleen. At the 12-month interval, group mean values for white blood cell counts in the high-dose animals were 2.3-2.5 times greater ($p < 0.05$) than in the controls. In addition, at the 12-month interval, a decrease in percent eosinophils (57% of controls) and in lymphocytes (78% of controls) and an increase in percent neutrophils and monocytes (1.6 times greater than the controls) was detected in the high-dose females. At the terminal sacrifice (18-months), moderate hypochromasia of erythrocytes was observed in the majority of the high-dose male mice. The

statistical significance of this morphological change was not determined, but was considered to be treatment related.

Differences observed in hematology in the 0.0025% and 0.0080% groups were judged not to be treatment-related because of a lack of dose response and histopathological correlation.

2. Clinical Chemistry - Several blood chemistry parameters were statistically different from the controls. Except for the increased activity detected in aspartate aminotransferase in the high-dose males at the 3-month interval, the minimal to moderate chemistry changes were not considered to be treatment-related due to a lack of histopathological correlation, reproducibility at later intervals, and dose response. At the 3-month interval, aspartate aminotransferase activity was 50% higher than concurrent controls in the high-dose males ($p < 0.05$). At the terminal sacrifice (18 months), enzyme activity in the high-dose males continued to be higher (20% greater than controls); this was not statistically significant. The increased enzyme activity in the high-dose males was considered to be related to the myopathy detected in these mice.

F. Sacrifice and Pathology:

1. Organ weights- At the 3-month interval, absolute and relative mean spleen weights in the high-dose males were 64% and 76% greater than the controls, respectively ($p < 0.05$). In the high-dose females at this same interval, absolute and relative spleen weights were 56% and 47% greater than the controls ($p < 0.05$), respectively. These findings were correlated to increased extramedullary hematopoiesis.

At the 3-month interval, absolute and relative mean liver weights were 38% and 32% greater than controls, respectively, ($p < 0.05$), in the high-dose females; at the 12-month interval, relative weights were 27% greater than controls ($p < 0.05$). At the 3, 12-, and 18-month intervals, mean relative liver weights in the high-dose males were 5-21% higher than concurrent controls ($p < 0.05$). No corroborative histopathological lesions were observed in the liver.

The following were not attributed to treatment: the higher relative mean brain weights (13-27% greater than controls; $p < 0.05$) observed in the high-dose males at the 18-month interval and in high-dose females at the 12-month interval, the lower mean absolute heart weights (90% of controls, $p < 0.05$) in the high-dose males at the 18-month interval, and the lower relative mean brain weights of low dose females observed at the 18-month interval (93% of controls, $p < 0.05$) were attributed to lower body weight. At the 18-month interval, the relative mean brain weights of the low dose females was significantly ($p < 0.05$) lower than controls, but a dose response was not observed.

2. Gross pathology - At the 3-month sacrifice, there were no treatment-related gross necropsy findings.

At the 12-and 18-month intervals, treatment-related gross necropsy findings were observed in the stomach mucosa and in generalized body fat content. At the 12-month sacrifice, 6/10 high-dose males and females each had diffuse thickening of the stomach glandular mucosa compared to 0/20 controls. At the 18-month sacrifice (males) and at the day 455 sacrifice (females), including animals that died prior to the terminal sacrifice, 31/50 high-dose males compared to 5/42 controls, and 32/50 high-dose females compared to 2/50 controls were also observed to have thickening of the stomach mucosa. These gross necropsy findings were confirmed by histopathological changes in the stomach mucosa including hyperplasia and inflammation. As a note, the high dose females that either died prior to terminal sacrifice or were sacrificed on day 455 were not microscopically examined and thus do not support this statement. At the 12-month sacrifice, a decrease in fat was observed in 8/10 high-dose females and 2/10 males compared to 0/20 controls. At the terminal sacrifice, a decrease in fat was observed in 24/50 high-dose males (11/49 controls) and 21/50 high-dose females (6/44 controls). Statistical analyses of the data were not performed.

Other necropsy findings observed both in the control and treated groups occurred with comparable frequency and are commonly seen in this strain/age of mice.

3. Microscopic pathology -

a) Non-neoplastic - Treatment-related non-neoplastic lesions in CD-1 mice fed XDE-105 for up to 18 months are presented in Tables 4 through 6. An increased incidence of hyperplasia and inflammation of the stomach was observed in animals treated at the 0.036% level. The severity of the stomach lesions increased with time. Splenic extramedullary hematopoiesis was increased at 3 months in the high-dose animals as a response to a transient hypochromic anemia. In addition, in animals treated at the 0.036% level, an increased incidence of vacuolation and/or degeneration/inflammation was observed in the following organs: kidneys, lungs, lymph nodes, pancreas, parathyroid glands, skeletal muscle, tongue, epididymides, ovaries, and uterus. The kidney lesions were most prominent at 3 months in the high-dose males and did not increase by the 18-month interval. The vacuolation in the multiple organs was only slight and the severity of the lesions did not increase with time.

Other non-neoplastic lesions, such as the vacuolation of the cervix and vagina in the high-dose females at the 3- and 12- month intervals, and the increased frequency of corneal mineralization in the mid-dose females at the 18-month interval were not considered to be toxicologically significant or treatment-related due to a lack of dose-response trend or because they were similar to those commonly seen in aging/aged mice.

There were no treatment related histopathologic changes in mice from the 0.0025% and 0.008% groups.

Table 4. Treatment-related non-neoplastic lesions in CD-1 mice fed XDE-105 for up to 3 months. [a]

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Group (%) | 0 | 0.0025 | 0.0080 | 0.036 | 0 | 0.0025 | 0.0080 | 0.036 |
| Number Examined | 10 | 10 | 10 | 10 | 9 | 10 | 10 | 10 |
| **Site** Lesion | | | | | | | | |
| **Kidneys:** Degeneration /regeneration of tubules (as) [b] | 0 | 0 | 2 | 10 | 2 | 3 | 1 | 7 |
| **Lungs:** Aggregates of alveolar macrophages (as) | 2 | 1 | 1 | 9 | 0 | 1 | 1 | 9 |
| **Lymph Nodes:** Vacuolation, macrophages (s) | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 7 |
| Sinus histiocytosis | 1 | 0 | 0 | 8 | 0 | 0 | 0 | 10 |
| **Pancreas:** Vacuolation, acini (as) | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 4 |
| **Parathyroid:** Vacuolation (s) | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 8 |
| **Skeletal muscle:** Myopathy (as) | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 |
| **Spleen:** Extramedullary hematopoiesis | 1 | 2 | 0 | 6 | 1 | 1 | 1 | 5 |
| **Stomach:** Hyperplasia (as) | 3 | 1 | 3 | 9 | 0 | 1 | 3 | 10 |
| Inflammation (as) | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 7 |
| **Tongue:** Myopathy (s) | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 4 |
| **Epididymides:** Vacuolation, epithelial cells (s) | 0 | 0 | 0 | 9 | NA [c] | NA | NA | NA |
| **Ovaries:** Vacuolation (s) | NA | NA | NA | NA | 0 | 0 | 0 | 7 |
| **Uterus:** Vacuolation, mucosa (s) | NA | NA | NA | NA | 0 | 0 | 0 | 9 |

a These data were extracted from study report, Table 61, pages 164-175.
b Severity reported as very slight (vs), slight (s), any severity (as) by study authors.
c NA= not applicable.



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Table 5. Treatment-related non-neoplastic lesions in CD-1 mice fed XDE-105 for up to 12 months. [a]

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Group (%) | 0 | 0.0025 | 0.0080 | 0.036 | 0 | 0.0025 | 0.0080 | 0.036 |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Site** Lesion | | | | | | | | |
| **Kidneys:** Tubule degeneration /regeneration (as) [b] | 6 | 5 | 4 | 10 | 6 | 3 | 4 | 7 |
| **Lungs:** Aggregates of alveolar macrophages (as) | 3 | 4 | 3 | 9 | 2 | 1 | 1 | 9 |
| **Lymph Nodes:** Sinus histiocytosis | 1 | 1 | 0 | 8 | 1 | 0 | 2 | 5 |
| **Pancreas:** Vacuolation, acini (s) | 1 | 0 | 0 | 7 | 2 | 0 | 0 | 8 |
| **Parathyroid:** Vacuolation (s) | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 7 |
| **Skeletal muscle:** Myopathy (s) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| **Stomach:** Hyperplasia, glandular mucosa (as) | 6 | 5 | 3 | 10 | 4 | 3 | 4 | 10 |
| Inflammation, mucosa (as) | 2 | 1 | 1 | 10 | 3 | 4 | 2 | 10 |
| **Tongue:** Myopathy (s) | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 6 |
| **Epididymides:** Vacuolation, epithelial cells (s) | 0 | 0 | 0 | 10 | NA [c] | NA | NA | NA |
| **Ovaries:** Vacuolation (s) | NA | NA | NA | NA | 1 | 0 | 0 | 8 |
| **Uterus:** Vacuolation, mucosa (s) | NA | NA | NA | NA | 0 | 0 | 0 | 9 |

a These data were extracted from study report, Table 62, pages 176-193.
b Severity reported as very slight (vs), slight (s), any severity (as) by study authors.
c NA = not applicable.

Table 6. Treatment-related non-neoplastic lesions in CD-1 mice fed XDE-105 for up to 18 months. [a]

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Group (%) | 0 | 0.0025 | 0.0080 | 0.036 | 0 | 0.0025 | 0.0080 | 0.036 |
| Number Examined | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 0 |
| **Site** Lesion | | | | | | | | |
| **Lungs:** Aggregates of alveolar macrophages (as) | 6 | 6 | 11 | 40 * | 10 | 3 | 8 | NA[c] |
| **Lymph Nodes:** Amyloid (as) | 7 | 6 | 3 | 11 | 5 | 6 | 6 | NA |
| Sinus histiocytosis | 1 | 0 | 3 | 13 * | 1 | 3 | 1 | NA |
| **Pancreas:** Vacuolation, acini (s) | 8 | 5 | 5 | 23 * | 5 | 7 | 9 | NA |
| **Parathyroid:** Vacuolation (s) | 3 | 2 | 5 | 40 * | 0 | 1 | 0 | NA |
| **Skeletal muscle:** Myopathy (as) | 0 | 0 | 0 | 5 * | 0 | 0 | 0 | 0 * |
| **Stomach:** Hyperplasia, glandular mucosa (as) | 26 | 26 | 26 | 49 * | 28 | 25 | 30 | NA |
| Inflammation, mucosa (as) | 12 | 17 | 12 | 43 * | 18 | 18 | 15 | NA |
| **Tongue:** Myopathy (s) | 0 | 0 | 0 | 24 * | 0 | 0 | 0 | NA |
| **Epididymides:** Vacuolation, epithelial cells (s) | 1 | 0 | 0 | 48 * | NA | NA | NA | NA |
| **Ovaries:** Cystic follicles | NA | NA | NA | NA | 8 | 7 | 11 | NA |

a These data were extracted from study report, Table 63, pages 194-232. The data include animals that died prior to terminal sacrifice. High-dose females were sacrificed on study day 455; no histopathological data were collected.
b Severity reported as very slight (vs), slight (s), any severity (as) by study authors.
c NA = not applicable.
* Significantly different from control mean by Yate's Chi-Square pairwise test, alpha = 0.10, two sided, alpha = 0.05, one sided

b) Neoplastic - No increases in the incidences of any neoplasm was observed in dosed animals. The incidence of hepatocellular adenoma was lower than concurrent controls (p=0.10 and 0.05) in the mid- and high-dose male groups. All tumors in the controls occurred at the expected incidence for mice

of this strain, age, and sex. Laboratory historical control data for neoplasms was not provided. Table 7 summarizes incidences of neoplastic lesions for selected tissues and organs.

Table 7. Summary of selected neoplastic lesions in CD-1 mice fed XDE-105 for up to 18 months. [a]

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Group (%) | 0 | 0.0025 | 0.0080 | 0.036 | 0 | 0.0025 | 0.0080 | 0.036 |
| Number Examined | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 0 |
| **Site** Lesion | | | | | | | | |
| **Liver: # Examined** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 0 |
| Hepatocellular adenoma: 1 or 2 | 10 | 7 | 3* | 2* | 0 | 1 | 3 | - |
| **Lungs: # Examined** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 0 |
| Bronchioloalveolar adenoma (1, 2, or 3) | 12 | 6 | 17 | 13 | 10 | 13 | 5 | - |
| Bronchioloalveolar adenoma and/or adenocarcinoma | 13 | 6 | 19 | 13 | 11 | 13 | 5 | - |
| **Mammary gland: #** | | | | | | | | |
| Examined | 13 | 1 | 2 | 14 | 50 | 9 | 49 | 0 |
| Adenoma | 0 | 0 | 0 | 0 | 1 | 0 | 0 | - |
| Adenocarcinoma | 0 | 0 | 0 | 0 | 2 | 0 | 0 | - |
| **Pituitary: # Examined** | 48 | 18 | 13 | 49 | 49 | 12 | 47 | 0 |
| Anterior (pars distalis) adenoma | 0 | 0 | 0 | 1 | 1 | 0 | 2 | - |
| **Uterus: # Examined** | - | - | - | - | 50 | 50 | 50 | 0 |
| Hemangioma | - | - | - | - | 1 | 1 | 4 | - |
| **Combined neoplasms:** | | | | | | | | |
| Total histiocytic sarcoma - any site | 1 | 1 | 0 | 0 | 0 | 1 | 0 | - |
| Total lymphosarcoma and/or leukemia/lymphoid cell - any site | 0 | 3 | 2 | 0 | 6 | 3 | 3 | - |
| Total hemangioma and/or hemangiosarcoma - any site | 1 | 0 | 0 | 1 | 4 | 6 | 9 | - |

a These data were extracted from study report, Table 66, pages 235-239. The data include animals that died prior to terminal sacrifice. High-dose females were sacrificed on study day 455; no histopathological data were collected.

* Significantly different from control mean by Yate's Chi-Square pairwise test, alpha = 0.10, two sided, alpha = 0.05, one sided, - indicates not applicable.

## III. DISCUSSION

A. Investigators Conclusions- The chronic NOEL is 0.008%, which is equivalent to 11.4 mg/kg/day for male mice and 13.8 mg/kg/day for females.

There was no evidence of a carcinogenic effect in mice treated with XDE-105 in the diet for up to 18 months.

B. Reviewer's Discussion/Conclusions- Male and female mice were fed diets containing XDE-105 at 0, 0.0025% (25 ppm), 0.008% (80 ppm), or 0.036% (360 ppm) for 18 months. The average calculated test substance consumption for the 0, 0.0025, 0.008, and 0.036% groups were: 0, 3.4, 11.4, or 50.9 mg/kg/day for males and 0, 4.2, 13.8, or 67.0 mg/kg/day for females. Dietary analyses at select study intervals confirmed that nominal diet concentrations of XDE-150 were achieved.

The high-dose females were terminated on day 455 of the study because the MTD in this group had been exceeded as evidenced by decreased feed consumption, increased body weight loss, and excessive mortality. The stomach was the most sensitive target organ in mice. At the 12-and 18-month intervals (day 455 for females), treatment-related gross necropsy findings were observed in the stomach mucosa of high-dose animals. Histopathological evaluation detected an increased incidence of stomach hyperplasia and inflammation in high-dose animals and in the high-dose males the severity of the stomach lesions progressed with time. Microscopic analyses were not conducted on high dose females that died prior to termination or on those that were sacrificed on day 455. Therefore, the histopathological data for these animals could not be compared with the gross pathology results.

No significant differences were observed in survival rates in male mice in any of the treated groups nor in the low- and mid-dose female groups throughout the study when compared to the respective control groups.

Several of the results obtained from organ weight determination and blood analyses in the high-dose animals were related to their lower feed consumption and resulting lower body weights. However, the higher white blood cell counts (2.3-2.5 times greater than controls; $p = 0.05$) detected in the high-dose animals at the 12-month interval was judged to be treatment related. At the terminal sacrifice (18-month), treatment related moderate hypochromasia of erythrocytes was also observed in the majority of the high-dose male mice. Aspartate aminotransferase activity in high-dose males at the 3-

month interval, was 50% higher than concurrent controls ($p = 0.05$); at the terminal sacrifice enzyme activity in high-dose males was 20% greater than controls.

At the 3-month interval, spleen weights (absolute and relative) were increased ($p = 0.05$) in animals dosed at 0.036%. An increased incidence of extramedullary hematopoiesis in the spleen was detected in the same animals. The increased liver weights ($p = 0.05$) observed in high-dose animals were not corroborated by macroscopic and histopathological changes. In the earlier subchronic test, increased liver weights and associated increases in alkaline phosphatase, alanine transferase, and aspartate transferase, and increased spleen weights were detected in mice dosed at 67.5 mg/kg/day (0.045% XDE-105).

The high-dose animals had treatment-related gross necropsy findings of the stomach mucosa and in generalized body fat content. In addition, the high-dose animals also had increased incidences of slight vacuolation and/or degeneration/inflammation of multiple organs. In the subchronic test, vacuolation of the same organs was observed in animals dosed at ≥22.5 mg/kg/day (0.015% XDE-105). However, these slight vacuolation changes that did not progress with time are probably of doubtful importance.

There were no treatment related histopathologic changes in mice from the 0.0025% and 0.008% groups and there were no treatment-related effects involving the eyes of any of the dosed animals.

In conclusion, the dose levels employed in this study were adequate to characterize the carcinogenic potential of XDE-150 in male CD-1 mice; however, sufficient numbers of high-dose females were not available to adequately evaluate late developing tumors. Chronic toxicity was characterized in females receiving the 0.036% diet by decreased feed consumption, increased body weight loss ($p = 0.05$), increased incidence of hyperplasia and inflammation of the stomach, and excessive mortality. In males receiving the 0.036% diet, chronic toxicity was characterized by increased inflammation of the stomach. No increases in the incidences of any neoplasm was observed in dosed animals.

The chronic LOEL is 0.036%, 50.9 mg/kg/day for male mice and tentatively 67.0 mg/kg/day for females.

C. Study deficiencies - The submitted study is classified as supplementary and does not satisfy the guideline requirements for a carcinogenicity study [§83-2 (b)] in mice. The study may be upgraded when data from an

ongoing Supplemental Study are provided. An EPA memo (ID 282443) dated 1/5/94 (Study pages 862-866) indicated that the Sponsor has initiated a supplemental study using 60 mice/sex/dose at 0.00%, 0.0008% and 0.024% XDE-105 in the diet.

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# DATA EVALUATION RECORD

SPINOSAD

Study Type: 83-4; Multigeneration Reproduction Study - Rat

Work Assignment No. 1-22D (MRID 43701506)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

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Disclaimer

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

EPA Reviewer: Roger Gardner Roger Gardner , Date 4-2-97
Review Section I, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Marion Copley
Review Section IV, Toxicology Branch I (7509C) , Date 5/27/97

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat

OPPTS Number: 870.3800 OPP Guideline Number: §83-4

DP BARCODE: D219011 SUBMISSION CODE: S492760
P.C. CODE: 110003 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-105 (Spinosad, 88.0% active ingredient, a.i.)

SYNONYMS: Factor A (76.1%) + Factor D (11.9%)

CITATION: Breslin, W.; Quast, J.; Vedula, U. (1994) XDE-105: Two Generation Dietary Reproduction Study in Sprague-Dawley Rats. Dow Chemical Co., Midland, Michigan. Lab Project Numbers: DR-0323-1194-008A0: DR-0323-1194-008L0: DR-0323-1194-008W1, December 20, 1994. MRID 43701506. Unpublished.

SPONSOR: DowElanco, 9330 Zionsville, Indianapolis, IN

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID 43701506) XDE-105 (88.0% spinosad a.i.) was administered to 30 Sprague Dawley rats/sex/dose in diet at target dose levels 0, 0.005, 0.02, and 0.2% w/w (equivalent to 0, 3, 10 and 100 mg/kg/day). Exposure to the $P_1$ animals began at 6 weeks of age and lasted for 10 weeks prior to the first mating to produce the $F_{1a}$ pups. Exposure to the $F_{1a}$ pups (30/sex) began at weaning and lasted for at least 12 weeks prior to mating to produce the $F_2$ pups. One week after weaning the $F_{1a}$ pups, the $P_1$ animals were mated again to produce an $F_{1b}$ generation. All animals were mated on a 1:1 ratio.

Parental toxicity was characterized in the high-dose animals by treatment-related increases in dystocia (↑3 and 17%) and vaginal bleeding after parturition (↑23 and 24%) and associated increases in mortality (↑7 and 10%, $P_1$ and $F_{1a}$ dams), increases in the absolute and/or relative weights of the heart, kidney, liver, spleen, and thyroid ($P_1$ and $F_{1a}$, both sexes), and histopathology in the lungs, mesenteric lymph nodes, spleen, and thyroid ($P_1$ and $F_{1a}$, both sexes), heart ($P_1$ males only), kidney, and prostate ($P_1$ and $F_{1a}$, males only) and in the stomach ($P_1$ and $F_{1a}$, females only). Histopathology in the lungs was characterized by an increase incidence of multifocal subacute to chronic inflammation of the interalveolar septae

along with multifocal aggregates of alveolar macrophages. For the spleen and mesenteric lymph nodes, the histopathology was described as sinus histiocytosis. The primary lesion in the thyroid was diffuse cytoplasmic vacuolation of the follicular epithelial cells with associated chronic active inflammation and necrosis. Treatment-related histopathologic lesions found exclusively in the high-dose males were degeneration of the myocardium with or without inflammation, tubular degeneration in the kidneys, and chronic active inflammation of the prostate. The treatment-related histopathologic lesion found exclusively in the high-dose females was characterized as dilation of the glandular crypts with cellular debris in the pyloric region of the stomach. There were no treatment-related effects noted in the reproductive function or performance of the high-dose $P_1$ or $F_{1a}$ adults. For the low or mid-dose $P_1$ or $F_{1a}$ adults, no treatment-related effects were noted in the clinical signs, mortality, food consumption, body weights, reproductive function and performance, organ weights, gross pathology, or histopathology. **The LOEL for systemic toxicity is 100 mg/kg/day based on increases in heart, kidney, liver, spleen, and thyroid weights (both sexes), corroborative histopathology in the spleen and thyroid (both sexes), heart and kidney (males only), and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. The NOEL for systemic toxicity is 10 mg/kg/day.**

Reproductive toxicity, which appears to be related to the systemic toxicity in the dams, was characterized in the high-dose offspring by decreases in the numbers of pups born alive (↓22-35%) and the mean litter sizes (↓23-38%) on days 1 and 4 ($F_{1a}$, $F_{1b}$), and body weight decreases (↓1-11%) throughout lactation ($F_{1a}$, $F_{1b}$, and $F_2$). There were no treatment-related gross pathologic changes noted in the offspring of any generation/treatment group. For the low- and mid-dose treatment groups, no treatment-related effects on the body weights, clinical signs, litter size, or survival indices were noted. **The LOEL for reproductive toxicity is 100 mg/kg/day based on decreases in litter size, survival ($F_2$ litters only), and body weights in the offspring and increased incidence of dystocia and/or vaginal bleeding after parturition with associated increases in mortality in the dams. The NOEL for reproductive toxicity is 10 mg/kg/day.**

The reproductive study in the rat is classified **Acceptable**, and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4) in rats.

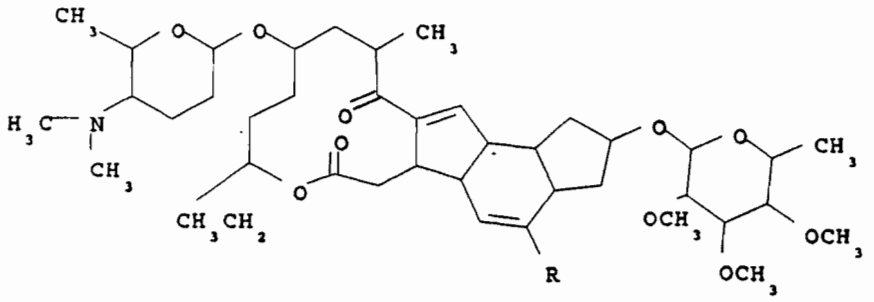
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

1. Test Material: XDE-105
Description: technical consisting of Factor A (76.1%) + Factor D (11.9%); white powder; determined stable for 2 years and 2 months under unspecified storage conditions
Lot/Batch #: DowElanco (AGR 293707)
Purity: 88.0% a.i.
CAS #: Not provided

FACTOR A - R = H; FACTOR D - R= $CH_3$



Vehicle: None

Test animals: Species: Rats
Strain: Sprague Dawley
Age at start of dosing: ($P_1$) approximately 6 wks
Weight at start of dosing:
($P_1$) Males: 141.7 - 202.8 g,
Females: 134.1 - 192.5 g
Source: Charles River Breeding Laboratory, Kingston, NY
Housing: Wire mesh, stainless steel cages or plastic cages with corn cob nesting material during gestation and lactation
Diet: Purina Certified Rodent Chow No. 5002, ad libitum
Water: municipal drinking water, ad libitum
Environmental conditions:
Temperature: approximately 22 °C
Humidity: 40 - 60%
Air changes: 12 - 15 changes/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period (P): 16 days

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure: One male was caged with one female from the same test group. Animals were housed together for 7 days and observed daily until sperm cells were found in a vaginal lavage sample or a copulation plug was observed. A second 7-day mating was attempted with an alternate male from the same treatment group for any female who did not show signs of mating within the initial mating period. If the second mating was unsuccessful, females were mated (7 days) for a third and final attempt. After mating, the $P_1$ females were placed in plastic cages with corn cob nesting material where they were kept through gestation and lactation. Because of a shortage in plastic cages, $F_{1a}$ (referred to as $P_2$ in the study report) dams were not placed in nesting cages until just prior to delivery. The $P_1$ females were mated twice to produce $F_{1a}$ and $F_{1b}$ litters. The $F_{1a}$ pups were mated to produce $F_2$ pups. All matings were conducted as described above. Sibling matings within the $F_{1a}$ generation were avoided.

2. Study schedule: Starting at 6 weeks of age, $P_1$ parental animals were given test diets for 10 weeks before they were mated to produce the $F_{1a}$ generation. Upon weaning at 3 weeks of age, $F_{1a}$ pups were selected to become the parents for the $F_2$ generation and were given the same concentration test diet as their $P_1$ dam. $F_{1a}$ animals were given test diets for at least 12 weeks prior to mating. One week after weaning the $F_{1a}$ pups, the $P_1$ animals were mated again to produce the $F_{1b}$ generation. Exposure of the test material to all animals was continuous in the diet throughout the study. Significant events and study weeks are presented in Attachment 1 to this DER (study report page 51).

3. Animal assignment: $P_1$ and $F_{1a}$ animals were randomly assigned (by weight for $P_1$ rats) to test groups as seen in Table 1.

TABLE 1. Animal Assignment.

| Test Group | Conc. in diet (%) | Dose in Diet[a] mg/kg/day | Animals/Group | | | |
|---|---|---|---|---|---|---|
| | | | $P_1$ Males | $P_1$ Females | $F_{1a}$ Males | $F_{1a}$ Females |
| Control | 0 | 0 | 30 | 30 | 30 | 30 |
| Low (LDT) | 0.005 | 3 | 30 | 30 | 30 | 30 |
| Mid (MDT) | 0.02 | 10 | 30 | 30 | 30 | 30 |
| High (HDT) | 0.2 | 100 | 30 | 30 | 30 | 30 |

a Test diets were administered to $P_1$ animals starting at approximately 6 weeks of age (10 weeks before mating) and to the $F_{1a}$ animals at 3 weeks of age (at least 12 weeks before mating) until sacrifice.

4. Dose selection rationale: The dose selection was based on a two subchronic dietary toxicity studies conducted with Fischer rats. In the first 90-day study, rats were dosed at 0, 0.05, 0.1, 0.2, and 0.4% XDE-105 in the diet (equivalent to 0, 30, 70, 150, or 300 mg/kg/day). The highest dose group (0.4% XDE-105) was terminated at 6 weeks because of high mortality and the cachectic condition of the surviving animals. The observed LOEL was 0.05% XDE-105 based on increases in the absolute and relative kidney and liver weights as well as various treatment-related histological changes in the lymphoid organs, kidneys, liver, heart, lung, pancreas, digestive tract, male and female reproductive organs, skeletal muscle, adrenal glands, and thyroid gland.

As a NOEL was not observed, a second subchronic study was conducted using Fischer rats dosed at 0, 0.003, 0.006, 0.012, and 0.06% XDE-105 in the diet (equivalent to 0, 2.4, 4.8, 9.5, and 47.4 mg/kg/day) for 13 weeks. The observed LOEL was 0.06% XDE-105 based on absolute and/or relative weight increases in the heart and liver of males and in the heart and spleen of females as well as histopathology of the thyroids in both males and females. The observed NOEL was 0.012% XDE-105.

Based on these results, the 2-generation reproductive study was conducted using doses of 0, 0.005, 0.02, 0.2% XDE-105 in the diet. These doses were selected to produce systemic toxicity in multiple organ systems at the high dose, slight toxicity at the mid dose, and a NOEL at the low dose.

5. Dosage preparation and analysis: Concentrated pre-mix formulations were prepared every 2-4 weeks by mixing appropriate amounts of test substance with Purina Certified Rodent Chow No. 5002. The pre-mix was serially diluted with the diet weekly to produce the test diets. The concentrations of test substance in the diets were adjusted weekly for both sexes to achieve targeted levels of 0, 3, 10, or 100 mg/kg/day. The concentrations were calculated from the previous week's mean body weight and food consumption data. The concentrations received during lactation were adjusted using historical food consumption data for lactating females. The weanlings received the same dietary concentrations given to the dams on the last week of gestation. Test diets were stored at ambient temperature.

Prior to the start of the study, stability of the test substance in the diet was evaluated for a period of up to 40 days. Homogeneity (top, middle, and bottom) was evaluated twice during the course of the study for each the low and high dose diets fed to males. Analyses for dietary concentrations were performed on samples of treated diet three to five times over the course of the study per dose level and generation (every 7-56 days).

Results (Data from study report pages 53-65)-
Homogeneity Analysis: 76-128% of nominal

Stability Analysis:
40 days: 95% of day 0 concentration
Concentration Analysis: See Table 2 below.

TABLE 2. Concentration analysis of XDE-105 doses levels administered.[a]

| Target dose level (concentration as % in diet) | % of nominal | |
|---|---|---|
| | Male | Female |
| 0.005 | 72-118 | 95-137[b] |
| 0.02 | 93-117 | 93-120 |
| 0.2 | 94-102 | 90-154[c] |

a Data extracted from the study report pages 54-59.
b One sample was found to be 137% of nominal. Upon reanalysis, this sample was determined to be 118% of nominal. All others were between 95-118% of nominal.
c Two samples were 154 and 139% of nominal. Upon reanalysis, these samples were determined to be 120 and 104% of nominal, respectively. All others were between 90 and 105% of nominal.

C. OBSERVATIONS

1. Parental animals: Adult animals were observed daily for changes in behavior, moribundity, and mortality. Observations for moribundity and mortality were made twice daily on the weekends. Body weights and food consumption data were recorded weekly during the study except during mating, when food consumption was not recorded as animals were cohabitating. For sperm-positive females, body weights were recorded on days 0, 7, 14, and 21 of gestation and food consumption was measured weekly. For lactating females body weights were recorded on days 1, 4, 7, 14, and 21 of lactation and food consumption was recorded once or twice during the first and second weeks and at 2-3 day intervals during the last week of lactation.

2. Litter observations: According to the report, the following litter observations (X) were made (see Table 3).

TABLE 3. $F_{1a}$, $F_{1b}$, and $F_2$ Litter Observations.[a]

| Observation | Time of observation (lactation day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 4[b] | Day 4[c] | Day 7 | Day 14 | Day 21 |
| Number of live pups | X | X | X | | X | X | X |
| Pup weight | | X | X | X | X | X | X |
| External alterations d | | | | | | | |
| Number of dead pups | X | X | X | | X | X | X |
| Sex of each pup (M/F) | | X | X | X | X | X | X |

a Data extracted from study report page 23.
b Before standardization (culling).
c After standardization (culling).
d The study report states that pups were observed for "abnormalities or demeanor changes...during the lactation period". Specific observation intervals were not indicated.

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were randomly killed and discarded. All dead pups were examined grossly.

3. Postmortem observations:

a) Parental animals: All surviving parental males and females (P1 and F1a) were sacrificed after the last litter of each generation was weaned. These animals were subjected to postmortem examinations as follows.

Gross necropsy was performed on all control and test animals and consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. Listed below in Table 4 are the tissues examined at necropsy. Tissues taken from the control and high-dose adults that were prepared for microscopic examination (X) and weighed (XX) are indicated. For 5/sex of the controls and high-dose animals in the $P_1$ group, all of the below listed tissues were examined histologically. Where treatment-related histological changes were noted in the high-dose animals, those tissues were examined in the low and mid-dose groups.

TABLE 4. Tissues examined at necropsy.

| | | | | | |
|---|---|---|---|---|---|
| | Adrenals | | Jejunum | X | Prostate |
| | Aorta | XX | Kidneys | | Rectum |
| | Auditory Sebaceous glands | | Lacrimal/ Harderian glands | | Salivary glands |
| | Bone (including Joint) | | Larynx | X | Seminal vesicles |
| | Bone Marrow | XX | Liver | | Skeletal Muscle |
| | Brain (cerebrum, brainstem, cerebellum) | | Lungs | | Skin |
| | Cecum | | Mammary gland | | Spinal cord (cervical, thoracic, lumbar) |
| X | Cervix | | Mediastinal lymph node and tissues | XX | Spleen |
| X | Coagulating glands | | Mesenteric lymph node and tissues | | Stomach |
| | Colon | | Nasal tissues | X | Testes |
| | Duodenum | | Oral tissues | | Thymus |
| X | Epididymides | X | Ovaries | XX | Thyroid gland |
| | Esophagus | X | Oviducts | | Tongue |
| | Eyes | | Pancreas | | Trachea |
| X | Gross lesions | | Parathyroid glands | | Urinary bladder |
| XX | Heart | | Peripheral Nerve | X | Uterus |
| | Ileum | X | Pituitary | X | Vagina |

b) Offspring: Ten pups/sex/group of the $F_{1a}$, $F_{1b}$, and $F_2$ offspring were sacrificed at 21 days of age and were subjected to postmortem gross examinations. Tissues were collected as described above for the adults and were preserved. However, terminal body weights and organ weights were not recorded and histological examinations were not performed on any tissue.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures. The significance level (p value) was 0.05 for all statistical analyses.

2. Indices:

Reproductive indices: The following reproductive indices as presented in the study report (page 108-113) were calculated for the $P_1$, $F_{1a}$, and $F_{1b}$ adults from the breeding and parturition records:



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**female mating index** = # of female with a sperm positive vaginal smear or pregnant without additional evidence of mating/# of females cohoused with males x 100%
**female conception index** = # of females delivering a litter/# of females mated x 100%
**female fertility index** = # of females delivering a litter/# of females cohoused with mates x 100%
**male mating index** = # of males which mated resulting in a sperm positive vaginal smear or pregnant female/total # of mates cohoused with females x 100%
**male conception index** = # of males which sired a litter/# of males mated x 100%
**male fertility index** = # of males which sired a litter/# of males cohoused with females x 100%
**gestation index** = # of females delivering a live litter/# of females delivering a litter x 100%

Offspring viability indices: The following viability indices as presented in the study report (page 108-113) were calculated for the $F_{1a}$ and $F_2$ litters from the lactation records:

**gestation survival index** = percentage of newborn pups that were alive at birth
**days 1 and 4 survival indices** = # of live pups on day 1 or 4/number of live pups on day 0 x 100%
**days 7, 14, and 21 survival indices** = # of live pups on day 7, 14, or 21/number of live pups on day 4 after culling x 100%

3. Historical control data: Historical data from the laboratory are presented in Table 5.

TABLE 5. Laboratory historical control data for reproductive and pup survival indices.[a]

| Parameter | # of Litters | Mean | Min | Max |
|---|---|---|---|---|
| Number of females | 13 | 28.85 | 15.00 | 30.00 |
| Female mating index (%) | 13 | 96.92 | 93.30 | 100.00 |
| Female conception index (%) | 13 | 83.59 | 66.70 | 100.00 |
| Female fertility index (%) | 8 | 78.34 | 66.70 | 100.00 |
| Male mating index (%) | 13 | 88.95 | 80.00 | 96.70 |
| Male conception index (%) | 13 | 86.91 | 67.90 | 100.00 |
| Male fertility index (%) | 10 | 78.57 | 63.30 | 96.70 |
| Gestation index (%) | 13 | 98.98 | 91.70 | 100.00 |
| Litter size - pups born live [b] | 12 | 13.87 | 11.50 | 15.20 |
| Gestation survival index (%) | 13 | 98.13 | 95.40 | 99.70 |
| Day 1 survival index (%) | 13 | 98.38 | 93.40 | 100.00 |
| Day 4 survival index (%) | 13 | 96.13 | 90.30 | 99.30 |
| Day 7 survival index (%) | 12 | 97.97 | 91.70 | 100.00 |
| Day 14 survival index (%) | 12 | 96.77 | 88.60 | 100.00 |
| Day 21 survival index (%) | 12 | 96.21 | 87.30 | 100.00 |
| Gestation length (days) | 13 | 21.78 | 21.50 | 22.10 |
| Time to mating (days) | 8 | 3.24 | 2.50 | 3.90 |

a These data were extracted from the study report page 114 unless otherwise noted.
b These data were extracted from the study report page 124.

## II. RESULTS

### A. PARENTAL ANIMALS

1. Mortality and clinical signs: Treatment-related clinical findings and increased incidence of mortality were noted only in the $P_1$ and $F_{1a}$ high-dose dams during the perinatal and lactation periods for the $F_{1a}$ and $F_2$ litters, respectively. Table 6 summarized data on mortality and clinical signs of toxicity.

Two high-dose $P_1$ females died early in the $F_{1a}$ lactation period, one form dystocia and one from renal failure/anorexia; a mid-dose female was sacrificed moribund because of ulcerated/lacerated mammary glands. No deaths occurred in the $F_{1b}$ lactation period. In the $F_{1a}$ parents, three high-dose females died during gestation, one from an unknown cause and the other two from pregnancy complications or dystocia as they both had multiple dead or degenerated pups in the uterus. Other deaths/moribund sacrifices in parents were not considered treatment related; they included a low dose $P_1$

male (nasal injury), one low-dose and one high dose $F_{1a}$ male (lymphosarcoma) and one control $F_{1a}$ female (nasal injury).

$P_1$ females at 100 mg/kg/day had an increased incidence of vaginal bleeding (7/29) in the $F_{1a}$ lactation period; this was not observed in the $F_{1b}$ lactation period. However, a similar frequency of vaginal bleeding (7/30) was observed in the $F_2$ lactation period for $F_{1a}$ dams. Dystocia was observed at parturition in one $P_1$ dam at $F_{1a}$ littering and in 5/30 $F_{1a}$ dams. The dystocia and vaginal bleeding are considered treatment related evidence of both maternal and reproductive toxicity.

TABLE 6. Mortality and Clinical Signs.[a]

| Observation | No. Observed/No. Examined (% incidence)[b] | | | |
|---|---|---|---|---|
| Dose Group (% in Diet) | 0 | 0.005 | 0.02 | 0.2 |
| $P_1$ Generation - Females | | | | |
| Spontaneous death | 0/30 (0) | 0/30 (0) | 0/30 (0) | 2/30 (7) |
| Moribund | 0/30 (0) | 0/30 (0) | 1/30 (3) | 0/30 (0) |
| Dystocia | 0/29 (0) | 0/30 (0) | 0/30 (0) | 1/29 (3) |
| Perineal soiling/vaginal bleeding | 0/29 (0) | 0/30 (0) | 0/30 (0) | 7/29 (24) |
| $P_1$ Generation - Males | | | | |
| Moribund | 0/30 (0) | 1/30 (3) | 0/30 (0) | 0/30 (0) |
| $F_{1a}$ Generation - Females | | | | |
| Spontaneous death | 0/30 (0) | 0/30 (0) | 0/30 (0) | 3/30 (10) |
| Moribund | 1/30 (3) | 0/30 (0) | 0/30 (0) | 0/30 (0) |
| Dystocia | 0/29 (0) | 0/30 (0) | 0/30 (0) | 5/30 (17) |
| Perineal soiling/vaginal bleeding | 0/29 (0) | 0/30 (0) | 0/30 (0) | 7/30 (23) |
| $F_{1a}$ Generation - Males | | | | |
| Moribund | 0/30 (0) | 1/30 (3) | 0/30 (0) | 1/30 (3) |

a Data extracted from study report pages 66, 67, 71, and 72.
b The %incidence was calculated by the reviewer.
c The mortality and clinical signs for the $P_1$ females is only for the pre-mating, gestation, and lactation periods associated with the $F_{1a}$ litter.

2. Body weight and food consumption: No treatment-related changes in food consumption, mean body weight, or body weight gain were noted for the low and mid-dose $P_1$ and $F_{1a}$ adults at any time during the study. During the pre-mating periods several slight equivocal changes were noted in the food consumption (g/animal/day), mean body weight, or body weight gains of the $P_1$ and $F_{1a}$ high-dose adults as described below.

Reported body weight and selected food consumption results for the pre-mating treatment period are summarized in Table 7.

For the high-dose $P_1$ males, slight decreases in food consumption (↓4%), body weights (↓3%), and body weight gains (↓3%) were noted relative to the controls. None of the changes in body weights, gains, or food consumption noted for the high-dose $P_1$ males reached statistical significance during the pre-mating period. However, the noted body weight decrease in the high-dose $P_1$ males was progressive and by study week 21 (pre-mating period for the $F_{1b}$ litter) the body weight depression was 7% relative to the controls and had reached statistical significance. No treatment-related changes in food consumption, mean body weight, or body weight gain were noted for the high-dose $P_1$ females during the $F_{1a}$ litter premating interval.

For the pre-mating period of the $F_{1a}$ adults, the food consumption males from all treatment groups was slightly increased (↑4-5%) relative to the controls. During this interval, the mean body weights of the high-dose $F_{1a}$ males were slightly lower (↓2%) relative to the controls; however, the body weight gains were comparable to the controls. The noted increases in food consumption and decreases in mean body weights for the $F_{1a}$ males did not reach statistical significance during the pre-mating period. No treatment-related changes in food consumption, mean body weight, or body weight gain were noted for the high-dose $F_{1a}$ females during the premating interval.

TABLE 7. Body Weight and Food Consumption - Pre-mating.[a]

| Observations/Study week | Dose Group (% in Diet) | | | |
|---|---|---|---|---|
| | Control | 0.005 | 0.02 | 0.2 |
| $P_1$ Generation Males - Pre-mating Period for the $F_{1a}$ Litter | | | | |
| Mean body weight (g) Week 10 | 562.3 | 562.2 | 567.8 | 547.8 |
| Mean weight gain (g) Weeks 1-10 | 383.2 | 382.2 | 389.9 | 370.1 |
| Mean food consumption (g/animal/day) Weeks 1-10 | 30.5 | 30.4 | 30.1 | 29.2 |
| $P_1$ Generation Females - Pre-mating Period for the $F_{1a}$ Litter | | | | |
| Mean body weight (g) Week 10 | 315.8 | 311.9 | 320.1 | 311.3 |
| Mean weight gain (g) Weeks 0-10 | 159.7 | 155.2 | 164.5 | 156.3 |
| Mean food consumption (g/animal/day) Weeks 0-10 | 21.0 | 21.1 | 21.2 | 20.7 |
| $F_{1a}$ Generation Males - Pre-mating | | | | |
| Mean body weight (g) Week 12 | 618.3 | 639.8 | 632.8 | 504.8 |
| Mean weight gain (g) Weeks 0-12 | 426.0 | 452.4 | 454.0 | 426.2 |
| Mean food consumption (g/animal/day) Week 0-12 | 30.5 | 31.9 | 31.9 | 31.6 |
| $F_{1a}$ Generation Females - Pre-mating | | | | |
| Mean body weight (g) Week 12 | 312.1 | 315.3 | 329.9 | 315.4 |
| Mean weight gain (g) Weeks 0-12 | 157.2 | 164.2 | 183 | 166.9 |
| Mean food consumption (g/animal/day) Week 0-12 | 22.2 | 22.2 | 22.6 | 22.7 |

a Mean weight gains were calculated by the reviewer using mean body weight data presented on study report pages 88, 91, 101, 103. The mean food consumption values were calculated by the reviewer using the mean weekly food consumption data presented on study report pages 74, 77, 83, and 85.

During gestation and lactation, several changes were noted in the food consumption, mean body weight, or body weight gains of the high-dose $P_1$ and $F_{1a}$ dams as described below.

For the $P_1$ dams receiving the 0.2% test diets, decreases in body weights (↓ ≤8%) and body weight gains (↓ ≤21%) were noted that were statistically significant by day 21 of gestation for both the $F_{1a}$ and $F_{1b}$ litters. The decrease in weight gain may be due in part to smaller litter sizes in the high-dose $P_1$ dams (10.9 and 10.1 pups/litter vs. 14.9 and 15.5 pups/litter in the controls, $F_{1a}$ and $F_{1b}$ litters, respectively). Food consumption was slightly decreased in the first 2 weeks of gestation (↓ ≤9%, $F_{1a}$ litter only) with a subsequent recovery by the end of gestation. During the lactation period for the $F_{1a}$ litter, there was a statistically significant dip in body weights (↓6%, day 4) with a subsequent recovery by the end of lactation. A similar trend was noted during the $F_{1b}$ lactation period, however, it was not statistically significant. Food consumption was slightly decreased in the first 2 weeks of lactation (↓ ≤16%, both generations) and, as with the body weight change, it was not noted by the end lactation. The noted decreases in food consumption did not reach statistical significance. (These data were extracted from the study report pages 79-82 and 93-100).

For the high-dose $F_{1a}$ dams, a slight decrease in body weights (↓3%) and body weight gains (↓5%) was noted by day 21 of gestation. During lactation period, there was a dip in body weights (↓3%, day 4) with a subsequent recovery by the end of lactation as was noted with the $P_1$ dams. These changes were not statistically significant. (These data were extracted from the study report pages 104-106). No treatment related effects on food consumption were noted for the high-dose $F_{1a}$ dams during gestation or lactation (study report pages 86 and 87).

3. Test Substance Intake: The study report states that the concentrations of the test material in the diets were calculated based on weekly body weights and feed consumption data.

4. Reproductive function:

a. Estrous cycle length and periodicity: No observations were made pertaining to the estrous cycle length and periodicity in this study. However, there were no indications of treatment-related female fertility abnormalities during the study (see Table 8a below).

b. Sperm measures: No sperm parameter observations were made in this study. However, there were no indications of treatment-related male fertility abnormalities during the study (see Table 8a below).

c. Sexual maturation ($F_1$): No observations were made pertaining to the sexual maturation rates of $F_{1a}$, $F_{1b}$, or $F_2$ litters.

5. Reproductive performance: There were no treatment-related effects noted in the reproductive performance of the $P_1$ or $F_{1a}$ adults. The reproductive indices, as described in section I.D.2. of this DER, were comparable with the controls for all treatment groups in the $P_1$ (for both $F_{1a}$ and $F_{1b}$ litters) and $F_{1a}$ adults with the following exception. For the $P_1$ mating period that resulted in the $F_{1a}$ litters, the conception index for the low dose males was statistically significantly decreased relative to the controls. This finding was not considered treatment-related as (i) the decreased conception index was within the historical control values (see Table 5 of this DER), (ii) there was no dose-response relationship, (iii) it was not repeated in the $F_{1b}$ and $F_2$ litters. There were no treatment-related effects on the indices in the second mating of the $P_1$ females; as expected, the male fertility indices were lower in all groups when compared to the first mating. Results for the $P_1$ and $F_{1a}$ parental animals are summarized from the report in Tables 8a and 8b.

TABLE 8a. Reproductive performance[a] for $P_1$ adults (first mating, $F_{1a}$ litter).

| Parameter[b] | Dose Level (% in Diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.2 |
| Number of females | 30 | 30 | 30 | 30 |
| Female mating index % | 96.7 (29/30) | 100 (30/30) | 100 (30/30) | 96.7 (29/30) |
| Female conception index % | 100 (29/29) | 80.0 (24/30) | 90.0 (27/30) | 82.8 (24/29) |
| Female fertility index % | 96.6 (29/30) | 80.0 (24/30) | 90.0 (27/30) | 80.0 (24.30) |
| Male mating index % | 90.0 (27/30) | 86.7 (26/30) | 90.0 (27/30) | 93.3 (28/30) |
| Male conception index % | 100 (27/27) | 76.9* (20/26) | 88.9 (24/27) | 78.6 (22/28) |
| Male fertility index % | 90.0 (27/30) | 66.7 (20/30) | 80.0 (24/30) | 73.3 (22/30) |
| Gestation index % | 100 (29/29) | 100 (24/24) | 100 (27/27) | 100 (24/24) |
| Gestation length (days) | 21.8 ± 0.5 | 21.8 ± 0.5 | 21.8 ± 0.4 | 21.7 ± 0.5 |
| Time to mating (days) | 3.3 ± 4.2 | 4.1 ± 4.8 | 4.3 ± 4.3 | 3.3 ± 3.5 |

a These data were extracted from the study report pages 108-109.
b Indices are expressed as % with the numbers of animals listed parenthetically.
* p=0.05



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TABLE 8b. Reproductive performance[a] for $F_{1a}$ adults.

| Parameter[b] | Dose Level (% in Diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.2 |
| Number of females | 29 | 30 | 30 | 30 |
| Female mating index % | 100 (29/29) | 100 (30/30) | 100 (30/30) | 100 (30/30) |
| Female conception index % | 82.8 (24/29) | 60.0 (18/30) | 90.0 (27/30) | 70.0 (21/30) |
| Female fertility index % | 82.8 (24/29) | 60.0 (18/30) | 90.0 (27/30) | 70.0 (21/30) |
| Male mating index % | 89.7 (26/29) | 86.7 (26/30) | 86.7 (26/30) | 90.0 (27/30) |
| Male conception index % | 84.6 (22/26) | 69.2 (18/26) | 88.5 (23/26) | 66.7 (18/27) |
| Male fertility index % | 75.9 (22/29) | 60.0 (18/30) | 76.7 (23/30) | 60.0 (18/30) |
| Gestation index % | 100 (24/24) | 100 (18/18) | 100 (27/27) | 100 (21/21) |
| Gestation length (days) | 21.7 ± 0.6 | 21.6 ± 0.5 | 21.9 ± 0.3 | 21.8 ± 0.5 |
| Time to mating (days) | 4.0 ± 4.7 | 4.0 ± 5.0 | 3.5 ± 3.1 | 3.0 ± 4.0 |

a These data were extracted from the study report pages 112-113.
b Indices are expressed as % with the numbers of animals listed parenthetically.

6. Parental postmortem results

a) Organ weights: The absolute and relative organ weights were comparable with the controls for the low and mid-dose $P_1$ males and females. For the high-dose $P_1$ males, the absolute and relative heart, liver, spleen, and thyroid weights and the relative kidney weights were statistically significantly increased. Corroborative histopathological changes were noted in the heart, spleen, thyroid, and kidney of the high-dose $P_1$ males. For the high-dose $P_1$ females, the absolute and relative weights of the heart, kidney, spleen, and thyroid and the relative liver weights were statistically significantly increased. Corroborative histopathological changes were noted in the spleen and thyroid of the high-dose $P_1$ females.

No treatment related changes in the absolute and relative organ weights were found for the low and mid-dose $F_{1a}$ males and females. For the high-dose $F_{1a}$ males and females, the absolute and relative heart, kidney, liver, spleen, and thyroid weights were statistically significantly increased. Corroborative histopathology was noted in the kidneys, spleen, and thyroid of the high-dose males and in the spleen and thyroid of the high-dose females. The absolute liver and heart weights were statistically significantly

increased in the mid-dose $F_{1a}$ females. However, these increases were not considered treatment-related as (i) the relative weights were unaffected, (ii) there were no corroborative histopathologic findings, and (iii) organ weights were not increase in the mid-dose $F_{1a}$ males or in the mid-dose $P_1$ animals.

As the organ weight findings for the $F_{1a}$ generation were consistent with the $P_1$ animals, the effects on organ weights were considered treatment-related. The mean absolute and relative weights for heart, liver, spleen, thyroid, and kidney are presented in Table 9 for the high-dose $P_1$ and $F_{1a}$ adults.

TABLE 9. Mean absolute and relative organ weights of the high-dose $P_1$ and $F_{1a}$ adults.[a]

| Organ [b] | Dose Level (% in Diet) | |
|---|---|---|
| | 0 | 0.2 |
| $P_1$-males | | |
| Heart [c] | 1.757 (0.249) | 1.978* (0.303)* |
| Liver | 18.269 (2.558) | 19.972* (3.047)* |
| Spleen [c] | 1.067 (0.150) | 1.326* (0.204)* |
| Thyroid [c] | 0.047 (0.007) | 0.103* (0.016)* |
| Kidney [c] | 4.504 (0.637) | 4.677 (0.722)* |
| $P_1$-females | | |
| Heart | 1.203 (0.330) | 1.343* (0.393)* |
| Liver | 10.235 (2.786) | 10.825 (3.167)* |
| Spleen [c] | 0.646 (0.177) | 0.860* (0.252)* |
| Thyroid [c] | 0.035 (0.010) | 0.044* (0.013)* |
| Kidney | 2.638 (0.722) | 2.934* (0.860)* |
| $F_{1a}$-males | | |
| Heart | 1.844 (0.276) | 2.154* (0.338)* |
| Liver | 18.129 (2.686) | 20.509* (3.154)* |
| Spleen [c] | 1.044 (0.156) | 1.404* (0.221)* |
| Thyroid [c] | 0.048 (0.007) | 0.075* (0.012)* |
| Kidney [c] | 4.378 (0.655) | 4.802* (0.750)* |
| $F_{1a}$-females | | |
| Heart | 1.177 (0.347) | 1.331* (0.396)* |
| Liver | 9.420 (2.771) | 10.746* (3.202)* |
| Spleen [c] | 0.669 (0.198) | 0.771* (0.230)* |
| Thyroid [c] | 0.033 (0.010) | 0.043* (0.013)* |
| Kidney | 2.446 (0.721) | 2.735* (0.816)* |

a These data were extracted from the study report pages 125-128.
b Absolute weights expressed in grams. Relative weights expressed in g/100g body weight and are listed parenthetically.
c Histopathologic lesions were noted at the high dose in these organs.
* p=0.05

b) Pathology

1) Macroscopic examination: An increased incidence of multifocal or generalized pale foci were noted in the lungs of the $P_1$ adults receiving the 0.2% diets [males: 20% (6/30) vs. 3% (1/30) for the controls; females: 30% (9/30) vs. 7% (2/30) for the controls, study report page 132-142]. As corroborative histopathology was noted in the lungs of these animals, it was considered a treatment-related effect. No significant gross pathologic findings were noted in the $P_1$ adults from the low- or mid-dose groups or in the $F_{1a}$ adults from any of the treatment groups.

2) Microscopic examination: No treatment-related histopathologic changes were noted in the low or mid-dose $P_1$ or $F_{1a}$ adults.

For the high-dose $P_1$ and $F_{1a}$ animals, histopathology was noted in the following organs: lungs, mesenteric lymph nodes, spleen, and thyroid (both sexes); heart ($P_1$ males only), kidneys, and prostate (males only); stomach (females only). Table 10 summarizes selected findings. Statistical analyses were not performed on these data. The histopathology characterization for each of these tissues is as described below.

Histopathology in the lungs was characterized by an increase incidence of multifocal subacute to chronic inflammation of the interalveolar septae along with multifocal aggregates of alveolar macrophages. For the spleen, the histopathology was described as sinus histiocytosis. Sinus histiocytosis was also found in the mesenteric lymph nodes of the control animals and at all dose levels. However, the aggregates of histiocytic cells were increased in number and in size in the high-dose animals relative to the controls and the other treatment groups.

For the thyroid, the primary lesion was noted as slight to moderate diffuse cytoplasmic vacuolation of the follicular epithelial cells. Associated with this was multifocal or focal chronic active inflammation and necrosis. The histopathologic changes were slightly more severe in the $P_1$ high-dose animals relative to the $F_{1a}$ high-dose adults. The T4 serum levels were measured in the $F_{1a}$ high-dose animals with the expectation that the noted morphological changes in the thyroids of the $P_1$ animals would also be observed in the $F_{1a}$ animals and would affect the T4 serum values. However, the serum T4 levels for the high-dose $F_{1a}$ animals were comparable with the controls (study report Tables 53-54, p. 129 and 130).

Treatment-related lesions found exclusively in the high-dose males were increased incidence of slight multifocal degeneration of the myocardium with or without inflammation ($P_1$ males only; 3/30 and 15/30 in controls and the high dose group, respectively), slight multifocal tubular degeneration in the kidneys (in the $P_1$ generation 6/30 and 12/30 for the control and high dose groups, respectively and in the $P_2$ generation respective incidences were 4/30 and 13/30), and slight chronic active inflammation of the prostate (in the $P_1$ generation 0/30 and 3/30 for the control and high dose groups, respectively and in the $P_2$ generation respective incidences were 3/30 and 10/30). For the $P_1$ males, myocardial degeneration was also found in controls and all treatment groups. However, the lesion was usually graded as very slight in the controls and in the animals receiving the 0.005 and 0.02% test diets, whereas the lesion was more commonly graded as slight in the high-dose males.

Very slight dilation of the glandular crypts with cellular debris in the pyloric region of the stomach was noted in the high-dose females only.

Corroborative increase in the organ weights were noted in the spleen and thyroid for both generations and sexes. For the males, corroborative increase in the weights of the hearts ($P_1$) and the kidneys ($P_1$ and $F_{1a}$) were noted.

TABLE 10a. Incidence of Selected Histologic Findings in Rats Fed XDE-105.[a] ($P_1$ Generation)

| Site & Lesion | No. Observed/30 Examined (% incidence)[b] | | |
|---|---|---|---|
| Dose Level (% in Diet) | 0 | 0.02 | 0.2 |
| $P_1$ - Males | | | |
| **Kidneys:**[c] multifocal tubular degeneration (s) | 6 (20) | 1 (3) | 12 (40) |
| **Lungs:** multifocal subacute to chronic inflammation in the interalveolar septae (vs and/or s[e]) | 4 (13) | 0 (0) | 10 (33) |
| multifocal aggregates of alveolar macrophages (vs, s, and/or m[e]) | 10 (33) | 10 (33) | 24 (80) |
| **Prostate:** multifocal and focal chronic active inflammation (vs and/or s[e]) | 1 (3) | 1 (3) | 14 (47) |
| **Spleen:**[c] sinus histiocytosis (vs and s) | 0 (0) | 0 (0) | 30 (100) |
| **Thyroid:**[c] focal/multifocal chronic active interstitial inflammation (vs, s[e], and/or m[e]) | 1 (3) | 0 (0) | 27 (90) |
| multifocal/focal necrosis (vs and s) | 0 (0) | 0 (0) | 12 (40) |
| diffuse epithelial cell vacuolation (vs, s[e], and/or m[e]) | 30 (100) | 30 (100) | 30 (100) |
| $P_1$- Females | | | |
| **Lungs:** multifocal subacute to chronic inflammation in the interalveolar septae (vs and/or s[e]) | 6 (20) | 4 (13) | 12 (40) |
| multifocal aggregates of alveolar macrophages (vs, s, and/or m[e]) | 6 (20) | 7 (23) | 19 (63) |
| **Spleen:**[c] sinus histiocytosis (vs and s) | 0 (0) | 0 (0) | 26 (87) |

| Site & Lesion | No. Observed/30 Examined (% incidence) [b] | | |
|---|---|---|---|
| Dose Level (% in Diet) | 0 | 0.02 | 0.2 |
| **Thyroid:** [c] | | | |
| focal/multifocal chronic active interstitial inflammation (vs and/or s[e]) | 0 (0) | 0 (0) | 11 (37) |
| multifocal necrosis (vs) | 0 (0) | 0 (0) | 4 (13) |
| diffuse epithelial cell vacuolation (vs, s[e], and/or m[e]) | 7 (23) | 5 (17) | 30 (100) |

a These data were extracted the study report pages 146-176.
b Percent incidence calculated by the reviewer.
c Corroborative organ weight increases were noted for these tissues.
d Severity reported by the study authors as very slight (vs), slight (s), and moderate (m).
e The most severe grading was found only in the high-dose animals as noted.

TABLE 10b. Incidence of Selected Histologic Findings in Rats Fed XDE-105.[a] ($F_{1a}$ Generation)

| Site & Lesion | No. Observed/30 Examined (% incidence) [b] | | |
|---|---|---|---|
| Dose Level (% in Diet) | 0 | 0.02 | 0.2 |
| $F_{1a}$- Males | | | |
| **Kidneys:** [c] | | | |
| multifocal tubular degeneration (s) | 4 (13) | 2 (7) | 13 (43) |
| **Lungs:** | | | |
| multifocal subacute to chronic inflammation in the interalveolar septae (vs and s[e]) | 4 (13) | 3 (10) | 14 (47) |
| multifocal aggregates of alveolar macrophages (vs, s, and/or m[e]) | 5 (17) | 7 (23) | 20 (67) |
| **Prostate:** | | | |
| multifocal and focal chronic active inflammation (vs, s and/or m[e]) | 5 (17) | 5 (17) | 12 (40) |
| **Spleen:** [c] | | | |
| sinus histiocytosis (vs, s and m) | 0 (0) | 0 (0) | 27 (90) |
| **Thyroid:** [c] | | | |
| focal/multifocal chronic active interstitial inflammation (vs, s[e], and/or m[e]) | 1 (3) | 0 (0) | 17 (57) |
| multifocal/focal necrosis (vs) | 0 (0) | 0 (0) | 6 (20) |
| diffuse epithelial cell vacuolation (vs, s and/or m[e]) | 29 (97) | 30 (100) | 30 (100) |

| Site & Lesion | No. Observed/30 Examined (% incidence) [b] | | |
|---|---|---|---|
| Dose Level (% in Diet) | 0 | 0.02 | 0.2 |
| $F_{1a}$- Females | | | |
| **Lungs:** | | | |
| multifocal subacute to chronic inflammation in the interalveolar septae (vs and/or s[e]) | 5 (17) | 3 (10) | 17 (57) |
| multifocal aggregates of alveolar macrophages (vs and/or s[e]) | 3 (10) | 2 (7) | 18 (60) |
| **Spleen:** [c] | | | |
| sinus histiocytosis (vs, s, and m) | 0 (0) | 0 (0) | 22 (73) |
| **Thyroid:** [c] | | | |
| focal/multifocal chronic active interstitial inflammation (vs and/or s[e]) | 0 (0) | 1 (3) | 11 (37) |
| multifocal/focal necrosis (vs) | 0 (0) | 0 (0) | 2 (7) |
| diffuse epithelial cell vacuolation (vs, s[e] and/or m[e]) | 2 (7) | 4 (13) | 30 (100) |

a These data were extracted the study report pages 146-176.
b Percent incidence calculated by the reviewer.
c Corroborative organ weight increases were noted for these tissues.
d Severity reported by the study authors as very slight (vs), slight (s), and moderate (m).
e The most severe grading was found only in the high-dose animals as noted.

B. OFFSPRING

1. Viability and clinical signs: Mean litter size and viability results from $F_{1a}$, $F_{1b}$, and $F_2$ pups during lactation are summarized in Tables 11a, 11b, and 11c.

For the animals receiving the 0.005 and 0.02% XDE-105 diets, no treatment-related effects on litter size or survival indices were noted for the $F_{1a}$, $F_{1b}$, or $F_2$ litters during lactation. Statistically significant decreases in the gestational survival indices for the $F_{1a}$ generation low-and mid-dose groups were noted (Table 11a), however, they were not considered treatment-related for the following reason: the values were within the laboratory's historical controls (see Table 5), decreases were not noted in the high dose group, the decreases were not dose-dependent, and lower gestational survival indices were not noted in the $F_{1b}$ or $F_2$ generations at these dose levels.

For high-dose $F_{1a}$, $F_{1b}$, and $F_2$ litters, the numbers of pups born alive and the mean litter sizes on days 1 and 4 were statistically significantly decreased relative to the

concurrent controls. In addition for the high-dose $F_{1a}$ and $F_{1b}$ litters, the mean numbers of pups born alive were below the laboratory historical controls (10.9 and 10.1, respectively vs. 11.5-15.2, see Tables 5, 11a and 11b). As the decreases in the numbers of pups born alive and the litter sizes were consistent among the two F1 generations and the $F_2$ generation, these findings were attributed to treatment with XDE-105.

For the high-dose litters, treatment-related decreases in survival indices were noted only in the $F_2$ generation. Statistically significant decreases in the gestation and day 4 survival indices were noted for the high-dose $F_2$ litters. The decreases were considered treatment-related because they were noted in multiple litters within the dose group/generation and the values were below the historical controls (gestation index of 94.7% vs. 95.4-99.7% and day 4 index of 90.0% vs. 90.3-99.3%, see Tables 5 and 11c). The survival indices for the high-dose $F_{1a}$ litters were comparable to the controls. For the $F_{1b}$ generation high-dose animals, the gestational survival index was lower than the historical controls (91.8% vs. 95.4-99.7%see Tables 5 and 11b). However, it was not considered statistically significant or treatment-related as it was attributed to the death of 16 pups from one litter.

TABLE 11a. Mean Litter Size and Viability of $F_{1a}$ generation.[a]

| Observation | Dose Level (% in Diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.2 |
| | $F_{1a}$ Generation | | | |
| Mean litter size | | | | |
| Day 0 | 14.9 | 13.5 | 14.7 | 10.9* |
| Day 1 | 14.7 | 13.5 | 14.4 | 10.7* |
| Day 4[b] | 14.5 | 13.3 | 13.9 | 10.6* |
| Day 4[c] | 8.0 | 7.8 | 7.6 | 7.6 |
| Day 7 | 8.0 | 7.8 | 7.6 | 7.3 |
| Day 14 | 8.0 | 7.7 | 7.6 | 7.3 |
| Day 21 | 8.0 | 7.7 | 7.6 | 7.3 |
| Number live pups | | | | |
| Day 0 | 431 | 324 | 397 | 262 |
| Day 1 | 427 | 323 | 389 | 257 |
| Day 4[b] | 421 | 319 | 374 | 244 |
| Day 4[c] | 231 | 187 | 206 | 174 |
| Day 7 | 231 | 186 | 205 | 168 |
| Day 14 | 231 | 185 | 205 | 168 |
| Day 21 | 231 | 185 | 205 | 168 |
| Number deaths | | | | |
| Days 0-4 | 10 | 5 | 23 | 18 |
| Days 4-21 | 0 | 2 | 1 | 6 |
| Survival indices | | | | |
| Gestation (%) | 99.5 (431/433) | 95.3* (324/340) | 97.1* (397/409) | 97.4 (262/269) |
| Day 1 (%) | 99.1 (427/431) | 99.7 (323/324) | 98.0 (389/397) | 98.1 (257/262) |
| Day 4 (%)[b] | 97.7 (421/431) | 98.5 (319/324) | 94.2 (374/397) | 98.0 (244/250) |
| Day 7 (%) | 99.6 (231/232) | 99.5 (186/187) | 99.5 (205/206) | 96.6 (168/174) |
| Day 14 (%) | 99.6 (231/232) | 98.9 (185/187) | 99.5 (205/206) | 96.6 (168/174) |
| Day 21 (%) | 99.6 (231/232) | 98.9 (185/187) | 99.5 (205/206) | 96.6 (168/174) |

a Data extracted from the study report number pages 108, 109, 115, and 537-540.
b Before standardization (culling)
c After standardization (culling)
* $p<0.05$
Shaded value is outside of the historical control value listed in Table 5 of this DER.



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TABLE 11b. Mean Litter Size and Viability of $F_{1b}$ generation.[a]

| Observation | Dose Level (% in Diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.2 |
| | $F_{1b}$ Generation | | | |
| Mean litter size | | | | |
| Day 0 | 15.5 | 12.6 | 15.1 | 10.1* |
| Day 1 | 15.5 | 12.5 | 15.1 | 9.7* |
| Day 4[b] | 15.4 | 12.5 | 14.8 | 9.6* |
| Day 4[c] | 7.7 | 7.3 | 7.9 | 6.5 |
| Day 7 | 7.6 | 7.2 | 7.7 | 6.4 |
| Day 14 | 7.6 | 7.2 | 7.7 | 6.4 |
| Day 21 | 7.5 | 7.2 | 7.7 | 6.4 |
| Number live pups | | | | |
| Day 0 | 373 | 277 | 333 | 201 |
| Day 1 | 371 | 275 | 332 | 193 |
| Day 4[b] | 369 | 274 | 326 | 192 |
| Day 4[c] | 185 | 160 | 174 | 129 |
| Day 7 | 183 | 158 | 170 | 128 |
| Day 14 | 174 | 158 | 170 | 128 |
| Day 21 | 150 | 158 | 169 | 128 |
| Number deaths | | | | |
| Days 0-4 | 4 | 3 | 7 | 9 |
| Days 4-21 | 35[d] | 2 | 5 | 1 |
| Survival indices | | | | |
| Gestation (%) | 99.5 (373/375) | 97.6 (277/281) | 99.4 (333/335) | 91.8 (201/219) |
| Day 1 (%) | 99.5 (371/373) | 99.3 (275/277) | 99.7 (332/333) | 95.0 (193/201) |
| Day 4 (%)[b] | 98.9 (369/373) | 98.9 (274/277) | 97.9 (326/333) | 95.5 (192/201) |
| Day 7 (%) | 98.9 (183/185) | 98.7 (158/160) | 97.7 (170/174) | 99.2 (128/129) |
| Day 14 (%) | 98.3 (174/177)[e] | 98.7 (158/160) | 97.7 (170/174) | 99.2 (128/129) |
| Day 21 (%) | 98.0 (150/153)[e] | 98.7 (158/160) | 97.1 (169/174) | 99.2 (128/129) |

a Data extracted from the study report pages 110, 111, 116, and 541-544.
b Before standardization (culling)
c After standardization (culling)
d This number is relatively high due to accidental deaths of pups after culling.
e The change in the denominator (number of live pups on day 4 after culling) was because of accidental deaths of pups after culling.
* $p<0.05$. Shaded values are outside of the historical control values listed in Table 5 of this DER.

TABLE 11c. Mean Litter Size and Viability of $F_2$ generation.[a]

| Observation | Dose Group (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 3 | 10 | 100 |
| $F_2$ Generation | | | | |
| Mean litter size | | | | |
| Day 0 | 15.2 | 13.8 | 14.6 | 11.9* |
| Day 1 | 15.0 | 13.7 | 14.4 | 11.6* |
| Day 4[b] | 14.9 | 13.6 | 14.3 | 10.9* |
| Day 4[c] | 7.7 | 7.8 | 8.0 | 7.3 |
| Day 7 | 7.7 | 7.8 | 7.9 | 7.4 |
| Day 14 | 7.7 | 7.8 | 7.9 | 7.4 |
| Day 21 | 7.7 | 7.8 | 7.9 | 7.4 |
| Number live pups | | | | |
| Day 0 | 364 | 249 | 393 | 249 |
| Day 1 | 361 | 246 | 388 | 244 |
| Day 4[b] | 358 | 244 | 386 | 217 |
| Day 4[c] | 185 | 140 | 215 | 145 |
| Day 7 | 185 | 140 | 212 | 140 |
| Day 14 | 185 | 140 | 212 | 140 |
| Day 21 | 185 | 140 | 212 | 140 |
| Number deaths | | | | |
| Days 0-4 | 6 | 5 | 7 | 32 |
| Days 4-21 | 0 | 0 | 3 | 5 |
| Survival indices | | | | |
| Gestation (%) | 100 (364/364) | 98.0 (249/254) | 98.5 (394/400) | 94.7* (249/263) |
| Day 1 (%) | 99.2 (361/364) | 98.8 (246/249) | 98.5 (388/394) | 98.0 (244/249) |
| Day 4 (%)[b] | 98.4 (358/364) | 98.0 (244/249) | 98.0 (386/394) | 90.0* (217/241)[d] |
| Day 7 (%) | 100 (185/185) | 100 (140/140) | 98.6 (212/215) | 100 (145/145) |
| Day 14 (%) | 100 (185/185) | 100 (140/140) | 98.6 (212/215) | 100 (145/145) |
| Day 21 (%) | 100 (185/185) | 100 (140/140) | 98.6 (212/215) | 100 (145/145) |

a Data extracted from the study report pages 112, 113, 121, and 561-564.
b Before standardization (culling)

c After standardization (culling)
d The change in the denominators (number of live pups on day 4 after culling) were because of the spontaneous death of two dams, one before and one after culling.
* Statistically different from control, $p<0.05$.
Shaded values are outside of the historical control values listed in Table 5 of this DER.

Significant clinical findings observed in the offspring during lactation were not considered signs of developmental toxicity; rather, were attributed to maternal neglect. These included increased incidence cannibalism, stomach void of milk, pups cold to the touch, and/or thin pups with decrease activity levels. These observations were not found in multiple litters within a generation/dose group. Most of the findings were noted in two $F_{1a}$ and three $F_2$ high-dose litters and in one $F_{1a}$ mid-dose litter. In each case, the dam of the litter was either in moribund condition or died during lactation. Clinical findings were unremarkable in the $F_{1b}$ litters for all dose levels and in the mid- and low-dose $F_2$ litters.

2. Body weight: Mean body weights for the $F_{1a}$, $F_{1b}$, and $F_2$ pups during lactation are summarized in Table 12. For the low and mid-dose treatment groups, no treatment-related effects on the mean pup body weights were noted for any generation during lactation.

Treatment-related body weight decreases during lactation were noted in the $F_{1a}$, $F_{1b}$, and $F_2$ high-dose litters. For the $F_{1a}$ and $F_{1b}$ generations, statistically significant decreases were noted in the mean body weights on lactation days 14 and 21 for both sexes (↓9-11%). For the $F_2$ generation, statistically significant decreases in the mean body weights were noted in the males only on lactation days 1 (↓7%)and 21 (↓8%). For the $F_2$ females, decreases (↓1-6%) in the mean pup body weights were also noted throughout lactation, however, it was not found statistically significant. As decreases in the mean pup body weights were consistently observed in the $F_{1a}$, $F_{1b}$, and $F_2$ generations, it was considered a treatment-related reproductive effect.

TABLE 12. Mean Body Weights (grams) in Pups During Lactation.[a]

| Day of lactation | Dose Level (% in Diet) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 0.005 | | 0.02 | | 0.2 | |
| | $F_{1a}$ Generation | | | | | | | |
| | Females | Males | Females | Males | Females | Males | Females | Males |
| Day 1 | 6.9 | 7.2 | 7.2 | 7.3 | 7.0 | 7.4 | 6.5 | 6.9 |
| Day 4[b] | 10.2 | 10.6 | 10.6 | 11.0 | 10.4 | 10.9 | 10.1 | 10.4 |
| Day 4[c] | 10.2 | 10.6 | 10.6 | 10.9 | 10.3 | 10.9 | 10.1 | 10.5 |
| Day 7 | 16.6 | 17.3 | 16.5 | 17.3 | 16.9 | 17.6 | 15.5 | 16.2 |
| Day 14 | 34.6 | 35.7 | 34.2 | 36.0 | 35.2 | 36.4 | 31.5* | 32.4* |
| Day 21 | 56.7 | 59.6 | 56.1 | 59.3 | 58.1 | 60.7 | 51.5* | 52.9* |
| | $F_{1b}$ Generation | | | | | | | |
| | Females | Males | Females | Males | Females | Males | Females | Males |
| Day 1 | 6.7 | 7.2 | 7.2 | 7.4 | 6.9 | 7.3 | 6.8 | 7.3 |
| Day 4[b] | 9.2 | 9.9 | 10.2 | 10.9 | 9.7 | 10.4 | 9.9 | 10.6 |
| Day 4[c] | 9.3 | 10.1 | 10.2 | 10.9 | 9.7 | 10.3 | 9.9 | 10.7 |
| Day 7 | 15.3 | 16.5 | 16.0 | 17.1 | 15.5 | 16.5 | 14.8 | 15.7 |
| Day 14 | 33.4 | 34.8 | 32.9 | 34.9 | 33.1 | 34.8 | 30.5* | 31.3* |
| Day 21 | 55.6 | 57.4 | 55.9 | 59.3 | 55.7 | 58.7 | 50.5* | 51.3* |
| | $F_2$ Generation | | | | | | | |
| | Females | Males | Females | Males | Females | Males | Females | Males |
| Day 1 | 6.7 | 7.2 | 6.7 | 7.1 | 6.9 | 7.3 | 6.5 | 6.7* |
| Day 4[b] | 9.8 | 10.3 | 10.0 | 10.7 | 10.2 | 10.8 | 9.6 | 9.9 |
| Day 4[c] | 9.7 | 10.2 | 10.1 | 10.7 | 10.2 | 10.8 | 9.6 | 9.9 |
| Day 7 | 16.0 | 16.8 | 16.9 | 18.0 | 17.0 | 18.0 | 15.8 | 16.2 |
| Day 14 | 34.2 | 35.3 | 35.0 | 36.5 | 35.2 | 36.6 | 32.6 | 33.3 |
| Day 21 | 55.9 | 58.0 | 57.7 | 60.9 | 57.0 | 59.7 | 52.3 | 53.4* |

a Data extracted from study report pages 117-120, 122, and 123.
b Before standardization (culling.
c After standardization (culling).
* $p<0.05$.

3. Offspring postmortem results:

a) Organ weights: Organ weights for the $F_{1a}$, $F_{1b}$ and $F_2$ weanlings were not recorded.

b) Pathology

1) Macroscopic examination: No treatment-related gross pathologic changes in the $F_{1a}$, $F_{1b}$, or $F_2$ weanlings were noted for any dose level.

2) Microscopic examination: Histopathologic examinations were not performed on any of the tissues collected from the $F_{1a}$, $F_{1b}$, or $F_2$ pups.

## III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The study authors concluded that the parental and reproductive/developmental LOEL was 100 mg/kg/day and that the NOEL was 10 mg/kg/day. The LOEL was based on decreased body weights or body weight gains, slightly decreased food consumption, increased incidence of vaginal bleeding, dystocia, and mortality during lactation, increased liver, kidney, heart, spleen, and thyroid weights, and histopathology of the lung, mesenteric lymph node, spleen, and thyroid (both sexes), heart ($P_1$ males only), kidney and prostate (males only), and stomach (females only). In addition, associated treatment-related effects on the offspring at the target 100 mg/kg/day dose level were noted as follows: decreased litter size, neonatal and gestational survival ($F_2$ litters only), and body weights.

B. REVIEWER'S DISCUSSION: Over the course of the 2-generation reproduction study, XDE-105 was administered continuously to Sprague Dawley rats at dose levels of 0.005, 0.02, and 0.2% in the diet (equivalent to 0, 3, 10, and 100 mg/kg/day). Exposure to the $P_1$ animals (30/sex) began at 6 weeks and lasted for 10 weeks prior to the first mating to produce the $F_{1a}$ pups. Exposure to the $F_{1a}$ pups (30/sex) began at weaning and lasted for at least 12 weeks prior to mating to produce the $F_2$ pups. One week after weaning the $F_{1a}$ pups, the $P_1$ animals were mated again to produce the $F_{1b}$ generation.

1. Parental Toxicity. Parental toxicity was characterized in the high-dose animals by treatment-related clinical findings, changes in organ weights, and gross and histopathologic lesions.

Significant clinical findings included treatment-related dystocia (↑3 and 17%) and/or vaginal bleeding after parturition (↑23 and 24%) and associated increases in mortality (↑7 and 10%) in both the high-dose $P_1$ ($F_{1a}$ litter only) and $F_{1a}$ dams (Table 6).

Several slight equivocal changes were noted in the food consumption, mean body weights, and body weight gains of the high-dose adults (Table 7). Throughout the study food consumption, body weights, and body weight gains were slightly decreased

in the high-dose $P_1$ males and mean body weights were slightly decreased in the $F_{1a}$ high-dose males.

For the $P_1$ and $F_{1a}$ dams receiving the 0.2% test diet, no treatment-related changes in body weight or food consumption were noted in either generation during the premating intervals. However, several changes in were noted in the high-dose dams during gestation and lactation. For the $P_1$ dams, decreases in body weights ($\leq \downarrow 8\%$) and body weight gains ($\leq \downarrow 21\%$) were noted that were statistically significant by day 21 of gestation for both the $F_{1a}$ and $F_{1b}$ litters. The decrease in weight gain may be due in part to smaller litter sizes in the high-dose $P_1$ dams. Food consumption was slightly decreased in the first 2 weeks of gestation ($\downarrow \leq 9\%$, $F_{1a}$ litter only) with a subsequent recovery by the end of gestation. During the lactation period for the $F_{1a}$ and $F_{1b}$ litters, there was a dip in body weights with a subsequent recovery by the end of lactation. Food consumption was slightly decreased in the first 2 weeks of lactation ($\downarrow \leq 16\%$, both generations) and, as with the body weight change, it was not noted by the end lactation.

For the high-dose $F_{1a}$ dams, a slight decrease in body weights ($\downarrow 3\%$) and body weight gains ($\downarrow 5\%$) was noted by day 21 of gestation. During lactation period, there was a dip in body weights ($\downarrow 3\%$, day 4) with a subsequent recovery by the end of lactation as was noted with the $P_1$ dams. In contrast to the high-dose $P_1$ dams, no effects on food consumption were noted for the high-dose $F_{1a}$ dams during gestation or lactation.

A treatment-related increase in the absolute and/or relative weights of the heart, kidney, liver, spleen, and thyroid were noted for the high-dose $P_1$ and $F_{1a}$ males and females (Table 9). Corroborative histopathological changes were noted in the spleen and thyroid for both generations and sexes. For the males, corroborative histopathological changes also were noted in the hearts ($P_1$) and the kidneys ($P_1$ and $F_{1a}$).

The only finding at necropsy was a treatment-related increased incidence of multifocal or generalized pale foci in the lungs of the $P_1$ adults receiving the 0.2% test diet. Corroborative histopathology was noted in the lungs of these animals.

Treatment-related histopathology (Table 10) was noted in the high-dose adults of both generations ($P_1$ and $F_{1a}$) and sexes in the lungs, mesenteric lymph nodes, spleen, and thyroid. Histopathology in the lungs was characterized by an increase incidence of multifocal subacute to chronic inflammation of the interalveolar septae along with multifocal aggregates of alveolar macrophages. For the spleen and mesenteric lymph nodes, the histopathology was described as sinus histiocytosis. Sinus histiocytosis was also found in the in the control animals and at all dose levels; however, the severity was increased at the high-dose. The primary lesion in the thyroid was diffuse

cytoplasmic vacuolation of the follicular epithelial cells with associated chronic active inflammation and necrosis.

Treatment-related histopathologic lesions found exclusively in the high-dose males were slight degeneration of the myocardium with or without inflammation ($P_1$ males only), tubular degeneration in the kidneys ($P_1$ and $F_{1a}$), and chronic active inflammation of the prostate ($P_1$ and $F_{1a}$). Treatment-related histopathologic lesions found exclusively in the high-dose females of both generations was characterized as very slight dilation of the glandular crypts with cellular debris in the pyloric region of the stomach.

There were no treatment-related effects noted in the reproductive function or performance of the high-dose $P_1$ or $F_{1a}$ adults as the following reproductive indices or parameters were comparable to the concurrent or historical controls: female and male mating, conception, and fertility indices, gestation index and length, and time to mating.

For the low or mid-dose $P_1$ or $F_{1a}$ adults, no treatment-related effects were noted in the clinical signs, mortality, food consumption, body weight, reproductive function and performance, organ weight, gross pathology, or histopathology.

2. Reproductive Toxicity. See "Executive Summary" above.

**DATA EVALUATION RECORD** C12237

XDE-105 (SPINOSAD)

Study Type: 83-5, 83-7; Two Year Chronic Toxicity, Neurotoxicity, and Oncogenicity Study in Fischer Rats

Work Assignment No. 1-22E (MRIDs 43701507, 43710503)

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Disclaimer

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

EPA Reviewer: R. Gardner, PhD
Review Section I, Toxicology Branch I (7509C) Date 7/22/96
EPA Secondary Reviewer: P. Hurley, Ph.D.
Review Section I, Toxicology Branch I (7509C) Date 6/21/96

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity/Oncogenicity Feeding Study - Rat and Neurotoxicity Study
OPPTS Number: 870.4300 OPP Guideline Number: §83-5, 83-7

DP BARCODE: D219011 SUBMISSION CODE: S492760
P.C. CODE: 110003 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-105 (88% ai)

SYNONYMS: Spinosad

CITATION: 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. MRID 43701507 and 43710503. Unpublished.

SPONSOR: DowElanco.

EXECUTIVE SUMMARY:

In a rat oncogenicity/chronic toxicity/neurotoxicity study (MRID 43701507 and 43701503), XDE-105 (Spinosad; 88% ai) was administered to Fischer 344 rats (65/sex/group) for up to 2 years at dietary levels of 0, 0.005, 0.02, 0.05, or 0.1% w:w (equivalent to 0, 2.4, 9.5, 24.1, or 49.4 mg/kg/day in males, and 0, 3.0, 12.0 30.3, or 62.8 mg/kg/day in females). A group of 15/sex/group, randomly designated as a satellite group were scheduled for sacrifice at 12 months. Ten satellites/sex/group underwent neurobehavioral testing at pre-test, 3, 6, 9, and 12 months, and a subset of 5/sex in the control and high dose group were assessed for neuropathology. The remaining satellite rats were evaluated for chronic toxicity at 12 months. The dosage equivalents in the satellites were 0, 4.6, 9.2, 23.0, or 46.0 mg/kg/day for males, and 0, 5.7, 11.4, 28.5, or 57.0 mg/kg/day for females. In the highest dosage group (0.1% XDE-105), there was excessive mortality and males were terminated at 102 weeks and females at 87.3 weeks. Since the MTD was exceeded at this dose, the 0.05% groups were evaluated as the high-dose for histologic findings and organ weights.

At 0.02% XDE-105, slight vacuolation of the follicular epithelial cells of the thyroid was observed in males (7/49) and females (34/50) scheduled for terminal sacrifice ($p < 0.05$). In the 0.05%

group, vacuolation of epithelial cells of the thyroid was seen at 12 months in all males (slight) and all females (slight to moderate); at terminal sacrifice, slight vacuolation of epithelial cells of the thyroid was observed in the majority of males and females and inflammation of the thyroid was seen in 3/50 males and 32/50 females. Absolute and relative weights of the thyroid were significantly increased (2-fold) in females at 24 months. The incidence of very slight inflammation of the lungs was increased in both sexes at 12 and 24 months; the increase in females was significant at 24 months (37/50 at 0.05% compared to 8/50 in controls; $p < 0.05$). In the 0.1% group, there was no effect on survival or weight gain in the first year of the study. By week 77, weight gain was depressed 14% (males) and 23% (females) compared to controls. At the 12-month sacrifice the following histopathologic changes were seen, slight degeneration of the heart in 3/10 males and 4/10 females, aggregates of reticuloendothelial (RE) cells in the larynx of 7/10 and spleen of 9/10 females, degeneration/regeneration of the glandular mucosa in the stomach of 9/10 females and inflammation of the lungs of 6/10 males and all females. Slight vacuolation of the kidney tubules was observed in 9/10 females. Vacuolation of follicular epithelia and moderate inflammation of the thyroid were present in the majority of rats at 0.1%. Absolute or relative weights of the heart, kidney, liver, spleen, and thyroid were significantly increased at 12 months. Gross findings in the main group (24 months) included decreased body fat, degenerative and inflammatory lesions of the heart, lungs, the glandular mucosa of the stomach, and the skeletal muscle, hydrothorax, and enlargement of the thyroid. Histologic examination of tissues of rats receiving 0.1% were not conducted at 24 months since the MTD had been exceeded. **The LOEL for systemic toxicity is 9.5 mg/kg/day, based on vacuolation of the epithelial follicular cells of the thyroid in both sexes. The NOEL is 3.0 mg/kg/day. Under the conditions of the study, there was no oncogenic response.**

In the chronic neurotoxicity portion of this study (MRID. 43710503), no effects on the Functional Observation Battery or on motor activity were observed after 3, 6, 9, or 12 months of dosing at a dietary level of 0.1% XDE-105 (equivalent to 46.0 mg/kg/day in males or 57.0 mg/kg/day in females). Histopathologic observations of the central and peripheral nervous system of the control and high dose groups revealed a number of lesions which were considered spontaneous and unrelated to dosing. These lesions were generally very slight and the incidences and severity in the high dose group were similar to that in the control group (both sexes). The positive control data provided appropriate positive responses. **A LOEL for neurobehavioral effects and neuropathic effects was not established. The NOEL for neurotoxicity is 0.1% XDE-105, the highest dose tested (46.0 mg/kg/day in males and 57.0 mg/kg/day in females).**

The chronic toxicity/oncogenicity study is **supplementary** and the chronic neurotoxicity study is **acceptable**. The chronic toxicity study may be upgraded pending submission of additional histopathology data from the high dose groups. The chronic neurotoxicity study satisfies the guideline requirements for a chronic neurotoxicity study (§83-7) in rodents. In the chronic/oncogenicity portion of the study, the test substance was evaluated at the maximally tolerated dose; however, unsufficient reasons were provided as to why the animals in the highest dose group were not microscopically examined after the 12 month sacrifice. The reason that the MTD was exceeded, thus, any microscopic data may not be useful may apply to the carcinogenicity section of the study, however, it does not necessarily apply to the chronic portion of the study. Without these data, a complete toxicological assessment of chronic toxicity cannot be conducted. The study stated that there was excessive mortality. This is true, however, mortality in the high dose groups did not start to increase over the other groups until after week 88 in males and after week 68 in females; even then mortality did not approach 50% until just before week 84 in females.

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

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test Material: XDE-105
   Description: White solid
   Lot/Batch #: ACD 13651; TSN AGR 293707
   Purity: 88% ai (76.1% Factor A and 11.9% Factor D)
   Stability of compound: Verified by analysis of purity (87.9-88.9% purity of active ingredients over 32 months)
   CAS #: Not provided
   Structure: Appendix

2. Vehicle and/or positive control: None

3. Test animals: Species: Rat
   Strain: Fischer 344
   Age and weight at study initiation: The rats were about 7 weeks old. Mean group weights at initiation were 140.4-142.7 g for males and were 98.6-99.6 g for females.
   Source: Charles River Laboratories, Kingston, NY.
   Housing: Animals were housed 2/cage in suspended steel cages with wire-meshed floors lined with animal cage-boards.

Diet: Purina Certified Chow #5002, ad libitum
Water: Tap water, ad libitum
Environmental conditions:
Temperature: 19.3-25.8 C
Humidity: 33-84%
Air changes: 10-12 changes/hour
Photoperiod: 12-hour dark/light
Acclimation period: 13 days

B. STUDY DESIGN

1. In-life dates: Start: 5/14/92 End: 5/17-20/93
[Neurotoxicity satellites: 5/16-18/94; main groups]

2. Animal assignment
Animals were assigned, using a randomized program based on body weights to give a homogeneous distribution between test groups, to the test groups in Table 1.

TABLE 1: STUDY DESIGN.

| Test Group | Conc. in Diet (%) | Dose to animal[a] (mg/kg/day) M/F | Main Study 24 months | | Interim Sac.[b] 12 months | |
|---|---|---|---|---|---|---|
| | | | Male | Female | Male | Female |
| Control | 0 | 0 | 50 | 50 | 15 | 15 |
| Low (LDT) | 0.005 | 2.4/3.0 | 50 | 50 | 15 | 15 |
| Intermed. | 0.02 | 9.5/12.0 | 50 | 50 | 15 | 15 |
| Mid (MDT) | 0.05 | 24.1/30.3 | 50 | 50 | 15 | 15 |
| High (HDT) | 0.1 | 49.4/62.8 | 50 | 50 | 15 | 15 |

[a] Based on 24 months
[b] 10/sex/group for FOB, and 5 for neuropathology

3. Dose selection rationale
The doses were based on results of two subchronic studies in Fischer 344 rats (MRID Nos.43566601; 43557504). In these studies, no effects on body weights were observed by 13 weeks. However, 0.1% XDE-105 caused histologic changes in multiple organs and it altered numerous clinical chemistry and hematology parameters. A decrease in body weight gain was expected at 0.1% in a chronic study. The choice of dose was agreed on during discussions with U.S. EPA, OPPTS.

4. Diet preparation and analysis

Premixes were prepared every 2-4 weeks by mixing an appropriate amount of test material with ground feed and ball-milling for 15 minutes. Diets were prepared at least every 2 weeks of the study by dilution of the premix with ground feed and mixing. Diets were stored at room temperature. Homogeneity was analyzed for the 0.005% diets (males and females separately) at nine locations in the mixer using three core samples (two from sides and one from middle) prior to initiation. Stability of test compound in diets was established in a previous study. Concentration in diets were measured at all levels at the start of the study and at least quarterly thereafter.

Results

Homogeneity Analysis: Diets at 0.005% were 0.00502% with a relative standard deviation (RSD) of 4.18% for males and 0.00461% with a RSD of 5.86% for females, indicating acceptable homogeneity.

Stability Analysis: In this study, test material over 40 days storage was 92-97% of the analyzed values at day 0.

Concentration Analysis: Mean percent of target and standard deviations (8-9 intervals of analysis) are shown in Table 2. All analyzed diets were within 10% of target.

TABLE 2. MEAN DIETARY CONCENTRATIONS (% OF TARGET).[a]

| Target (w:w) | 0.005% | 0.02% | 0.05% | 0.1% |
|---|---|---|---|---|
| Males | 100 ± 8.0 | 99 ± 5.7 | 98 ± 3.1 | 101 ± 3.8 |
| Females | 99 ± 6.0 | 97 ± 6.9 | 98 ± 4.7 | 99 ± 3.8 |

[a] Data obtained from Table 6, pages 65-66, in study report DR-0323-1194-005.

5. Statistics

Bartlett's test for equality of variances was applied to body weight, organ weight, clinical chemistry, appropriate hematology and urinary data. This was followed by parametric or non-parametric ANOVA followed respectively by Dunnett's test or the Wilcoxon Rank-Sums test. Food consumption outliers were identified by a statistical sequential test. Differences in mortality were tested by the Gehan-Wilcoxon test. Histologic findings were evaluated by pairwise chi-square comparison with Yate's continuity correction, and for linear trend using the Cochran-Armitage trend test.

C. METHODS

1. Observations
Animals were inspected at least twice daily for morbidity, moribundity and mortality as well as clinical signs of toxicity or behavioral changes. Clinical examinations were conducted on individual animals prior to the study and weekly thereafter. In addition to external examination of appearance, respiration, behavior and nervous system function was examined (including examination for tremors, convulsions, salivation and diarrhea). Palpable masses were also recorded as to time of onset and progression or disappearance.

2. Body weight
Animals were weighed weekly for the first 13 weeks and monthly thereafter.

3. Food consumption and compound intake
Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g/animal/day. Food efficiency (feed consumption/day ÷ body weight gain/day) was calculated weekly for the first 13 weeks of the study. Time-weighted average compound intake values (mg/kg/day) were calculated from the feed consumption, body weight data, and targeted concentrations in the diet throughout the study.

4. Ophthalmoscopic examination
Eyes were examined for all animals prior to the start of the study using a pen-light and prior to the scheduled sacrifices by placing a moistened slide on the cornea and visualization through fluorescent light.

5. Blood
Blood was collected from fasting animals via the orbital sinus for hematology and clinical analysis from 10 rats/sex/group (satellites) after 6 and 12 months and from the first 10 and 20 rats/sex/group, respectively, at 18 and 24 months. The CHECKED (X) parameters were examined.

a. Hematology

| | | | |
|---|---|---|---|
| x | Hematocrit (HCT)* | x | Leukocyte differential count* |
| x | Hemoglobin (HGB)* | | Mean corpuscular HGB (MCH) |
| x | Leukocyte count (WBC)* | | Mean corpusc. HGB conc.(MCHC) |
| x | Erythrocyte count (RBC)* | | Mean corpusc. volume (MCV) |
| x | Platelet count* | | Reticulocyte count |
| | Blood clotting measurements* | | Blood smears |
| | (Thromboplastin time) | x | RBC morphology |
| | (Thromboplastin time) | x | Platelet morphology |
| | (Clotting time) | x | WBC morphology |
| | (Prothrombin time) | | |

* Required for chronic studies based on Subdivision F Guidelines.

b. Clinical Chemistry

| | ELECTROLYTES | | OTHER |
|---|---|---|---|
| x | Calcium* | x | Albumin* |
| x | Chloride* | x | Blood creatinine* |
| | Magnesium | x | Blood urea nitrogen* |
| x | Phosphorus* | x | Total Cholesterol |
| x | Potassium* | x | Globulins |
| x | Sodium* | x | Glucose* |
| | ENZYMES | x | Total bilirubin |
| x | Alkaline phosphatase (ALP) | x | Total serum protein (TP)* |
| | Cholinesterase (ChE) | x | Triglycerides |
| x | Creatine phosphokinase | | Serum protein electrophores |
| | Lactic acid dehydrogenase (LDH) | | |
| x | Serum alanine amino-transferase (also SGPT)* | | |
| x | Serum aspartate amino-transferase (also SGOT)* | | |
| | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

* Required for chronic studies based on Subdivision F Guidelines.

6. Urinalysis

Urine was collected from non-fasted animals (same animals and same intervals as blood sampling). The CHECKED (X) parameters were examined.

| | | | |
|---|---|---|---|
| x | Appearance* | x | Glucose* |
| | Volume* | x | Ketones* |
| x | Specific gravity* | x | Bilirubin* |
| x | pH | x | Blood* |
| x | Sediment (microscopic)* | | Nitrate |
| x | Protein* | x | Urobilinogen |

* Required for chronic studies

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed:

| | DIGESTIVE SYSTEM | | CARDIOVASC./HEMAT. | | NEUROLOGIC |
|---|---|---|---|---|---|
| X | Tongue | X | Aorta* | XX | Brain* |
| | Salivary glands* | XX | Heart* | X | Periph.nerve* |
| X | Esophagus* | X | Bone marrow* | X | Spinal cord (3 levels)*[T] |
| X | Stomach* | X | Lymph nodes* | | |
| X | Duodenum* | XX | Spleen* | X | Pituitary* |
| X | Jejunum* | X | Thymus* | X | Eyes (optic n.)*[T] |
| X | Ileum* | | | | |
| X | Cecum* | | UROGENITAL | | GLANDULAR |
| X | Colon* | | | | |
| X | Rectum* | XX | Kidneys*+ | XX | Adrenal gland* |
| XX | Liver*[+] | X | Urinary bladder* | X | Lacrimal gland[T] |
| | Gall bladder* | XX | Testes*[+] | X | Mammary gland[T] |
| X | Pancreas* | X | Epididymides | XX | Parathyroids*[++] |
| | | X | Prostate | XX | Thyroids*[++] |
| | | X | Seminal vesicle | | |
| | | XX | Ovaries*[+] | | |
| | RESPIRATORY | X | Uterus* | | |
| | | X | Cervix | | |
| X | Trachea* | X | Vagina | | |
| X | Lung* | | Penis | | |
| X | Nose (Nasal Cavity) | | | | OTHER |
| | Pharynx | | | | |
| X | Larynx | | | X | Bone* |
| X | Oral tissues | | | X | Skeletal muscle* |
| | | | | X | Skin* |
| | | | | X | All gross lesions and masses* |

* Required for chronic studies based on Subdivision F Guidelines.
\+ Organ weight required in chronic studies.

All tissues at the high dose were preserved for possible future examination; a full complement of tissues was examined histologically for the control and mid-dose groups at 24 months. In the intermediate- and low-dose groups, liver, kidney, lungs, mesenteric lymph nodes, thyroid with parathyroid, tongue, stomach, mammary glands, larynx, spleen, prostate and gross lesions were examined microscopically.

At the 12-month sacrifice, a full complement of tissues was examined for the control and 0.1% groups.

## II. RESULTS

### A. OBSERVATIONS

1. Mortality - Survival through week 52 was 98-100% in all groups. Mortality rates of the groups receiving 0.1% XDE-125 were 60% in females compared to 6% for controls at termination (88 weeks)and mortality rate at 102 weeks was 80% for the 0.1% males compared to 26% for controls, which exceeds the FIFRA guideline requirements. The lower dose (0.05%) was therefore evaluated as the high dose in the study. At week 105, survival in the 0.05% group was 76% in males and 84% in females. Survival data are summarized in Table 3.

TABLE 3. SURVIVAL (%) IN RATS FED XDE-105.[a]

| Week | Males (% in Diet) | | | | | Females (% in Diet) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | .005 | .02 | .05 | 0.1 | 0 | .005 | .02 | .05 | 0.1 |
| 52 | 100 | 100 | 100 | 100 | 98 | 100 | 100 | 100 | 100 | 100 |
| 76 | 100 | 94 | 98 | 98 | 96 | 98 | 98 | 100 | 100 | 82 |
| 84 | 94 | 94 | 96 | 94 | 94 | 96 | 96 | 96 | 100 | 48 |
| 88 | 90 | 92 | 94 | 88 | 86 | 94 | 94 | 96 | 100 | 40 |
| 92 | 90 | 90 | 92 | 88 | 74 | 92 | 94 | 96 | 96 | - |
| 100 | 78 | 76 | 80 | 78 | 30 | 82 | 94 | 92 | 88 | - |
| 105 | 72 | 62 | 66 | 76 | - | 70 | 86 | 88 | 84 | - |

[a] Data obtained from Tables 7-8, pages 68-69, in study report DR-0323-1194-005.

2. Toxicity - There were no clinical observations related to dietary levels of 0.005%, 0.02%, or 0.05% XDE-105. In the high-dose group (0.1%), thin appearance, rapid respiration, and perineal soiling were observed. The animals were debilitated and excessive toxicity was the basis for early sacrifice of males (714 days) and females (611 days).

### B. BODY WEIGHT

Mean body weights were significantly lower than controls for males at 0.1% beginning at week 25 and were 5%, 9.5%, 15%, and 18% lower at weeks 53, 77, 93, and 101, respectively. In females at 0.1%, mean body weights were significantly decreased beginning at 4 weeks, and were 2%, 10%, 14%, and 17% less than controls at weeks 4, 53, 77, and 85,



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respectively ($p < 0.05$). Mean weight gain data at selected intervals are summarized in Table 4.

TABLE 4. MEAN WEIGHT GAIN (G).[a]

| Weeks | Dietary Level (%) | | | | | Change in high dose (%) |
|---|---|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 | 0.10 | |
| Males | | | | | | |
| 0-13 | 169 | 171 | 171 | 169 | 165 | -2% |
| 0-25 | 224 | 224 | 226 | 223 | 214* | -4.5% |
| 0-53 | 285 | 285 | 283 | 283 | 266* | -7% |
| 0-77 | 299 | 298 | 301 | 291 | 259* | -13% |
| 0-85 | 291 | 296 | 299 | 286 | 240* | -18% |
| 0-97 | 280 | 275 | 278 | 267 | 209* | -25% |
| Females | | | | | | |
| 0-6 | 56 | 54 | 56 | 55 | 53* | -5% |
| 0-13 | 82 | 79 | 82 | 80 | 77* | -6% |
| 0-25 | 97 | 95 | 99 | 98 | 95 | -2% |
| 0-53 | 113 | 111 | 121* | 117 | 109 | -4% |
| 0-77 | 152 | 146 | 158 | 153 | 117* | -24% |
| 0-85 | 159 | 156 | 166 | 159 | 116* | -27% |

[a] Data obtained from Tables 13-14, pages 84-97, in study report DR-0323-1194-005.
* Significantly different from control value, $p < 0.05$.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: There were no important effects on food consumption in either sex at any dose.

2. Compound consumption: Time-weighted average compound consumption in mg/kg/day were summarized in Table 1 of this report.

3. Food efficiency: No meaningful differences in food efficiency were observed at any dose during weeks 1-13.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related effects in either sex.

E. BLOOD WORK

1. Hematology - No important effects on red blood cell parameters were observed. Mean HCT levels were lower than controls (4%; $p < 0.05$) in high dose females at 12 months, but RBC and HGB were not different. Mean WBC counts were higher than control in 0.1% females at 18 months ($5 \times 10^3/mm^3$ compared to $3.6 \times 10^3/mm^3$); percent neutrophils were increased and lymphocytes decreased. This may be related to inflammation of the lungs and thyroid.

2. Clinical chemistry - Serum alkaline phosphatase (AP) levels were significantly increased compared to controls at 6 and 12 months for females in the 0.1% group and at 18 months for males in the 0.05% and 0.1% groups. Aspartic amino-transferase (AST) activity was significantly increased in the 0.1% group for males (12 mos) and females (18 mos). BUN levels were slightly but non-significantly increased compared to controls at all intervals in males and females in the 0.1% group but no kidney lesions accompanied the changes. Other changes in clinical chemistry parameters that reached a level of significance were not considered related to dosing since they were slight, not dose related, or consistent over time. Table 5 summarizes data for ALP and AST activity.

TABLE 5. ASPARTIC-AMINOTRANSFERASE AND ALKALINE PHOSPHATASE ACTIVITIES.[a]

| Month | Males/Dose (%) | | | Females/ Dose (%) | | |
|---|---|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0 | 0.05 | 0.1 |
| AST (U/L) | | | | | | |
| 6 | 96 ± 13 | 80 ± 8* | 100 ± 18 | 82 ± 21 | 73 ± 11 | 84 ± 19 |
| 12 | 89 ± 11 | 88 ± 19 | 120 ± 29* | 86 ± 26 | 75 ± 8 | 101 ± 19 |
| 18 | 79 ± 18 | 70 ± 7 | 63 ± 16[b] | 79 ± 17 | 78 ± 9 | 109 ± 19* |
| 24 | 72 ± 11[b] | 64 ± 21[b] | --- | 71 ± 16[b] | 63 ±10[b] | --- |
| ALP (U/L) | | | | | | |
| 6 | 79 ± 7 | 79 ± 7 | 86 ± 1 | 46 ± 7 | 50 ± 7* | 59 ± 12* |
| 12 | 64 ± 10 | 67 ± 7 | 67 ± 4 | 34 ± 5 | 37 ± 3 | 45 ± 8 |
| 18 | 54 ± 7 | 67 ± 5* | 77 ± 8* | 43 ± 6 | 47 ± 10 | 54 ± 19 |
| 24 | 57 ± 22 | 61 ± 17[b] | --- | 44 ± 11[b] | 49 ± 5[b] | --- |

[a] Data obtained from Tables 61-68, pp 180-187, in study report DR-0323-1194-005.
[b] Means and standard deviations were recalculated by the reviewers, omitting statistically identified outliers.
* Significantly different from control value, analysis by study laboratory ($p < 0.05$).

F. URINALYSIS: - No changes in urinary parameters that were observed in dosed groups were considered related to dosing.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - Table 6 summarizes selected organ weight data in males and females in the control, 0.05% and 0.1% groups at 12 and 24 months. No changes in absolute or relative (to body weight) data were seen at the lower doses.

TABLE 6. ABSOLUTE AND RELATIVE ORGAN WEIGHT (%).[a]

| Organ | Males (Dietary level-%) | | | Females (Dietary level-%) | | |
|---|---|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0 | 0.05 | 0.1 |
| | 12 Months | | | | | |
| Heart g | 1.01 | 1.03 | 1.12* | 0.64 | 0.68* | 0.74* |
| % | 0.249 | 0.265* | 0.291* | 0.32 | 0.34* | 0.39* |
| Kidney g | 2.37 | 2.32 | 2.61* | 1.39 | 1.47 | 1.65* |
| % | 0.58 | 0.60 | 0.68* | 0.69 | 0.73 | 0.88* |
| Liver g | 10.12 | 10.12 | 10.64 | 5.38 | 5.65 | 6.21* |
| % | 2.48 | 2.60 | 2.67* | 2.66 | 2.81 | 3.30* |
| Spleen g | 0.70 | 0.66 | 0.91* | 0.43 | 0.47 | 0.67* |
| % | 0.17 | 0.17 | 0.24* | 0.21 | 0.23* | 0.36* |
| Thyroid | 0.029 | 0.031 | 0.106* | 0.020 | 0.022 | 0.059* |
| % | 0.007 | 0.008 | 0.028* | 0.010 | 0.010 | 0.032* |
| | 24-Months | | | | | |
| Heart g | 1.12 | 1.15 | - | 0.85 | 0.92* | - |
| % | 0.30 | 0.31 | | 0.34 | 0.37* | - |
| Kidney g | 2.91 | 3.03 | - | 1.98 | 2.16* | - |
| % | 0.79 | 0.83 | | 0.80 | 0.87* | - |
| Thyroid | 0.037 | 0.042* | - | 0.031 | 0.062* | - |
| % | 0.010 | 0.012* | | 0.012 | 0.025* | - |

[a] Data obtained from Tables 77-80, pages 196-203, in study report DR 0323-1194-105.

* Significantly different from control, $p < 0.05$.

The following histologic changes correlated with organ weight changes:

- Degeneration of heart muscle fibers with interstitial accumulation of macrophages in males and females at 0.1% at 12 months;
- Vacuolation of tubular epithelium of kidneys and increase in absolute and relative kidney weights in 0.1% females at 12 months.
- Increase in reticuloendothelial cells and increase in absolute and relative liver weights in 0.1% females at 12 months;

- Increase in extramedullary hemopoietic cells and increased absolute and relative spleen weight in 0.1% males at 12 months;
- Vacuolation of thyroid follicular cells and inflammation of thyroid in 0.1% males and females at 12 months and in 0.05% males and females at 24 months.

2. Gross pathology - At the 12-month sacrifice, the lungs of 8/10 females at 0.1% XDE-125 had multiple pale foci. Other gross lesions, including a kidney nodule in a 0.1% male (cause of death), were spontaneous, at a low incidence, and not dose or compound related. Tables 7A and 7B present selected data for gross lesions in the rats (50/group/sex) in the main study.

TABLE 7A. GROSS NECROPSY FINDINGS IN MALES AT 24 MONTHS (MAIN GROUPS).[ab]

| Organ/Finding | Dose (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 | 0.1 |
| Perineal staining | 0 | 4 | 7 | 4 | 10 |
| Decreased fat | 4 | 7 | 4 | 3 | 32 |
| Heart (Thrombus) - Left atrium | 0 | 0 | 1 | 0 | 21 |
| Lungs- | | | | | |
| Area pale,multifocal | 0 | 0 | 0 | 0 | 12 |
| Focus,pale multifocl | 0 | 0 | 1 | 1 | 13 |
| Mammary glands - Hyperplasia | 6 | 5 | 5 | 2 | 0 |
| Stomach - Glandular erosion/ulcer | 13 | 11 | 4 | 5 | 16 |
| Thorax - Hydrothorax/serosang. | 0 | 0 | 1 | 0 | 19 |
| Thyroid - Increased size | 0 | 1 | 0 | 1 | 24 |

[a] Data were obtained from Table 82, pp 206-228, in study report DR-0323-1194-005.

[b] The study included 50 animals/sex/group, all modes of death or sacrifice.

TABLE 7B. GROSS NECROPSY FINDINGS IN FEMALES AT 24 MONTHS (MAIN GROUPS).[ab]

| Organ/Finding | Females/Dose (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 | 0.1 |
| Perineal staining | 5 | 6 | 6 | 2 | 17 |
| Decreased fat | 9 | 4 | 4 | 4 | 20 |
| Heart (Thrombus) - Left atrium | 0 | 0 | 0 | 0 | 11 |
| Lungs- | 0 | 0 | 0 | 0 | 8 |
| Area pale,multifocal | 5 | 2 | 0 | 11 | 19 |
| Focus,pale multifocl | - = | | | | |
| Mammary glands - Hyperplasia | 21 | 17 | 30 | 23 | 1 |
| Stomach - Glandular erosion/ulcer | 8 | 5 | 3 | 3 | 6 |
| Thorax - Hydrothorax/serosang. | 0 | 0 | 0 | 0 | 14 |
| Thyroid - Increased size | 0 | 0 | 1 | 0 | 12 |
| Uterus - Mass/nodule | 14 | 11 | 19 | 20 | 15 |

[a] Data were obtained from Table 82, pp 206-228, in study report DR-0323-1194-005.

[b] The study included 50 animals/sex/group, all modes of death or sacrifice.

The incidence of many lesions was increased primarily in the 0.1% group of both sexes (decreased fat, perineal staining, thrombi in the left atrium of the heart, increased size of the thyroid, pale foci (multifocal) in the lungs, erosion/ulcer of the glandular stomach, and serosanguinous hydrothorax). Some lesions were increased in the 0.05% group but not in the 0.1% group (brain compression in males due to a pituitary mass, mammary gland hyperplasia). Lesions that were frequent in all groups including controls were masses or nodules in the testis or uterus, female mammary gland hyperplasia, and increased spleen size.

3. Microscopic pathology

a) Non-neoplastic - Tables 8A and 8B, and 9A and 9B, summarize the incidence of nonneoplastic lesions at 12 months and for rats scheduled for sacrifice at 24 months, respectively.

TABLE 8A. NON-NEOPLASTIC HISTOLOGIC LESIONS IN MALES AT 12 MONTHS.[a]

| Organ: Lesion, severity[b] | Dose (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 | 0.1 |
| HEART: Degeneration, sl. | 0 | 0 | 0 | 0 | 3 |
| KIDNEYS: Tubular vacuolat. | 0 | 0 | 0 | 0 | 0 |
| LIVER : Aggregates of RE cells, v sl./sl. | 7 | 5 | 4 | 5 | 9 |
| LARYNX | | | | | |
| Aggregates of RE cells, v.sl./sl. | 0 | 0 | 0 | 0 | 2 |
| Degen. muscle fibers v.sl. | 0 | 0 | 0 | 0 | 2 |
| LUNGS: Inflammation, v.sl. | 1 | 1 | 0 | 2 | 6 |
| STOMACH | | | | | |
| Glandular degen./regeneration v.sl. | 0 | 0 | 0 | 0 | 0 |
| SPLEEN: Increased hematopoiesis | 0 | 0 | 0 | 0 | 0 |
| THYROID | | | | | |
| Vacuol. epithelia | | | | | |
| Sl. | 0 | 0 | 0 | 10 | 8 |
| Mod. | 0 | 0 | 0 | 0 | 2 |
| Inflammation,mod. | 0 | 0 | 0 | 0 | 9 |

[a] Data were obtained from Table 83, pages 229-242, in study report DR-0323-1094-005.

[b] Severity - v.sl. = very slight; sl. = slight; mod. = moderate.

TABLE 8B. NON-NEOPLASTIC HISTOLOGIC LESIONS IN FEMALES AT 12 MONTHS.[a]

| Organ: Lesion, severity[b] | Dose (%) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 | 0.1 |
| HEART: Degeneration, sl. | 0 | 0 | 0 | 0 | 4 |
| KIDNEYS: Tubular vacuolat. | 0 | 0 | 0 | 0 | 9 |
| LIVER: Aggregates of RE cells, v sl./sl. | 8 | 5 | 4 | 5 | 10 |
| LARYNX | | | | | |
| Aggregates of RE cells, v.sl./sl. | 0 | 0 | 1 | 0 | 7 |
| Degen. muscle fibers, v.sl. | 0 | 0 | 0 | 0 | 2 |
| LUNGS: Inflammation, v.sl. | 1 | 1 | 1 | 3 | 10 |
| STOMACH | | | | | |
| Glandular degen./regeneration v.sl. | 0 | 0 | 0 | 0 | 9 |
| SPLEEN: Increased hematopoiesis | 0 | 0 | 0 | 0 | 10 |
| THYROID | | | | | |
| Vacuol. epithelia | | | | | |
| Sl. | 0 | 0 | 0 | 5 | 0 |
| Mod. | 0 | 0 | 0 | 5 | 10 |
| Inflammation, mod. | 0 | 0 | 0 | 0 | 7 |

[a] Data were obtained from Table 83, pages 229-242, in study report DR-0323-1094-005.

[b] Severity - v.sl. = very slight; sl. = slight; mod. = moderate.

At 12 months, most histologic changes were observed only in the 0.1% group - slight degeneration of the myocardium of the heart in both sexes with accumulation of macrophages and replacement of muscle cells with connective tissue; slight to very slight aggregates of RE cells and degeneration of muscle fibers in the larynx in males and females; and degeneration/regeneration of the mucosa of the glandular stomach in females. Aggregation of RE cells in the lymph nodes was seen in all rats including controls but the severity was moderate in 9/10 males and 9/10 females at 0.1%. Very slight inflammation of the lungs (subacute to chronic and multifocal) was seen in 6 males and 10 females at the highest dose. Changes in the thyroids consisted of moderate inflammation in 0.1% males and females and slight to moderate epithelial vacuolation in all rats at 0.05 and 0.1% with a greater severity in females than in males.

At 24 months, tissues at 0.1% were not routinely examined, since the MTD was exceeded. Selected non-neoplastic lesions are presented in Table 9.

TABLE 9A. NONNEOPLASTIC HISTOLOGIC LESIONS IN MAIN MALE GROUPS AT 24 MONTHS.[a]

| | Dose (% in diet) | | | |
|---|---|---|---|---|
| Organ: Lesion, severity[b] | 0 | 0.005 | 0.02 | 0.05 |
| LUNGS: Inflammation | | | | |
| Multifocal, v. sl. | 6 | 8 | 7 | 9 |
| Focal/multifocal | 14 | 17 | 13 | 16 |
| THYROID | | | (49)[c] | |
| Vacuolation epithelial cells, v. sl./ sl. | 0 | 0 | 7* | 48* |
| Inflammation | 0 | 0 | 0 | 3 |
| BRAIN: Trapezoid body, degen. of individual fibers, v.sl. multifoc | 2 | (22) 0 | (22) 0 | 4 |
| ADRENAL: Focus of altered cells, v. sl./sl. | 18 | (23) 7 | (20) 5 | 23 |
| LYMPH NODES: mesenteric aggregates of RE cells | | | | |
| v. sl. | 38 | 42 | 45* | 24* |
| slight | 7 | 6 | 3 | 24* |
| EYES | | (25) | (20) | |
| Retinal degeneration | 29 | 12 | 2 | 34 |
| Inflammation | 8 | 11 | 11 | 10 |

[a] Data obtained from Table 84, pages 243-290, in study report DR-0323-1194-005.

[b] Grades of severity: v.sl. = very slight; sl. = slight; mod. = moderate.

[c] Number of tissues (X) examined when 50/sex/group were not available.

* Significantly different than control incidence, $p < 0.05$.

TABLE 9B. NONNEOPLASTIC HISTOLOGIC LESIONS IN MAIN FEMALE GROUPS AT 24 MONTHS.[a]

| Organ: Lesion, severity[b] | Dose (% in diet) 0 | 0.005 | 0.02 | 0.05 |
|---|---|---|---|---|
| LUNGS: Inflammation | | | | |
| Multifocal, v. sl. | 2 | 4 | 2 | 28* |
| Focal/multifocal | 8 | 16 | 13 | 37* |
| THYROID | | | | |
| Vacuolation epithelial cells, v. sl./ sl. | 6 | 6 | 34* | 42* |
| Inflammation | 1 | 0 | 0 | 32* |
| BRAIN: Trapezoid body degen. of individual fibers, v.sl. multifoc | 0 | (9)[c] 0 | (10) 0 | 0 |
| ADRENAL: Focus of altered cells, v. sl./sl. | 29 | (10) 5 | (8) 1 | 34 |
| LYMPH NODES: mesenteric aggregates of RE cells | | | | |
| v. sl. | 41 | 41 | 40 | 22* |
| slight | 3 | 4 | 8 | 22* |
| EYES | | (11) | (13) | |
| Retinal degeneration | 46 | 8 | 13 | 46 |
| Inflammation | 8 | 7 | 6 | 14 |

[a] Data obtained from Table 84, pages 243-290, in study report DR-0323-1194-005.

[b] Grades of severity: v.sl. = very slight; sl. = slight; mod. = moderate.

[c] Number of tissues (X) examined when 50/sex/group were not available.

* Significantly different than control incidence, p <0.05.

Inflammation of the lungs, characterized by thickening of the septa, foamy cells, macrophages with pigment and occasional cholesterol clefts, occurred at a significantly increased incidence in 0.05% females as compared to controls. Increases were not seen in dosed males or were they significant in females at 0.005% or 0.02%. Lungs of nine females in the 0.1% group (eight spontaneous deaths and a moribund sacrifice) were examined histologically; all had slight alveolar histiocytosis, inflammation and mineralization of blood vessels.

There was a high incidence of slight vacuolation of epithelial cells of the thyroid follicles in males and females at 0.02% and 0.05% XDE-105 and subacute to chronic inflammation (predominantly very slight to slight) was significantly increased in females at 0.05%.

Very slight multifocal degeneration of individual nerve cells in the trapezoid body of the brain was observed in four males at 0.05% compared to two control males. There was a slight increase in foci of altered cells in the adrenals of both sexes in the 0.05% group. Aggregates of RE cells in the mesenteric lymph nodes were seen in the majority of rats in all groups; the severity of the finding was somewhat greater in the 0.05% groups (ie., slight vs very slight). This could be compound related. Seven females in the 0.05% group had hyperplasia of the myeloid bone marrow; this finding was considered secondary to other lesions and not compound related.

b) Neoplastic - Tables 10A and B summarize selected incidence data for neoplasms. There was no treatment-related oncogenic response.

TABLE 10A. NEOPLASTIC LESIONS IN MALE RATS FED XDE-125 FOR 2 YEARS.[a]

| Organ: Neoplasm, severity[b] | Dose (% in diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 |
| PANCREAS: | | (20)[c] | (18) | |
| Islet cell adenoma | 8 | 1 | 2 | 13 |
| PITUITARY (anter.): | | (35) | (32) | |
| Adenoma | 23 | 22 | 22 | 24 |
| MAMMARY GLANDS: | | (31) | (28) | (49) |
| Adenocarcinoma | 0 | 1 | 0 | 0 |
| Fibroadenoma | 1 | 4 | 4 | 4 |
| RETICULOENDOTHELIAL: | (49) | (40) | (37) | (49) |
| Granulocytic leukemia | 19 | 15 | 6 | 6 |
| TESTIS: | | (47) | (47) | |
| Leydig cell tumor | 45 | 44 | 45 | 47 |

[a] Data obtained from Table 86, pages 292-298, in study report DR-0323-1194-0054.

[b] Grades of severity: v.sl. = very slight; sl. = slight; mod. = moderate.

[c] Number of tissues (X) examined when 50/sex/group were not available.

TABLE 10B. NEOPLASTIC LESIONS IN FEMALE RATS FED XDE-125 FOR 2 YEARS.[a]

| Organ/Neoplasm | Females/Dose (%in diet) | | | |
|---|---|---|---|---|
| | 0 | 0.005 | 0.02 | 0.05 |
| PANCREAS: | | (7) | (6) | |
| Islet cell adenoma | 3 | 0 | 0 | 1 |
| PITUITARY (anter.): | | (27) | (38) | |
| Adenoma | 27 | 15 | 27 | 20 |
| MAMMARY GLANDS: | (49) | (28) | (38) | |
| Adenocarcinoma | 0 | 1 | 0 | 0 |
| Fibroadenoma | 8 | 2 | 6 | 2 |
| RETICULOENDOTHELIAL: | (49) | (39) | (36) | (49) |
| Granulocytic leukemia | 11 | 5 | 8 | 8 |
| UTERUS: | | (26) | (31) | |
| Endometrial stromal polyp | 18 | 18 | 25 | 23 |

[a] Data obtained from Table 86, pages 292-298, in study report DR-0323-1194-0054.
[b] Grades of severity: v.sl. = very slight; sl. = slight; mod. = moderate.
[c] Number of tissues (X) examined when 50/sex/group were not available.

## NEUROTOXICITY

CITATION: Spencer, P.J., and B.L. Yano. (1995) XDE-105: Chronic neurotoxicity study in Fischer rats. The Toxicology Research Laboratory, Dow Chemical Company, Midland, MI. Laboratory Study No. DR-0323-1194-005N. April 28, 1995. MRID 43710503. Unpublished.

### I. MATERIALS AND METHODS

A. MATERIALS

Data are presented in the preceding chronic/oncology study review.

B. STUDY DESIGN

Fifteen animals/sex/group were assigned to the study (satellite animals) for assessment of chronic toxicity and chronic neurotoxicity at 1 year. Ten rats/sex/dose group were for the FOB and motor activity portion of the study (assessed at pretest and during months 3,6,9 and 12); five/sex/group were assigned to the neuropathology portion. The dose equivalents in the groups were 0,. 4.6, 9.2, 23.0, and 46.0 mg/kg/day for males and 0. 5.7, 11.4, 28.5, and 57.0 mg/kg/day for female groups.

C. METHODS

1. Body weights

Body weights taken nearest the time to each FOB testing were analyzed statistically and reported.

2. The following Functional Observation Battery (FOB) parameters were recorded:

| Measurements/Counts | Recorded As |
|---|---|
| Hindlimb grip performance | Grams force |
| Forelimb grip performance | Grams force |
| Landing foot splay | Distance between hindfeet(cm) |
| **Hand-held observations** | |
| General (thin,fat red ocular/nasal crusts | Description |
| Palpebral closure | Rank |
| Pupil size | Normal,Increased or Decreased |
| Lacrimation | Rank |
| Salivation | Rank |
| Abnorm. of skin or haircoat | Description |
| Perianal staining | Present or Absent |
| Tremors,convulsions, muscle tone | Description |
| Abnormal respiration | Description |
| Reactivity to handling | Rank |
| **Open-field observations** | |
| Level of activity | Rank |
| Responsiveness to sharp noise | Rank |
| Responsiveness to touch | Normal, Increased, Decreased |
| Responsiveness to tail pinch | Rank |
| Abnormal behavior-stereotypy,circling | Description |
| Gait abnormalities | Rank |
| Urine volume in FOB | Rank |
| Fecal boluses in FOB | Rank |

One observer conducted all of the FOB observations. The rats were selected in a random manner in such a way that the observer did not know the dose group of the rat.

Hindlimb grip performance was conducted as follows. The rat's fore legs were placed on a bench and the hind legs were set on a horizontal screen attached to a strain gauge. The observer then pulled back smoothly and firmly on the rat's tail until its grip on the screen was broken. The average of 3 trials was used for statistical analysis.

Forelimb grip strength was similarly measured with the rat's fore feet on the screen and the hind feet placed on a smooth plastic surface. Individual grip performance (grams of pull) was divided by the rat body weight, resulting in a measure of grams pull/gram body weight.

Landing foot splay was determined as follows. The tarsal joint pads of the rear feet were marked with ink and the animal dropped from a distance of about 30 cm onto the recording sheet. This was repeated 3 times and the distance from center to center of the ink marks was measured. The average of 3 splay values was used for statistical analysis

Motor Activity procedures are as follows. Motor activity cages (chambers) consisted of a circular clear plastic alley. An infrared photobeam bisected the alley so that the beam crossed the alley in 2 locations. The chambers were isolated from each other in a quiet dim room. Each animal was tested individually and test sessions consisted of six 8-minute epochs, totalling 48 min of testing. Total activity counts for each epoch were recorded, Each beam break that lasted more than 100 msec constituted an activity count. This duration was set to discount activities such as tail flicking, rearing, head bobbing etc. Cages were calibrated prior to each testing day and photocells were adjusted to assure an equivalence of devices.

3. Statistical Analysis

Statistical analyses were conducted on body weights, grip performance (ave. of 3), landing foot splay (ave. of 3). Motor activity counts were expressed as square roots to minimize problems of heterogeneity of variance and departure from normality.

The incidence of ranked FOB observations, between control and dose were evaluated by a test of proportions and only those findings with a critical z-score for an alpha=0.02 were reported. Ranked scores for each observation were converted to composite scores as the sum of (rank x incidence) for that observation (overall summarization for each sex for each observation at each dose level).

For continuous data, means and standard deviations were calculated and homogeneity of variance confirmed with the F-max test.

Repeated-measures ANOVA, a multivariate analysis, was used to account for all time periods and both sexes. This increases the degrees of freedom (e.g.,18 degrees of freedom/dose level for 10M/10F). The following interactions were examined:

Treatment x Time
Treatment x Time x Sex
Treatment x Time x Epoch (motor activity only)

The inclusion of preexposure data in the analysis makes relevant only the analysis which includes factors of both treatment and time. To reduce the rate of false declarations, the type I error rate (alpha) per comparison was set at 0.02. Probability values were reported without correction.

4. Historical Control Data

Positive control data for FOB parameters were provided:

Control- saline
Chlorpromazine - 4 mg/kg ip; examinations 10-20 min post-dosing
d-Amphetamine - 8 mg/kg ip; examinations 15-20 min post-dosing
Atropine/Physostigmine - 2 mg/kg/0.75 mg/kg after 5 min, both ip - examination after 10-30 min.

5. Neuropathology

Rats (5/sex/group) were anesthetized, the thorax opened and they were perfused with phosphate buffer containing 0.7% $NaNO_3$, followed by a buffered solution of 1.5% glutaraldehyde/4% formaldehyde. The remaining 5 rats/sex/group were sacrificed and tissues fixed as usual in formaldehyde. A complete gross necropsy was conducted on both groups of rats.

Tissues for neuropathologic examination were embedded in paraffin (brain) or plastic (nerves). Neural tissues were examined from males and females in the control- and high-dose groups. Nine transverse sections of the brain were prepared at various levels. Text Figure IV-1 from the study (shown in Appendix 1 of the DER) illustrates the levels of the sections and the structures expected in each of the sections. Tissues from the central nervous system were stained with H&E; peripheral nerves and dorsal root ganglia were treated with osmium before embedding in plastic and sections were stained with toluidine blue.

The following table lists the nervous system tissues that were examined microscopically.

Neuropathology - Tissues Examined

Brain
-Olfactory bulb
-Cerebrum,frontal,parietal,temporal, occipital
-Thalamus/Hypothalamus
-Midbrain
-Pons
-Cerebellum
-Medulla-gracilis/cuneatus,reticular nuclei, trapezoid body
Pituitary
Trigeminal ganglia and nerve
Spinal cord (cross & oblique sect)
Cervical Swelling (C3-C6)
Lumbar Swelling (L1-L4)

Dorsal Root Ganglia
Cervical and Lumbar
Dorsal and Ventral Roots
Cervical and Lumbar
Peripheral nerves (cross and longitudinal section)
Proximal Sciatic
Tibial
Sural
Optic
Eyes
Skeletal muscle
Anterior tibial and Gastrocnemius

Positive Control neuropathology incidence data were provided. These included data from rats treated with 55 mg/kg acrylamide (5x/week for 3 weeks) or a single dose of 7 mg/kg trimethyl tin (Study Report, pp 506-517).

## II. RESULTS

### A. Functional Observation Battery

Treatment did not affect body weights, hand-held or open field observations, hindlimb grip strength, forelimb grip strength, or landing foot splay in either males or females at any dose group.

Composite scores (rank of effect x no. of rats) that were subjectively disparate were urination in open field in 0.005% males at preexposure and 3 months (19-20 vs 11 for controls; level of activity for 0.05% females at 6 months (28 vs 21 for controls); responsiveness to tail pinch in dosed females at 6 months (30-31 vs 24 for controls); level of activity in 0.05% males at 12 months (26 vs 18 for controls); and responsiveness to sharp noise in 0.005% males at 12 months (29 vs 22 for controls).

Significant observation with the test for proportions ($p < 0.02$) were moderate activity and responsiveness to tail pinch in 0.05% females at 6 months; minimal defecation in 0.1% males at 12 months; moderate activity in 0.05% males and moderate response to sharp noise in 0.005% males at 12 months. The random pattern of these differences between sex and dose group and lack of dose response supports the absence of treatment related effects.

### B. Motor Activity

No effect was observed at any time on any aspect of motor activity in either sex. Significant effects unrelated to treatment (time or sex) show that the lack of treatment effects were unlikely to be due to an overall lack of statistical power to detect an effect.

### C. Neuropathology

Neuropathology findings are summarized in Table 12.

TABLE 12. NEUROHISTOLOGIC PATHOLOGY FINDINGS.[a]

| Site/Finding | Males/Dose | | Females/Dose | |
|---|---|---|---|---|
| | 0 | 0.1 | 0 | 0.1 |
| Brain-Medulla oblongata | | | | |
| Swollen axons, very sl., foc./multifocal | | | | |
| Nucleus gracilis | 3 | 1 | 4 | 4 |
| Trigeminal nerve | 1 | 0 | 1 | 2 |
| Reticular nucleus | 1 | 0 | 0 | 0 |
| Degeneration indiv. nerve fibers, v. sl. | | | | |
| Trapezoid body | 4 | 4 | 0 | 2 |
| Brain-Thalamus/Hypothalamus | | | | |
| Atrophy-Optic tract, moderate | 1 | 0 | 0 | 0 |
| Cranial Nerve-Optic | | | | |
| Atrophy, unilateral/moderate | 1 | 0 | 0 | 0 |
| Dorsal Root Ganglia with roots-lumbar | | | | |
| Degeneration-individual n. fibers, very slight, multifocal | 1 | 1 | 0 | 0 |
| Eyes | | | | |
| Mineralization-blood vessels/sclera very sl., unilateral/bilateral | 5 | 4 | 2 | 3 |
| Skeletal muscle | | | | |
| Focal degeneration, v. slight-ant. tibial | 0 | 0 | 0 | 1 |
| Spinal cord-cervical | | | | |
| Swollen axons- focal, very slight multifocal | 1 | 3 | 4 | 3 |
| Degeneration indiv. n. fibers, very sl. | 5 | 5 | 5 | 5 |
| Spinal cord-lumbar | | | | |
| Swollen axons- focal, very slight multifocal | 2 | 1 | 1 | 2 |
| Degeneration indiv. n. fibers, very sl. | 2 | 1 | 1 | 1 |
| Trigeminal ganglia | | | | |
| Degeneration indiv. nerve fibers, focal multifocal, very slight | 1 | 0 | 2 | 0 |

[a] Data were obtained from Table IV-2, pages 92-96, in Study No. DR-0323-1194-005N.

XDE-125 was not neuropathic. Spontaneous lesions that occurred were mineralization of the cerebellum, swollen areas in the region of the gracilis nucleus and various other areas; degeneration of individual nerve fibers of the medulla oblongata, trigeminal ganglia, sciatic nerve, dorsal root ganglia,and spinal cord; unilateral retinal degeneration and optic nerve atrophy. These lesions were generally very

slight in severity and their incidence did not indicate that they were treatment related.

## III. DISCUSSION/CONCLUSIONS

### A. Investigator's Conclusion

The LOEL for systemic toxicity was 0.02% XDE-105 in the diet based on slight vacuolation of the follicular epithelial cells of the thyroid in males and females and the NOEL after 24-months dietary exposure was 0.005% XDE-105 for male and female Fischer 344 rats, which is equivalent to 2.4 or 3.0 mg/kg/day, respectively. The highest dietary level (0.10%) was considered to have exceeded the MTD. There was no carcinogenic response. The NOEL for chronic neurotoxicity based on FOB evaluation, motor activity and neuropathologic evaluation was 0.01% XDE-105, equivalent to 50.7 mg/kg/day in males and 63.8 mg/kg/day for female rats.

### B. Reviewer's Discussion

With the exception of lack of histopathology data for the high dose groups after the 12-month sacrifice in the chronic study, the study was adequately conducted and reported and individual animal data supported the summary data. The thyroid was the most sensitive target organ in rats and the severity of toxicity increased with time of dosing. At 12 months, vacuolation of the follicular epithelial cells of the thyroid was observed in all males and females at 0.05% and 0.10% doses. The severity of the finding was slight in most males but moderate in 5/10 females at 0.05% and all females at 0.10% XDE-105. Moderate inflammation of the thyroid was observed in the majority of rats at the 0.10% dose at 12 months. Absolute and relative thyroid weights were significantly increased in both sexes at 0.10%. In the second year of the study, the size of the thyroid observed at gross necropsy was increased in 24/50 males and 12/50 males at 0.10%. Thyroid weights were not evaluated in the 0.10% group at terminal sacrifice. Absolute and relative thyroid weights were significantly increased (2-fold) compared to controls in the 0.05% females at terminal sacrifice. In the main study, vacuolation of the epithelial cells of the thyroid was seen in 14 and 96% of the males and 68 and 84% of the females at 0.02% and 0.05%, respectively, and thyroid inflammation was present in 64% of the females in the 0.05% group.

Weight gain was not affected at dietary levels of 0.05% or less nor was it affected at 0.10% in the first year of the study. Survival did not decrease in the 0.10% group until after 76 weeks in females and 88 weeks in males. The most

frequent findings for spontaneous deaths were atrial thrombosis, hydrothorax, and heart disease. There was general debility of the rats probably first indicated by decrease in fat depots.

Clinical laboratory findings did not indicate any important effects on hematology parameters. However, in an earlier subchronic rat study at higher doses (0.2% and 0.4%) profound anemia and a compensatory hematopoietic effect were observed. A slight increase in BUN levels, which reached a level of significance in high dose females (6, 12,and 18 months), were not considered of toxicologic importance since the magnitude of the effect was small and it was not accompanied by pathology at 12 months. In a previous subchronic study, renal effects were seen in females at a 0.2% dose. A significant increase an ASAT was observed in 0.1% males at 12 months and in 0.1% females at 18 months. This was not associated with liver toxicity but may be associated with increased heart weights and slight degeneration of heart muscle in the 0.10% males and females observed at 12 months. Increases in alkaline phosphatase activity were seen in 0.10% females at 6 months and in 0.05% and 0.10% males at 18 months. These levels were close to the upper range of historical controls and the toxicologic importance of the increases are doubtful.

At the 12-month sacrifice, histologic findings in organs other than the thyroid were predominantly in the 0.1% group, and several were accompanied by increases in organ weights. Aggregates of reticuloendothelial cells were seen in several organs in rats receiving 0.10% - the spleen of 5/10 females and the larynx of 7/10 females. In the mesenteric lymph nodes, RE cell aggregation was of moderate severity in 9/10 males and 9/10 females whereas the finding was very slight in the majority of rats in control, and groups less than 0.1%. Inflammation of the lungs was seen in 0.1% males and females and glandular degeneration of the stomach in 0.1% females.

At 24 months, thyroid changes were seen at 0.02% and 0.05 % in both sexes; inflammation of the lungs was significantly increased in both sexes at 0.05% and the severity of aggregation of RE cells in the mesenteric lymph nodes was increased at 0.05%. Other histologic findings were probably not related to dosing.

The neurotoxicity study was adequately conducted and reported. The methodology for the FOB and motor activity was validated using positive controls and the same technician conducted the tests with positive controls and the test compound. The evaluator was given the animals for testing not knowing from which test group they were assigned. The equipment for the motor tests was calibrated and adjusted

daily for uniformity. The expected responses were found for the positive controls. Histopathologic validation of various types of neurotoxicants were conducted for evaluation purposes. The statistical analysis of the behavioral and motor responses was rigorous. No evidence of neurotoxicity or neuropathology was found with XDE-105. In previous studies, a single dose of up to 2000 mg/kg/day produced no neurotoxic effect and in a supplementary subchronic study in rats, 0.06% XDE-105 (42.7 or 52.1 mg/kg/day in males and females respectively) did not cause any neurotoxicity.

## IV. STUDY DEFICIENCIES

The one major deficiency in the chronic portion of the study is that microscopic examinations were not conducted in the high dose groups after the 12-month sacrifice. As a minor deficiency, blood clotting parameters were not measured.

012237

Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. KB 5/27/97
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Acute Oral Toxicity Study
Guideline 81-1
Species: Rat

EPA Identification Nos.: EPA MRID No. 43770701
EPA Pesticide Chemical Code: 110003
Submission No. S492760
Data Package No. D219011

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): R42590

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN.

Title of Report: XDE-105: The Acute Toxicity of XDE-105 Administered Orally to Fischer 344 Rats.

Author(s): Wright, F.L., M.J. Keton, and D.W. Grothe

Report Issued: November 9, 1992

Executive Summary: The acute oral $LD_{50}$ for male and female Fischer 344 rats is >2000 mg/kg of body weight based on results from this study (MRID 43770701). The results of this study should be considered with a more recent acute oral toxicity study with rats and mice (MRID 43414515) which indicated that four of five male and one of five female rats died after receiving a single oral dose of 5000 mg/kg. **The more recent report noted that the $LD_{50}$ value for male Fischer 344 rats is 3738 mg/kg, and it is apparently based on data from the study reviewed in this DER (MRID 43770701) along with results of the more recent study (MRID 43414515). The results from the two studies place XDE-105 into Toxicity Category III.**

Core Classification: This study by itself does not satisfy §81-1 guideline requirements for an acute oral toxicity study and is classified as Supplimentary. It is classified as acceptable when the results are combined with those from a more recently submitted study (MRID 43414515).

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## Materials and Methods

A. Test Animals: Male and female Fischer 344 rats were used. They were acclimated for a period of approximately 6 days. The animals were about 8 to 9 weeks of age at the start of the test, and were obtained from Harlan Sprague Dawley, Inc., Indianapolis, Indiana.

B. Test Substance: XDE-105 (78.2% a.i.) was supplied as a solid (Lot no. ACD13453).

C. Test substance preparation: The report described preparation of the test substance for dosing as follows:

> A suspension of XDE-105 was prepared once on the day of dosing at a concentration of 102.3 mg/ml in 10% (w/v) aqueous acacia solution...A few drops of a comercially available food-grade simethicone emulsion were added to the suspension and the control solution to reduce foaming.

D. Experimental design: Animals were randomly assigned to test groups as follows:

| Test Group | Dose Level (mg/kg)* | Number Assigned | |
|---|---|---|---|
| | | Males | Females |
| Control | 0 | 5 | 5 |
| Treatment | 2000 | 5 | 5 |

* Diet was provided *ad libitum* but was withdrawn for at least 16 hours prior to dosing by gavage (25 ml/kg).

E. Observations: The observations procedures were described in the report as follows:

> Animals were observed for changes in behavior and appearance. Clinical observations were recorded at 1-hour intervals for the first 6 to 7 hours after dosing, then daily for the subsequent 2 weeks of observation.
>
> Individual body weights were recorded and mean body weights and mean body weight gains for the group (by sex) were calculated weekly.
>
> A gross pathologic examination was performed...on all test animals. This procedure included asystematic evaluation of the general body condition and external body orifices as well as the tissues and organs within the thoracic and abdominal cavities.



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## Reported Results

A. Clinical signs and mortality: None of the test animals died. Clinical signs frequently observed during the observation period included soft stool, hypoactivity and hunched posture on the first day in males and posterior soiling and poor grooming in females on the first day. Most of the effects persisted for two days (males) or up to 4 days in females.

B. Body weights: Group mean body weights and body weight gains showed no effects of treatment.

C. Gross necropsy: Necropsy observations indicated no effects at termination of the study according to the report.

## Discussion

A. Authors' Conclusions: The authors' summary of the results was reported as follows:

> There were no deaths among vehicle-control or treated animals. No lesions were seen in the rats that were necropsied at termination. Treatment-related signs of toxicity were prevalent throughout Test Day 1. Males appeared normal on Test Day 2; females by Test Day 4. Mean body weight and body weight gain values for treated animals were similar to vehicle-control animals at the conclusion of the study.
>
> The median lethal dose of XDE-105, when administered orally to Fischer 344 rats, was >2000 mg/kg of body weight for males and females.

B. Reviewer's Discussion and Conclusions: See "Executive Summary" above.

012237

Primary Review by: Roger Gardner Roger Gardner 5/22/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Developmental Toxicity
Guideline §83-3 (Range-finding)
Species: Rat

EPA Identification No.s: EPA MRID No. 43770702
EPA Pesticide Chemical Code: 110003
Submission No. S492760
Data Package No. D219011

Test Material: XDE-105

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-002 and -002A

Testing Facility: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan

Title of Report: XDE-105: Oral Gavage Teratology Probe Study in Sprague-Dawley Rats

Author(s): Vedula, U., B.L. Yano, and W.J. Breslin

Report Issued: December 18, 1992

Executive Summary: XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 10 mated Sprague-Dawley strain rats by gavage at dose levels of 0, 10, 50, or 150 mg/kg/day (Part 1) or 0, 200, 250 or 300 mg/kg/day (Part 2) from gestation day 6 through 16 (gestation day 0 was the day mating occurred) (MRID 43770702). This study was conducted to define a dose range for the main rat study reviewed previously (see MRID 43557505). Females were observed for changes in appearance or behavior, and body weight and food consumption were determined at intervals during gestation. Animals were sacrificed on gestation day 16, and at necropsy, gross observations of organs and organ weights of the kidneys, spleen, heart and liver were obtained, and reproductive observations were made. Histological observations of the liver, kidney, spleen, thyroid gland, trachea, ovaries, oviducts, uterus, cervix and vagina from two pregnant rats given 0 mg/kg/day and five preganant rats givent 200 mg/kg/day were recorded.

Maternal toxicity was reported at doses of 200, 250 and 300 mg/kg/day and was characterized by decreased body weight at Days 9, 12 and 16 of gestation (5-8% less than controls for all treated groups), and decreased body weight gain for Days 6-9, 6-16, and 0-16 of gestation which were not consistent with increased dose or the decreased mean body weights for treated groups in comparison with controls. Along with these marginal weight

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and weight gain decreases, there were no clinical signs reported, and organ weights were not affected by administration of XDE-105. There were also no increases in the incidence of gross observations that could be related to administration of the test substance, and histological observations were limited to two control group animals and 5 animals from the 200 mg/kg/day dose group.

The limited results of this study suggest that the 200 mg/kg/day dose level, which was the highest dose tested in a definitive developmental toxicity study (MRID 43557505), was not high enough to cause maternal toxicity in pregnant Sprague-Dawley rats. But based on results from a rabbit developmental toxicity study (MRID 43414521), the insufficient dose range evaluated in the definitive study should not be considered a critical deficiency. The highest dose in the rabbit study of 50 mg/kg/day approached a toxic level, which suggests that the rabbit is more sensitive to XDE-105 toxicity, and repeating the rat developmental toxicity study (MRID 43557505) would not add significantly to the data required for developmental toxicity testing.

Core Classification: This study does not, by itself, satisfy §83-3 guideline requirements for a rat developmental toxicity study but should be classified as acceptable supplementary data. The study was intended to define the dose range to be evaluated in a definitive developmental toxicity study.

## Materials and Methods

With the exceptions noted below, the protocol and observations for the range-finding study are similar to those of the definitive study reviewed in another DER (MRID 43557505).

A. Study Design: The report described the experimental design as follows:

> Initially, groups of 10 adult female Sprague-Dawley rats were administered XDE-105 by oral gavage on Days 6-15 of gestation at dose levels of 0, 10, 50,or 150 mg/kg/day (part I). However, the high dose of 150 mg/kg/day did not produce maternal effects sufficient to meet the maximum tolerated dose criteria. Therefore, a repeat of this probe study was conducted at dose levels of 0, 200, 250, or 300 mg/kg/day (Part II).

B. Observations: Observations of the test animals were described in the report as follows:

> All animals were observed daily during the study for alterations in behavior or demeanor. Bodyweights were recorded on Day 0, daily during Days 6 through 16 of gestation...Feed and water consumption was determined at 3-4 day intervals beginning on Day 0.
>
> On Day 16 of gestation all animals which survived the dosing regimen were submitted for a complete necropsy...The animals were weighed (and sacrificed)...Eyes were examined *in situ* by visual inspectin using a glass slide technique. Weights of the liver, kidneys, heart and spleen were reocrded at the time of necropsy and organ-to-body weight ratios calculated. Thyroid glands for all dams were weighed after fixation and organ to body weight ratios calculated...In order to more accurately assess the usage of 200 mg/kg/day as a top dose level for a subsequent gavage teratology study in rats, sections of liver, kidney, spleen, thyroid gland, trachea, ovaries, oviducts, uterus, cervix and vagina from two

pregnant rats given 0 mg/kg/day and five preganant rats givent 200 mg/kg/day were preserved...

Gross examination of the uterus for implantations and resorptions, and the ovaries for number of corpora lutea was performed...The uteri of apparently non-pregnant animals were stained...and examined for evidence of early resorptions. The number of corpora lutea was not recorded for animals that were not visibly pregnant at necropsy.

## Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report described these observations as follows:

All rats survived both test periods (Part I and Part II) and no treatment-related changes in behavior or demeanor were observed at any dose level.

2. Body Weight and Food Consumption: The report described maternal body weight resulst as follows:

Decreases in body weights were noted on various days during the dosing period in dams given 200 mg/kg/day or greater, with statistically identified decreases occurring on Days 9, 12, and 16 in dams given 200 or 300 mg/kg/day and Day 12 in dams given 250 mg/kg/day. Body weight gains of pregnant animals given 200 or 300 mg/kg/day were significantly lower than control values on Days 6-9, resulting in a significant decrease over the entire dosing period. Although not statistically identified, body weight gains of animals given 250 mg/kg/day were slightly decreased over these intervals as well. Body weight effects noted in animals given 200, 250 or 300 mg/kg/day were associated with slight decreases in feed consumption on Days 6-9 and 9-12. No significant effects were observed on body weights, body weight gains, or feed consumption in animals given 10, 50 or 150 mg/kg/day.

| | Dose level (mg/kg/day) | | | |
|---|---|---|---|---|
| Observation | 0 | 200 | 250 | 300 |
| Mean body weight (g) on gestation day | | | | |
| 0 | 276.4 | 274.5 | 267.6 | 270.9 |
| 6 | 321.4 | 315.6 | 311.3 | 315.6 |
| 9 | 338.9 | 312.0* | 322.9 | 318.3* |
| 12 | 358.4 | 300.9* | 336.6* | 332.9* |
| 16 | 391.6 | 367.0** | 368.8 | 360.4** |



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| Observation | Dose level (mg/kg/day) 0 | 200 | 250 | 300 |
|---|---|---|---|---|
| Mean body weight gain gestation days | | | | |
| 0 - 6 | 45.0 | 41.1 | 43.7 | 44.7 |
| 6 - 9 | 17.5 | -31.6* | 11.6 | 2.7* |
| 9 - 12 | 19.5 | 18.9 | 13.7 | 14.7 |
| 12 - 16 | 33.2 | 36.1 | 32.3 | 27.4 |
| 6 - 16 | 70.2 | 51.4** | 57.6 | 44.8** |
| 0 - 16 | 115.2 | 95.5** | 101.2 | 89.5** |

* Significantly different from controls, Dunnett's Test ($p \leq 0.05$).
** Significantly different from controls, Wilcoxon's Test ($p \leq 0.05$).

There were no statistically significant differences indicated in tabulated data from the report with respect to group mean feed and water consumption determinations.

3. Organ Weights: The investigators noted no significant organ weight changes.

4. Necropsy Observations: The report described gross observations as follows:

> No treatment-related effects were noted at dose levels of 10, 50, or 150 mg/kg/day. A dose-related increase in the incidence of increased cecal size was observed in animals given 200, 250, or 300 mg/kg/day (0, 2, 4 and 5 of 10 animals in the control 200, 250, and 300 mg/kg/day groups, respectively)...Other observations were interpreted to be spontaneous changes not attributed to XDE-105 and included decreased amount of fat, mottled kidneys, pale liver, somach focus - dark, and hemorrhage in the thoracic cavity.

Limited histological observations were described in the report as follows:

> Treatment-related effects occurred in the thyroid glands of all five females examined in the 200 mg/kg/day group and consisted of a very slight to slight vacuolation of the epithelial cells lining the follicles (0/2 and 5/5 examined in the control and 200 mg/kg/day dose groups, respectively). These changes were accompanied by an apparent decrease in the staining intensity of the colloid (no numerical data provided). Other organs examined were not affected by exposure to XDE-105.

5. Uterine Observations; The report indicated that there were no treatment-related effects on pregnancy rate or the numbers of corpora lutea, implantations, resorptions or pre- or postimplantation losses. These results are sumarized from the report as follows:

| Observation | Phase I: Dose level (mg/kg/day) 0 | 10 | 50 | 150 |
|---|---|---|---|---|
| Number of animals | 10 | 10 | 10 | 10 |
| Number died: | 0 | 0 | 0 | 0 |
| Pregnant | 0 | 0 | 0 | 0 |
| Non-pregnant | 1 | 0 | 0 | 0 |
| With viable fetuses at termination | 9 | 10 | 10 | 10 |
| Corpora lutea/dam | 18.1 | 16.8 | 18.6 | 19.3 |
| Implantations/dam | 18.1 | 17.1 | 16.5 | 17.8 |
| Resorptions/litter | 1.0 | 0.9 | 1.6 | 1.2 |
| Mean litter size | 17.1 | 16.2 | 14.9 | 16.6 |

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

| Observation | Part II: Dose level (mg/kg/day) 0 | 200 | 250 | 300 |
|---|---|---|---|---|
| Number of animals | 10 | 10 | 10 | 10 |
| Number died: | 0 | 0 | 0 | 0 |
| Non-pregnant | 1 | 0 | 0 | 1 |
| With viable fetuses at termination | 9 | 10 | 10 | 9 |
| Corpora lutea/dam | 17.8 | 18.2 | 17.9 | 17.4 |
| Implantations/dam | 16.7 | 17.1 | 17.5 | 15.3 |
| Resorptions/litter | 0.7 | 0.9 | 0.8 | 0.8 |
| Mean litter size | 16.0 | 16.2 | 16.7 | 14.6 |

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

## Discussion

A. Authors' Conclusions: The authors' conclusions were reported as follows:

> ...oral administration of XDE-105 by gavage resulted in maternal toxicity at dose levels of 200 mg/kg/day and greater. Rats gavaged at 200, 250, and 300 mg/kg/day exhibited

> decreased body weights, body weight gains, and feed consumption. Of the five dams examined histologically that were given 200 mg/kg/day, all exhibited vacuolation of the epithelial cells lining the follicles of the thyroid gland. No significant maternal effects were observed at 10, 50, or 150 mg/kg/day and no adverse effects on water consumption, organ weight, or embryonal/fetal parameters at any dose level tested. Therefore, the maternal no-observed-effect level (NOEL) for this study was 150 mg/kg/day and the embryonal/fetal NOEL was 300 mg/kg/day, the highest dose level tested.

B. Reviewer's Discussion and Conclusions: The Data Evaluation Record for the main study concluded:

> Marginal maternal toxicity was reported at the highest dose tested and was indicated by decreased body weight gain (46% less than that for the control group during gestation days 6-9 and 11% less for the day 9-12 interval), and slightly reduced body weight at Day 12 (high dose group animals weighed an average of 4% less than the control group animals). These weight differences were not noted to occur with dose-related absolute and relative liver, kidney, heart, and spleen weight changes, and no animals were described in the report as having dose-related clinical signs. **The NOEL for maternal toxicity may be ≥200 mg/kg/day.**
>
> The highest dose tested (200 mg/kg/day) may not be adequate, but the range-finding study should be submitted for review before the study can be upgraded or a decision on the adequacy of dosing can be mead.

In the range-finding study, decreased body weight was noted at Days 9, 12 and 16 of gestation when group mean values were 5-8% less than control mean values for the 200, 250, and 300 mg/kg/day dose groups, respectively. Decreased body weight gain for Days 6-9 of gestation were not consistent with increased dose or the decreased mean body weights for treated groups in comparison with controls. The 200, 250 and 300 mg/kg/day dose groups showed gains of -31.6, 11.6 and 2.7 g for Days 6-9 of gestation compared with a mean weight gain of 17.5 g. in control animals. The corresponding mean weights for the control, 200, 250 and 300 mg/kg/day dose groups at Day 9 were 338.9, 312, 322.9, and 318.3 g., respectively. Although the weight gain values varied widely from group to group and were statistically significantly different from controls for the 200 and 300 mg/kg/day dose groups, those differences were not associated with a statistically significant difference between group mean weights for the treated and control groups at Day 9.

By the end of the study the overall weight gain was 17, 12, and 22% less than that for controls at Day 16 of gestation for the 200, 250, and 300 mg/kg/day dose groups, respectively, while the respective group mean body weights at Day 16 were 6, 6 and 8% less than the control mean. Again these results are not clearly dose-related.

In addition to these marginal weight decreses, there were no clinical signs reported, and organ weight results did not show any dose-related effects in treated groups when



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compared to the control group. There were also no increases in the incidence of gross observations that could be related to administration of the test substance.

Although an acute oral toxicity study was conducted with Fischer 344 rats rather than the Sprague-Dawley strain rats used in this range-finding study and the definitive developmental toxicity study, the clinical signs noted (MRID 43770701) within a day following a 2000 mg/kg/day dose were posterior soiling and poor grooming in females. One female given a single 5000 mg/kg dose of XDE-105 died a week after treatment (MRID 43414515); clinical signs appeared within a day after treatment and persisted throughout the 14-day observation period after dosing. Signs included lacrimation, salivation, chromorhinorrhea, chromodacryorrhea, rapid respiration, urine and fecal soiling in the perineal area, incoordination and decreased activity. However, none of these signs were observed in this range-finding study after repeated dosing at levels up to approximately 8% of the oral LD50. Therefore, it is likely that a broader dose range could have been evaluated in, and a higher dose could have been tested in the definitive developmental toxicity study.

Based on results from a rabbit developmental toxicity study (MRID 43414521), the insufficient dose range evaluated in the definitive study should not be considered a critical deficiency. The highest dose in the rabbit study of 50 mg/kg/day approached a toxic level, which suggests that the rabbit is more sensitive to XDE-105 toxicity, and repeating the rat developmental toxicity study (MRID 43557505) would not add significantly to the data required for developmental toxicity testing.



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