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

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JUL 6 1995

MEMORANDUM

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

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

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Actions Requested: DowElanco is requesting an Experimental Use Permit for spinosad on cotton (232 pounds active ingredient for the season) with a crop destruct provision. Data supporting this EUP request were previously submitted (D210443, D210471, D210473 and D212834), and are reviewed here (Data Evaluation Records are attached).

Recommendations and Conclusions

1. There are adequate data to support the request for an EUP for the use of NAF-85 on cotton provided treated crops are destroyed after the experimental program has been completed.

2. There are insufficient data to support an exemption from tolerances for food uses and waivers of chronic studies on Spinosad (see discussion on page 19 below).

3. The acute toxicity categories for the technical grade active ingredient (XDE-105) and the formulated product (NAF-85) are as follows:

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Toxicity	Toxicity Category	
	XDE-105	NAF-85
Oral	IV*	IV
Dermal	III	Iii
Inhalation	IV	IV
Eye Irritation	IV	IV
Dermal Irritation	IV	IV
Dermal Sensitization	No	No

* Results indicated a lower LD_{50} in rats (3738 mg/kg/day) based on data from a previous unsubmitted study. The previous study is needed to support an appropriate Toxicity Category.

4. The lowest no-observed-effect level (NOEL) for subchronic toxicity was established in a three-month feeding study with dogs at 4.9 mg/kg/day (150 ppm in the diet). The LEL is 9.73 (♂) or 10.47 (♀) mg/kg/day (300 ppm) based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption and biochemical evidence of anemia and possible liver damage.

5. The NOEL for dermal and systemic toxicity in a 21-day dermal study in rabbits was 1000 mg/kg/day (limit dose).

6. Developmental toxicity studies established the NOELs for maternal and developmental toxicity at ≥50 mg/kg/day. in rabbits (highest dose tested) and ≥200 mg/kg/day in rats (highest dose tested. The range-finding studies in rabbits and rats should be submitted for review before the adequacy of doses tested in both species can be determined.

7. A series of mutagenicity studies including an *in vitro* forward mutation assay (mouse lymphoma cells), *in vitro* chromosome aberration assay (CHO cells), an *in vivo* micronucleus assay (mice), and an *in vitro* unscheduled DNA synthesis assay (primary rat hepatocytes) showed no mutagenic activity associated with XDE-105.

8. A comparison of the NOEL from the subchronic feeding study in dogs with dietary exposures is not done for this action because there is a provision in the EUP to destroy treated crops.

9. The Occupational and Residential Exposure Branch (OREB) worker exposures estimates can be used to determine Margins of Exposure (MOEs) based on the NOEL of 4.9 mg/kg/day for toxicity and are as follows:

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Individual	Margins of Exposure
Cotton	
Mixer/loader	2882
Applicator	
Ground boom	7903
Aerial	24,500
Orchards & vegetables	
Mixer/loader	331
Applicator	1968

I. Background

In a letter dated November 30, 1994, DowElanco requested consideration of submitted mammalian toxicology data to support new registrations for technical grade Spinosad (XDE-105) and for use of its formulation, NAF-85, on cotton as an insecticide. The registrant also petitioned for an exemption from a requirement for tolerances on all crops, and a waiver request for certain toxicology studies was included in the submission.

Spinosad, a fermentation product of *Saccharopolyspora spinosa*, is unusual in that the active ingredient (XDE-105) as a component of end use products either remains associated with dead cell mass (NAF-144, 2.6% a.i.) or is extracted, purified and formulated (NAF-85, 44% a.i.). The killed *S. spinosa* product (NAF-144) most resembles a biological pesticide and is being considered elsewhere by the Biopesticide Pollution Prevention Division (BPPD) while the extraction products (XDE-105 technical and the NAF-85 formulation) are considered as a conventional pesticide in this review. Although acute toxicity studies on NAF-144 have been submitted to BPPD, mammalian toxicity data on the active ingredient (XDE-105) will be used to support evaluation of potential health hazards associated with uses of both the NAF-144 and NAF-85 products.

A. Mammalian Toxicity Data Requirements

1. Data Requirements by Proposed Use

Bischoff (1994) noted:

> DowElanco and EPA determined that neither the microbial nor the conventional pesticide data requirement schemes were appropriate for the raw fermentation product and that a customized set of data requirements was needed.

Previous Toxicology Branch 1 memoranda discussed data requirements for indoor and outdoor residential and non-food crop

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uses (D192601) and ant bait stations (D189488). The minimum data recommended for the proposed residential and non-food uses was:

§81-1 through -6	Acute toxicity battery
§82-1, -3, -4	Subchronic feeding, dermal, inhalation studies
§83-3 (one species)	Developmental toxicity study
§84-2 through -4	Mutagenicity studies

For the ant bait station use, the recommended minimum data a self contained and unbreakable station would be:

§81-1 through -6	Acute toxicity battery
§84-2 through -4	Mutagenicity studies

For the killed microbial formulation (NAF-144) the recommendations were (D198678):

> While the company has suggested that they register their product as a killed microbial, SAB does not foresee the need for inactivation of the microbe for other than proprietary reasons. The tiered scheme developed under Subdivision M was designed to test the pathogenicity/infectivity of live microbes and toxins present in the TGAI or elaborated during the test exposure. The dosing level involved for testing killed products would not necessarily be analogous. It would probably be preferable to do the tests with the limit doses for biochemical pesticides (e.g., 5000mg/kg or, 3 mg/L inhalation, or 2000mg/kg dermal).
>
> SAB would recommend the inclusion of a test with the live microbe (perhaps pulmonary pathogenicity/infectivity) to insure there is no possibility of adverse effects with exposure to the live organism. This is cogent considering *S. spinosa*'s ability to grow at high temperatures (35°C) and an extensive literature demonstrating the involvement of thermophilic actinomycetes in bagassosis and hypersensitive pneumonitis.
>
> ...special consideration can be given to this formulation (NAF-144) based on the data set currently generated for the extracted active ingredient XDE-105 as a conventional chemical pesticide... The tolerance for the killed microbial product will reflect the available amount of the active ingredient and will most likely be linked to the final tolerance for XDE-105.

2. Submitted Mammalian Toxicity Data

The following mammalian toxicity data have been submitted in support of proposed uses of the purified fermentation product NAF-85 on cotton:

XDE-105 (Technical Grade Active Ingredient)

81-1	Acute oral toxicity study	83-3	Developmental toxicity study
81-2	Acute dermal toxicity study	84-2	Gene mutation assay in bacteria
81-3	Acute inhalation toxicity study	84-2	Gene mutation assay in mammalian cells
81-4	Primary eye irritation study	84-4	*In vitro* cytogenetics assay
81-5	Primary dermal irritation study	84-4	Micronucleus assay
81-6	Dermal sensitization study	84-4	Unscheduled DNA synthesis assay
82-1	Subchronic feeding study		

NAF-85 (End Use Product)

81-1	Acute oral toxicity study	81-4	Primary eye irritation study
81-2	Acute dermal toxicity study	81-5	Primary dermal irritation study
81-3	Acute inhalation toxicity study	81-6	Dermal sensitization study

These study reports were screened and accepted for review (D210443, D210471, and D210473), but additional study reports, which were cited by the registrant were requested. These additional studies included

81-7	Acute neurotoxicity in rats	82-1	13-Week dietary study in mice
82-5	13-Week dietary/neurotoxicity in rats	82-2	21-Day dermal in rabbits
		83-3	Teratology in rats

These additional reports were also screened and accepted for review (D212834).

3. Waiver requests for mammalian toxicity data

The registrant specifically requested the waiver of additional subchronic and chronic toxicity studies used to support registration of a terrestrial food use. DowElanco believes, "...the relevant human health...questions for many raw and purified fermentation products are similar or identical to those for microbial or biochemical pesticides," and DowElanco requested that the Agency consider a tiered assessment scheme like those used in the assessment of biological pesticides.

DowElanco further noted, "...a customized data set may not always be sufficient to adequately characterize a purified fermentation product," and avermectin was discussed as an example as follows:

> Avermectin is...relevant to spinosad because of the structural similarity of the two active ingredients. Both are macrocyclic lactones. However, their toxicological properties,..., are vastly different. For example,... the acute and subchronic toxicity of avermectin is at least 1 to 5 orders of magnitude higher than spinosad. Results provided in the customized data set, along with the summary data, verify the fundamental differences between these pesticides and support a tiered testing approach...
>
> No significant acute mammalian toxicity was observed in any of the tests for either test material (XDE-105 or NAF-85). A NOEL of 4.9

mg/kg was established in an oral subchronic toxicity study in dogs and significant toxicity occurred only in the highest dose level tested (29 mg/kg/day). No teratogenicity was demonstrated in rabbits at dose levels that induced maternal toxicity, and the fetal NOEL was 50 mg/kg/day. No mutagenic activity was observed in multiple *in vivo* and *in vitro* tests. The lack of significant toxicity observed in these studies supports the conclusion that additional toxicology...testing, equivalent to Tier II and Tier III testing for biological pesticides can be waived.

To further support the waiver request and provide additional assurance of the lack of adverse effects associated with the fermentation product, DowElanco has submitted additional information summarizing the results of additional mammalian subchronic tests...conducted with spinosad (see page 5 above for list of studies and discussions on pages 9 through 14 below).

C. Labeling

The proposed label recommends ground or aerial application at rates from 1.4 to 3.6 fl. oz. NAF-85 (44.2% active ingredient, 480 g/l SC) for spinosad.

Precautionary labeling includes the signal word "CAUTION" and the statement, "Keep Out of Reach of Children..." Other precautionary statements include:

Harmful if absorbed through the skin.

Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling.

Recommended Personal Protective Equipment (PPE) includes:

Applicators and other handlers must wear:
- Long-sleeved shirt and long pants
- Water proof gloves
- Shoes plus socks

Other recommendations for users are:

Users should:
- Users should wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

D. Previously Submitted Toxicology Data

Dose selection and progress reports for long-term feeding studies in rats and mice have been considered by TB-1. Dose levels of 0%, 0.005%, 0.0025%, 0.008%, or 0.036% in the diet of mice and 0%, 0.01%, 0.02%, 0.05%, and 0.1% in the diet of rats were

described as appropriate for the long-term feeding studies in both species (D177673).

1. Rat Chronic Feeding/Oncogenicity Study

A letter Dated July 20, 1992 (from Dr. J. F. Quast to Dr. R. F. Bischoff, Product Registration Manager, DowElanco), was submitted to the Agency and considered by TB-1 (D181573). The registrant's letter indicated, "EPA says the 0.1% may be too high (approximately 50 mg/kg/day) for the rat chronic feeding/ oncogenicity study." Based on more severe toxicity reported to occur after 13 weeks than that noted after 2 weeks as shown in subchronic feeding studies at similar dose levels, the following protocol adjustments were proposed in the registrant's letter:

a. to use four dose levels in the rat study rather than the recommended three levels based on the uncertainty suggested by the two subchronic feeding studies;

b. the next lowest dose level of 0.05% (approximately 25 mg/kg/day), which would serve as a maximum tolerated dose if the highest dose tested was associated with excessive toxicity; and

c. reduce the lowest dose tested from 0.01% (about 5 mg/kg/day) to 0.005% in the diet (approximately 2.5 mg/kg/day).

A meeting was proposed for a time when the interim results (one year sacrifice) become available to discuss the concerns for dose selection [illegible] that information available.

2. Mouse Oncogenicity Study

There are similar issues with the mouse oncogenicity protocol except that:

a. dose levels are slightly different in the mouse (0.036% or 30 mg/kg/day is the highest dose level tested), and

b. the responses in mice after 2 weeks and 13 weeks are more consistent, and the need to change the dose levels as was proposed for the rat is not as great.

The mouse study also has an interim sacrifice which can be used to support further discussions on the selection of dose levels.

Subsequently, a progress report was considered (D193954). After 10 months, male and female mice given the 0.036% diet showed increased mortality, and a supplementary study was started with 60 males given the equivalent of 1.1 mg/kg/day and 60 females given the equivalent of 1.5 mg/kg/day. A concurrent control group containing 46 males and 36 females was also added. An

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interim report at the 12-month necropsy in the supplemental study will be submitted for review.

Finally, the registrant recently reported that the highest dose group (0.036% diet) would be terminated in the main mouse oncogenicity study (D197109).

II. Summary of New Information

A. Mammalian Toxicology Data

The following summaries are from Data Evaluation Records (DER), which are attached, on submitted toxicity studies supporting the new registrations requested.

1. Toxicity Studies on the End Use Product

Results of acute toxicity studies on NAF-85 are summarized as follows:

NAF-85 (44% formulation)

Study	Results	Tox. Category
81-1 Acute Oral LD_{50} (rat)[1]	LD_{50} > 5000 mg/kg ♂ LD_{50} > 5000 mg/kg ♀ LD_{50} > 5000 mg/kg ♂+♀	IV
81-2 Acute Dermal LD_{50} (rabbit)[2]	LD_{50} > 2000 mg/kg ♂ LD_{50} > 2000 mg/kg ♀ LD_{50} > 2800 mg/kg ♂+♀	III
81-3 Acute Inhalation LD_{50} (rat)[3]	LC_{50} > 5 mg/l ♂ LC_{50} > 5 mg/l ♀ LC_{50} > 5 mg/l ♂+♀	IV
81-4 Primary Eye Irritation (rabbit)[4]	Slight conjunctival irritation.	IV
81-5 Primary Dermal Irritation (rabbit)[5]	Slight transient erythema and edema.	IV
81-6 Dermal Sensitization (guinea pig)[6]	Not a sensitizer in a Buehler test.	

[1] MRID No. 43414509
[2] MRID No. 43414510
[3] MRID No. 43414511
[4] MRID No. 43414512
[5] MRID No. 43414513
[6] MRID No. 43414514

There were no toxic signs observed in the above acute studies at limit doses (highest dose levels tested).

2. Toxicity Studies on the Technical Grade Active Ingredient

a. Acute Toxicity

Results of acute toxicity studies on XDE-105 are summarized as follows:

XDE-105 (88-90.4% a.i.)

Study	Results	Tox. Category
81-1 Acute Oral LD_{50} (rat)[1]	LD_{50} = 3738 mg/kg ♂ LD_{50} > 5000 mg/kg ♀ LD_{50} > 5000 mg/kg ♂+♀	IV
81-2 Acute Dermal LD_{50} (rabbit)[2]	LD_{50} > 2000 mg/kg ♂ LD_{50} > 2000 mg/kg ♀ LD_{50} > 2800 mg/kg ♂+♀	III
81-3 Acute Inhalation LD_{50} (rat)[3]	LC_{50} > 5.18 mg/l ♂ LC_{50} > 5.18 mg/l ♀ LC_{50} > 5.18 mg/l ♂+♀	IV
81-4 Primary Eye Irritation (rabbit)[4]	Slight conjunctival irritation.	IV
81-5 Primary Dermal Irritation (rabbit)[5]	No erythema and edema.	IV
81-6 Dermal Sensitization (guinea pig)[6]	Not a sensitizer in a Buehler test.	

[1] MRID No. 43414515: The reported results indicated a lower LD_{50} value for male Fischer 344 rats (3738 mg/kg/day) apparently based on data from a previous study with Fischer 344 rats at a 2000 mg/kg dose level (cited in the study report as Wright, et al., 1992b). The report of the previous study is needed to support the determination that the acute oral LD_{50} = 3738 mg/kg for male rats and to upgrade the study classification.
[2] MRID No. 43414516
[3] MRID No. 43414517
[4] MRID No. 43414518
[5] MRID No. 43414519
[6] MRID No. 43414520

There were no toxic signs observed in the above acute studies at limit doses (highest dose levels tested).

b. Acute Neurotoxicity Study (MRID 43557501)

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 was 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.

This study does not satisfy §81-8 guideline requirements for an acute oral mammalian neurotoxicity study and is classified as Core Supplementary (MRID 43557501). There were no positive control data from the testing laboratory presented in the report.

c. Subchronic Feeding Studies

i. 13-Week Feeding Study in Rats (MRID No. 43566601)

Male and female Fischer 344 rats were fed XDE-105 in their diets at concentrations of 0%, 0.05%, 0.1%, 0.2%, and 0.4%. The actual doses, based on food consumption were 0, 33.9, 68.5, 133.5, and 273.1 mg/kg/day in males, and 0, 38.8, 78.1, 151.6, and 308.2 mg/kg/day in females. The high-dose was discontinued on day 44 due to excessive mortality.

Clinical signs seen in the high-dose rats (0.4%) included deep, rapid, or labored breathing, thinness, chromorhinorrhea, piloerection, hypothermia, and distended penis. Only sporadic clinical signs were seen in the 0.2% rats. Body weights gain for both sexes was reduced in the high-dose group, while the other groups gained weight at a rate comparable to controls. Food consumption and food efficiency were significantly decreased in both sexes.

Biologically significant hematologic anomalies were found in both sexes only in the 0.4% and 0.2% dose groups (there were no concurrent controls for the 0.4% rats). Anemia was indicated by decreased erythrocyte counts, hemoglobin concentration, hematocrit and erythrocyte indices as well as the release of reticulocytes and nucleated erythrocytes. The erythrocytes were small and hemoglobin-deficient. Other abnormal findings include polychromasia, hypochromasia, anisocytosis (excessive size variation), poikilocytes (unusual shapes), microcytes (unusually small cells), and stomatocytes. Thus, a profound effect on erythropoiesis dictated the release of immature cells. In the 0.2% groups, both sexes had mild leukocytosis due to neutrophilia and lymphocytosis. Monocyte counts doubled in both sexes. Thrombocyte counts were unaffected.

Clinical chemistry anomalies in the high-dose males and females included increases in blood urea nitrogen, total bilirubin, alkaline phosphatase, ALT, AST, GGT, inorganic phosphorus, sodium, potassium and cholesterol (males only), and decreases in creatinine, triglycerides (males only), albumin, globulin, and total protein.

Fewer anomalies were seen in the 0.2% rats. Alkaline phosphatase and ALT values were scarcely affected, but AST values were

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elevated in both sexes due to trauma in cardiac and skeletal muscle. Significantly elevated BUN and inorganic phosphorus values were evidence of renal damage. Neither creatinine nor bilirubin levels were altered. Mildly elevated cholesterol levels in both sexes were likely renal-related.

Females may be more sensitive than males for renal effects. Markedly reduced urine pH in the 0.2% rats (pH of 6.4 and 5.4, M/F, compared to 8.2 in the controls) along with elevated inorganic phosphorus levels suggest a condition of metabolic acidosis, an acidic shift in the blood's acid/base status due to a loss of base (e.g. bicarbonate) through the kidneys, or retention of nonvolatile acids (as seen in salicylate poisoning). The compensatory action is to raise the blood pH through CO_2 elimination via the lungs. This, combined with anemic anoxia, would account for the deep, rapid, or labored breathing in the high-dose rats.

The principal target organs/tissues appear to be the bone marrow, liver, kidneys, heart, skeletal muscle, and thyroid, with the thyroid being the most sensitive indicator. The most universal histopathologic finding in this study was vacuolation, which was found in the kidney, liver, heart, spleen, lymph node, thymus, pancreas, stomach, oviduct, uterus, vagina, epididymis, adrenals, and thyroid. Other effects noted in the high dose group included increased incidences of multifocal hepatocytic granuloma and progressive cardiomyopathy in both sexes, skeletal muscle multifocal degeneration and multifocal regeneration in females, splenic histiocytosis in both sexes, slightly increased splenic weights in females (27%), lymph node enlargement in females, and lymph node histiocytosis in both sexes)

Based on these results, the NOEAL for systemic toxicity is 0.05% (33.9 and 38.8 mg/kg/day in males and females, respectively, and the LEL is 0.1% (68.5 and 78.1 mg/kg/day in males and females, respectively.

ii. Second 13-Week Feeding Study in Rats (MRID No. 43557502)

Male and female Fischer 344 rats were fed XDE-105 in their diets at concentrations of 0 (sham control), 0.003%, 0.006%, 0.012%, and 0.06%. The actual doses, based on food consumption, were 0, 2.2, 4.3, 8.6, and 42.7 mg/kg/day in males, and 0, 2.6, 5.2, 10.4 and 52.1 mg/kg/day in females. There were two "recovery" groups assigned to the control and high-dose which received control diets for another 4 weeks.

There were no deaths, no compound-related clinical signs, or ophthalmologic lesions, and no effect on body weights, food consumption, food efficiency, clinical pathology or gross pathology. Very slight to slight thyroid follicle epithelial

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cell vacuolation was seen in 10/10 males and 8/10 females in the high dose group. A similar incidence was seen in the recovery group, although severity was reduced.

Based on these results, **the NOEAL for systemic toxicity is 0.012% (8.6 and 10.4 mg/kg/day in males and females, respectively; the NOAEL is .06% (42.7 and 52.1 mg/kg/day for males and females, highest dose tested) based on thyroid follicular cell vacuolation; and an LEL was not established in this study.**

iii. 13-Week Feeding Study in Dogs (MRID No. 43444102)

Spinosad was tested in a 13-week oral feeding study in male and female Beagle dogs. The chemical was mixed in the diet at the following dose levels: 0, 150, 300, 1350/900 ppm (males) or 900 ppm (females). These levels corresponded to 0, 4.89, 9.73 or 33.4 mg/kg/day for the low, mid- and high dose males and 0, 5.38, 10.47 or 29.9 mg/kg/day for the low, mid- and high dose females, respectively. The concentration of 1350 ppm was reduced from 1350 ppm to 900 ppm in males on day 38.

At 300 ppm and above, cytoplasmic vacuolation or vacuolated cell aggregation was observed in a variety of tissues and atrophic gastric mucosa in both sexes. At 1350/900 ppm, arteritis was also observed in both sexes. In addition, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis, cellular depletion in the bone marrow and thymic atrophy were observed. Clinical signs included periocular sebum, decreased spontaneous motor activity, unsteady standing posture and watery, red/black stools and/or loose stools. One male was sacrificed in extremis. Decreases in mean body weights and food consumption were observed in both sexes, particularly males. Evidence of anemia was found in the hematological examinations (decreases in hematocrit, hemoglobin and erythrocytes) as well as decreases in white blood cell counts, lymphocytes and reticulocytes. Decreases in albumin and A/G ratio and increases in globulin, total cholesterol, GOT, ALP and GPT were also observed. The increases in the latter three were slight, in only one sex and in one case due to only one dog. There were increases in several organ weights, although most in only one sex and/or in only absolute or relative weights. Increases in spleen and liver weights were supportive of the microscopic and/or clinical chemistry results. **The NOEL is 150 ppm (4.89 (♂) or 5.38 (♀) mg/kg/day) and the LEL is 300 ppm (9.73 (♂) or 10.47 (♀) mg/kg/day based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption and biochemical evidence of anemia and possible liver damage.**

iv. !3-Week Feeding Study in Mice (MRID No. 43566602)

Groups of 10 male and 10 female CD-1 strain mice were given diets containing XDE-105 at 0, 0.005%, 0.015%, 0.045% or 0.12% (0, 7.5, 22.5, 67.5, or 180 mg/kg/day) for 13 weeks.

The incidence of mortalities at the 180 mg/kg/day dose level resulted in termination of that group after 6 weeks of the study. Effects associated with the highest dose tested included changes consistent with hepatobiliary disturbance, iron deficient anemia, inflammation (i.e., marked neutrophilic and lymphocytic leukocytosis), and loss of or decreased production of albumin as well as necrosis in liver, lymph node and lung. Although there were no control animals concurrently sacrificed, the effects noted in animals from the 180 mg/kg/day group were consistent with a dose-related response.

At dose levels ≥22.5 mg/kg/day, cells of the lymphoid organs, liver, kidney, stomach, ovary, female reproductive tract, and epididymis had cytoplasmic vacuolation. Other tissues less severely affected at these dose levels included the heart, lung, pancreas, adrenal cortex, bone marrow, tongue, and pituitary gland.

The 67.5 mg/kg/day dose level was associated with significantly increased absolute and relative liver weight in both sexes. Terminal body weight for male mice was also significantly decreased. Clinical chemistry results were consistent with the liver weight increases at the 67.5 mg/kg/day dose level in that activity of alkaline phosphatase, alanine transferase, and aspartate transferase were greater than control values in males and females. Histologically, the incidence of slight centrilobular hepatocellular cytomegaly was increased in males, and an increase in the incidence and severity of centrilobular hepatocellular vacuolation in the liver of females was observed.

The 67.5 mg/kg/day dose level was also associated with significantly increased absolute and relative spleen weights in female mice. Related effects included statistically significant decreases for males in hemoglobin, packed cell volume, mean corpuscular volume, and mean corpuscular hemoglobin, and statistically significant decreases for females in mean corpuscular volume and mean corpuscular hemoglobin. Hematopoiesis was noted in the spleen of 1 of 10 males and no females from the 67.5 mg/kg/day dose group, and lymphatic vacuolation (minimal, slight and/or moderate grades) was observed in males from the 22.5 and 67.5 mg/kg/day dose groups; these changes were also observed in females from the 67.5 mg/kg/day dose group. Bone marrow necrosis was reported in males and females from the 67.5 mg/kg/day doe group, and no similar lesions were noted in the control group animals.

Other effects of XDE-105 in mice included skeletal muscle myopathy which was observed in the quadriceps of males and females from the 67.5 and 180 mg/kg/day groups. Increased severity and incidence of gastric glandular dilation was also noted in male and female mice.

Based on these results, **the NOAEL for XDE-105 was established at 7.5 mg/kg/day, and the LOAEL was determined to be 22.5 mg/kg/day in mice.**

d. Subchronic Dermal Toxicity Study (MRID 43557503)

Groups of 5 male and 5 female New Zealand White strain rabbits were given 15 dermal applications of XDE-105 at 0, 100, 500, or 1000 mg/kg/day for 21 days. Under the conditions of the test, dermal application of XDE -105 at doses up to 1000 mg/kg/day (a limit dose), there was no evidence of treatment-related toxicity. Therefore, **the NOEL for dermal and systemic toxicity in this study was 1000 mg/kg/day.**

e. Subchronic Neurotoxicity (MRID 43557504)

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.**

This study does not satisfy §82-7 guideline requirements for a subchronic mammalian neurotoxicity study and is classified as Core Supplementary (MRID 43557504). This study was an extension of a subchronic feeding study, and there were no positive control data from the testing laboratory presented in the report..

f. Developmental Toxicity

i. Rabbits (MRID No. 43414521)

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. 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.

Maternal toxicity was reported 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). These high dose group results may not be toxicologically significant because (1) the compared weight gains represent 1-2% of the body weights of the test animals, (2) the compensatory changes noted in food consumption occurred at the lowest dose tested as well as the 50 mg/kg/day dose level and they were not dose related, and (3) statistically significant differences were not consistently found for body weight or food consumption results. In addition, there were no significant differences noted with respect to absolute and relative liver and kidney weights, and no animals were characterized in the report as thin in appearance. The incidence of animals with decreased fecal output could be interpreted as coincidental. Although the incidence of aborted pregnancies was higher in the 50 mg/kg/day dose group than historical control values, the absence of similar observations at doses from 50 to 400 mg/kg/day in 28 animals from a range-finding study summarized in the report dose not support the conclusion that XDE-105 causes abortions in rabbits. **The NOEL for maternal toxicity may be ≥50 mg/kg/day.**

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

ii. Rats (MRID No. 43557505)

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. 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 did not 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.**

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

g. Mutagenicity Studies

i. Ames assay (MRID No. 43414522)

The mutation rates observed after treatment of *Salmonella typhimurium* strains (TA1535, TA1537, TA98, and TA100) and one strain of *Escherichia. coli* (WP2/uvrA⁻) with XDE-105 increased in a dose-related manner when compared to the vehicle control. The colonies were shown in a replica plate assay to be predominately auxotrophs and not revertants. No growth of auxotrophs is expected in the Ames assay, but their presence in this assay suggests that XDE-105 supported their growth. The investigators noted that trace amounts of histidine and other amino acids were present in the test substance, which is a fermentation product. Therefore, a Ames assay with XDE-105 may not be appropriate, and this assay is considered to be unacceptable.

ii. Mouse lymphoma cell assay (MRID No. 43414523)

Spinosad did not induce forward mutations in mouse lymphoma L5178Y $Tk^{+/-}$ cells at concentrations of 0, 1, 5, 10, 15, 20 or 25 µg/ml without metabolic activation or at concentrations of 15 through 50 µg/ml with metabolic activation.

iii. Chromosome aberration assay in vitro (MRID No. 43414524)

Spinosad did not increase the number of CHO cells with chromosome aberrations at concentrations of 20, 26, or 35 µg/ml without metabolic activation or at concentrations of 100, 250 or 500 µg/ml with metabolic activation.

iv. Micronucleus test in vivo (MRID No. 43414525)

Spinosad did not increase the frequency of micronuclei in replicate assays with bone marrow cells from ICR mice treated with doses of 0, 500, 1000 or 2000 mg/kg/day for two consecutive days. There was a statistically significant increase in the incidence of MPCE in males reported for the first assay, but values for all animals were within the historical control range. However, there was no significant increase in the incidence of MPCE in the replicate assay. The increase noted in the first assay was attributed to an unusually low result for the concurrent control group in the first assay.

v. Unscheduled DNA synthesis assay (MRID No. 43414526)

Spinosad did not induce unscheduled DNA synthesis (UDS) in adult rat hepatocytes *in vitro* at concentrations of 0.01 to 5 µg/ml. Concentrations from 10 to 1000 µg/ml of XDE-105 were cytotoxic.

B. Risk Characterization

For purposes of a preliminary characterization of risks associated with proposed uses of spinosad, the critical NOEL for subchronic toxicity was established in a three-month feeding study with dogs (MRID 43444102) at 4.9 mg/kg/day (150 ppm in the diet of males, see page 12 above). The LEL is 9.73 (♂) or 10.47 (♀) mg/kg/day (300 ppm) based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption and biochemical evidence of anemia and possible liver damage. Similar effects have been reported in mice (MRID 43566602) and rats (MRIDs 43557502 and 43566601).

Based on a conversation with Mark Dow (OREB May 31, 1995, see attached memorandum dated June 9, 1995), preliminary exposure estimates for workers using spinosad on cotton as part of the proposed EUP program are based on the following assumptions:

- The highest label rate = 3.6 fl. oz./A
- Maximum plot size = 25 A
- Clothing is as listed on page ? above.
- Mixing/loading = open pour
- Ground boom application = open cab
- Fixed-wing aerial application = closed cockpit

Exposures were estimated as µg/day using PHED version 1.1, and they are compared with the NOEL of 4.9 mg/kg/day such that MOE = NOEL (mg/kg/day) ÷ exposure (µg/kg/day) where the body weight is assumed to be 70 kg for humans. Exposure estimates were also provided for use of the NAF-144 formulation which was to be applied at a maximum rate of 0.16 lb. a.i./A applied in orchards and on other vegetable crops. Exposure estimates and MOEs are as follows:

Individual	Exposure (µg/kg/day)	Margins of Exposure
<u>Cotton</u>		
Mixer/loader	1.7	2882
Applicator		
Ground boom	0.62	7903
Aerial	0.2	24,500
<u>Orchards & vegetables</u>		
Mixer/loader	14.8	331
Applicator	2.5	1968

III. Discussion

A. Data Requirements

The data requirements for the requested EUP are summarized as follows:

Spinosad Technical Grade of the Active Ingredient
(Requirements for an Experimental Use Permit - Food Use)

Guideline	Study	Required	Satisfied	Comment
81-1	Acute oral toxicity	Yes	No	1
81-2	Acute dermal toxicity	Yes	Yes	
81-3	Acute inhalation toxicity	Yes	Yes	
81-4	Primary eye irritation	Yes	Yes	
81-5	Primary dermal irritation	Yes	Yes	
81-6	Dermal sensitization	Yes	Yes	
81-7	Acute delayed neurotoxicity	No	--	
81-8	Acute neurotoxicity (mammalian)	No	No	2
82-1a	Subchronic feeding (rodent)	Yes	Yes	3, 4
82-1b	Subchronic feeding (nonrodent)	Yes	Yes	3, 4
82-2	21-Day dermal	No	Yes	
82-3	90-Day dermal	No	--	
82-4	90-Day inhalation	No	--	
82-7	90-Day neurotoxicity	No	No	2
83-1a	Chronic feeding (rodent)	No	--	5
83-1b	Chronic feeding (nonrodent)	No	--	
83-2a	Oncogenicity (mouse)	No	--	5
83-2b	Oncogenicity (rat)	No	--	5
83-3a	Developmental (1st species)	Yes	No	4, 6
83-3b	Developmental (2nd species)	Yes	No	4, 6
83-4	Multigeneration reproduction	No	--	5
83-5	Chronic feeding/oncogenicity	No	--	5
84-2a	Gene mutation	Yes	Yes	
84-2b	Struc. chromosome aberration	Yes	Yes	
84-2c	Other genotoxicity	Yes	Yes	
85-1	Metabolism	No	--	5

1 The reported results indicated an LD_{50} value for male Fischer 344 rats was calculated from data in a previous study combined with the submitted study. The report of the previous study is needed to support the determination that the acute oral LD_{50} = 3738 mg/kg for male rats and to upgrade the study classification for MRID 43414515.

2 Neurotoxicity studies were conducted and submitted, but the studies did not use positive controls, so they are considered to be supplementary information.

3 Includes two rat feeding studies (MRIDs 43566601 and 43557502) and one mouse feeding study (MRID 43566602).

4 Only one study with one of the two required species is needed to support an EUP.

5 A study is in progress.

6 The highest dose tested in this study may not be adequate, but the appropriate range-finding study should be submitted for review before the study can satisfy the requirement.

Spinosad NAF-85 (44% formulation)
(Requirements for an Experimental Use Permit - Food Use)

Guideline	Study	Required	Satisfied	Comment
81-1	Acute oral toxicity	Yes	Yes	
81-2	Acute dermal toxicity	Yes	Yes	
81-3	Acute inhalation toxicity	Yes	Yes	
81-4	Primary eye irritation	Yes	Yes	
81-5	Primary dermal irritation	Yes	Yes	
81-6	Dermal sensitization	Yes	Yes	

B. Data Waivers and Tolerance Exemptions

Using a tiered approach as requested by the registrant (see page 5 above) and the submitted data, a waiver of long-term studies is inappropriate. The acute toxicity studies (MRIDs 43414509-43414520) suggest Toxicity Categories III and IV (limit doses from 2000 to 5000 mg/kg), and the acute neurotoxicity study in rats (MRID 43557501) showed no effects at single doses as high as 2000 mg/kg. However, repeated dose studies such as the developmental toxicity studies (MRIDs 43414521 and 43557505) and the 13-week feeding study in dogs (MRID 43444102) indicate that NOELs may range from ≥50 mg/kg/day for the shorter developmental studies to <10 mg/kg/day in the 13-week studies. The difficulties encountered with the long term feeding studies in rats and mice described above (see pages 6-8) further emphasize the need for a conventional battery of mammalian toxicity studies to support food-use registrations of spinosad. Therefore, data waivers and exemptions from tolerances are not recommended.

The current request for an experimental use permit on cotton contains a crop destruct condition. However,

References

43414509 Gilbert, K.S. NAF-85: Acute Oral Toxicity Study in Fischer 344 Rats. Unpublished report no(s). DR-0341-0784-001A submitted by DowElanco dated July 22, 1994. Prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414510 Gilbert, K.S. NAF-85: Acute Dermal Toxicity Study in New Zealand White Rabbits. Unpublished Report No(s). DR-0341-0784-001D dated July 22, 1994, submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414511 Beekman, M.J. NAF-85: Acute Aerosol Inhalation Toxicity Study with Fischer 344 Rats. Unpublished Report No(s). DR-0341-0784-003 dated July 15, 1994 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414512 Gilbert, K.S. NAF-85: Primary Eye Irritation Study in New Zealand White Rabbits. Unpublished Report No(s). DR-0341-0784-001C dated July 22, 1994 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414513 Gilbert, K.S. NAF-85: Primary Dermal Irritation Study in New Zealand White Rabbits. Unpublished Report No(s). DR-0341-0784-001B July 22, 1994 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414514 Gilbert, K.S. NAF-85: Dermal Sensitization Potential in Hartley Albino Guinea Pig. Unpublished Report No(s) DR-0341-0784-001E submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

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.

43414516 Gilbert, K.S. XDE-105: Acute Dermal Toxicity Study in New Zealand White Rabbits. Unpublished Report No(s) DR-0323-1194-017D submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414517 Wolff, R.K., D.L. Allen, G.D. Williams, and D.W. The Acute Inhalation Toxicity in Fischer 344 Rats of Technical XDE-105. Unpublished Report No(s) R33491 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN..

43414518 Gilbert, K.S. XDE-105: Primary Eye Irritation Study in New Zealand White Rabbits. Unpublished Report No(s)

DR-0323-1194-017C submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414519 Gilbert, K.S. XDE-105: Primary Dermal Irritation Study in New Zealand White Rabbits. Unpublished Report No(s) DR-0323-1194-017B submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43414520 Gilbert, K.S. XDE-105: Dermal Sensitization Potential in Hartley Albino Guinea Pig. Unpublished Report No(s) DR-0323-1194-017E 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.

43414522 Garriott, M.L., M.A. Rexroat, and D.W. Grothe The Effect of XDE-105 on the Induction of Reverse Mutations in *Salmonella typhimurium* and *Escherichia coli*. Unpublished Report No(s) 910820AMS3162, 910917AMS3162, and 910924AMS3162 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

43414523 Garriott, M.L., K.C. Michaelis, and D.W. Grothe The Effect of XDE-105 on the Induction of Forward Mutations at the Thymidine Kinase Locus of L5178Y Mouse. Unpublished Report No(s) 910910MLA3162 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

43414524 Garriott, M.L., D.E.F. Kindig, and D.W. Grothe The Effect of XDE-105 on the In Vitro Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells. Unpublished Report No(s) 910918CAB3162, 911009CAB3162 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

43414525 Garriott, M.L., j.d. Brunny, D.E.F. Kindig, and D.W. The Effect of XDE-105 on the In ViVo Induction of Micronuclei in Bone Marrow of ICR Mice. Unpublished Report No(s) 910916MNT3162, 911007MNT3162 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

43414526 Garriott, M.L., D.J. Yount, and D.W. Grothe The Effect of XDE-105 on the Induction of Unscheduled DNA Synthesis in Adult Rat Primary Hepatocytes. Unpublished Report No(s) 910806UDS3162, 910827UDS3162 submitted by DowElanco, prepared by Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN..

43444102 Harada, T XDE-105: 13-Week Oral Subchronic Toxicity Study in Dogs. Unpublished Report No(s). IET 91-0079 submitted by DowElanco, prepared by The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan

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.

43557502 Albee, R.R., N.M. Berdaxco, and B.L. Yano XDE-105: 13-Week Dietary Toxicity and 4-Week Recovery Studies in Fischer 344 Rats. Unpublished Report No(s) DR-0323-1194-001A submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

43557503 Vedula, U., and B.L. Yano XDE-105: Probe and 21-Day Repeated Dose Dermal Toxicity Study in New Zealand White Rabbits. Unpublished Report No(s) DR-0323-1194-018 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.

43566601 Grothe, D.W., S.M. Boss, and C.L. Gries. A Subchronic Toxicity Study in Fischer 344 Rats Administered XDE-105 in the Diet for 3 Months. Unpublished Report No(s) R20690 submitted by DowElanco, prepared by Toxicology Research Laboratories, Lilly Research Laboratories.

4356662 Grothe, D.W., S.M. Boss, and C.L. Gries A Subchronic Toxicity Study in CD-1 Mice Administered XDE-105 in the Diet for 3 Months. Unpublished Report No(s) MO 1290 submitted by DowElanco, prepared by The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, Michigan.

Bischoff, R. F. November 30, 1994. "Data Waiver Request for Environmental Fate, Residue and Additional Toxicology Studies Used to Support New Registrations Involving Use of *S. spinosa* Fermentation Based Products." Report No. RFB112794 submitted by DowElanco, Indianapolis, IN.

D177673 Reddy, G.G., TB-1/HED. Memorandum dated May 18, 1992, to G. LaRocca, IRB, Registration Division

D181573 Reddy, G.G., TB-1/HED. Memorandum dated September 10, 1992, to G. LaRocca, IRB, Registration Division

D189488 Reddy, G.G., TB-1/HED. Memorandum dated June 14, 1993, to G. LaRocca, IRB, Registration Division

D192601 Reddy, G.G., TB-1/HED. Memorandum dated October 4, 1992, to G. LaRocca, IRB, Registration Division

D193954 Reddy, G.G., TB-1/HED Memorandum dated October 21, 1993, to G. LaRocca, IRB, Registration Division

D197109 Reddy, G.G., TB-1/HED. Memorandum dated January 5, 1994, to G. LaRocca, IRB, Registration Division

D198678 Kough J.L., SAB/HED. Memorandum dated March 18, 1994, to W. Nelson, IRB, Registration Division

D210443, D210471, and D210473 Gardner, R., TB-1/HED. Memorandum dated February 9, 1995, to A. Hayward, PM Team 13, IRB, Registration Division

D212834 Gardner, R., TB-1/HED. Memorandum dated February 9, 1995, to A. Hayward, PM Team 13, IRB, Registration Division

011597

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

JUN 9

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Spinosad EUP Worker Exposure Estimates

FROM: Mark I. Dow, Ph.D., Section Head
Special Review and Registration Section II

TO: Roger Gardner, Toxicologist
Review Section 1, Toxicology Branch 1
Health Effects Division (7509C)

THRU: Larry C. Dorsey, Chief
Occupational and Residential Exposure Branch
Health Effects Division (7509C)

Steven M. Knott for

This is formal conveyance of the exposure figures you were informally given on 31 May 95. As a result of our discussion late on 30 May 95 with Debra Edwards and Karl Baetcke, we were tasked with providing exposure and risk assessments for Spinosad used in Experimental Use Permits on cotton, apple, celery, lettuce, potato and tomato.

Estimates of exposure are:

Cotton -	applicator/aerial	0.21 µg/Kg bw/day
	/groundboom	0.62 µg/Kg bw/day
	mixer/loader	1.7 µg/Kg bw/day
Apple -	applicator/groundboom	14.8 µg/Kg bw/day
	mixer/loader	2.49 µg/Kg bw/day

Estimates of applicator and mixer/loader exposure were provided for air and ground application for cotton and ground application for apple. The ground apparatus is ground boom for cotton and airblast for apple. Further, the estimates are derived using label parameters relative to application rates,

work clothing and open cab machinery. The maximum label rates of application were used. Plot size is assumed to be 25 acres. In terms of exposure estimates, this is considered "conservative" (i.e., plots are most likely not larger in size). Worker/handler units of exposure are derived from the Pesticide Handler Exposure Database version 1.1.

Estimates of exposure were not provided for application to celery, lettuce, potato and tomato as OREB believes exposures from such applications would not be significantly different from the estimates for cotton.

The informal presentation of exposure estimates did not list mixer/loader exposure for application to apple. It is included here. Calculations and "factors"/assumptions are presented in Appendix I. OREB is confident in the PHED data used to derive worker/handler units of exposure.

cc: Chem file - spinosad PC code 110003
Correspondence file
M Dow

APPENDIX I 011597

Estimates of Handler Exposure to Spinosad Applied to Cotton and Apples

Assumptions and Calculations:

4 lb ai/gal ec
Rate: 0.11 lb ai/A = highest label rate for cotton = 3.6 fl oz A
4 lb ai/gal ÷ 128 fl oz/gal = 0.031 lb ai/fl oz
3.6 fl oz/A * 0.031 lb ai/fl oz = 0.11 lb ai/A

0.16 lb ai/A (Label states maximum rate) for apples

Plot size: 25 A assumed (under the stated conditions of the EUP, 25 A is considered highly conservative i.e., under the petition as presented, plots are not thought to exceed 25 A).

PPE/Clothing: minimum work clothing as indicated by the Worker Protection Standard = Long sleeved shirt, Long pants and Gloves (pilots exempt from wearing gloves)

Worker body weight = 70 Kg

Units of Exposure/PHED Version 1.1:
Total Units = Dermal and Inhalation

15.36 µg/lb ai applied = groundboom-open cab

5.29 µg/lb ai applied = fixed wing aerial

258.31µg/lb ai applied = airblast-open cab

43.566µg/lb ai handled = mixer/loader - open pour

Am't Handled/Applied per day
Cotton: 0.11 lb ai/A * 25 A/day = 2.81 lb ai/day
Apples: 0.16 lb ai/A * 25 A/day = 4.0 lb ai/day

Spinosad/Cotton/EUP

Applicator/Groundboom/Open cab

15.36 µg/lb ai applied * 2.81 lb ai applied/day = 43.2µg/day

$\frac{43.2\ \mu g/day}{70\ Kg\ bw}$ = **0.62 µg/Kg bw/day**

Applicator/Fixed wing Aerial (Closed cockpit)

5.29 µg/lb ai applied * 2.81 lb ai applied/day = 14.9 µg/day

$\frac{14.9\ \mu g/day}{70\ Kg\ bw}$ = **0.21 µg/Kg bw/day**

Mixer/Loader/Open-pour

43.566µg/lb ai handled * 2.81 lb ai handled/day = 122.4µg/day

$\frac{122.4\mu g/day}{70\ Kg\ bw}$ = **1.7µg/Kg bw/day**

APPLES

Applicator/Airblast/Open cab

258.31 µg/lb ai applied * 4.00 lb ai/day = 1033.24 µg/day

$\frac{1033.24\ \mu g/day}{70\ Kg\ bw}$ = **14.8 µg/Kg bw/day**

Mixer/Loader/Open-pour

43.566 µg/lb ai handled * 4.00 lb ai/day = 174.3 µg/day

$\frac{174.3\ \mu g/day}{70\ Kg\ bw}$ = **2.49 µg/Kg bw/day**

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

.ARIO: Long pants, long sleeves, no gloves **AERIAL APPLICATION** (Fixed-Wing)

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs
HEAD (ALL)	Other	.13	.5177	192.2156	.264	5
NECK.FRONT	Other	.015	.0521	195.2015	.0291	5
NECK.BACK	Other	.011	.0419	186.1575	.0225	5
UPPER ARMS	Other	.291	.9343	237.2257	.4677	3
CHEST	Other	.355	.9494	263.6086	.5048	4
BACK	Other	.355	.9412	266.054	.4963	4
FOREARMS	Other	.121	.4256	241.7998	.2089	2
THIGHS	Other	.382	1.5121	235.4011	.6974	2
LOWER LEGS	Other	.238	.8535	236.9069	.4225	2
FEET	Other	.131	.4126	110.5914	.2588	2
HANDS	Lognormal	1.4273	8.4156	235.7182	1.5231	4
TOTAL DERM:	3.5521	3.4563	15.056		4.8956	

95% C.I. on Mean: Dermal: [-188.3558, 218.4678] .

Data File: APPLICATOR

Number of Records: 57
Subset Name: AERIAL.CLSD1.APPL

D INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for AERIAL.CLSD1.APPL

With Dermal Grade Uncovered Equal to "A" "B" "C"
Subset originated from AERIAL.CLSD.APPL
With Application Method Equal to 5 and (Aerial-Fixed Wing)
With Cab Type Equal to 3 4 (Closed Cab)
Subset originated from APPL.FILE

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

ARIO: Long pants, long sleeves, no gloves

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs.
HEAD (ALL)	Other	.13	.6226	192.1137	.2781	38
NECK.FRONT	Other	.015	.0624	196.3141	.0309	38
NECK.BACK	Other	.011	.0478	196.4435	.0221	38
UPPER ARMS	Other	.291	1.108	235.2617	.5291	26
CHEST	Other	.355	1.4052	235.4042	.6481	24
BACK	Other	.355	1.4052	235.4042	.6481	24
FOREARMS	Other	.1815	.5626	217.4724	.2671	20
THIGHS	Other	.382	1.5121	235.4011	.6974	24
LOWER LEGS	Other	.238	.9245	230.1785	.4514	26
FEET	Lognormal	.393	.5121	84.4171	.3603	11
HANDS	Lognormal	2.0725	10.1256	211.6615	3.1144	34
TOTAL DERM:	5.4332	4.424	18.2881		7.047	

95% C.I. on Mean: Dermal: [-226.218, 262.7942]

Number of Records: 38
Data File: APPLICATOR Subset Name: AERIAL.CLSD2.APPL

■

ID INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for AERIAL.CLSD2.APPL

With Hand Grade Equal to "A" "B" and
With Hand Measuring Method Equal to 1 2 (Handrinse, Glove)
Subset originated from AERIAL.CLSD.APPL
With Application Method Equal to 5 and (Aerial-Fixed Wing)
With Cab Type Equal to 3 4 (Closed Cab)
Subset originated from APPL.FILE

011597

SUMMARY STATISTICS FOR INHALATION EXPOSURES

	DISTRIB. TYPE	NANOGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs
EXPOSURE	Lognormal	102.2994	580.6471	157.8565	143.2315	32

95% C.I. on Geo. Mean: [3.458, 5932.7595]
Number of Records: 32
Data File: APPLICATOR Subset Name: AERIAL.CLSD3.APPL

<< Specifications >>
Subset Specifications for AERIAL.CLSD3.APPL

With Airborne Grade Equal to "A" "B" "C"
Subset originated from AERIAL.CLSD.APPL
With Application Method Equal to 5 and (Aerial-Fixed Wing)
With Cab Type Equal to 3 4 (Closed Cab)
Subset originated from APPL.FILE

CALCULATIONS

521 ug/lb ai sprayed - 1.5231 ug/lb ai sprayed = 2.029 ug/lb ai sprayed
(dermal unit exposure w/o hands)

2.029 ug/lb ai sprayed + 3.1144 ug/lb ai sprayed = 5.1434 ug/lb ai sprayed
TOTAL dermal unit exposure

5.1434 ug/lb ai sprayed + 0.1432 ug/lb ai sprayed (inhalation) = 5.2866 ug/lb ai sprayed
TOTAL UNIT EXPOSURE (combined dermal and inhalation)

** Final Draft version of PHED v.1.1 used

Groundboom - Open Cab - long Pants long Sleeves gloves

011597

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

SCENARIO: Long pants, long sleeves, gloves (GROUNDBOOM APPLICATION)

`TCH CATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs.
.AD (ALL)	Lognormal	.78	2.5874	139.8663	.9707	31
NECK.FRONT	Lognormal	.3	1.5763	167.9503	.3296	23
NECK.BACK	Lognormal	.242	.9753	181.4416	.2219	27
UPPER ARMS	Lognormal	1.455	1.6914	88.749	1.1637	32
CHEST	Lognormal	1.42	1.8282	95.4874	1.2005	40
BACK	Normal	1.775	2.0057	88.3183	1.3235	40
FOREARMS	Lognormal	.605	2.7301	419.1055	.564	32
THIGHS	Lognormal	1.146	3.3025	180.0273	1.2698	31
LOWER LEGS	Other	.714	2.2285	167.1842	.8001	33
FEET						0
HANDS						0
TOTAL DERM:		8.4399	8.437	18.9254	7.8438	

95% C.I. on Mean: Dermal: [-141.4485, 179.2993]

Number of Records: 42
Data File: APPLICATOR Subset Name: BRUCE1.APPL

ADD INHALATION | CHANGE HEAD | LB AI TO KG AI | EXIT

<< Specifications >>
Subset Specifications for BRUCE1.APPL

With Dermal Grade Uncovered Equal to "A" "B"
`ubset originated from BRUCE.APPL
:h Application Method Equal to 2 3 and (Groundboom Tractor, Groundboom Truck)
.th Cab Type Equal to 1 2 (Open Cab)
Subset originated from APPL.FILE

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

SCENARIO: Long pants, long sleeves, gloves

TCH CATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs.
HEAD (ALL)	Lognormal	3.51	14.5427	256.3981	3.1175	60
NECK.FRONT	Lognormal	.6225	1.5156	134.3626	.5743	48
NECK.BACK	Lognormal	.484	1.5962	277.5153	.3815	55
UPPER ARMS	Lognormal	1.3095	1.627	95.5501	1.0466	22
CHEST	Lognormal	2.13	6.0169	190.4768	2.0825	59
BACK	Lognormal	2.485	6.384	193.3835	2.2369	59
FOREARMS	Lognormal	.968	3.8282	263.411	1.0872	47
THIGHS	Other	.382	3.82	360.4738	.796	25
LOWER LEGS	Other	.833	2.1745	152.035	.9379	44
FEET	Lognormal	1.048	20.3196	211.3742	1.9643	9
HANDS	Lognormal	13.6511	43.3524	188.4279	6.2868	21
TOTAL DERM:	19.9926	27.4231	105.1771		20.5115	

95% C.I. on Mean: Dermal: [-1363.2173, 1573.5715]

Number of Records: 65
Data File: APPLICATOR Subset Name: BRUCE2.APPL

ADD INHALATION | CHANGE HEAD | LB AI TO KG AI | EXIT

<< Specifications >> Subset

Specifications for BRUCE2.APPL

With Hand Grade Equal to "A" "B" "C" and
With Hand Measuring Method Equal to 1 2
Subset originated from BRUCE.APPL
With Application Method Equal to 2 3 and (Groundboom Tractor, Groundboom Truck)
With Cab Type Equal to 1 2 (Open Cab)
Subset originated from APPL.FILE

SUMMARY STATISTICS FOR INHALATION EXPOSURES

	DISTRIB. TYPE	NANOGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs.
EXPOSURE	Lognormal	915.7671	1673.8958	156 4139	637.7581	22

95% C.I. on Geo. Mean: [30.3269, 13411.6863]
Number of Records: 22
Data File: APPLICATOR Subset Name: BRUCE3.APPL

<< Specifications >>
Subset Specifications for BRUCE3.APPL

With Airborne Grade Equal to "A" "B"
Subset originated from BRUCE.APPL
With Application Method Equal to 2 3 and (Groundboom Tractor, Groundboom Truck)
With Cab Type Equal to 1 2 (Open Cab)
Subset originated from APPL.FILE

CALCULATIONS

8.4399 ug/lb ai sprayed + 6.2868 ug/lb ai sprayed = 14.7267 ug/lb ai sprayed
TOTAL dermal unit exposure

14.7267 ug/lb ai sprayed + 0 637 ug/lb ai sprayed = 15.3644 ug/lb ai sprayed
(inhalation) **TOTAL UNIT EXPOSURE**
(combined dermal and inhalation)

**PHED version 1.1 used

Liquid-Open Mixing - long pants - long sleeves - gloves

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES 011597

SCENARIO: Long pants, long sleeves, gloves **LIQUID/OPEN MIXING**

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI MIXED Median	Mean	Coef of Var	Geo. Mean	Obs.
HEAD (ALL)	Other	2.99	128.9568	493.8357	4.0992	121
NECK.FRONT	Lognormal	1.695	23.2318	360.9199	1.74	103
NECK.BACK	Lognormal	.341	15.7106	381.706	.5427	109
UPPER ARMS	Other	.582	157.6735	903.2036	1.4925	90
CHEST	Other	3.905	19.2219	262.7404	3.4337	89
BACK	Other	.8875	11.009	221.7177	1.8891	88
FOREARMS	Other	.6655	4.4266	211.9821	.8927	84
THIGHS	Lognormal	3.82	16.8134	196.8466	4.0237	71
LOWER LEGS	Other	.952	38.271	819.5203	1.1162	81
FEET	Lognormal	5.371	346.998	180.1404	19.5296	25
~~HANDS~~	~~Lognormal~~	~~3.5883~~	~~34.7596~~	~~316.3227~~	~~3.5782~~	~~80~~
TOTAL DERM:	39.3962	24.7973	797.0722		42.3376	

95% C.I. on Mean: Dermal: [-12060.5932, 13654.7376]

Number of Records: 137

Data File: MIXER/LOADER Subset Name: OPN1.LIQ.MLOD

D INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for OPN1.LIQ.MLOD

With Dermal Grade Uncovered Equal to "A" "B"
Subset originated from OPN.LIQ.MLOD
With Liquid Type Equal to 1 2 3 4 5 and
With Mixing Procedures Equal to 1
Subset originated from MLOD.FILE

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

SCENARIO: Long pants, long sleeves, gloves

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI MIXED Median	Mean	Coef of Var	Geo. Mean	Obs
HEAD (ALL)	Lognormal	1.885	144.2756	493.6915	3.2991	8
NECK.FRONT	Lognormal	1.2675	22.6456	409.0843	1.0823	8
NECK.BACK	Lognormal	.2255	16.0728	418.1281	.3845	8
UPPER ARMS	Other	.582	211.2486	781.2068	1.8065	6
CHEST	Other	5.68	22.1621	251.7974	4.2547	7
BACK	Other	.71	15.265	208.1592	2.2097	6
FOREARMS	Other	.726	5.8497	182.5683	1.172	6
THIGHS	Lognormal	3.82	16.6769	197.9019	3.8973	6
LOWER LEGS	Other	.714	42.9788	773.9239	1.1099	7
FEET	Lognormal	5.371	346.998	180.1404	19.5296	2
HANDS	Lognormal	11.5385	58.2033	228.9173	6.7068	5
TOTAL DERM:	43.3116	32.5195	902.3764		45.4524	

95% C.I. on Mean: Dermal: [-14732.9293, 16537.6826]

Data File: MIXER/LOADER

Number of Records: 112
Subset Name: OPN2.LIQ.MLOD

D INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for OPN2.LIQ.MLOD

With Hand Grade Equal to "A" "B" and
With Hand Measuring Method Equal to 1 2
Subset originated from OPN.LIQ.MLOD
With Liquid Type Equal to 1 2 3 4 5 and
With Mixing Procedures Equal to 1
Subset originated from MLOD.FILE .

SUMMARY STATISTICS FOR INHALATION EXPOSURES

	DISTRIB. TYPE	NANOGRAMS PER LB AI MIXED Median	Mean	Coef of Var	Geo. Mean	Obs
EXPOSURE	Other	1041.6667	3519.5428	233.2266	598.9849	8

95% C.I. on Geo. Mean: [8.0354, 44650.2369]
Number of Records: 92
Data File: MIXER/LOADER Subset Name: OPN3.LIQ.MLOD

<< Specifications >>
Subset Specifications for OPN3.LIQ.MLOD

With Airborne Grade Equal to "A" "B"
Subset originated from OPN.LIQ.MLOD
With Liquid Type Equal to 1 2 3 4 5 and
With Mixing Procedures Equal to 1
Subset originated from MLOD.FILE

CALCULATIONS

39.3962 ug/lb ai mixed - 3.5782 ug/lb ai mixed = 35.818 ug/lb ai mixed
(dermal unit exposure w/o hands)

35.818 ug/lb ai mixed + 6.7068 ug/lb ai mixed = 42.5248 ug/lb ai mixed
TOTAL dermal unit exposure

42.5248 ug/lb ai mixed + 1.0416 ug/lb ai mixed (inhalation) = 43.5664 ug/lb ai mixed
TOTAL UNIT EXPOSURE
(combined dermal and inhalation)

**PHED version 1.1 used

Airblast - Open Cab - long pants long sleeves gloves

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES 011597

SCENARIO: Long pants, long sleeves, gloves **AIRBLAST-OPEN**

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs
HEAD (ALL)	Lognormal	257.14	797.0776	153.0421	185.8648	4
NECK.FRONT	Lognormal	17.7	38.0282	145.7142	12.5667	3
NECK.BACK	Lognormal	9.537	28.3626	157.8159	3.8584	4
UPPER ARMS	Lognormal	4.656	42.3987	265.4846	6.4049	4
CHEST	Lognormal	5.325	22.2689	175.6535	5 6753	4
BACK	Lognormal	3.55	15.0209	172.0057	4.3627	4
FOREARMS	Lognormal	1.21	7.4511	148.7525	2.0066	3
THIGHS	Lognormal	27.122	58.6432	186.3098	18.7807	3
LOWER LEGS	Lognormal	9.282	17.7886	126.359	7.5149	3
FEET						
HANDS	Lognormal	3.8	7.4605	109.8921	2.4137	3
TOTAL DERM:	254.4487	339.322	1034.5003		254.4487	

95% C.I. on Mean: Dermal: [-10919.1483, 12988.1489]

Number of Records: 48
Data File: APPLICATOR Subset Name: JIM2.APPL

D INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for JIM2.APPL

With Dermal Grade Uncovered Equal to "A" "B"
Subset originated from JIM.APPL
With Application Method Equal to 1 and (Airblast)
With Cab Type Equal to 1 2 (Open Cab)
Subset originated from APPL.FILE

SUMMARY STATISTICS FOR CALCULATED DERMAL EXPOSURES

SCENARIO: Long pants, long sleeves, gloves

PATCH LOCATION	DISTRIB. TYPE	MICROGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs
HEAD (ALL)	Lognormal	73.71	816.985	156.115	152.7534	3
NECK.FRONT	Lognormal	4.5825	34.8283	164.1831	8.1666	3
NECK.BACK	Lognormal	5.907	25.1205	178.8273	6.7222	3
UPPER ARMS	Lognormal	6.2565	49.179	260.7302	11.7972	3
CHEST	Lognormal	6.745	24.566	173.0005	7.5932	3
BACK	Lognormal	5.325	16.1525	206.386	5.6735	3
FOREARMS	Other	1.815	6.8106	140.434	3.4333	2
THIGHS	Lognormal	5.73	35.5461	249.8887	9.0206	3
LOWER LEGS	Lognormal	7.616	17.4505	134.4466	6.252	2
FEET						
HANDS	Lognormal	10.6667	8.5261	74.1769	2.4288	1
TOTAL DERM:	212.2225	128.3537	1035.1646		~~213.8408~~	

95 [illegible]. on Mean: Dermal: [-11940.9957, 14011.3249]

Data [illegible]le: APPLICATOR Number of Records: 40
Subset Name: JIM3.APPL

[illegible] INHALATION CHANGE HEAD LB AI TO KG AI EXIT

<< Specifications >>
Subset Specifications for JIM3.APPL

With Hand Grade Equal to "A" "B" and
With [illegible]and Measuring Method Equal to 1 2 (gloves, handrinse)
Sub[illegible]originated from JIM.APPL
Wi[illegible]plication Method Equal to 1 and (Airblast)
Wi[illegible]b Type Equal to 1 2 (Open Cab)
Sub[illegible]originated from APPL.FILE

SUMMARY STATISTICS FOR INHALATION EXPOSURES

	DISTRIB. TYPE	NANOGRAMS PER LB AI SPRAYED Median	Mean	Coef of Var	Geo. Mean	Obs
EXPOSURE	Other	3849.6377	8068.4196	221.0318	2847.4731	

95% C.I. on Geo. Mean: [113.8844, 71195.9313]
Number of Records: 47
Data File: APPLICATOR Subset Name: JIM4.APPL

<< Specifications >>
Subset Specifications for JIM4.APPL

With Airborne Grade Equal to "A" "B"
Subset originated from JIM.APPL
With Application Method Equal to 1 and (Airblast)
With Cab Type Equal to 1 2 (Open Cab)
Subset originated from APPL.FILE

CALCULATIONS

254.4487 ug/lb ai sprayed - 2.4137 ug/lb ai sprayed = 252.035 ug/lb ai sprayed
(dermal unit exposure w/o hands)

252.035 ug/lb ai sprayed + 2.4288 ug/lb ai sprayed = 254.4638 ug/lb ai sprayed
TOTAL dermal unit exposure

254.4638 ug/lb ai sprayed + 3.8496 ug/lb ai sprayed = 258.3134 ug/lb ai sprayed
(inhalation) **TOTAL UNIT EXPOSURE**
(combined dermal and inhalation)

PHED version 1.1 used

011597

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. 5/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

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

EPA Identification Nos.: EPA MRID No. 434145-09
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0341-0784-001A

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

Title of Report: NAF-85: Acute Oral Toxicity Study in Fischer 344 Rats.

Author(s): Gilbert, K.S.

Report Issued: July 22, 1994

Executive Summary: The acute oral LD_{50} of NAF-85 in male and female Fischer 344 rats is > 5000 mg/kg of body weight. These results place NAF-85 into Toxicity Category IV for acute oral toxicity.

Core Classification: This study satisfies §81-1 guideline requirements for an acute oral toxicity study and is classified as acceptable (MRID 43414515).

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 10 weeks of age at the start of the test, and were obtained from Charles River Laboratories, Inc., Kingston, New York.

B. Test Substance: NAF-85 (43.4% a.i.) was supplied as a liquid (Lot no. B372-41).

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

> Five male and five female rats received 5000 mg of undiluted NAF-85 per kg body weight by single-dose gavage.

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

Test Group	Dose Level (mg/kg)*	Number Assigned	
		Males	Females
---	5000	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 made 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: None of the test rats died. Clinical signs frequently observed during the observation period included lacrimation, salivation, chromorhinorrhea, chromodacryorrhea, and urine and fecal soiling in the perineal area. Most of the effects were noted within three hours after dosing and persisted through day 4 of the observation period.

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

Mean body weight (g)					
Males			Females		
Day 2	Day 8	Day 15	Day 2	Day 8	Day 15
209.9 n=5	217.7 n=5	235.9 n=5	134.6 n=5	139.1 n=5	154.8 n=5

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

> No treatment-related observations were made at necropsy.

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-85 for male and female Fischer 344 rats was greater than the limit dose of 5000 mg/kg.

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

Primary Review by: Roger Gardner Ron Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl A. Baetcke 6/25/95
Toxicology Branch I/HED

011597

DATA EVALUATION RECORD

Study Type: Acute Dermal Toxicity Study
Guideline 81-2
Species: Rabbit

EPA Identification Nos.: EPA MRID No. 434145-10
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (proposed common name for Factor A + Factor D);

Sponsor: DowElanco

Study Number(s): DR-0341-0784-001D

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

Title of Report: NAF-85: Acute Dermal Toxicity Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: July 22, 1994

Executive Summary: The acute dermal toxicity of NAF-85 was evaluated in male and female rabbits by applying a single topical doses of 2,000 mg/kg of body weight (MRID 43414510). All 5 male and 5 female animals survived through the two-week observation period. Therefore, the acute dermal LD_{50} >2000 mg/kg in male and female rabbits.

There were no indications of systemic toxicity including clinical signs or necropsy findings associated with topical administration of NAF-85 to test animals. However, the test substance was associated with erythema which persisted for five days following dosing.

Based on the results of this study NAF-85 should be classified into Toxicity Category III for acute dermal toxicity.

Core Classification: This study satisfies §81-2 guideline requirements for an acute dermal toxicity study and should be classified as Core Guideline.

Materials and Methods

A. Test Animals: Male and female New Zealand White strain adult rabbits were used. They weighed between 2.39 and 2.57 kg, and were acclimated for a period of 7 days. The rabbits were obtained from Hazleton Research Products, Inc., Kalamazoo, MI.

On the day before the study began, the trunks of animals selected for the study were clipped free of hair.

B. Test Substance: NAF-85 (43.4% a.i.) was supplied as a liquid (Lot no. B372-41).

C. Vehicle: None

D. Study Design: The test procedure was described in the report as follows:

> Five rabbits per sex were treated with 2000 mg of NAF-85 per kg body weight. The test material was applied to a piece of gauze covering a piece of non-absorbant cotton. The size of the gauze/cotton was 10 by 14 cm (approximately 10% of the surface area of the rabbits). The test material impregnated gauze patch was held in place by an elastic rabbit jacket. The wrappings were removed after approximately a 24-hour exposure period and observations were recorded for irritation at the application site. The skin was wiped thoroughly with water and dried with a soft disposable towel. The rabbits were fitted with a plastic collar to prevent ingestion of residual material. The collars were removed when study personnel had determined that the skin and fur, at the application site, were dry. Careful in-life observations were made and recorded frequently the day of dosing and at least once each workday throughout the two-week observation period. Routine monitoring on weekends was limited to animal husbandry procedures required to ensure availability of feedand water. The rabbits were weighed prestudy, the day of treatment, and on test days 2, 8, and 15.
>
> Pathology
> At study termination...(a) complete necropsy examination was conducted...

Reported Results

Results were described in the report as follows:

> All rabbits survived the 2000 mg/kg limit test..., therefore, no other dose levels were tested.

> All animals were observed with erythema at the application site, immediately after test material reomoval. All erythema was resolved by test day five...No clinical signs indicative of systemic toxicity were noted. In addition, all rabbits gained body weight during the two-week observation period.
>
> There were no treatment related observations made at necropsy...

Discussion

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

> Under the conditions of this study, the acute dermal LD_{50} of NAF-85 was greater than the 2000 mg/kg limit dose, for male and female New Zealand White rabbits.

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

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Acute Inhalation Toxicity Study
Guideline §81-3
Species: Rat

EPA Identification Nos.: EPA MRID No. 434145-11
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0341-0784-003

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

Title of Report: NAF-85: Acute Aerosol Inhalation Toxicity Study with Fischer 344 Rats.

Author(s): Beekman, M.J.

Report Issued: July 15, 1994

Executive Summary: The acute inhalation LC_{50} in Fischer 344 strain male and female rats is > 5 mg/L. The MMAD ≈ 2.88 μm, GSD ≈ 2.26 μm, and 13.6% of the particles ere < 1.3 μm in diameter.

These results place NAF-85 into Toxicity Category IV for acute inhalation toxicity.

Core Classification: This study satisfies §81-3 guideline requirements for an acute inhalation toxicity study and should be classified as Core Guideline (MRID 434145-11).

Materials and Methods

A. Test Animals: Male and female Fischer 344 rats were used. They were acclimated for a period of 3 weeks, and on the day prior to exposure animals were acclimated to the nose-only cones. The animals were 11 weeks of age at the start of the test, and were obtained from Charles River Laboratories, Inc., Kingston, New York.

B. Test Substance: NAF-85 (44.6% a.i.) was supplied as a liquid (Lot no. B372-129).

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

> The viscous nature of the test material did not allow for normal metering of the liquid into the aerosol generation apparatus. Therefore, the material was diluted with distilled, deionized water (1 gram water: 2 grams formulation).

The report described conditions for generation of the test atmosphere as follows:

> Chambers: A 42 liter nose-only chamber (30 cm dia x 60 cm high) was sued for this study. Compressed air supplied to the chamber was controlled by a system designed to maintain temperature at approximately 22°C. The air flow was maintained at approximately 30 liters per minute, which was sufficient to provide the normal concentration of oxygen to the animals. The chamber was maintained at a slight negative pressure relative to the surrounding area.
>
> Generation System: Liquid aerosols of NAF-85 were generated by metering the test material with an FMI pump into a stainless steel...spray nozzle. The test material mixed with compressed air in the spray nozzle, and aerosol was sprayed into the chamber. Since the mixture contained materials of varying vapor pressure, the test material was not recycled.
>
> Chamber Monitoring: Air flow through the chamber was determined by a manometer. The manometer was calibrated with a gas meter prior to the start of the study. Ambient room temperature, chamber relative humidity and chamber air flow values were recorded approximately every 30 minutes during the 4-hour exposure period.
>
> Mass and Nominal Concentration: The mass concentration of aerosol in the chamber was determined gravimetrically 5 times during the 4-hour exposure period. Each gravimetric sample was taken by drawing air samples through a vertical stainless steel tube which projected into the animal breathing zone. Aerosol particles were collected on Teflon filters with a pore size of 0.45 microns.
>
> A substantial portion of the exposure atmosphere consisted of vapor (primarily water), therefore, vapor samples were collected using silica gel sorbent tubes in-line with the Teflon filters. Background measurements of water vapor in the chamber were taken prior to starting the exposure.
>
> The time-weighted average (TWA) exposure concentration was calculated by combining the aerosol and vapor gravimetric measurements for each sample, and then subtracting the average background value.

Particle Size Determination: The aerodynamic particle size was determined 3 times during the exposure period by drawing samples from the animal breathing zone through a six-stage Cascade Impactor. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg) were determined for each sample as well as for all samples.

D. Treatment: The treatment procedure was described in the report as follows:

> Groups of 5 male and 5 female rats were exposed to 7.5 mg/l of diluted NAF-85 which, after correcting for dilution yielded 5 mg/liter of respirable aerosol (limit test...and MMAD ≤ 4 μ) for a 4 hour period.

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

> Animals were weighed and examined prior to exposure to the test material. All animals were observed during the exposure period and daily during the two-week post-exposure period. The observations included an evaluation of the fur, eyes, mucous membranes and respiration. Behavior pattern and nervous system activity were assessed by specific observation for tremors, convulsions, salivation, lacrimation and diarrhea, as well as lethargy and other signs of altered central nervous system function...All rats were weighed on test days 2, 4, 8, 11 and 15 during the two-week post-exposure period.
>
> All rats were submitted for a complete gross necropsy on test day 15...

Reported Results

A. Test atmosphere: Mean temperature in the test chamber was 21°C and the mean relative humidity was 69.3% during the exposure period. The investigators described the NAF-85 aerosol concentrations in the test chamber as follows:

> The combined time-weighted average (TWA) concentration of aerosol and vapor was 7.5 mg/l. The nominal concentration was 13.1 mg/l. After correcting for the dilution, the actual TWA of NAF-85 in the test atmosphere was 5.0 mg/l, and the corrected nominal concentration was 8.7 mg/l. The difference between the analytical and nominal concentrations was most likely due to loss of material in the generation apparatus and exposure chamber.
>
> Two background gravimetric samples were taken approximately two hours after loading the animals in the nose-only chamber and prior to aerosol generation. The average background concentration of 2.5 mg/l (primarily water vapor) was subtracted from the combined aerosol and vapor concentration for each sample to determine the test material concentration.

> The first gravimetric sample was obtained 85 minutes into the exposure and indicated that the test atmosphere concentration was low. However, prior to taking this sample, the test atmosphere appeared to be at or above the 5 mg/l limit test.
>
> Approximately 30 minutes into the exposure, a particle size sample was obtained, and the mass of this sample was very near the mass obtained with later particle size samples when the concentration was greater than 5 mg/l. This indicated that at 30 minutes into the exposure, the test atmosphere was most likely above the 5 mg/l limit test concentration. This conclusion is supported in that the relative humidity of the chamber after starting exposure was high. This indirectly indicated a substantial concentration of water vapor in the test atmosphere.
>
> The low concentration at approximately 85 minutes into the exposure may have been caused by a clogged spray nozzle. The clean-out needle in the spray nozzle was used repeatedly after obtaining the first gravimetric sample. Subsequently, the test atmosphere was well above 5 mg/l later in the exposure period.
>
> Based on the portion of the T.W.A. that was contained in the vapor traps, approximately 98% of the atmosphere was vapor. On average approximately 94% of the trapped vapor was collected on the first sampling tube which indicated minimal breakthrough to the second sampling tube. Non-volatile components accounted for at least 29% (w/w) of the composition of the diluted NAF-85 formulation. therefore, the substantial vapor component of the generated test atmosphere (98%) may have been due to loss of aerosol particles in the glassware leading to the exposure chamber or in the chamber itself.

The average MMAD reported for particles in the test atmosphere was 2.88 μ with an average geometric standard deviation of 2.26. There were 13.6% of the particles with a MMAD $\leq$1.3 μ according to the report.

B. Mortality and clinical signs: According to the report, there were no deaths during the study. Clinical signs included transient soiling and rough hair coat during and until the fourth day of the study. These signs were observed in both sexes and were considered normal responses for animals in nose-only exposures.

C. Body weights: Group mean body weights exhibited slight changes which were described in the report as follows:

> The mean body weights of male and female rats were slightly decreased from pre-exposure values through test day 4 (maximum of 4 and 3%, respectively)...The mean body weights of both male and female rats had reached or surpassed pre-exposure values by test day 8.

D. Gross necropsy: There were no significant observations noted at gross necropsy according to the report.

Discussion

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

> Based on the results of this study, and accounting for the dilution of the test material, the four hour LC50 was greater than 5 mg/l aerosolized NAF-85.

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

011597

Primary Review by: Roger Gardner Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl D. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Primary Eye Irritation Study
Guideline §81-4
Species: Rabbit

EPA Identification Nos.: EPA MRID No. 434145-12
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (proposed common name for Factor A + Factor D);

Sponsor: DowElanco

Study Number(s): DR-0341-0784-001C

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

Title of Report: NAF-85: Primary Eye Irritation Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: July 22, 1994

Executive Summary: The test material (NAF-85) when instilled in the eyes of rabbits (0.1 ml/eye) produced only slight, transient conjunctival irritation (5/6 animals after 1 hour; 3/6 animals after 24 hours; and 0/6 at 48 hours). All treated eyes had returned to a normal appearance by 72 hours after treatment. These results indicate that NAF-85 should be classified into Toxicity Category IV for eye irritation. (MRID 434145-18).

Core Classification: This study satisfies §81-4 guideline requirements for a primary eye irritation study and should be classified as Core Guideline (MRID 434145-12).

Materials and Methods

A. Test Animals: Three male and three female New Zealand White strain rabbits were used. They weighed between 2.37 and 2.73 kg., and were acclimated for a period of two weeks. The rabbits were obtained from Hazleton Research Products, Inc., Lalamazoo, MI.

On the day before the study began, the eyes of test animals were examined using the fluorescein dye technique.

B. Test Substance: NAF-85 (43.4% a.i.) was supplied as a liquid (Lot no. B372-41).

C. Vehicle: The test substance was administered as received at the laboratory.

D. Treatment: The treatment procedure was described in the report as follows:

> A 0.1 ml. aliquot of NAF-85 was instilled into the conjunctial sac of the right eye of three male and three female rabbits. The left eye remained untreated and served as a control. The eyes of all rabbits remained unwashed post- treatment. The behavior of each rabbit was observed immediately post-treatment for indications of pain or discomfort. Both eyes of each rabbit were examined with a penlight at 1, 24, 48 and 72 hours post-instillation for conjunctival redness and chemosis, discharge, corneal opacity, and reddening of the iris. The study was completed 72 hours post-treatment. Rabbits were weighed on the day of treatment and at the study completion.

The Draize scoring system was listed in the report as follows:

(1) Cornea

(A) Opacity: Degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	2*
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*

(B) Area of corneal involvement:

One quarter (or less), but not 0	1
Greater than one quarter, but less than half	2
Greater than half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

(A) x (B) x 5; Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combinations of any thereof), iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

(A) x 5: Total Maximum = 10

(3) Conjunctivae

(A) Redness: (refers to peripheral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1*
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1*
Obvious swelling with partial eversion of the lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed	4*

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

[(A) + (B) + (C)] x 2: Total Maximum = 20

The total score for the eye is the sum of all the scores obtained from the cornea, iris and conjunctivae.

* Indicates a positive effect.

Reported Results

Selected eye irritation scores are summarized from the report as follows:

Observation time	Animal number	Conjunctivae			Corneal opacity	Reddening of iris
		Redness	Chemosis	Discharge		
1 Hour	93A5527	0	1	0	0	0
	93A5528	0	0	0	0	0
	93A5529	1	0	0	0	0
	93A5530	1	0	0	0	0
	93A5531	1	0	0	0	0
	93A5532	1	0	0	0	0
24 Hours	93A5527	0	0	0	0	0
	93A5528	0	0	0	0	0
	93A5529	1	0	0	0	0
	93A5530	1	0	0	0	0
	93A5531	1	0	0	0	0
	93A5532	0	0	0	0	0

Discussion

A. Authors' Conclusions: The authors' conclusion was that NAF-85 was slightly irritating to the eyes of test animals.

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

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. 6/25/95
Toxicology Branch I/HED

011597

DATA EVALUATION RECORD

Study Type: Primary Dermal Irritation Study
Guideline §81-5
Species: Rabbit

EPA Identification No.s: EPA MRID No. 434145-13
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0341-0784-001B

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

Title of Report: NAF-85: Primary Dermal Irritation Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: July 22, 1994

Executive Summary: Application of 0.5 ml NAF-85 to an area of clipped skin of New Zealand White strain rabbits (3 per sex) under 4-hour semioccluded conditions resulted in slight erythema reactions in 4 rabbits within an hour after dosing. The irritation persisted for 24 hours in one female rabbit. Based on these results, technical grade Spinosad should be classified into Toxicity Category IV for primary dermal irritation (MRID 43414513).

Core Classification: This study satisfies 152B-14 (§81-5) guideline requirements for a primary dermal irritation study and should be classified as Core Guideline (MRID 431036-09).

Materials and Methods

A. Test Animals: Male and female New Zealand White strain adult rabbits were used. They weighed between 2.27 and 2.75 kg, and were acclimated for a period of 7 days. The rabbits were obtained from Hazleton Research Products, Inc., Kalamazoo, MI.

On the day before the study began, a 10 X 10 cm area on the backs of animals selected for the study were clipped free of hair.

B. Test Substance: NAF-85 (43.4% a.i.) was supplied as a liquid (Lot no. B372-41).

C. Vehicle: None.

D. Treatment: The treatment procedure was described in the report as follows:

> A 0.5 ml aliquot of the test material was applied to a Hill Top Chamber. This was held in place with an elastic rabbit jacket. The jacket and chamber were removed after four hours and the back was wiped with a damp disposable towel to remove any residual test substance.

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

> The application sites were graded for erythema and edema within thirty minutes, and 24, 48 and 72 hours after test material removal. Animals were weighed on the day of treatment, and at study termination.

The Draize scoring system was listed in the report as follows:

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised approximately 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Reported Results

The irritation scores were summarized in the report as follows:

	Erythema				Edema			
		Hours				Hours		
Sex	30-60 Minutes	24	48	72	30-60 Minutes	24	48	72
♀	0	0	0	0	0	0	0	0
♀	1	0	0	0	0	0	0	0
♀	1	0	0	0	0	0	0	0
♂	1	0	0	0	0	0	0	0
♂	0	0	0	0	0	0	0	0
♂	1	1	0	0	0	0	0	0

The average primary irritation score for the 30-60 minute observation was 0.67. (This value is the total dermal irritation score (erythema and edema combined) divided by the number of test sites examined at each time.)

Discussion

A. Authors' Conclusions: The authors' concluded that the test material caused slight dermal irritant in 4 of 6 rabbits within the first hour after dosing. Slight irritation persisted in one rabbit 24 hours after treatment.

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

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. 6/25/95
Toxicology Branch I/HED

011597

DATA EVALUATION RECORD

Study Type: Dermal sensitization Study
Guideline §81-6
Species: Guinea Pig

EPA Identification No.s: EPA MRID No. 434145-14
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: NAF-85

Synonyms: Spinosad (Factor A + Factor D)

Sponsor: DowElanco

Study Number(s): DR-0341-0784-001E

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

Title of Report: NAF-85: Dermal Sensitization Potential in Hartley Albino Guinea Pig

Author(s): Gilbert, K.S.

Report Issued: July 22, 1994

Executive Summary: Application of undiluted NAF-85 (0.4 ml) to an area of clipped skin of ten male Hartley albino guinea pigs for approximately six hours in an irritation screen, during the induction phase, and at the challenge phase of a sensitization study resulted in no erythema or edema reactions. Based on these results, NAF-85 is not a skin sensitizer (MRID 434145-14).

Core Classification: This study satisfies §81-6 guideline requirements for a dermal sensitization study and should be classified as Core Guideline (MRID 431036-14).

Materials and Methods

A. Test Animals: Male Hartly albino strain guinea pigs were used. They weighed between 389 and 431 g, and were acclimated for a period of 7 days. The guinea pigs were obtained from Charles River Breeding Laboratories, Inc., Kingston, NY.

B. Test Substance: NAF-85 (43.4% a.i.) was supplied as a liquid (Lot no. B372-41).

C. Vehicle: None.

D. Study Design: The study design was described in the report as follows:

> Screen Phase
> A preliminary skin irritation screen was conducted in order to determine if a slightly irritating dose was achievable as well as to establish the highest non-irritating concentration of the test material. Guinea pigs wee clipped free of hair, on the left and right side (and may have been clipped on their back), the day prior to dosing. Single application of 0.4 ml of neat NAF-85 was topically applied to the skin of guinea pigs for six hours, in up to three application sites per animal. Multiple animals were used in verifying that 100% was the highest non-irritating d ıe level. The following day the application sites were depilated with Neet hair cream remover. Skin irritation readings were recorded approximately 24 and 48 (sic) after test material removal.
>
> Induction Phase
> The left side of ten guinea pigs per treatment group were clipped free of hair the day prior to study initiation. A single 0.4 ml aliquot of neat NAF-85,...was applied to the left side of ten male guinea pigs in Hill Top Chambers. A 10% solution of DER 331 epoxy resin in dipropylene glycol monoethyl ether (DPGME) was used as a positive control and applied in Hill Top Chambers to another group of ten male guinea pigs. The chamber was secured with Vetrap® which was held in place with Elastin™ and removed after approximately a six-hour exposure period. Observations for erythema/edema were recorded the following day. The animals were clipped and the respective groups were treated with the test material or positive control in the same manner at weekly intervals for a total of three consecutive weeks.
>
> Challenge Phase
> Approximately two weeks after the last induction application, the right side of the animals was clipped free of hair. The test material, a 0.4 ml aliquot of neat NAF-85,...or the positive control, was applied to the right side of the guinea pigs in the same manner as in the induction phase. Also, ten naive animals were dosed, five received a 0.4 ml aliquot of neat NAF-85 and five received 0.4 ml of 10% DER 331. The test material was removed after approximately a six-hour exposure period. The following day the application sites were depilated with Neet hair cream remover. The application sites were observed and graded for sensitization response or irritation approximately 24 and 48 hours after the challenge application. The animals were observed at random, such that treatment group and naive animals were unknown. A material is considered a sensitizer if a positive response (erythema and/or edema scored to be 1.0 or greater) is observed following 48 hours after dosing in two or more of the challenge animals with no evidence of irritation in the naive animals.

The Draize scoring system was listed in the report as follows:

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised approximately 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Reported Results

None of the animals treated with NAF-85 in this study exhibited signs of dermal irritation during the screen, induction or challenge phases of the study. The report indicated that 9/10 positive control animals exhibited a sensitization response when challenged.

Discussion

A. Authors' Conclusions: The authors' concluded that the test material was not a dermal sensitizer.

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

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. 6/25/95
Toxicology Branch I/HED

011597

DATA EVALUATION RECORD

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.

Core Classification: This study does not satisfy §81-1 guideline requirements for an acute oral toxicity study and is classified as Core Supplimentary (MRID 43414515).

The results place XDE-105 into Toxicity Category IV for acute oral toxicity, but the reported results indicated a lower LD_{50} value for male Fischer 344 rats (3738 mg/kg/day) apparently based on data from a previous study with Fischer 344 rats at a 2000 mg/kg dose level (cited in the study report as Wright, et al., 1992b). The report of the previous study is needed to support the determination that the acute oral LD_{50} = 3738 mg/kg for male rats and to upgrade the study classification.

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 viuceral 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 n=5	149.4 n=1	179.9 n=1	130.1 n=5	127.5 n=4	148.4 n=4
Mice	31.9 n=5	30.6 n=5	32.8 n=4	24.7 n=5	22.7 n=5	23.6 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.

011597

Primary Review by: Roger Gardner Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl P. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Acute Dermal Toxicity Study
Guideline 81-2
Species: Rabbit

EPA Identification Nos.: EPA MRID No. 434145-16
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (proposed common name for Factor A + Factor D);

Sponsor: DowElanco

Study Number(s): DR-0323-1194-017D

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

Title of Report: XDE-105: Acute Dermal Toxicity Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: August 2, 1994

Executive Summary: The acute dermal toxicity of XDE-105 was evaluated in male and female rabbits by applying a single topical doses of 2,000 mg/kg of body weight (MRID 43414516). All 5 male and 5 female animals survived through the two-week observation period. Therefore, the acute dermal LD_{50} >2000 mg/kg in male and female rabbits.

There were no indications of systemic toxicity including clinical signs or necropsy findings associated with topical administration of XDE-105 to test animals.

Based on the results of this study XDE-105 should be classified into Toxicity Category III for acute dermal toxicity.

Core Classification: This study satisfies §81-2 guideline requirements for an acute dermal toxicity study and should be classified as Core Guideline.

Materials and Methods

A. Test Animals: Male and female New Zealand White strain adult rabbits were used. They weighed between 2.36 and 2.63 kg, and were acclimated for a period of 7 days. The rabbits were obtained from Hazleton Research Products, Inc., Kalamazoo, MI.

On the day before the study began, the trunks of animals selected for the study were clipped free of hair.

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

C. Vehicle: The report noted that each dose was moistened with 4 ml distilled water before application.

D. Study Design: The test procedure was described in the report as follows:

> Five rabbits per sex were treated with 2000 mg of XDE-105 per kg body weight...The test material was applied to a piece of gauze covering a piece of non-absorbant cotton. The size of the gauze/cotton was 10 by 14 cm (approximately 10% of the surface area of the rabbits). The test material impregnated gauze patch was held in place by an elastic rabbit jacket. The wrappings were removed after approximately a 24-hour exposure period and observations were recorded for irritation at the application site. The skin was wiped thoroughly with water and dried with a soft disposable towel. Careful in-life observations were made and recorded frequently the day of dosing and at least once each workday throughout the two-week observation period. Routine monitoring on weekends was limited to animal husbandry procedures required to ensure availability of feedand water. The rabbits were weighed prestudy, the day of treatment, and on test days 2, 8, and 15.
>
> Pathology
> At study termination...(a) complete necropsy examination was conducted...

Reported Results

Results were described in the report as follows:

> All rabbits survived the 2000 mg/kg limit test..., therefore, no other dose levels were tested.
>
> No clinical signs indicative of systemic toxicity were noted. No significant dermal alterations were noted during the in-life phase. In addition, all rabbits gained body weight during the two-week observation period.

There were no treatment related observations made at necropsy...

Discussion

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

> Under the conditions of the study, the acute dermal LD_{50} of XDE-105 was greater than the 2000 mg/kg limit dose, for male and female New Zealand White rabbits.

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

011597

Primary Review by: Roger Gardner Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl H. Baetcke 6/26/96
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Acute Inhalation Toxicity Study
Guideline 81-3
Species: Rat

EPA Identification Nos.: EPA MRID No. 434145-17
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): R33491

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN.

Title of Report: The Acute Inhalation Toxicity in Fischer 344 Rats of Technical XDE-105

Author(s): Wolff, R.K., D.L. Allen, G.D. Williams, and D.W. Grothe

Report Issued: July 23, 1994

Executive Summary: The acute inhalation LC_{50} in Fischer 344 strain male and female rats is > 5.18 mg/L. The MMAD = 6.50 µm at the highest level tested because the test substance is a sticky material that tends to coagulate at high atmospheric concentrations. At a lower concentration of 0.90 mg/L the MMAD was 2.96 µm with a GSD = 2.77 µm and 13.5% of the particles <1 µm.

The results place XDE-105 into Toxicity Category IV for acute inhalation toxicity.

Core Classification: This study satisfies §81-3 guideline requirements for an acute inhalation toxicity study and should be classified as Core Guideline (MRID 434145-17).

Materials and Methods

A. Test Animals: Male and female Fischer 344 rats were used. They were acclimated for a period of 3 weeks, and on the day prior to exposure animals were acclimated to the nose-only cones. The animals were 11 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 (88.0% a.i.) was supplied as a solid (Lot no. ACD13651).

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

> **Aerosol Generation and Exposure Methods**
> The test material proved extremely difficult to generate as an aerosol due to its sticky nature. Several attempts to generate the material using a screw-feed apparatus feeding into an air-powered jet mill dry powder disperser were unsuccessful. Even after milling and dessicating, the material still clogged the screw-feed device within minutes. After several attempts, using the dessicated, air-milled material, reasonable concentrations were achieved using a Wright Dust Feed II. However, even with a cyclone between the generator and the exposure chamber to exclude particles over 2 μm MMEAD, it was not possible to achieve 25% of the particles < 1 μm at concentrations above 0.5 mg/l. Total airflow through the system, operated under subatmospheric conditions, was 40 l/min. for Group 1 and 15 l/min. for Group 2...The air supply was drawn from a compressed air line with no additional conditioning. Humidifying the air would likely increase coagulation effects and further hinder the aerosol generation process...Group 2 was run without a cyclone. The rats were restrained in acrylic "nose-only" exposure tubes which were fitted to ports of a 41L cylindrical stainless steel exposure chamber. The equilibrium time (t_{99}) for the exposure system was calculated to be 4.7 minutes for Group 1 and 12.6 minutes for Group 2.
>
> **Exposure Concentration**
> For Group 1, the exposure concentration was determined on a nominal, total gravimetric, and activity basis. The nominal concentration was calculated from generator output (mg/min) divided by total ariflow through the inhalation chamber (L/min). The total gravimetric concentration was determined from a minimum of eight gravimetric samples. The gravimetric samples were taken at the "respiratory level" of the animals during the exposure. The samples were then submitted for assessment of XDE-105 activity. The gravimetric sampling rate was 1 L/min. The duration of each gravimetric sample was 1 minute. The concentration used was targeted at the maximum concentration which would yield 25% of the particles <1 μm...For Group 2, the exposure concentration was tartgeted at the limit concentration of 5 mg/L...

Particle Size Determination
Particle size determination was determined by averaging two samples taken at the "respiratory level" of the animals during the exposure period. A Sierra Model 218K Ambient Cascade Impactor...was used to collect the samples. The samples were also submitted for analysis of XDE-105 activity. The sample flow rate was 3 L/min. with a duration of 3.9 or 4.9 minutes for Group 1 and 0.6 or 0.7 minutes for Group 2.

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

All animals were observed for sign of toxicity during and immediately after exposure, approximately 1 hour postexposure then twice daily Monday through Friday and once daily on weekends. Toxicity was evaluated by body weight changes, physical signs, changes in behavior, mortality and gross pathological examination.

Reported Results

A. Test atmosphere: The exposure concentrations and particle size results are summarized from the report as follows:

Condition	Group 1	Group 2
Mean concentration (mg/L)		
Total gravimetric	0.90±0.55	5.18±0.90
Nominal	11.95	25.67
mg XDE-105/L	0.67±0.39	4.57±0.97
Particle size		
MMEAD[a]	2.96±2.77	6.5
AMEAD[a]	2.67±2.54	--[b]
% <1 μm	13.5[b]	--[b]

[a] MMEAD = mass median equivalent aerodynamic diameter; AMEAD = activity median equivalent aerodynamic diameter
[b] See discussion below.

The investigators noted that the 25% criterion for particle sizes <1 μm was not achieved in this study and added the following discussion in the report:

...pilot studies conducted inour laboratory could not achieve lower particle sizes and still maintain a concentration above 0.5 mg/L. The analysis of Hinds (1982) indicates that particle number concentrations of 10^6 and 10^7 particles per cubic centimeter are likely to be present at mass concentrations of 5 mg/L., and that these aerosols would show the effects of coagulation. Coagulation of particles will result in increases in particle size with increases in mass concentration. This

effect has been seen in other studies. Wolff, *et al.* (1990) reported particle sizes for inert dusts of 2.5, 4.6, and 6.7 μm at concentrations of 0.25, 1 and 5 mg/L, respectively. Because the number concentrations were higher at higher mass concentrations, coagulation became important, and increased the particle size from low to high mass concentrations. for this reason, the particle size of the high concentration exposure (5.18 mg/L) was large (6.50 μm MMEAD).

B. Mortality and clinical signs: According to the report, there were two deaths during exposure (one female from Group 1 and one female from Group 2). Clinical signs included poor grooming in both animals and chromodacryorrhea in the Group 1 animal. The investigators noted tha all surviving Group 1 animals appeared normal by Day 4 and surviving Group 2 animals appeared normal by Day 5.

C. Body weights: Group mean body weights exhibited slight changes which were described in the report as follows:

> Mean body weights for surviving animals exceeded pre-exposure levels upon termination of the study. LMean body weight gain for surviving Group 1 animals were 62 g and 26 g for males and females, respectively. Mean body weight gain values for Group 2 were 51 g for males and 20 g for females.

D. Gross necropsy: There were no significant observations noted in surviving animals at gross necropsy according to the report. Observations in the two animals that died were discussed in the report as follows:

> Changes were limited to a slight red nasal discharge and red moist lungs in one female which died on the day of exposure to 0.90 mg compound XDE-105/L of air. One female also died during the exposure to 5.18 mg compound XDE-105/L of air and the changes were limited to the presence of a white powdery substance around the nares, and soiling of the head, neck, and the inguinal region.

Discussion

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

> Exposure to an aerosol of XDE-105 at 0.90 mg/L of air produced 5% mortality (1/20 animals; 1 of 10 females). The same compound at 5.18 mg/L., also produced 5% mortality (1/20 animals and 1/10 females). The 4-hour median lethal concentration of technical XDE-105 was estimated to be >5.18 mg/L of air.

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

C. References:

Hinds, W.C. 1982. Coagulation. In *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*. pp 233-248. John Wiley & Sons, New York.

Wolff, R., M. Collins, K. Carlson, R. Tamura, and M. Djorato. 1990. Effects of high exposure concentrations of inhaled low toxicity dusts on pulmonary function in guinea pigs. *Toxicologist* 10:207.

011597

Primary Review by: Roger Gardner Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl V. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Primary Eye Irritation Study
Guideline §81-4
Species: Rabbit

EPA Identification Nos.: EPA MRID No. 434145-18
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (proposed common name for Factor A + Factor D);

Sponsor: DowElanco

Study Number(s): DR-0323-1194-017C

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

Title of Report: XDE-105: Primary Eye Irritation Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: August 2, 1994

Executive Summary: The test material (XDE-105) when instilled in the eyes of rabbits (0.1 g/eye) produced only slight, transient conjunctival irritation (6/6 animals after 1 hour; 1/6 animals after 24 hours; and 0/6 at 48 hours). All treated eyes had returned to a normal appearance by 72 hours after treatment. These results indicate that XDE-105 should be classified into Toxicity Category IV for eye irritation. (MRID 434145-18).

Core Classification: This study satisfies §81-4 guideline requirements for a primary eye irritation study and should be classified as Core Guideline (MRID 434145-18).

Materials and Methods

A. Test Animals: Three male and three female New Zealand White strain rabbits were used. They weighed between 2.45 and 2.67 kg., and were acclimated for a period of two weeks. The rabbits were obtained from Hazleton Research Products, Inc., Lalamazoo, MI.

On the day before the study began, the eyes of test animals were examined using the fluorescein dye technique.

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

C. Vehicle: The test substance was administered as received at the laboratory.

D. Treatment: The treatment procedure was described in the report as follows:

> A 0.1 g. aliquot of XDE-105 was instilled into the conjunctial sac of the right eye of three male and three female rabbits. The left eye remained untreated and served as a control. The eyes of all rabbits remained unwashed post- treatment. The behavior of each rabbit was observed immediately post-treatment for indications of pain or discomfort. Both eyes of each rabbit were examined with a penlight at 1, 24, 48 and 72 hours post-instillation for conjunctival redness and chemosis, discharge, corneal opacity, and reddening of the iris. The study was completed 72 hours post-treatment. Rabbits were weighed on the day of treatment and at the study completion.

The Draize scoring system was listed in the report as follows:

(1) Cornea

(A) Opacity: Degree of density (area most dense taken for reading)

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1*
Easily discernible translucent areas, details of iris slightly obscured	
Opalescent areas, no details of iris visible, size of pupil barely discernible	3*
Opaque, iris invisible	4*

(B) Area of corneal involvement:

One quarter (or less), but not 0	1
Greater than one quarter, but less than half	2
Greater than half, but less than three-quarters	3
Greater than three-quarters, up to whole area	4

(A) x (B) x 5; Total Maximum = 80

(2) Iris

(A) Values

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combinations of any thereof), iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

(A) x 5: Total Maximum = 10

(3) Conjunctivae

(A) Redness: (refers to peripheral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1*
More diffuse, deeper crimson red, individual vessels not easily discernible	2*
Diffuse beefy red	3*

(B) Chemosis

No swelling	0
Any swelling above normal (includes nictitating membrane)	1*
Obvious swelling with partial eversion of the lids	2*
Swelling with lids about half closed	3*
Swelling with lids about half closed to completely closed	4*

(C) Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

[(A) + (B) + (C)] x 2: Total Maximum = 20

The total score for the eye is the sum of all the scores obtained from the cornea, iris and conjunctivae.

* Indicates a positive effect.

Reported Results

The eye irritation scores were presented in the report as follows:

Spinosad (XDE-105) §81-4: Rabbit

Observation time	Animal number	Conjunctivae			Corneal opacity	Reddening of iris
		Redness	Chemosis	Discharge		
1 Hour	94A0966	2	2	0	0	0
	94A0967	2	2	0	0	0
	94A0968	1	1	0	0	0
	94A0969	1	1	0	0	0
	94A0970	2	1	0	0	0
	94A0971	2	1	0	0	0
24 Hours	94A0966	0	0	0	0	0
	94A0967	0	0	0	0	0
	94A0968	1	0	0	0	0
	94A0969	0	0	0	0	0
	94A0970	0	0	0	0	0
	94A0971	0	0	0	0	0

Discussion

A. Authors' Conclusions: The authors' conclusion was that XDE-105 was a slightly irritating to the eyes of test animals.

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

011597

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Primary Dermal Irritation Study
Guideline §81-5
Species: Rabbit

EPA Identification No.s: EPA MRID No. 434145-19
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-0323-1194-017B

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

Title of Report: XDE-105: Primary Dermal Irritation Study in New Zealand White Rabbits

Author(s): Gilbert, K.S.

Report Issued: August 2, 1994

Executive Summary: Application of moistened XDE-105 (0.5 g) to an area of clipped skin of rabbits (3 per sex) under 4-hour semioccluded conditions resulted in no erythema or edema reactions. Based on these results, technical grade Spinosad should be classified into Toxicity Category IV for primary dermal irritation (MRID 434145-19).

Core Classification: This study satisfies 152B-14 (§81-5) guideline requirements for a primary dermal irritation study and should be classified as Core Guideline (MRID 431036-09).

Materials and Methods

A. Test Animals: Male and female New Zealand White strain adult rabbits were used. They weighed between 2.16 and 2.50 kg, and were acclimated for a period of 7 days. The rabbits were obtained from Hazleton Research Products, Inc., Kalamazoo, MI.

On the day before the study began, a 10 X 10 cm area on the backs of animals selected for the study were clipped free of hair.

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

C. Vehicle: 0.g g test substance was moistened with 0.4 ml water and applied to the test sites.

D. Treatment: The treatment procedure was described in the report as follows:

> ...the test material...was applied to a Hill Top Chamber. This was held in place with an elastic rabbit jacket. The jacket and chamber were removed after four hours and the back was wiped with a damp disposable towel to remove any residual test substance.

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

> The application sites were graded for erythema and edema within thirty minutes, and 24, 48 and 72 hours after test material removal. Animals were weighed on the day of treatment, and at study termination.

The Draize scoring system was listed in the report as follows:

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised approximately 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Spinosad (XDE-105) §81-5: Rabbit

Reported Results

The irritation scores were summarized in the report as follows:

	Erythema				Edema			
		Hours				Hours		
Sex	30-60 Minutes	24	48	72	30-60 Minutes	24	48	72
♀	0	0	0	0	0	0	0	0
♀	0	0	0	0	0	0	0	0
♀	0	0	0	0	0	0	0	0
♂	0	0	0	0	0	0	0	0
♂	0	0	0	0	0	0	0	0
♂	0	0	0	0	0	0	0	0

The average primary irritation score for the 30-60 minute observation was 0. (This value is the total dermal irritation score (erythema and edema combined) divided by the number of test sites examined at each time.)

Discussion

A. Authors' Conclusions: The authors' concluded that the test material was not a primary dermal irritant.

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

011597

Primary Review by: Roger Gardner 5/30/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Dermal Sensitization Study
Guideline §81-6
Species: Guinea Pig

EPA Identification No.s: EPA MRID No. 434145-20
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-0323-1194-017E

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

Title of Report: XDE-105: Dermal Sensitization Potential in Hartley Albino Guinea Pig

Author(s): Gilbert, K.S.

Report Issued: August 2, 1994

Executive Summary: Application of undiluted XDE-105 (0.4 g) to an area of clipped skin of ten male Hartley albino guinea pigs for approximately six hours in an irritation screen, during the induction phase, and at the challenge phase of a sensitization study resulted in no erythema or edema reactions. Based on these results, XDE-105 is not a skin sensitizer (MRID 434145-20).

Core Classification: This study satisfies §81-6 guideline requirements for a dermal sensitization study and should be classified as Core Guideline (MRID 431036-20).

Materials and Methods

A. Test Animals: Male Hartly albino strain guinea pigs were used. They weighed between 347 and 413 g, and were acclimated for a period of 7 days. The guinea pigs were obtained from Charles River Breeding Laboratories, Inc., Kingston, NY.

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

C. Vehicle: None/

D. Study Design: The study design was described in the report as follows:

Screen Phase
A preliminary skin irritation screen was conducted in order to determine if a slightly irritating dose was achievable as well as to establish the highest non-irritating concentration of the test material. Guinea pigs wee clipped free of hair, on the left and right side (and may have been clipped on their back), the day prior to dosing. Single application of 0.4 g of neat XDE-105 was topically applied to the skin of guinea pigs for six hours, in up to three application sites per animal. Multiple animals were used in verifying that 100% was the highest non-irritating dose level. The following day the application sites were depilated with Neet hair cream remover. Skin irritation readings were recorded approximately 24 and 48 (sic) after test material removal.

Induction Phase
The left side of ten guinea pigs per treatment group were clipped free of hair the day prior to study initiation. A single 0.4 g aliquot of neat XDE-105,...was applied to the left side of ten male guinea pigs in Hill Top Chambers. A 10% solution of DER 331 epoxy resin in dipropylene glycol monoethyl ether (DPGME) was used as a positive control and applied in Hill Top Chambers to another group of ten male guinea pigs. The chamber was secured with Vetrap® which was held in place with Elastin™ and removed after approximately a six-hour exposure period. Observations for erythema/edema were recorded the following day. The animals were clipped and the respective groups were treated with the test material or positive control in the same manner at weekly intervals for a total of three consecutive weeks.

Challenge Phase
Approximately two weeks after the last induction application, the right side of the animals was clipped free of hair. The test material, a 0.4 g aliquot of neat XDE-105,...or the positive control, was applied to the right side of the guinea pigs in the same manner as in the induction phase. Also, ten naive animals were dosed, five received a 0.4 g aliquot of neat XDE-105 and five received 0.4 g of 10% DER 331. The test material was removed after approximately a six-hour exposure period. The following day the application sites were depilated with Neet hair cream remover. The application sites were observed and graded for sensitization response or irritation approximately 24 and 48 hours after the challenge application. The animals were observed at random, such that treatment group and naive animals were unknown. A material is considered a sensitizer if a positive response (erythema and/or edema scored to be 1.0 or greater) is observed following 48 hours after dosing in two or more of the challenge animals with no evidence of irritation in the naive animals.

The Draize scoring system was listed in the report as follows:

(1) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(2) Edema formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges are well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised approximately 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Reported Results

None of the animals treated with XDE-105 in this study exhibited signs of dermal irritation during the screen, induction or challenge phases of the study. The report indicated that 9/10 positive control animals exhibited a sensitization response when challenged.

Discussion

A. Authors' Conclusions: The authors' concluded that the test material was not a dermal sensitizer.

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

Primary Review by: Roger Gardner Roger Gardner 6/12/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

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 does not satisfy §81-8 guideline requirements for an acute oral mammalian neurotoxicity study and is classified as Core Supplementary (MRID 43557501). There were no positive control data from the testing laboratory presented in the report..

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 obse vations 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 $SPF_{p \times q}$ 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 (α) per comparison was set at 0.02...The corrections for multiple statistical analyses were applied to α 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)
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.

* 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.

Reviewed by: John E. Whalan
Section I, Tox. Branch I (H7509C)
Secondary reviewer: Roger L. Gardner
Section I, Tox. Branch I (H7509C)

GUIDELINE: 82-1

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study in Rats

MRID NO: 435666-01

CHEM. ID NO.: 110003

TEST MATERIALS: XDE-105 (77.6% Factors A + D; Lot No. ACD13453)

SYNONYMS: Spinosad, LY232105, 232105

STUDY NUMBER(S): R20690

SUBMITTED BY: DowElanco

TESTING FACILITY: Toxicology Research Laboratories, Lilly Research Laboratories

TITLE OF REPORT: A Subchronic Toxicity Study in Fischer 344 Rats Administered XDE-105 in the Diet for 3 Months.

AUTHOR(S): D.W. Grothe, S.M. Boss, and C.L. Gries

REPORT ISSUED: November 20, 1992

SUMMARY: Male and female Fischer 344 rats were fed XDE-105 in their mash feed at concentrations of 0 (sham control), 0.05%, 0.1%, 0.2%, and 0.4%. The actual doses, based on food consumption were 0, 33.9, 68.5, 133.5, and 273.1 mg/kg/day in males, and 0, 38.8, 78.1, 151.6, and 308.2 mg/kg/day in females. The high-dose was discontinued on day 44 due to excessive mortality. The clinical findings reveal a steep dose response curve.

Clinical signs seen in the high-dose rats included deep, rapid, or labored breathing, thinness, chromorhinorrhea, piloerection, hypothermia, and distended penis. Only sporadic clinical signs were seen in the 0.2% rats. Body weights for both sexes remained static in the high-dose, while e other groups thrived. Food consumption was markedly lower (≤49% in males, ≤48% in females), and food efficiency was profoundly affected in both sexes.

Biologically significant hematologic anomalies were found in both sexes only in the 0.4% and 0.2% dose groups (there were no concurrent controls for the 0.4% rats). Anemia was manifest by decreased RBC, HGB, Hct, and erythrocyte indices, and the release of reticulocytes and nucleated erythrocytes. The erythrocytes were small and hemoglobin-

deficient. Other abnormal findings include polychromasia, hypochromasia, anisocytosis (excessive size variation), poikilocytes (unusual shapes), microcytes (unusually small cells), and stomatocytes. Thus, a profound effect on erythropoiesis dictated the release of immature cells. In the 0.2% groups, both sexes had mild leukocytosis (+26% and +34%, M/F) due to neutrophilia (+11% and +58%, M/F) and lymphocytosis (+26% and +25%, M/F). Monocyte counts doubled in both sexes. Thrombocyte counts were unaffected.

Clinical chemistry anomalies in the high-dose males and females included increases in BUN, total bilirubin, alkaline phosphatase, ALT, AST, GGT, inorganic phosphorus, sodium, potassium and cholesterol (males only), and decreases in creatinine, triglycerides (males only), albumin, globulin, and total protein.

As would be expected, fewer anomalies were seen in the 0.2% rats. Alkaline phosphatase and ALT values were scarcely affected, but AST values were elevated in both sexes due to trauma in cardiac and skeletal muscle. Significantly elevated BUN and inorganic phosphorus values were evidence of renal damage. Neither creatinine nor bilirubin levels were altered. Mildly elevated cholesterol levels in both sexes were likely renal-related. It appears that females are more sensitive than males for renal effects. No other biologically significant clinical chemistry anomalies were seen in this or lower dose groups.

Markedly reduced urine pH in the 0.2% rats (pH of 6.4 and 5.4, M/F, compared to 8.2 in the controls) along with elevated inorganic phosphorus levels suggest a condition of metabolic acidosis, an acidic shift in the blood's acid/base status due to a loss of base (e.g. bicarbonate) through the kidneys, or retention of nonvolatile acids (as seen in salicylate poisoning). The compensatory action is to raise the blood pH through CO_2 elimination via the lungs. This, combined with anemic anoxia, would account for the deep, rapid, or labored breathing in the high-dose rats.

The principal target organs/tissues appear to be the bone marrow, liver, kidneys, heart, skeletal muscle, and thyroid, with the thyroid being the most sensitive indicator. The most universal histopathologic finding in this study was vacuolation, which was found in the kidney, liver, heart, spleen, lymph node, thymus, pancreas, stomach, oviduct, uterus, vagina, epididymis, adrenals, and thyroid.

NOAEL = 0.05% (33.9 and 38.8 mg/kg/day, M/F; Lowest Dose Tested) - adrenal cortical vacuolation in males, lymph node histiocytosis in both sexes.

LEL = 0.1% (68.5 and 78.1 mg/kg/day, M/F) - elevated BUN (+64%) and inorganic phosphorus (+18%) in female rats; moderately reduced urinary pH in females (6.6 compared to 8.2 in the controls, probably due to metabolic acidosis); multifocal hepatocytic granuloma and multifocal Kupffer cell vacuolation in females; progressive cardiomyopathy in both sexes, skeletal muscle multifocal degeneration and multifocal regeneration in females, splenic histiocytosis in both sexes, slightly increased splenic weights in females (27%), lymph node enlargement in females, and lymph node histiocytosis in both sexes.

STUDY CLASSIFICATION: This study is **Acceptable**, and satisfies data requirement 82-1 for a Subchronic Oral Toxicity study. There was no description of the physical state of the test article (powder, liquid, color, etc.). There were no concurrent clinical pathology control values with which to compare the high-dose group on study day 44. At study termination (and day 44 for the high-dose group), the rats were sacrificed by unspecified means. This study received Quality Assurance review.

****

PROTOCOL: Randomly assigned groups of 10 male (118.6 ±7.0 g) and 10 female (95.9 ± 4.3 g) Fischer 344 rats were fed XDE-105 in their mash feed at doses of 0% (sham control), 0.05%, 0.1%, 0.2%, and 0.4%. These doses were based on results from a 2-week pilot study. The high-dose was discontinued on day 44 due to excessive mortality. Formulated feed was prepared every 4 weeks. Both the test article and the formulated feed were stored in tightly sealed containers at room temperature. Formulated feed was evaluated for stability and homogeneity at the time of preparation, and after 7, 14, and 32 days.

The rats were individually housed in stainless steel and Lexan® cages with wire mesh floors. Disposable waste trays were changed at least weekly, and the rats were transferred to clean cages monthly. Food and water were available *ad libitum*.

The rats received ophthalmologic examinations prior to study commencement and near termination (except for the high-dose group which was terminated early). Body weights and food consumption were measured weekly.

The clinical pathology parameters listed below were measured for all rats at termination (day 44 for the high-dose rats). All rats, except the high-dose rats, were fasted before sampling. After the rats were anesthetized with ether, hematology samples were collected from the orbital plexus, and samples for clinical chemistry (including activated partial thromboplastin time and prothrombin time) were collected from the abdominal aorta at necropsy.

Hematology

Erythrocyte count	Mean corpuscular hemoglobin concentration
Hematocrit	Erythrocyte morphology
Hemoglobin	Platelet count
Reticulocytes	Leukocyte count
Mean corpuscular hemoglobin	Differential leukocyte count
Mean corpuscular volume	

Clinical Chemistry

Alkaline phosphatase (AP)	Glucose
Alanine transaminase (ALT)	Bilirubin, total
Aspartate transaminase (AST)	Total protein
Gamma glutamyltransferase	Prothrombin time (PT)
Lactic dehydrogenase (LDH)	Activated partial thromboplastin time (APPT)
Albumin	Sodium
Globulin	Chloride
Cholesterol, total	Phosphorus, inorganic
Triglycerides	Potassium
Blood urea nitrogen	Calcium
Creatinine	
Creatine phosphokinase	

Urinalysis was performed near the end of the study for 5 rats/sex/dose, except for the high-dose. Urine was collected for individuals over a 5 hour interval. No other details were provided on the collection protocol.

Urinalysis

Volume	Ketone bodies
Specific gravity	Bilirubin
pH	Blood
Color	Urobilinogen
Clarity	Sodium
Protein	Potassium
Glucose	Chloride
Creatinine	

At study termination (and day 44 for the high-dose group), the rats were sacrificed by unspecified means. All rats, including those that died or were sacrificed moribund, were necropsied and examined for gross lesions. The following tissues were evaluated histopathologically and graded for severity (minimal, slight, moderate, and marked):

Histopathology

Skin	Tongue	Oviduct
Mammary gland	Esophagus	Cervix
Bone marrow	Stomach	Vagina
Spleen*	Duodenum	Pituitary gland
Lymph node (mesenteric)	Jejunum	Adrenal gland*
	Ileum	Thyroid & parathyroid*
Bone (sternum, femur)	Cecum	Thymus
Femoro-tibial joint	Colon	Brain:*
Skeletal muscle	Rectum	Cerebrum
Trachea	Kidney*	Cerebellum
Lung (w/bronchi)*	Urinary bladder	Brain stem
Heart*	Prostate*	Spinal cord
Aorta	Testis*	Sciatic nerves
Salivary gland	Epididymis	Eye
Liver*	Seminal vesicle	Harderian glands
Pancreas	Uterus*	Unusual lesions
	Ovaries*	

* Weighed at necropsy (all except the 0.4% high-dose group)

Ultrastructural evaluation, via electron microscope, was made of thyroid, spleen, liver, kidney (renal cortex), and lung sections from 5 animals/sex in the control, 0.2% and 0.4% groups.

RESULTS:

Test Article: Analyses demonstrated that the test article was both stable and homogeneous in feed at room temperature over a period of 32 days. Dose concentration was within 6.6% of nominal for each formulation. Calculated doses, corrected for the potency of active materials, were as follows:

	Doses (mg/kg/day)	
% of Diet	**Males**	**Females**
0	0	0
0.05%	33.9	38.8
0.1%	68.5	78.1
0.2%	133.5	151.6
0.4%	273.1	308.2

Survival and Clinical Signs: During weeks 6 and 7, 5 males and 5 females in the high-dose groups had either died or were sacrificed moribund, so all surviving rats were sacrificed on day 44. The only other death was a 0.1% female which died during the second week for reasons unrelated to the test article. Aside from a few sporadic signs, clinical signs were observed almost exclusively in the 0.4% dose groups, and included deep, rapid, or labored breathing, thinness, chromorhinorrhea, piloerection, hypothermia, and distended penis. There were no dose-related ophthalmologic lesions. In the 0.2% rats, 1/10 females had chromodacryorrhea, 1/10 males had pale eyes, and 3/10 females had ventral/perineal soiling.

Body Weights: The body weights of the high-dose males and females remained static while the other dosed and control groups gained weight at comparable rates. By day 42, the high-dose weights were 55% and 44% less than control weights for males and females, respectively.

Food Consumption: Food consumption was severely lower in the high-dose rats — as much as 49% in males and 48% in females, compared to controls. Consumption was also reduced somewhat in the 0.2% groups — as much as 11% in males and 17% in females, compared to controls, with improvement as the study progressed. Food efficiency was profoundly affected in the high-dose rats of both sexes. In the 0.2% rats, food efficiency was consistent until the final 3 weeks when there was a decrease of 10-13%, compared to controls. The females in this dose group were slightly more affected, with decreases ranging from 7-19%.

Clinical Pathology: The high-dose rats were bled before being sacrificed on day 44. Their clinical pathology can only be discussed in general terms since no concurrent blood was collected from the controls, and no urine was collected for the high-dose rats.

Hematology: Biologically significant hematologic anomalies were found only in the high-dose and 0.2% dose groups. In the high-dose groups, there was moderate anemia for both sexes as expressed in the erythrocyte count, hematocrit and hemoglobin values, and the erythrocyte indices. Most significant, however, was the fact that 6% of the erythrocytes in males and 10% of the erythrocytes in females were nucleated. Reticulocytes would also have been elevated had they been counted. Other abnormal findings include polychromasia, anisocytosis (excessive size variation), and the presence of stomatocytes. This suggests a profound effect on erythropoiesis that required the early release of immature cells. Even in the absence of control data, it is apparent that the males and females had neutrophilia and lymphopenia. There may have been a slight thrombocytopenia.

A similar response was seen in the 0.2% rats. The reticulocyte to erythrocyte ratios were 11% in males and 6% in females. A significant number of nucleated erythrocytes were found in males, but not in females. The following table lists the percentage difference in erythrocyte values, compared to controls; the bolded values are considered biologically significant:

0.2% Group Hematology

Parameter	Δ% - Males	Δ% - Females
RBC	-11%	+20%
HGB	**-40%**	**-8%**
Hct (PCV)	**-33%**	**-5%**
MCV	**-25%**	**-21%**
MCH	**-33%**	**-23%**
MCHC	-11%	-3%

RBC, HGB, and Hct values are typically within a few percent of each other, but that is clearly not the case in this study. Also, a marked anemia in males is countered by a marked polycythemia in females (RBC increased by 20%). The polycythemia is likely an artifact caused by dehydration. This would explain the upward skewing of the RBC, HGB, and Hct values while maintaining similar ratios to those seen in the males. If the females were properly hydrated, their values would resemble those of the males. Water consumption was not measured, and urine volumes are unreliable due to renal trauma, so in the absence of evidence to the contrary, it is reasonable to assume the apparent polycythemia was an aberration, and that the females were as anemic as the males.

Despite these irregularities, it can be seen that hematopoiesis was markedly compromised. The number of erythrocytes was only slightly reduced, but these cells were small and deficient in hemoglobin. Other abnormal findings in the 0.2% groups include anisocytosis, polychromasia, hypochromasia, poikilocytes (unusual shapes), and microcytes (unusually small cells).

In the 0.2% groups, the males and females had mild leukocytosis (+26% and +34%, M/F) due to neutrophilia (+11% and +58%, M/F) and lymphocytosis (+26% and +25%, M/F). Monocyte counts doubled in both sexes. Thrombocyte counts were unaffected. A slight decrease in prothrombin time and partial thromboplastin time was not clinically significant.

Clinical Chemistry: In the high-dose, the males and females had similar anomalies. These included increases in BUN, total bilirubin, alkaline phosphatase, ALT, AST, GGT, inorganic phosphorus, sodium, potassium and cholesterol (males only), and decreases in creatinine, triglycerides (males only), albumin, globulin, and total protein.

As would be expected, fewer anomalies were seen in the 0.2% rats. The following table lists the percentage difference in key clinical chemistry values, compared to controls; the bolded values are considered biologically significant:

0.2% Group Clinical Chemistry

Parameter	Δ% - Males	Δ% - Females
ALP	+2%	+15%
ALT	+52%	+20%
AST	**+219%**	**+174%**
BUN	+11%	**+57%**
Creat	-7%	+6%
Chol.	**+37%**	**+30%**
Trig.	**-34%**	-8%
I. Phos	+11%	**+35%**

The fact that alkaline phosphatase and ALT values were scarcely affected indicates a lack of liver involvement. Thus, the elevated AST values in both sexes were not due to liver pathology, but rather to trauma in some other tissue, such cardiac or skeletal muscle. BUN values were significantly elevated in the females only. Considering that BUN does not tend to elevate until about 75% of the nephrons have been damaged, these values indicate renal damage. The elevated phosphorus levels in females is further evidence of renal damage. It is surprising that neither creatinine or bilirubin levels were altered. The mildly elevated cholesterol levels in both sexes were likely renal-related.

Biologically significant elevated BUN (+64%) and inorganic phosphorus (+18%) levels were seen in the 0.1% female rats. This suggests that the females are more sensitive than males for renal effects. No other biologically significant anomalies were seen in this or lower dose groups.

Urinalysis: Urinary pH for each of the groups is presented below; the boided values are considered biologically significant:

Urinary pH

Dose (%)	Males	Females
0	8.2	8.2
0.05%	8.2	8.0
0.1%	7.8	**6.6**
0.2%	**6.4**	**5.4**

Urine specific gravity and volume were not significantly affected. Urine potassium was elevated 25% in males and 28% in females in the 0.2% groups, compared to controls, but it is not clear whether this was a dose-related effect. All other parameters were within normal limits.

Gross Pathology: The dose related gross lesions are presented below; the bolded values are considered biologically significant:

Gross Lesions

Organ/Lesion	Control	0.05%	0.1%	0.2%	0.4%
			Males		
WHOLE ANIMAL					
Thinness					**10/10**
KIDNEY					
Whole tissue alteration				**2/10**	**1/10**
LIVER					
Hepato-diaphragmatic nodule				1/10	
Whole tissue alteration					**5/10**
LUNG					
Whole tissue alteration					**10/10**
SPLEEN					
Enlarged				**10/10**	
LYMPH NODE					
Enlarged				**9/10**	
STOMACH					
Contents				**10/10**	
Lesion				**10/10**	
CECUM					
Contents		2/10	**6/10**	**6/10**	
Distension					**7/10**
TESTIS					
Enlarged				**8/10**	
PENIS					**7/7**
Distension					
THYROID					
Whole tissue alteration				**10/10**	

Gross Lesions

Organ/Lesion	Control	0.05%	0.1%	0.2%	0.4%
			Females		
WHOLE ANIMAL					
Thinness					10/10
KIDNEY					
Whole tissue alteration				4/10	
LIVER					
Hepato-diaphragmatic nodule				2/10	
Whole tissue alteration					1/10
LUNG					
Whole tissue alteration				9/10	9/10
SPLEEN					
Enlarged				10/10	
LYMPH NODE					
Enlarged			7/10	10/10	
STOMACH					
Contents				10/10	
[illegible]				10/10	
CECUM					
Contents			1/10	5/10	
Distension					6/10
OVARY					
Cyst	1/10		3/10	2/10	
THYROID					
Whole tissue alteration				10/10	

Organ Weights: Terminal organ weights were recorded for all but the high-dose rats. The following tables present the percentage deviation from control organ weights. Percentage deviation for organ weights relative to brain weights are virtually identical to the absolute organ weights (within a few percent), so they are not included in this DER. The bolded values are considered biologically significant:

Organ Weights - Absolute (Δ%)

Organ	0.05%	0.1%	0.2%
	Males		
Body Weight	+8%	+4%	-12%
Kidneys	+10%	+11%	+10%
Liver	+11%	+13%	+7%
Heart	+7%	+6%	**+15%**
Spleen	+4%	+7%	**+82%**
Testes	+14%	+10%	+9%
Prostate	+14%	+13%	**-17%**
Adrenals	+4%	+11%	**+21%**
Thyr/Parathyr.	-3%	+26%	**+116%**
Brain	+1%	+1%	0%

Organ Weights - Absolute (Δ%)

Organ	0.05%	0.1%	0.2%
	Females		
Body Weight	+2%	-2%	-13%
Kidneys	+6%	+14%	+17%
Liver	+8%	+17%	**+28**
Heart	+10%	+13%	**+19%**
Spleen	+3%	**+27%**	**+91%**
Ovaries	+4%	+10%	+11%
Uterus	-23%	-22%	**-31%**
Adrenals	+5%	+8%	**+21%**
Thyr/Parathyr.	+10%	+22%	**+86%**
Brain	+1%	-2%	0%

Organ Weights - Relative to Body Weight (Δ%)

Organ	0.05%	0.1%	0.2%
	Males		
Body Weight	+8%	+4%	-12%
Kidneys	+2%	+7%	+26%
Liver	+3%	+9%	**+21%**
Heart	0%	+3%	+32%
Spleen	-4%	+3%	**+107%**
Testes	+5%	+5%	+22%
Prostate	+6%	+10%	-4%
Adrenals	-4%	+6%	**+38%**
Thyr/Parathyr.	-10%	+22%	**+144%**
Brain	-6%	-3%	+14%

Organ Weights - Relative to Body Weight (Δ%)

Organ	0.05%	0.1%	0.2%
	Females		
Body Weight	+2%	-2%	-13%
Kidneys	+4%	+16%	+35%
Liver	+6%	+20%	**+48**
Heart	+8%	+16%	**+37%**
Spleen	0%	**+30%**	**+121%**
Ovaries	+1%	+12%	+27%
Uterus	-26%	-21%	-21%
Adrenals	+2%	+10%	**+39%**
Thyr/Parathyr.	+7%	+25%	**+113%**
Brain	+1%	-1%	15%

Histopathology: The following table summarizes the dose-related histopathologic lesions and their severities:

Histopathologic Lesions

Organ/Lesion	Control		0.05%		0.1%		0.2%		0.4%	
	M	F	M	F	M	F	M	F	M	F
KIDNEY										
Cortical tubular necrosis, minimal								1/10	6/10	2/10
slight								1/10		
Multifocal cortical tubular vacuolation, minimal							5/10	2/10		3/10
slight							5/10	6/10	8/10	5/10
moderate								2/10		
LIVER										
Multifocal granuloma, minimal						2/10	5/10	10/10	5/10	3/10
slight						5/10	3/10		4/10	0
moderate						2/10			1/10	1/10
Multifocal hematopoiesis, minimal	1/10						3/10			
Hepato-diaphragmatic nodule							1/10	2/10		
Chronic multifocal inflammatory necrosis, minimal									2/10	2/10
slight										1/10
moderate									2/10	
Multifocal hepatocellular vacuolation, minimal								1/10	2/10	3/10
slight									7/10	7/10
moderate									1/10	
Multifocal vacuolation, Kupffer cell, minimal						2/10	8/10		2/10	3/10
slight						7/10		10/10	7/10	7/10
moderate									1/10	
HEART										
Progressive cardiomyopathy, minimal	2/10	2/10	1/10	1/10	3/10	1/10	4/10	4/10	2/10	
slight					1/10	1/10	1/10	3/10		
Multifocal vacuolation, minimal								1/10	1/10	
slight						1/10		1/10	2/10	1/10
LUNG										
Alveolar macrophages, minimal								2/10		
slight		1/10					1/10	1/10		1/10
moderate								1/10	3/10	1/10
moderate								8/10	7/10	8/10
Acute multifocal inflammation, slight									8/10	7/10

Organ/Lesion	Control		0.05%		0.1%		0.2%		0.4%	
	M	F	M	F	M	F	M	F	M	F
OVIDUCT										
Mucosal vacuolation, slight								1/10		9/10
UTERUS										
Atrophy										2/10
Mucosal vacuolation, slight						2/10		8/10		8/10
moderate								1/10		1/10
VAGINA										
Mucosal vacuolation, slight										3/10
TESTIS										
Bilateral hypospermatogenesis, moderate									2/10	
marked									8/10	
EPIDIDYMIS										
Mucosal vacuolation, moderate									10/10	
SKELETAL MUSCLE										
Multifocal degeneration, minimal						4/10	2/10	1/10		
slight						2/10	5/10	7/10	3/10	3/10
moderate							3/10	2/10	6/10	7/10
Multifocal regeneration, slight						2/10	5/10	8/10	2/10	1/10
moderate							5/10	2/10	3/10	8/10
BONE MARROW										
Hypocellularity, slight									4/10	4/10
moderate						1/10			6/10	4/10
ADRENALS										
Cortical vacuolation, slight	9/10		9/10		7/10	1/9	3/10	4/10	10/10	8/10
THYROID										
Diffuse follicle epithelial cell										
vacuolation, minimal			2/10							
slight			4/10							
moderate					5/10	1/9				
marked					5/10	8/9	10/10	10/10	10/10	10/10
Acute focal inflammation, slight						1/9	2/10	1/10		
Acute multifocal inflammation, slight							5/10	1/10		

Organ/Lesion	Control		0.05%		0.1%		0.2%		0.4%	
	M	F	M	F	M	F	M		M	F
SPLEEN										
Lymphoid atrophy									1/10	1/9
lymphoid vacuolar change, slight									2/10	2/9
moderate									8/10	7/9
Hematopoiesis, slight								6/10		
moderate							10/10	3/10		
Histiocytosis, minimal	2/10	1/10	3/10		6/10	3/10				
slight					3/10	5/10	2/10			
moderate							8/10	8/10		
marked								2/10		
LYMPH NODE										
Lymphoid vacuolar change, slight								3/10	9/10	5/10
moderate										4/10
Histiocytosis, minimal	2/10	2/10	4/10	1/10						
slight		2/10	3/10	5/10	4/10	3/10	1/10		1/10	1/10
moderate		1/10			5/10	6/10	9/10	9/10	5/10	4/10
marked								1/10	2/10	4/10
Lymphocytic necrosis, slight									3/10	1/10
THYMUS										
Atrophy									6/9	6/9
Lymphoid vacuolar change, slight								1/10	2/9	4/9
moderate									3/9	1/9
Histiocytosis, slight							1/10	1/10	1/9	2/9
moderate									3/9	
PANCREAS										
Diffuse acinar atrophy, slight						1/10		1/10	2/10	3/9
Diffuse acinar vacuolation, slight									10/10	9/9
STOMACH										
Glandular dilation, slight	1/10				1/10		2/10		8/10	5/10
Diffuse mucosal fibrosis, slight									7/10	2/10
Focal hyperkeratosis, moderate							7/10	7/10		
Diffuse glandular epithelial vacuolation										
minimal										1/10
slight									7/10	6/10
moderate									2/10	

Ultrastructural Evaluation: The following text is reproduced from the study report (page 5b)

> Rats given 0.2% or 0.4% XDE-105 had a treatment-related occurrence of cytoplasmic lamellar inclusion bodies in the liver, kidney, thyroid, spleen, and lung. The change was characterized primarily by membranous whorls of various sizes, but also included some irregular electron-dense aggregates within vacuoles. The most pronounced effects were in epithelial cells. Follicular cells in the thyroid gland were engorged with cytoplasmic lamellar inclusion bodies, which occupied most of the cytoplasmic volume. Pronounced changes also occurred in the kidney in both proximal and distal tubular epithelial cells, with approximately half of the cytoplasmic volume occupied by inclusions in some epithelial cells. Occasional cytoplasmic lamellar inclusion bodies, graded slight, also occurred in the following: hepatocytes in liver; pneumocytes and macrophages in lung; and lymphoid, reticular, and endothelial lining cells in the spleen. Occasional changes also occurred in interstitial cells of each of the tissues but these changes were substantially less than the changes in epithelial cells. Tissues from control rats had minimal occurrence of cytoplasmic lamellar inclusion bodies, these structures being infrequent and small. Other doses were not evaluated in this study.

DISCUSSION: The high-dose of 0.4% (273.1 and 308.2 mg/kg/day in males and fema respectively) was lethal to half the males and females, so the remaining rats were terminated a 44 days on study. This group was replete with toxic signs involving multiple target organs. The lower dose, 0.2%, was considerably less toxic. The broad difference in toxic responses expresse closely grouped doses suggests a steep dose response curve for this test article.

Clinical Signs: Clinical signs seen in the high-dose rats included deep, rapid, or labored breath thinness, chromorhinorrhea, piloerection, hypothermia, and distended penis. None of these si were seen in the 0.2% rats.

Body Weights and Food Consumption: Body weights for both sexes remained static in the high-d while the other dosed and control groups thrived. By day 42, the high-dose weights were 5 44% less than control weights for males and females, respectively. As would be expected consumption was markedly lower (as much as 49% in the males and 48% in the females, compa to controls), and food efficiency was profoundly affected in both sexes.

Thus, the 0.2% dose appeared to be far less toxic than the 0.4% dose. Still, some of the differen in toxic expression can be attributed to the dosing at 0.4% over 44 days versus dosing at 0.2% o 90 days. The 0.2% group had more time to express toxicity, as can be seen in the clinical pathol and histopathology data. Unfortunately, a direct comparison of clinical pathology data between high-dose group and the other groups is impossible since there were no concurrent controls, and urinalysis at all for the high-dose rats.

Metabolic Acidosis: Inorganic phosphorus levels, which are regulated by the kidneys, were eleva in the females (0.1% and 0.2% levels). Urinary pH was moderately reduced in the 0.1% fema

and markedly reduced (pH of 6.4 and 5.4 in males and females, respectively) in the 0.2% rats. Tl suggests a condition of metabolic acidosis, an acidic shift in the blood's acid/base status due to a lc of base (e.g. bicarbonate) through the kidneys, or retention of nonvolatile acids (as seen in salicyla poisoning). The compensatory action is to raise the blood pH through CO_2 elimination via the lun This, combined with anemic anoxia, would account for the deep, rapid, or labored breathing in t high-dose rats. One can only wonder what the urinary pH must have been in the high-dose rat

Target Organs/Tissues: The principal target organs/tissues appear to be the bone marrow, liv kidneys, heart, skeletal muscle, and thyroid. The most universal histopathologic finding in this stu was vacuolation, which was found in the kidney, liver, heart, spleen, lymph node, thymus, pancre stomach, oviduct, uterus, vagina, epididymis, adrenals, and thyroid.

Bone Marrow: The early stages of anemia were detected in high-dose rats at 44 days as evidenc by a modest decrease in erythrocytes, hematocrit, and hemoglobin, and the release of numerc nucleated erythrocytes (6% of RBC's in males, and 10% in females). Had they been measur reticulocytes would also have been significantly elevated. The erythrocytes were small a hemoglobin deficient. Abnormal erythrocyte morphology included polychromasia, anisocytosis, a stomatocytes. Thus, the hematopoietic tissues were releasing immature and defective erythrocyt This was confirmed histopathologically. Slight to moderate bone marrow hypocellularity was se in every high-dose rat. Both sexes had neutrophilia and lymphopenia, and a slight thrombocytopen

Anemia was more fully expressed in the 0.2% rats at 90 days. Although the number of erythrocy was only slightly reduced, these cells were small and deficient in hemoglobin. The reticuloc erythrocytes ratios were 11% in males and 6% in females. A significant number of nucleat erythrocytes were found in males, but not in females. Other abnormal findings in the 0.2% grou include anisocytosis, polychromasia, hypochromasia, poikilocytes, and microcytes. The males a females had mild leukocytosis (+26% and +34%, M/F) due to neutrophilia (+11% and +58 M/F) and lymphocytosis (+26% and +25%. M/F). Monocyte counts doubled in both sex Thrombocyte counts were unaffected. Extramedullary hematopoiesis, which was not observed in t high-dose rats, was seen in the livers (male only) and spleen (both sexes) of the 0.2% rats. Gr splenic enlargement and enlarged lymph nodes was seen in all males and females at this do Although hypocellularity of the bone marrow was not found in this group, it is clear that the bo marrow was compromised.

Liver: Liver trauma was indicated in the high-dose by increases in alkaline phosphatase, ALT, AS GGT, and cholesterol, gross findings of whole tissue alteration, and by histopathologic findings minimal to moderate multifocal granuloma, minimal to moderate chronic multifocal inflammat necrosis, and minimal to moderate multifocal vacuolation of the hepatocytes and Kupffer ce. Increased inorganic phosphorus and decreased triglycerides, albumin, and globulin were probal due to malnutrition, but they could also be attributed to hepatocellular insult.

Alkaline phosphatase and ALT were not significantly elevated in the 0.2% rats. Nevertheless, li lesions were found at this dose, including minimal to moderate multifocal granuloma, hepa diaphragmatic nodules in 1 male and 2 females, and minimal to moderate multifocal Kupffer c

vacuolation. Male liver weights were not significantly affected, but female liver weights were slightly elevated (absolute: +28%; relative: +48%). Liver lesions seen in the 0.1% females (only) included minimal to moderate multifocal granuloma and minimal to slight multifocal Kupffer cell vacuolation. There were no lesions found in the 0.05% groups.

Kidney: The increased BUN, total bilirubin, sodium, and potassium levels in the high-dose rats were probably due to a mixture of renal failure and malnutrition. These rats were found to have minimal cortical tubular necrosis and minimal to slight multifocal cortical tubular vacuolation.

The 0.2% rats had elevated BUN (females only), gross whole tissue alteration in 2/10 males and 4/10 females, minimal to slight cortical tubular necrosis in 2/10 females, and multifocal cortical tubular vacuolation in both sexes. Kidney weights were slightly increased, but not to a biologically significant degree. No lesions were seen in lower doses.

Heart and Skeletal Muscle: In the 0.2% rats, alkaline phosphatase and ALT values were scarcely affected, but AST values were significantly elevated in both sexes. This pattern suggests significant trauma in a tissue other than the liver — such as cardiac and/or skeletal muscle. This was confirmed histopathologically. Progressive cardiomyopathy was seen in all groups, including controls, but dose related increases in incidence and severity were seen in the 0.1%, 0.2%, and 0.4% groups. Minimal to slight multifocal vacuolation was also seen in these groups. Heart weights were increased in these groups (absolute: +15% and +19%, M/F; relative: +32% and +37%, M/F). Skeletal muscle lesions were also seen in the 0.1%, 0.2%, and 0.4% groups, and included minimal to moderate multifocal degeneration and slight to moderate multifocal regeneration.

Thyroid: All males and females in the 0.2% groups had gross findings of whole tissue alteration of the thyroid. Thyroid weights were increased at this dose (absolute: +116% and +86%, M/F; relative: +144% and +113%, M/F). Diffuse follicle epithelial cell vacuolation, ranging from minimal to slight, was seen in 6/10 males in the 0.05% group, and in all rats in the higher dose level at severities ranging from moderate to marked. This appears to be the most sensitive indicator of toxicity for this chemical. Slight acute focal and multifocal inflammation was seen in 7/10 males and 2/10 females in the 0.2% group.

Several other pathologic lesions of note (other than vacuolation) are as follows:

Lung: Minimal to moderate alveolar macrophages in the 0.2% (mostly females) and 0.4% groups and slight acute multifocal inflammation in the 0.4% rats. Gross whole tissue alteration was seen in nearly all the high-dose males and 0.2% males.

Spleen: Lymphoid atrophy was seen in one male and one female in the high-dose group. Minimal to marked histiocytosis was seen in the controls and the 0.5%, 0.1%, and 0.2% groups. Spleen weights were markedly increased in the 0.2% groups (absolute: +82% and +91%, M/F; relative: +107% and +121%, M/F), and slightly increased in the 0.1% females (absolute: +27%; relative: +30%).

Lymph Node: Slight lymphocytic necrosis was found in 3 males and 1 female in the high-dose group. Minimal to marked histiocytosis was seen in all groups, but primarily in the 0.1%, 0.2%, and 0.4% groups. Gross lymph node enlargement was seen in all rats in the 0.2% dose groups, and in 7/1 females in the 0.1% dose group.

Thymus: Slight to moderate histiocytosis was seen in the 0.2% and 0.4% groups.

Pancreas: Slight diffuse acinar atrophy was found in the high-dose group.

Stomach: Slight glandular dilation was seen in the high-dose and in 2/10 females dosed at 0.2%. Slight diffuse mucosal fibrosis was seen in the high-dose, and moderate focal hyperkeratosis was seen in the 0.2% group. Gross findings of stomach lesions (not otherwise specified) and stomach contents were reported for the 0.2% group.

Uterus: Uterine atrophy was found in 2/10 high-dose females.

Testis: Moderate to marked bilateral hypospermatogenesis was seen in all high-dose males. Although no gross lesions were observed in this group, 8/10 males had enlarged testes in the 0.2% group.

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Reviewed by: John E. Whalan 9 Jun 1995
Section I, Tox. Branch I (H7509C)
Secondary reviewer: Roger L. Gardner 6/12/95
Section I, Tox. Branch I (H7509C)

GUIDELINE: 82-1

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity Study in Rats with Recovery Phase

MRID NO: 435575-02

CHEM. ID NO.: 110003

TEST MATERIALS: XDE-105 (88% Factors A + D; Lot No. AGR 293707)

SYNONYMS: Spinosad, LY232105, 232105

STUDY NUMBER(S): DR-0323-1194-001A

SUBMITTED BY: DowElanco

TESTING FACILITY: Toxicology Research Laboratories, The Dow Chemical Company

TITLE OF REPORT: XDE-105: 13-Week Dietary Toxicity and 4-Week Recovery Studies in Fischer 344 Rats.

AUTHOR(S): B.L. Yano and D.M. Bond

REPORT ISSUED: September 15, 1994

SUMMARY: Male and female Fischer 344 rats were fed XDE-105 in their mash feed for 13 weeks at concentrations of 0 (sham control), 0.003%, 0.006%, 0.012%, and 0.06%. The actual doses; based on food consumption were 0, 2.2, 4.3, 8.6, and 42.7 in males, and 0, 2.6, 5.2, 10.4, and 52.1 in females. There were two "recovery" groups assigned to the control and high-dose which received control feed for another 4 weeks.

There were no deaths, no compound-related clinical signs or ophthalmologic lesions, and no effect on body weights, food consumption, food efficiency, clinical pathology, or gross pathology. Very slight to slight thyroid follicle epithelial cell vacuolation was seen in 10/10 males and 8/10 females in the high-dose group. A similar incidence was seen in the recovery group, although severity was reduced somewhat.

NOEL = 0.012% (8.6 and 10.4 mg/kg/day, M/F)
NOAEL = 0.06% (42.7 and 52.1 mg/kg/day, M/F; Highest Dose Tested) - thyroid follicle epithelial cell vacuolation.
LEL - Not defined

STUDY CLASSIFICATION: The rationale for selecting doses too low to elicit frank toxicity is unclear. Since an LEL value could not be defined, this study is unacceptable on its own, but combined with the previous study (Study No. R20690; MRID No. 435666-01) it allows the defining of a NOEL value and offers limited information on reversibility in the thyroid. Thus, the two studies combined are **Acceptable**, and satisfy data requirement 82-1 for a Subchronic Oral Toxicity study.

There was no description of the physical state of the test article (powder, liquid, color, etc.). The histopathology severity scale for not described. Although the group sizes were 20/sex for the control and high-dose and 10/sex for all other groups, the body weight, food consumption, and feed efficiency tables present the group sizes as 25/sex and 15/sex, respectively. This error had no impact on the interpretation of the study. This study received Quality Assurance review.

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PROTOCOL: Randomly assigned groups of male and female Fischer 344 rats (6-8 weeks old) were fed XDE-105 in their mash feed for 13 weeks at doses of 0% (sham control), 0.003%, 0.006%, 0.12%, and 0.06%. Ten rats/sex were randomly assigned to each dose level. There were two additional "recovery" groups of 10 rats/sex assigned to the control and high-dose which received control feed for another 4 weeks. The doses, which were based on a previous subchronic study in rats (Study No. R20690; MRID No. 435666-01), were justified as follows:

> The high dosage was expected to produce clear evidence of toxicity based upon the results of the subchronic study previously conducted. The remaining dose levels were expected to provide dose-response data for the treatment-related effect observed in the high-dose group and to ensure definition of a NOEL for the test material.

There was no reason to expect the 0.06% dose to produce clear evidence of toxicity since the 0.05% dose was virtually non-toxic in the previous study. The only biologically significant effects seen at 0.05% were adrenal cortical vacuolation in males and lymph node histiocytosis in both sexes — findings so trivial that TB-I defined this as the NOAEL dose.

Premix was prepared every 3-4 weeks, and feed was formulated every two weeks. Formulated feed was evaluated for stability and homogeneity at the start of the study. Dose concentration analyses were performed at the beginning, middle, and end of the study.

The rats were individually housed in stainless steel cages with wire mesh floors. Food and water were available *ad libitum*. The rats were observed daily for clinical signs. Once weekly, the rats received detailed evaluations of their health status. Body weights and food consumption were measured weekly during the dosing and recovery periods. The rats received ophthalmologic examinations prior to study commencement and at necropsy. The

clinical pathology parameters listed below were measured at week 13. After fasted rats were anesthetized with methoxyflurane, blood was collected from the orbital sinus. Urine was collected from non-fasted rats during week 12 by manual compression of the abdomen.

Hematology

Erythrocyte count	Leukocyte morphology
Hematocrit	Platelet count
Hemoglobin	Leukocyte count
Nucleated erythrocytes	Differential leukocyte count
Erythrocyte morphology	

Clinical Chemistry

Thyroxin (T_4)	Creatine phosphokinase (CK)
Alkaline phosphatase (AP)	Glucose
Alanine transaminase (ALT)	Bilirubin, total
Aspartate transaminase (AST)	Total protein
Albumin	Sodium
Globulin	Chloride
Cholesterol, total	Phosphorus, inorganic
Triglycerides	Potassium
Blood urea nitrogen	Calcium
Creatinine	

Urinalysis

Specific gravity	Ketone bodies
pH	Bilirubin
Color	Occult blood
Appearance	Urobilinogen
Protein	Sediment
Glucose	characterization

No hematology or urinalysis studies were performed for the recovery groups because there were no anomalies at week 13, however, measurements were made of creatinine in the males, and total protein, albumin, globulin, and cholesterol in the females.

At study termination, the rats were anesthetized by methoxyflurane inhalation, their tracheas were clamped to prevent aspiration of blood, and then they were decapitated. All rats, including those that died or were sacrificed moribund, were necropsied and examined for gross lesions. The following tissues were evaluated histopathologically and graded for severity (minimal, slight, moderate, and marked) for all high-dose and control rats:

Histopathology

Skin	Pancreas	Oviduct
Mammary gland	Tongue	Cervix
Bone marrow	Esophagus	Vagina
Spleen*	Stomach	Pituitary gland
Lymph node (mesenteric, mediastinal)	Duodenum	Adrenal gland*
	Jejunum	Thyroid & parathyroid*
	Ileum	Thymus
Mesenteric tissues	Cecum	Auditory sebaceous glands
Mediastinal tissues	Colon	Brain:*
Bone (w/joint)	Rectum	Cerebrum
Skeletal muscle	Kidneys*	Cerebellum
Nasal tissues	Urinary bladder	Brain stem
Larynx	Prostate	Spinal cord (3 sections)
Trachea	Testes*	Peripheral nerve
Lung (w/bronchi)	Epididymis	Eyes
Heart*	Seminal vesicle	Lacrimal and harderian glands
Aorta	Coagulating glands	
Salivary gland	Uterus	Unusual lesions
Liver*	Ovaries*	

* Weighed at necropsy

The tissues examined for the control and high-dose recovery groups included thyroid gland, parathyroid gland, esophagus, larynx, and trachea. The tissues processed and examined for the low and intermediate dose groups were the lungs, liver, kidneys, thyroid, spleen, thymus, lymph nodes, and any unusual gross lesions.

RESULTS:

Test Article: Analyses demonstrated that the test article was both stable and homogeneous in feed at room temperature over a period of 40 days. Dose concentration was within 8% of nominal for each formulation. Calculated doses were as follows:

% in Diet	Male Dose (mg/kg/day)	Female Dose (mg/kg/day)
0%	0	0
0.003%	2.2	2.6
0.006%	4.3	5.2
0.012%	8.6	10.4
0.06%	42.7	52.1

Survival and Clinical Signs: There were no deaths and no compound-related clinical signs or ophthalmologic lesions. A transitory mass/nodule was observed between days 65-79 in the midline of female 91a4138 in the high-dose group, but was not considered compound-related.

Body Weights, Food Consumption, and Feed Efficiency: The test article had no effect on body weights, food consumption, or food efficiency for any group..

Clinical Pathology: No compound-related anomalies were revealed in the hematology, clinical chemistry, or urinalysis data.

Gross Pathology: In the high-dose, one male had a multifocal focus on its kidney and a female had strangulated or necrotic fat within the abdominal cavity. Neither of these lesions can be considered dose-related.

Organ Weights: The following tables present the percentage deviation from control absolute organ weights. Relative organ weights are not presented because terminal body weights were nearly identical for all groups.

Organ Weights - Absolute (Δ%)

Organ	0.003%	0.006%	0.012%	0.06%	0.06% (Recovery)
			Males		
Heart	-2%	+4%	+6%	+10%	-1%
			Females		
Heart	0	+4%	+3%	+9%	+2%
Spleen	+2%	+7%	+3%	+9%	+2%

None of these values are considered biologically significant. The increased spleen weight in the high-dose females was modest and not dose-related. The only organ weights of possible interest are the 10% and 9% increases in heart weights in males and females. respectively. These anomalies were reversible in both sexes. Thyroid weights, which were doubled in the previous study, were unaffected.

Histopathology: There were very few histopathologic findings of note. Three heart lesions, which are not dose-related, are presented only because of the slight increase in heart weights. The tables on the following page summarizes the dose-related histopathologic lesions and their severities.

Histopathologic Lesions

Organ/Lesion	Control		0.003%		0.006%		0.012%		0.06%	
	M	F	M	F	M	F	M	F	M	F
HEART										
Degeneration with or without inflammation, very slight	7/10	5/10							8/10	7/10
Focal hypertrophy, blood vessels slight	0/10	1/10							0/10	0/10
Mineralization, blood vessels very slight	2/10	0/10							4/10	1/10
LUNG										
Alveolar histiocytosis, very slight	0/10	0/10	0/10	0/10	1/10	1/10	0/10	0/10	0/10	3/10
THYROID										
Follicle epithelial cell vacuolation, very slight	3/10	2/10	2/10	2/10	3/10	2/10	4/10	2/10	1/10	3/10
slight	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	9/10	5/10

Histopathologic Lesions - Recovery Groups

Organ/Lesion	Control		0.06%	
	M	F	M	F
THYROID				
Follicle epithelial cell vacuolation, very slight	0/10	1/10	5/10	6/10
slight	0/10	0/10	4/10	1/10

DISCUSSION: The purposes of the study were to, "Evaluate the systemic toxicity potential of the test material in rats following dietary subchronic exposure and to evaluate potential recovery." Neither of these purposes were achievable because the doses were too low. It was possible, however, to define a NOEL — something the previous study lacked.

There were no deaths, no compound-related clinical signs or ophthalmologic lesions, and no effect on body weights, food consumption, food efficiency, clinical pathology, or gross pathology. A slight increase in heart weight in males (10%) and females (9%) cannot be considered biologically significant since there were no collaborating clinical chemistry or histopathology data. Heart weights were normal in the recovery group.

The only finding of any significance is very slight to slight thyroid follicle epithelial cell vacuolation in the thyroid in 10/10 males and 8/10 females in the high-dose group. The pathologist reported that these cells, "appeared somewhat enlarged and the colloid within the follicles occasionally had a decreased staining intensity as compared to the control rats." This lesion did not fully reverse the recovery group. Very slight to slight vacuolation was seen in 9/10 males and 7/10 females in the high-dose recovery group, but severity was reduced compared to the rats in the main study. Thyroxin levels were unaffected. Based on these findings, it is reasonable to assume that minimal to marked vacuolation in the previous study would be equally slow to reverse.

Primary Review by: Roger Gardner Roger Gardner 6/12/95 011597
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl D. Baetcke 6/26/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Subchronic Feeding Study
Guideline 82-1
Species: Mice

EPA Identification No.s: EPA MRID No. 43566602
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): MO 1290

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

Title of Report: A Subchronic Toxicity Study in CD-1 Mice Administered XDE-105 in the Diet for 3 Months.

Author(s): Grothe, D.W., S.M. Boss, and C.L. Gries

Report Issued: December 4, 1992

Executive Summary: Groups of 10 male and 10 female CD-1 strain mice were given diets containing XDE-105 at 0, 0.005%, 0.015%, 0.045% or 0.12% (0, 7.5, 22.5, 67.5, or 180 mg/kg/day) for 13 weeks. Test animals were observed daily for clinical signs and mortality, and they were weighed weekly. Food consumption was not determined. Prior to sacrifice at 13 weeks, animals were fasted overnight, and blood samples were collected for hematological and biochemical observations. After sacrifice animals were subjected to macroscopic and microscopic examination, and selected organs were weighed.

The incidence of mortalities at the 180 mg/kg/day dose level resulted in termination of that group after 6 weeks of the study (3/10 males and 2/10 females died). The authors concluded that effects associated with the highest dose tested included changes consistent with hepatobiliary disturbance, iron deficient anemia, inflammation (i.e., marked neutrophilic and lymphocytic leukocytosis), and loss of or decreased production of albumin as well as necrosis in liver, lymph node and lung. Although there were no control animals concurrently sacrificed, the effects noted in animals from the 180 mg/kg/day group were consistent with a dose-related response.

At dose levels ≥22.5 mg/kg/day, cells of the lymphoid organs, liver, kidney, stomach, ovary, female reproductive tract, and epididymis had cytoplasmic vacuolation. Other tissues less severely affected at these dose levels included the heart, lung, pancreas, adrenal cortex, bone marrow, tongue, and

pituitary gland. Four of 10 males in the 22.5 mg/kg/day dose group had minimal and/or slight lymphoid vacuolar change compared with none in the control group.

The 67.5 mg/kg/day dose level was associated with significantly increased absolute and relative liver weight in both sexes (1.8 g compared with 1.4 g in treated and control group males, respectively, p≤0.05; 1.5 g vs. 1.2 g in treated and control group females, respectively, p≤0.05). Terminal body weight for male mice was significantly decreased (34.6 g for 67.5 mg/kg group and 37.5 g for controls). Clinical chemistry results were consistent with the liver weight increases at the 67.5 mg/kg/day dose level in that activity of alkaline phosphatase, alanine transferase, and aspartate transferase were 36%, 123%, and 81% greater than control values in males, respectively. The respective values in females from the 67.5 mg/kg/day dose group were 11%, 146% and 120% greater than control values. Histologically, the incidence of slight centrilobular hepatocellular cytomegaly was increased in males (6/10 at 67.5 mg/kg/day compared to 0/10 in the control group), and an increase in the incidence and severity of centrilobular hepatocellular vacuolation in the liver of females was observed (1 of 10 with minimal severity in the 22.5 mg/kg/day dose group and 3 of 9 or 4 of 9 with minimal and slight severity in the 67.5 mg/kg/day dose groups as compared to 0 of 10 in the control group).

The 67.5 mg/kg/day dose level was also associated with significantly increased absolute and relative spleen weights in female mice (68 vs. 106.3 mg in control and 67.5 mg/kg dose groups, respectively, p≤0.05). Related effects noted in the report included statistically significant (p≥0.05) decreases for males in hemoglobin (14% less than control value), packed cell volume (11% less than control value), mean corpuscular volume (9% less), and mean corpuscular hemoglobin (10% less than controls), and statistically significant decreases for females in mean corpuscular volume (10% less than controls) and mean corpuscular hemoglobin (5% less than controls). Hematopoiesis was noted in the spleen of 1 of 10 males and no females from the 67.5 mg/kg/day dose group, and lymphatic vacuolation (minimal, slight and/or moderate grades) was observed in 4 and 10 of 10 males from the 22.5 and 67.5 mg/kg/day dose groups, respectively; these changes were observed in 7 of 10 females from the 67.5 mg/kg/day dose group. Control group males and females had no lymphoid vacuolar changes according to the report. Bone marrow necrosis was reported in 6 of 10 and 5 of 9 males and females from the 67.5 mg/kg/day doe group, respectively, and no similar lesions were noted in the control group animals.

Other effects of XDE-105 in mice included skeletal muscle myopathy which was observed in the quadriceps in 3 and 5 of 10 male mice from the 67.5 and 180 mg/kg/day groups, respectively, and in females the respective incidences were 1 of 9 and 4 of 10. Severity and incidence of gastric glandular dilation was also noted to increase in male and female mice. For example, in males the incidence of minimal glandular dilation was 2/10, 3/10, and 1/10 in the control, 7.5 and 22.5 mg/kg/day dose groups, respectively; the respective incidences of slight glandular dilation were 4/10 and 3/10 in the 22.5 and 67.5 mg/kg/day groups; and the incidence of moderate glandular dilation in male mice from the was 3/10 and 6/10 in the 67.5 and 180 mg/kg/day groups. Marked gastric glandular dilation was observed in 4 of 10 males each in the 67.5 and 180 mg/kg/day dose groups.

Based on these results, the NOAEL for XDE-105 was established at 7.5 mg/kg/day, and the LOAEL was determined to be 22.5 mg/kg/day in mice.

Core Classification: This study satisfies §82-1 guideline requirements for a rodent subchronic feeding study and should be classified as Core Guideline (MRID 43566602).

Materials and Methods

A. Test Animals: Male and female CD-1 strain mice were used. They were 4 to 5 weeks of age on arrival at the laboratory and were acclimated for a period of one week. The animals weighed 29.9 ± 1.8 g (males) or 22.2 ± 1.3 g (females) and were obtained from Charles River Laboratories, Inc., Portage Michigan.

B. Test Substance: Technical grade XDE-105 (77.6% a.i.) was supplied as a powder (lot no. ACD13453).

C. Vehicle: Diet (Purina Rodent Chow® #5002)

D. Diet Preparation: The test diets were prepared every 4 weeks during the study, and they were stored in closed containers at room temperature.

Test diets were analyzed for homogeneity and concentration of XDE-105 prior to initiation of the study. They were also stored under ambient laboratory conditions and analyzed for stability over 7, 14, and 32 day storage intervals. Samples of test diets were also collected and analyzed near the beginning, middle and end of the treatment period according to the report.

According to the report, test mixtures were found to be homogeneous and test concentrations were within 10% of nominal values.

E. Study Design: Animals were randomly assigned to test groups so as to achieve homogenous distribution by body weight. They were assigned as follows:

Test Group	Dose Level (ppm)*	Number Assigned	
		Males	Females
Control	0	10	10
Low Dose	50	10	10
Low-mid Dose	150	10	10
High-mid Dose	450	10	10
High Dose	1200	10	10

* Diets were provided *ad libitum* for three months; diet was withdrawn overnight prior to making clinical pathology observations.

F. Observations: The animals were observed daily for clinical signs and mortality, and they were examined weekly for muscle tone, appearance of coat and eyes, respiration, posture, locomotion, excreta, and presence of external lesions. They were weighed weekly during the study. Food consumption was not determined during the study.

After the animals were fasted overnight and prior to termination of the study, blood samples were collected from the orbital sinus. Hematological observations included the following:

Leukocyte count	Hematocrit	Differential leukocyte counts
Erythrocyte count	MCV, MCH, MCHC	
Hemoglobin	Platelets	

Additional blood samples were taken by cardiac puncture from some animals for clinical chemistry determinations. The following biochemistry parameters were also measured:

Alkaline phosphatase	Alanine amino-transferase (ALT)	Albumin
Total bilirubin	Urea nitrogen	Globulin
Aspartase amino-transferase (AST)	Creatinine	Cholesterol
	Total protein	Triglycerides
		Glucose

After three months on test diets the animals were sacrificed and subjected to necropsy. Macroscopic examinations of each animal were conducted and the following organs were weighed: brain, ovaries, testes, heart, kidneys, liver, and spleen. The following organs were fixed for possible microscopic examination:

Adrenals	Ovary	Salivary gland
Aorta	Testis with epididymis	Sciatic nerve
Bone with bone marrow	Gross lesions	Skeletal muscle
Bone marrow smear	Heart	Skin
Brain	Kidney	Spinal cord
Eye with optic nerve	Hardarian gland	Spleen
Tongue	Liver	Thymus
Esophagus	Lung with bronchi	Thyroid/para-thyroid glands
Stomach	Lymph nodes	Trachea
Duodenum	Mammary gland	Urinary bladder
Jejunum	Pancreas	Uterus
Ileum	Pituitary	Uterus, cervix
Cecum	Prostate with seminal vesicle	Vagina
Colon		
Rectum		

Samples of liver, kidney and lung were collected from 3 animals/sex/group in the control and 450 ppm dose groups and processed for electron microscopy.

G. Statistical Analysis: The report describe statistical methods as follows:

> ...(Dunnett's test) was used in the analysis of differences between control and treated group means for parameters for which data are generally distributed normally (body weight, weight gain, hematology, clinical chemistry, and organ weight). The homogeneity of variances was tested by...(Bartlett's test). All references to statistical significance in this report represent $p \leq 0.05$.

Reported Results

A. In-Life Observations:

1. Clinical Signs and Mortality: The report indicated:

> Due to the death of three males and two females in the 0.12% group (1200 ppm) after 6 weeks of treatment and cachexia of other surviving animals in the group were necropsied on Test Day 44. Survival was 100% in all other dose groups at the end of 3 months of treatment. One female in the 0.045% group (450 ppm), however, was found to be missing at the time of necropsy on Test Day 93. This female was observed and weighed on Test Day 92 and likely escaped during transportation or immediately prior to necropsy.

The investigators noted an increase in the incidence of animals with hypoactivity, rough hair coat, rapid respiration and thinness in the 1200 ppm dose group. The incidence of selected clinical signs in the other test groups is summarized from the report as follows:

	Dose (ppm)			
Observation	0	50	150	450
Males				
Rough/oily hair coat	0	3	5	5
Soiling ventral/perineal	0	0	1	3
Alopecia	2	2	1	5
Females				
Rough/oily hair coat	0	0	0	2
Thinness	0	0	0	1
Hypothermia	0	0	0	1
Alopecia	0	0	0	6

2. Body Weight: The report described body weight results as follows:

> After 6 weeks of treatment, weight gain decreases of 230% and 165%, relative to controls, were observed in males and females, respectively, of the 0.12% group (1200 ppm). Significant mean

body weight and weight gain decreases relative to controls were also observed in males of the 0.045% group (450 ppm) through most of the study. By study termination, mean weight gain was 33% lower than that in control males. Mean body weight and weight gain for females in the 0.045% group were not significantly different than controls; however, values for this group were slightly lower than those of the controls during most of the study. At study termination, mean weight gain was 11% lower than that in control females. Mean body weight and weight gain for animals in the 0.005% and 0.015% groups were comparable to those of controls throughout the study.

These results are summarized from the report as follows:

	Dose level (ppm)**			
Mean Body Weight (g)	0	50	150	450
Males at week				
0	29.6	29.8	30.5	29.3
4	33.7	33.7	33.9	32.0
8	36.3	36.8	36.5	34.2*
13	37.5	37.1	36.7	34.6*
Females at week				
0	22.2	21.6	22.5	22.5
4	26.0	24.9	25.8	25.1
8	28.8	27.3	29.0	27.6
13	29.3	28.7	30.6	28.8

* Significantly different from controls ($p \leq 0.05$)
** Excludes 1200 ppm dose group since it was terminated after 6 weeks.

B. Clinical Pathology:

1. Hematology: Hematology results were described in the report as follows:

Male and female mice in the 0.12% group had microcytic, hypochromic anemia and marked neutrophilic leukocytosis. Minimal changes occurred in mice in the 0.045% group. Male and female mice given 0.12% XDE-105 in the diet had mildly to moderately lowered mean packed cell volume (PCV) (approximately 0.7 times control value), hemoglobin (HGB), erythrocyte count, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Females also had lowered mean corpuscular hemoglobin concentration (MCHC). Male mice in the 0.045% group had minimally lowered PCV, HGB (also male mice in the 0.015% group), MCV, and MCH. Female mice from this group had minimally lowered mean MCV and MCH.

Male and female mice given 0.12% XDE-105 had markedly higher total leukocyte (up to 75,000 to 100,000/µl in some

individuals) and neutrophil counts, and moderately higher lymphocyte and monocyte counts. Neutrophils had cytoplasmic basophilia and nuclear hypersegmentation (indication of degeneration and prolonged circulation). Males in the 0.045% group had minimally lowered mean lymphocyte counts and females had mildly higher group mean neutrophil counts.

These results are summarized from the report as follows:

Observation	Dose (ppm) 0	150	450	1200*
Males				
Erythrocyte count (10^6 per μl)	10.632	9.824	10.347	8.651
Hemoglobin (HBG) (g/dl)	16.630	15.340*	14.560*	11.143
Packed cell volume (PCV) (%)	53.74	50.07	47.71*	36.56
Mean corpuscular volume (MCV) (fl)	50.57	51.12	46.24*	42.2
Mean corpuscular hemoglobin (MCH) (pg)	15.640	15.680	14.110*	12.88
Mean corpuscular hemoglobin conc. (%)	30.930	30.650	30.550	28.750
Leukocyte count ($10^3/\mu$l)	4.71	3.20	3.12	57.4
Neutrophils ($10^3/\mu$l)	1.060	0.650	1.030	48.7
Monocytes ($10^3/\mu$l)	0.040	0.010	0.020	0.471
Females				
Erythrocyte count (10^6 per μl)	10.468	10.282	11.252	9.770
Hemoglobin (HBG) (g/dl)	16.520	16.370	15.811	11.712
Packed cell volume (PCV) (%)	52.44	51.42	51.06	40.75
Mean corpuscular volume (MCV) (fl)	50.22	50.18	45.42*	41.69
Mean corpuscular hemoglobin (MCH) (pg)	15.810	15.980	15.078*	11.962
Mean corpuscular hemoglobin conc. (%)	31.510	31.840	30.989	28.750
Leukocyte count ($10^3/\mu$l)	3.86	4.23	3.62	62.9
Neutrophils ($10^3/\mu$l)	0.720	0.880	1.244*	50.4
Monocytes ($10^3/\mu$l)	0.000	0.030	0.044	0.225

* Statistically significantly different from controls, $p \leq 0.05$.
* Samples collected on test day 43 and no statistical comparisons with control values were made.

2. Clinical Chemistry: The report described these results as follows:

Male and female mice in the 0.12% group had significantly higher group mean alkaline phosphatase (2 to 3 times controls), alanine transferase (10 to 15 times control), and aspartate transferase (4 to 8 times control) enzyme activity; minimally higher globulin levels; and mildly lower albumin levels. Males also had minimally to mildly lower glucose, blood urea nitrogen, bilirubin, cholesterol and triglyceride values, and females had minimally to mildly lower group mean glucose and bilirubin values. Males in the 0.045% group had minimally higher alkaline phosphatase, alanine transferase, and aspartate transferase enzyme activity and lower albumin, and females had mildly higher alanine transferase and aspartate transferase enzyme activity.

These results are summarized from the report as follows:

Spinosad §82-1: Mouse

Observation	Dose (ppm) 0	150	450	1200*
Males				
alkaline phosphatase (IU/l)	83.8	92.4	114.4*	229.3
alanine transferase (IU/l)	16.0	19.I	35.6*	245
aspartate transferase (IU/l)	78.3	75.I	141.6*	518
globulin (g/dl)	2.540	2.478	2.460	2.883
albumin (g/dl)	3.100	2.944	2.810	2.233
blood urea nitrogen (mg/dl)	24.77	30.I4	27.37	19.80
bilirubin (mg/dl)	0.2390	0.2278	0.2140	0.1550
cholesterol (mg/dl)	129.1	119.6	153.6	96.0
triglycerides (mg/dl)	79.3	59.0	64.9	43.0
Females				
alkaline phosphatase (IU/l)	131.3	166.1	146.2	207
alanine transferase (IU/l)	27.1	27.7	66.8*	301
aspartate transferase (IU/l)	139.8	130.9	307.4*	572
globulin (g/dl)	2.220	2.320	2.322	3.100
albumin (g/dl)	3.130	3.210	3.100	2.160
glucose (mg/dl)	148.7	145.3	156.2	62.8
bilirubin (mg/dl)	0.2250	0.2180	0.2244	0.1460

* Statistically significantly different from controls, $p \leq 0.05$.
* Samples collected on test day 43 and no statistical comparisons with control values were made.

B. Necropsy:

1. Gross Observations: Treatment-related gross observations were described in the report as follows:

> Changes in lymphoid organs...were noted in animals of the 0.12% group as enlarged spleens.
>
> In the kidney,...changes were noted as pale kidneys in the 0.12% group.
>
> Hepatic lesions...(in most animals from the 0.12% group were) 1- to 3-cm diameter pale, caseous lesion(s) within the liver. This necrotic focus often resulted in peritoneal inflammation with adhesions to surrounding viscera; diagnosed as secondary peritonitis.
>
> Lymph node involvement,..., consisted of severe necrosis in mesenteric lymph nodes of animals from the 0.12% group, resulted grossly in slight to moderate enlargement...

2. Organ Weights: Results of organ weight observations were described in the report as follows:

> Animals in the 0.12% group were killed because of cachectic body condition before the test was concluded, and organ weights were not obtained.

Significant treatment-related organ weight increases affected the kidney, liver and spleen.

Selected organ weight results are summarized from the report as follows:

Observation	Dose (ppm) 0	50	150	450
	Males			
Terminal body weight (g)	37.5	37.1	36.7	34.6*
Liver weight (g)	1.4	1.4	1.5	1.8*
Liver/body weight (%)	0.4	0.4	0.4*	0.5*
Kidney (mg)	498.4	501.9	541.3	540.6
Kidney/body weight (%x10)	133.3	135.6	147.6	154.7
Spleen (mg)	80.8	64.5	77.0	94.3
Spleen/body weight (x100)	21.5	17.4	21.0	27.1
	Females			
Terminal body weight (g)	29.4	28.6	30.7	28.6
Liver (g)	1.2	1.2	1.3*	1.5*
Liver/body weight (g/10g)	0.4	0.4	0.4*	0.5*
Kidney (mg)	331.9	354.1	347.3	360.0
Kidney/body weight (mg/10g)	113.2	123.9	113.5	127.6*
Spleen (mg)	68.5	66.2	84.4	106.3*
Spleen/body weight (mg/10g)	23.3	23.1	27.2	37.1*

* Statistically significantly different from controls, $p \leq 0.05$.

3. Microscopic Observations: Treatment-related effects were described in the report as follows:

Changes in lymphoid organs...were of two types: lymphoid cell vacuolar changes and lymphoid organ histiocytosis. Lymphoid cell vacuolar change was characterized by lymphoid cells (lymphocytes or lymphoblasts) with large vacuoles within the cytoplasm, giving a moth-eaten appearance to the organs on scanning magnification. Lymphocytic necrosis was a feature of the vacuolation with pyknotic nuclei evident within some vacuoles...The degree of severity was increased in the 0.045% and 0.12% groups. Lymphoid organ histiocytosis was characterized by collections of histiocyte-macrophage-type cells with faintly vacuolar cytoplasm within the sinusoids of lymphoid organs...with the severity being increased in the 0.045% and 0.12% groups.

In the kidney, cortical tubules contained large cytoplasmic vacuoles which were up to 10 micrometers in diameter...(In some animals) the vacuoles were accompanied by clusters of cortical tubules undergoing regenerative changes, and tnese were diagnosed as vacuolar degeneration...(Some animals) had renal tubule vacuolation or vacuolar degeneration.

Hepatic lesions consisted of slight centrilobular cytomegaly, vacuolation in hepatocytes and Kupffer cells, and massive hepatic necrosis...Hepatocellular vacuolation was characterized by hepatocytes which contained fine cytoplasmic vacuoles. In marked and moderate cases, as seen in the 0.12% group, all viable hepatocytes were diffusely involved. In minimum and slight cases, the distribution was centrilobular...

Hepatic necrosis occurred only in the 0.12% group and was considered to be the proximate cause of death in the five dead animals...Histologically, the necrosis was characterized by foci of coagulation necrosis and fibrosis surrounded by thick bands of nuclear debris and necrotic inflammatory cells. The necrotic and inflammatory zones were surrounded by areas of degenerative, enlarged, finely vacuolated hepatocytes. In areas which were not necrotic, there were multifocal areas of acute inflammation, considered to be partly a reflection of septicemia but also a reaction to the necrotic process.

The incidence of these lesions are summarized from the report as follows:

	Dose (ppm)				
Observation	0	50	150	450	1200
	Males				
Spleen: No. examined	10	10	10	10	10
Lymphoid vacuolar change; minimal	0	0	3	2	0
slight	0	0	1	6	4
moderate	0	0	0	2	6
Hematopoiesis; moderate	0	0	0	1	9
Multifocal lymphocytic necrosis; slight	0	0	0	10	6
Lymph Node: No. examined	9	7	9	8	10
Lymphoid vacuolar change; minimal	0	0	1	0	4
slight	0	0	0	6	1
moderate	0	0	0	1	3
Histiocytosis; slight	0	0	1	2	5
moderate	0	0	0	5	4
Acute inflammation; slig	0	0	0	0	2
moderate	0	0	0	0	0
Necrosis; severe	0	0	0	0	10
Lymphocytic necrosis; slight	0	0	1	0	0
Multifocal lymphocytic necrosis; slight	0	0	0	5	3
moderate	0	0	0	2	0

Observation	Dose (ppm) 0	50	150	450	1200
	Males				
Kidney: No. examined	10	10	10	10	10
Cortical tubular regeneration/ minimal	2	1	3	0	0
slight	0	0	0	2	0
moderate	0	0	0	5	2
Multifocal cortical tubular regeneration; marked	0	0	0	1	3
Cortical tubular vacuolation; minimal	0	0	0	2	0
slight	0	0	0	6	0
moderate	0	0	1	1	7
Liver: No. examined	10	10	10	10	10
Centrilobular cytomegaly; slight	0	0	1	6	2
Multifocal necrosis; sever	0	0	0	0	10
Centrilobular hepatocellular vacuolation; minimal	0	0	0	0	3
slight	0	0	0	0	5
Diffuse hepatocellular vacuolation; marked	0	0	0	0	10

The incidence of selected lesions in the spleen, lymph nodes, kidneys. and liver of female mice are summarized from the report as follows:

Observation	Dose (ppm) 0	50	150	450	1200
	Females				
Spleen: No. examined	10	10	10	10	10
Lymphoid vacuolar change; minimal	0	0	0	2	0
slight	0	0	0	5	4
moderate	0	0	0	0	5
Hematopoiesis; moderate	0	0	0	0	8
Multifocal lymphocytic necrosis; slight	0	0	0	0	0
Lymph Node: No. examined	10	9	10	8	10
Lymphoid vacuolar change; slight	0	0	2	6	4
moderate	0	0	0	1	1
Hematopoiesis; slight	0	0	0	4	10
moderate	0	0	0	2	7
Multifocal lymphocytic necrosis; slight	0	0	2	7	4
moderate	0	0	0	0	1

Observation	Dose (ppm) 0	50	150	450	1200
	Females				
Kidney: No. examined	10	10	10	9	10
Cortical tubular regeneration;					
minimal	0	1	1	1	0
slight	0	0	0	1	0
Multifocal cortical tubular					
regeneration; slight	0	0	0	1	0
marked	0	0	0	0	1
Cortical tubular vacuolation;					
minimal	0	0	0	1	0
slight	0	0	0	1	3
moderate	0	0	0	0	6
Liver: No. examined	10	10	10	9	10
Multifocal necrosis; sever	0	0	0	0	8
Centrilobular hepatocellular					
vacuolation; minimal	0	0	0	3	0
slight	0	0	0	4	0
Diffuse hepatocellular					
vacuolation; marked	0	0	0	0	9
minimal	0	0	1	0	0
slight	0	0	0	3	0
moderate	0	0	0	0	1
Kupffer cell vacuolation;					
minimal	0	0	0	0	4

Additional microscopic observations were described in the report as follows:

Cardiac lesions were characterized by mild degrees of vacuolation of myocytes or intramural histiocytes and macrophages. This was not a prominent lesion but was noted in 13 of 20 animals in the 0.12% group.

Pulmonary lesions consisted of collections of intra-alveolar foamy macrophages. Alveolar macrophages occurred in 18 of 19 and 19 of 20 animals in the 0.045% and 0.12% groups, respectively. The degree of involvement was primarily mild. Only in one male from the 0.045% group was there a moderate macrophages occurrence...

Splenic involvement, other than the changes in lymphoid cell already discussed, consisted of extramedullary hematopoiesis. This occurred in 2 of 19 and 15 of 20 animals from the 0.045% and 0.12% groups, respectively.

Lymph node involvement, other than the histiocytosis and the changes in lymphoid cells already discussed, consisted of severe necrosis in mesenteric lymph nodes of animals from the 0.12% group, resulted grossly in slight to moderate enlargement...

The report also noted increased incidences of vacuolation in acinar cells of the pancreas, adrenocortical cells in the zona reticularis, the ovary, skeletal muscle cells in the tongue, and in mucosal cells in the female reproductive tract and in the epididymides. The incidence of these observations was similar to those summarized in the table below (see page 10 above).

Other lesions attributed by the investigators to treatment with XDE-105 were described in the report as follows:

> Seventeen of 19 and 14 of 20 animals in the 0.045% and 0.12% groups, respectively, had pancreatic effects. In these animals small, cytoplasmic vacuoles occurred in the acinar cells. The degree of vacuolation ranged from slight to moderate.
>
> Lesions in the tongue consisted of mild degrees of skeletal muscle myopathy and vacuolation. The myopathy was characterized by individual regenerative or degenerative muscle fibrils. Vacuolation occurred in myocytes and also histiocytes between muscle bundles. Slight chronic inflammation occurred in some of the affected tongues. Myopathy of the tongue occurred in 9 of 19 and 14 of 20 animals from the 0.045% and 0.12% groups, respectively.
>
> In the stomach a complex of lesions occurred which was diagnosed as glandular dilation. It was characterized by a multifocal distribution of marked glandular dilation scattered randomly throughout the depth of the mucosa. In addition, there were combinations of the following changes; inflammation in the lower mucosa, hyaline droplet formation in scattered dilated and undilated glands, mineralized debris and sloughed cells within the dilated glands, and necrosis of superficial epithelium usually in areas of hyaline droplet formation. Also noted in the mucosa were histiocytes with foamy cytoplasm. Seven of 20, 19 of 19, and 20 of 20 animals from the 0.015%, 0.045% and 0.12% groups had gastric glandular dilation, respectively. Histiocytic cells with foamy cytoplasm occurred in the gastric mucosa in 6 of 19 and 13 of 20 animals from the 0.045% and 0.12% groups, respectively.
>
> Treatment-related vacuolation occurred in the ovary. Mild vacuolation is normal in cells within aretic follicles and regressing corpora lutea. In treated animals the vacuolation was very prominent and also involved cells between the follicles and corpora lutea in the interstitial tissue of the ovary. The interstitial vacuolation appeared to involve thecal cells and interstitial histiocytes. Minimal and slight degrees of vacuolation within the ovary were observed in 7 of 10, 10 of 10, and 9 of 10 females in the 0.0%, 0.005%, and 0.015% groups, respectively. These mild changes were considered to be physiological and unrelated to treatment. Moderate or marked degrees of vacuolation in the ovary were considered to be treatment-related and were observed in 9 of 9 and 9 of 10 females in the 0.045% and 0.12% groups, respectively.

The entire female genital tract; oviduct, uterus, cervix and vagina, had treatment-related changes characterized as mucosal vacuolation and submucosal histiocytosis. Mucosal cells in the oviduct were swollen with fine, cytoplasmic vacuolation in 7 of 9 and 8 of 10 females in the 0.045% and 0.12% groups, respectively. In the uterus, cytoplasmic vacuolation affected the epithelium of the lumen and submucosal glands in 9 of 9 and 8 of 10 females in the 0.045% and 0.12% groups, respectively. Epithelial vacuolation of the cervix was observed in 4 of 9 and 5 of 10, and of the vagina in 3 of 9 and 7 of 10 females from the 0.045% and 0.12% groups, respectively. Submucosal histiocytes with finely vacuolated cytoplasm were noted in the uterus of 5 of 9 and 7 of 10 females in the 0.045% and 0.12% groups, respectively, and also in the cervix of one female in the 0.12% group.

A prominent change occurred in the epididymis in 1 of 10 and 10 of 10 males in the 0.045% and 0.12% groups, respectively. Vacuolation in the mucosal cells of the epididymis was characterized by large and frequent vacuoles in the tubular epithelial cytoplasm. The vacuoles were up to 5 to 10 micrometers in diameter.

Slight myopathy of the quadriceps muscle occurred in 7 of 19 and 11 of 20 animals from the 0.045% and 0.12% groups, respectively. The myopathy was characterized by multifocal fibrils undergoing degeneration or regeneration.

Mild necrosis occurred in the bone marrow of 1 of 20 and 11 of 19 animals from the 0.015% at (sic) the 0.045% groups, respectively. The necrosis was characterized by scattered pyknotic nuclei in the marrow of femoral and sternal sections.

Slight cytoplasmic vacuolation of adrenocortical cells in the zona reticularis was observed in 2 of 10 and 8 of 10 males in the 0.045% and 0.12% groups, respectively. Slight cytoplasmic vacuolation of cells in he pituitary gland was noted in two males and two females from the 0.12% group.

Discussion

A. Authors' Conclusions: The authors' discussion and conclusions were reported as follows:

Results from this subchronic toxicity study indicated that a dietary level of 0.12% XDE-105 was not tolerated by the test animals. The most important effect of XDE-105 on CD-1 mice was in many tissues (histiocytic [macrophage-type] and epithelial cells of organs such as the liver, kidney, lung, and cardiac and skeletal muscle) at levels ≥0.015%. The presence of vacuolation correlated with increases in the weight of the liver, spleen and kidney. Clinical chemistry parameters were also affected. Secondarily, there were decreases in growth.

Lesions in this study with no apparent direct relation to the vacuolation were splenic hematopoiesis, skeletal muscle myopathy, hepatocellular cytomegaly, gastric glandular

dilation, bone marrow necrosis, and massive necrosis in liver, lymph node and lung...

Administration of 0.12% XDE-105 in the diet caused changes in clinical pathology parameter values consistent with hepatobiliary disturbance, iron deficient anemia, inflammation (i.e., marked neutrophilic and lymphocytic leukocytosis), and loss of or decreased production of albumin.

...Based on the above information, the no-adverse-effect level for XDE-105 was determined to be 0.005%.

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

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Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 5/27/95
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 5/30/95
Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Feeding - dog (82-1b)

SHAUGHNESSY NO./TOX. CHEM. NO.: 110003 / New Chemical

ACCESSION NO./MRID NO.: 434441-02

DP BARCODE/SUBMISSION NO.: D209722 / S477588

TEST MATERIAL: XDE-105

SYNONYMS: Spinosad (proposed common name for Factor A + Factor D)

LABORATORY PROJECT ID NUMBER: IET 91-0079

SPONSOR: DowElanco Division, Dow Chemical Japan Ltd., Seavans North, Tokyo, Japan

TESTING FACILITY: The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan

TITLE OF REPORT: XDE-105: 13-Week Oral Subchronic Toxicity Study in Dogs

AUTHOR(S): Takanori Harada

REPORT ISSUED: September 1, 1994

CONCLUSION: XDE-105 (Spinosad, 88.0% pure) was tested in a 13-week oral feeding study in male and female Beagle dogs. The chemical was mixed in the diet at the following dose levels: 0, 150, 300, 1350/900 ppm (males) or 900 ppm (females). These levels corresponded to 0, 4.89, 9.73 or 33.4 mg/kg/day for the low, mid- and high dose males and 0, 5.38, 10.47 or 29.9 mg/kg/day for the low, mid- and high dose females, respectively. The concentration of 1350 ppm was reduced from 1350 ppm to 900 ppm in males on day 38.

At 300 ppm and above, cytoplasmic vacuolation or vacuolated cell aggregation was observed in a variety of tissues in both sexes as well as atrophic gastric mucosa. At 1350/900 ppm, arteritis was observed in a variety of tissues in both sexes. In addition, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed. Clinical signs included periocular sebum, decreased spontaneous motor activity, unsteady standing posture and watery, red/black stools and/or loose

stools. One male was killed in extremis. Decreases in mean body weights (19% for males, 12% for females) and food consumption were observed in both sexes, particularly males. Evidence of anemia was found in the hematological examinations (decreases in hematocrit, hemoglobin and erythrocytes) as well as decreases in white blood cell counts, lymphocytes and reticulocytes. Decreases in albumin and A/G ratio and increases in globulin, total cholesterol, GOT, ALP and GPT were also observed. The increases in the latter three were slight, in only one sex and in one case due to only one dog. There were increases in several organ weights, although most in only one sex and/or in only absolute or relative weights. Increases in spleen and liver weights were supportive of the microscopic and/or clinical chemistry results. **The NOEL is 150 ppm (4.89 (♂) or 5.38 (♀) mg/kg/day) and the LEL is 300 ppm (9.73 (♂) or 10.47 (♀) mg/kg/day based on microscopic changes in a variety of tissues, clinical signs of toxicity, decreases in mean body weights and food consumption and biochemical evidence of anemia and possible liver damage.**

Classification: Core Guideline

Testing Guideline Satisfied: 82-1(b)

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: XDE-105 (chemical name not available - huge molecule)

Description: Off-white to pale yellow powder
Lot #: AGR293707
Purity: 88.0%
Source: Not stated, assumed Sponsor
Vehicle: Test diet
Positive Control: N/A

2. Test Animals

Species and Strain (sexes): Male and female Beagle dogs
Age: 5-6 months at receipt; 6-7 months at initiation
Weight(s): 5.4 - 7.2 kg at receipt
Source(s): Ohito Biotech Center Inc. (Shuzenzi-cho, Tagata-gun, Shizuoka)

3. Procedure

a. Dietary Preparation: A specified amount of the test material was mixed with part of the basal diet in a mortar as a "pre-mixture". The pre-mixture was then blended with the remaining part of the basal diet with a mixer.

Frequency of preparation: Once prior to initiation of treatment and every 4 weeks thereafter.

Storage conditions: The prepared diets were placed in plastic bags, sealed, placed in plastic containers and stored in a dark and cold (about 4 °C) room until used. The prepared diet was then transferred into an aluminum container and retained in the animal room at room temperature until provided to the animals (up to 10 days).

Stability Analyses: Stability of the test substance in the diet was conducted on a sample dietary level of 200 ppm. The samples had been stored in a dark and cold room for 5 weeks with a subsequent 10-day storage at room temperature. Stability studies were also conducted on the moistened diet containing the test substance that had been stored at room temperature for 24 hours.

Homogeneity Analyses: Homogeneity of the test substance in the test diets was determined on samples taken from the top, middle and bottom of the mixer. These samples were taken from all dose groups on one date and for the high dose group on a second date.

Concentration Analyses: Concentration analyses for all dose groups were conducted on the same dates as the homogeneity analyses plus two additional dates. These analyses were conducted on the samples taken for the homogeneity analyses plus additional samples taken from the middle of the mixer. On the dates when no homogeneity analyses were conducted, the samples for the concentration analyses were taken from the middle of the mixer.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of a 4-week range finding study in which dogs were fed XDE-105 at one of the following dose levels: 0, 200, 2000 or 4000 ppm (one dog/sex/dose). In that study, the dogs fed 4000 ppm were sacrificed *in extremis* due to deteriorated physical condition. At 2000 ppm, the following effects were observed in both dogs: "body weight loss, lower food consumption, anemia, various abnormalities in blood biochemical parameters and pathological changes in various organs". No effects were observed at 200 ppm.

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c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	mg/kg/day	Main Study 3 months male	female
Contr.	0	0	4	4
1	150	5	4	4
2	300	10	4	4
3	1350/900[a]	45/30	4	
4	900	30		4

[a]The dietary concentration for the high dose males was reduced from 1350 to 900 ppm on day 38 because one male dog died from weakness caused by the test substance.

d. Clinical Observations and Mortality: All animals were observed once daily for morbidity, mortality and clinical signs of toxicity. A careful physical examination was conducted once/week.

e. Body Weight Determinations: Body weights were recorded at initiation of treatment, weekly during the treatment period and at termination.

f. Food and/or Water Consumption: Food consumption was measured daily. Chemical intake was calculated from the body weight and food consumption data and the nominal dose level.

g. Ophthalmological Examinations: These examinations were conducted prior to initiation and after 13 weeks of treatment. The following items were examined: eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body and fundus.

h. Clinical Pathology: (*) recommended by Guidelines

1) Hematology:

Collection times for blood (including # of animals): Hematological examinations were conducted on all surviving animals prior to initiation and at weeks 7 and 13. Blood samples were withdrawn from the cephalic vein of each animal following overnight starvation.

The following CHECKED (X) parameters were examined:

X		X	
x	Hematocrit (HCT)*	x	Mean corpuscular HGB (MCH)
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB conc. (MCHC)
x	Leukocyte count (WBC)*	x	Mean corpuscular volume (MCV)
x	Erythrocyte count (RBC)*	x	Reticulocytes
x	Platelet count*		
	Total plasma protein (TP)		
x	Leukocyte differential count*		

2) Clinical Chemistry:

The following CHECKED (X) parameters were examined:

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorus*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
	Enzymes:	x	Total bilirubin*
x	Alkaline phosphatase	x	Total protein*
	Cholinesterase	x	Triglycerides
x	Creatinine phosphokinase*	x	A/G Ratio
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	Gamma-glutamyl transpeptidase (GGTP)		

3) Urinalysis:

Collection times for urine (including # of animals):
Urinalysis examinations were conducted prior to treatment and after 13 weeks of treatment. For each animal, urine was pooled for 24 hours.

The following CHECKED (X) parameters were examined:

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

i. Gross Necropsy:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to end of exposure period and were subjected to complete gross pathological examinations: One animal was found moribund at week 5. This animal was anesthetized with pentobarbital sodium and then euthanized by exsanguination from the carotid artery. The animal was subjected to a complete autopsy that included the examinations for the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents.

Animals (groups) sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals were euthanized as above and subjected to gross examinations.

j. Histopathology:

Animals (groups) which died or were sacrificed in moribund condition and/or were sacrificed as part of an interim group prior to the end of the exposure period and were subjected to microscopic examination: all animals.

Animals (groups) which were sacrificed at the end of the treatment/observation period and were subjected to microscopic examination: all animals.

CHECKED (X) tissues were preserved for histopathological examination and (XX) tissues were weighed upon removal from the animal. The (*) tissues were recommended by the Guidelines.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	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*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)*
x	Jejunum*	x	Thymus*		Glandular
x	Ileum*		Urogenital	xx	Adrenals*
x	Cecum*	xx	Kidneys*		Lacrimal gland
x	Colon*	x	Urinary bladder	x	Mammary gland*
x	Rectum*	xx	Testes*	xx	Parathyroids*
xx	Liver*	x	Epididymides	xx	Thyroids*
x	Gall bladder*	x	Prostate		Other
xx	Pancreas*		Seminal vesicle	x	Bone*
	Respiratory	xx	Ovaries	x	Skeletal muscle*
x	Trachea*	x	Uterus*	x	Skin
x	Lung*			x	All gross lesions and masses
				x	Faucial tonsils

k. Statistical Analyses: According to the report, "the following statistical methods were used to determine the significance of the results:

Statistical methods	Data for analysis
Multiple comparison test: Dunnett's or Scheffe's method	Body weight Urine volume Urine specific gravity Hematology Blood biochemistry Organ weights
Mann-Whitney's U test	Food consumption Urinalysis (except urine volume and specific gravity)
Fisher's exact probability test	Clinical signs Mortality Ophthalmology Pathology

B. RESULTS:

1. Dietary Preparation: The stability of the test substance in the diet at 200 ppm for the samples that had been stored in a dark and cold room for 5 weeks with a subsequent 10-day storage at room temperature was 93% of the nominal value. The stability of the test substance in the moistened diet that had been stored at room temperature for 24 hours was 98% of the nominal value. The tables supporting these values were not provided in the report. For homogeneity of the test substance in the diet, the coefficient of variation for each dose level was within 1.1%. Tables were provided in the report to support this value. The mean concentrations of the test chemical in the diet at the nominal levels of 150, 300, 900 or 1350 ppm were 144, 290, 879 or 1335 ppm, respectively. The values were within 96-99% of the target concentrations. These values were supported by tables in the report.

2. Clinical Observations and Mortality: In the 1350/900 ppm group, clinical signs included periocular sebum, decreased spontaneous motor activity, unsteady standing posture and watery, red stools in males and loose stools in females. The authors of the report believed that the abnormalities in motor activity in the males was due to severe inanition due to markedly lower food consumption. One high dose male was found moribund and was killed in extremis at week 5 because of "unfavorable prognosis". The dog had exhibited marked body weight loss, lower food consumption, decreased spontaneous motor activity and unsteady standing posture. The report stated that no clinical signs or deaths were observed in any of the other treated groups. However, it is noted that vomit: foamy fluid was observed in at least one animal in all treated male groups and in all female groups, including controls. The following table summarizes the observed clinical signs of interest.

Clinical Observations

Clinical sign	Dose (ppm)	0			150			300			1350/900		
	Treatment Weeks	1-4	5-8	9-13	1-4	5-8	9-13	1-4	5-8	9-13	1-4	5-8	9-13
		Males											
	No. of animals examined	4	4	4	4	4	4	4	4	4	4	4[a]	3
Vomit: Foamy fluid		0	0	0	1	1	1	2	1	2	2	2	1
Periocular region: sebum		0	0	0	0	0	0	0	0	0	1	1	2
Decreased spontaneous motor activity		0	0	0	0	0	0	0	0	0	1	2	0
Unsteady standing posture		0	0	0	0	0	0	0	0	0	1	1	0
Watery, black stools		0	0	0	0	0	0	0	0	0	0	1	0
		Females											
	No. of animals examined	4	4	4	4	4	4	4	4	4	4	4	4
Loose stool		1	0	0	0	0	0	0	0	0	4	1	0
Vomit: foamy fluid		2	1	1	2	1	0	2	0	0	2	0	0
Vaginal bloody discharge		0	0	1	0	0	2	0	0	1	0	0	1

[a]One animal died at week 5.
[b]Number of animals

3. Body Weight Determinations: A decrease in mean body weight was observed in the high dose male group, beginning at approximately week 7 at lasting throughout the rest of the study. By the end of the study, high dose males weighed approximately 19% less than the control group. One of the three high dose males was within the control range, but the other two weighed significantly less than the control dogs. In females, this decrease was not as evident; the mean body weight of high dose females was approximately 88% of the control group by study termination. Two of the 4 females were within the range of the control animals and 2 were somewhat less than the control animals. The following table summarizes mean body weights for both sexes.

Mean Body Weights (kg)				
Dose Level (ppm) Weeks	0	150	300	1350/900[a]
Males				
0	7.7 ± 0.7	7.7 ± 0.5	7.7 ± 0.6	7.8 ± 0.4
4	8.7 ± 0.8	8.8 ± 0.4	8.7 ± 0.6	8.3 ± 0.9
8	9.4 ± 0.7	9.6 ± 0.3	9.5 ± 0.5	8.3 ± 1.6[b]
13	10.1 ± 0.7	10.3 ± 0.2	10.2 ± 0.5	8.2 ± 2.4[b]
Females				
0	7.5 ± 1.1	7.4 ± 0.6	7.4 ± 0.7	7.4 ± 0.6
4	8.3 ± 1.2	8.1 ± 0.8	8.1 ± 0.8	8.2 ± 0.7
8	8.8 ± 1.4	8.4 ± 1.2	8.5 ± 1.0	8.1 ± 1.0
13	9.3 ± 1.7	8.7 ± 1.4	8.9 ± 1.0	8.2 ± 1.2

[a] 1350/900 in males only; 900 ppm in females throughout the entire study.
[b] Only three animals were available for measurement.

4. Food and/or Water Consumption: Food consumption appeared to be less than the control groups for both sexes at the high dose. The following table summarizes food consumption for both sexes.

Mean Food Consumption (g/dog/day)[a]				
Dose Level (ppm) Weeks	0	150	300	1350/900[b]
Males				
1	300	300	282	300
4	300	300	300	235
8	300	300	300	261[c]
13	300	300	300	229[c]
Females				
1	300	300	288	300
4	300	300	293	294
8	300	300	293	249
13	298	300	300	252

[a] Calculated from the following formula: [feeding amount (300 g diet + 300g water) - food residue] / 2

[b] 1350/900 in males only; 900 ppm in females throughout the entire study.

[c] Only three animals were available for measurement.

The mean chemical intake values were calculated to be 0, 4.89, 9.73 or 33.4 mg/kg/day for the low, mid- and high dose males and 0, 5.38, 10.47 or 29.9 mg/kg/day for the low, mid- and high dose females, respectively.

5. Ophthalmological Examinations: No treatment-related effects were observed.

6. Hematology: In the high dose group of both sexes, significant decreases in hematocrit and hemoglobin were observed. In males, these were accompanied by decreases in red blood cells, lymphocytes and reticulocytes. White blood cell counts and platelets were decreased also, although not statistically significantly so. In females, there was a significant decrease in mean corpuscular hemoglobin. Reticulocytes were increased and erythrocytes, white blood cells, lymphocytes and platelets were decreased, although not statistically significantly so. The above observations indicate possible anemia. The following table summarizes selected results from male and female dogs at study initiation and after 13 weeks.

Selected Group Mean Hematology Data at 0 and 13 Weeks									
Dose (ppm)	# Animals	Ht (%)	Hb (g/dl)	RBC (10^6/mm^3)	WBC	Lymph	PLT (10^3/mm^3)	Retics. (/10^3RBC)	MCH (pg)
Males									
Week 0									
0	4	38.3	13.2	5.89	10.8	4.5	292	-	22.4
150	4	36.4	12.6	5.53	10.9	5.5	330	-	22.7
300	4	38.0	13.1	5.89	11.7	4.3	349	-	22.3
1350/ 900	4	34.8	12.1	5.32	9.6	3.7	316	-	22.8
Week 13									
0	4	43.0	14.7	6.49	10.9	4.5	307	13 ± 5	22.7
150	4	41.6	14.2	6.26	11.4	4.0	302	7 ± 3	22.7
300	4	43.0	14.7	6.63	9.2	3.7	300	8 ± 5	22.2
1350/ 900	3	31.4**	10.6**	5.12**	8.4	2.0*	279	2 ± 2*	20.8
Females									
Week 0									
0	4	40.5	14.1	6.26	10.4	4.9	373	-	22.5
150	4	42.0	14.3	6.36	9.8	3.5	335	-	22.5
300	4	41.7	14.2	6.28	9.5	3.8	343	-	22.7
900	4	43.7	15.0	6.65	8.9	3.4	322	-	22.5
Week 13									
0	4	45.2	15.5	6.81	11.8	4.1	383	8 ± 3	22.8
150	4	47.6	16.1	7.13	10.6	3.5	332	12 ± 4	22.7
300	4	46.1	15.8	6.84	7.8	3.1	292	10 ± 5	23.0
900	4	40.2*	13.4*	6.20	7.3	2.6	282	20 ± 14	21.6**

*Statistically significant ($p < 0.05$); **Statistically significant ($p < 0.01$)

7. Clinical Chemistry: In the high dose groups, statistically significant decreases in albumin and A/G ratio were observed in both sexes. Significant increases in globulin (♂), total cholesterol (♂), TG (♂) and GOT (♀) were also observed. In addition, non-statistically significant increases in alkaline phosphatase were observed in both sexes. This increase in males was probably not biologically significant. In females, the ALP value in 1/4 dogs was close to the control range and elevated in 3/4 dogs. GPT was also elevated in high dose females, although all of this elevation was due to one dog. This dog had normal values at initiation of treatment. The following table summarizes selected clinical chemistry values for male and female dogs at initiation of treatment and at study termination.

Selected Group Clinical Chemistry Data at 0 and 13 Weeks									
Dose (ppm)	# Animals Examined	Alb (g/dl)	Glob (g/dl)	A/G ratio	T. Chol (mg/dl)	TG (mg/dl)	ALP (U/l)	GOT (U/l)	GPT (U/l)
Males									
0 Weeks									
0	4	3.94	2.51	1.19	133	38	102	31	41
150	4	3.05	2.51	1.22	127	39	128	32	36
300	4	2.98	2.78	1.09	136	34	90	29	29
900/1350	4	2.94	2.29	1.29	112	31	97	29	39
13 Weeks									
0	4	3.21	2.55	1.26	128	39	69	35	47
150	4	3.18	2.62	1.22	129	38	75	37	45
300	4	3.30	2.84	1.19	147	37	65	33	46
900/1350	4	2.49**	3.65**	0.68**	162*	52*	93	67	70
Females									
0 Weeks									
0	4	3.05	2.27	1.35	116	32	111	33	38
150	4	2.98	2.40	1.25	114	35	95	32	35
300	4	3.14	2.45	1.30	135	44	113	33	37
900	4	3.17	2.29	1.39	130	40	112	30	49
13 Weeks									
0	4	3.35	2.56	1.32	124	46	61	34	44
150	4	3.28	2.76	1.20	136	44	62	32	36
300	4	3.30	2.63	1.29	156	45	78	37	39
900	4	2.60**	3.19	0.83**	145	59	161 ± 143	172± 227*	451 ± 789

*Significantly different from control group ($p<0.05$); **Significantly different from control group ($p<0.01$)

8. Urinalysis: In high dose females, there appeared to be a slight decrease in urinary pH at study termination. No other abnormalities were observed. The following table summarizes the results.

pH Values at 0 and 13 Weeks									
			pH						
Week	Dose (ppm)	# Animals Examined	5.0	6.0	6.5	7.0	7.5	8.0	8.5
Males									
0	0	4				1[a]	1	1	1
	150	4			1	1			2
	300	4			1				3
	1350/900	4					1		3
13	0	4						1	3
	150	4							4
	300	4							4
	1350/900	3			1		1		1
Females									
0	0	4						1	3
	150	4						2	2
	300	4					1	1	2
	900	4						1	3
13	0	4							4
	150	4						1	3
	300	4				1	1		2
	900	4			1	1	1	1*	

[a]Number of animals
*Significantly different from control at 5% level of probability.

9. Gross Pathology: Most macroscopic lesions were observed in high dose animals and at no other dose level, including controls in either sex.

Males:

The following were seen at the high dose level in one animal each: emaciation, atrophy of the thymus, enlargement of the lymph nodes, yellow and brown spots on the lungs, black sandy contents of the gall bladder, edema and brownish color in the pancreas, enlargement of the kidneys (considered to be congenital), red spot on the thyroid, sebum in the eye and hematoma-like mass in the thoracic cavity.

The following were seen at the high dose level in two animals each: whitish granular mucosa of the stomach, distended stomach with diet and pale liver and kidneys.

Females:

The following were observed at the high dose level in one animal each: enlargement of the spleen, distension of the stomach with diet, mud-like contents in the large intestine, pale and/or enlarged liver and black sandy contents in the gallbladder.

Whitish granular mucosa/whitish mucosa of the stomach was observed in 3 high dose animals.

10. Organ Weights: Statistically significant increases in absolute and relative pancreatic weights were observed in high dose males. Increases were also observed in high dose females, although not statistically significantly so. Absolute and relative spleen weights were increased in both sexes at the high dose. These increases were not statistically significant. Liver weights were also slightly increased in both sexes, although only relative liver weights in high dose females were statistically significant. Thyroid weights were increased in high dose males; relative thyroid weights were statistically significantly increased. The following table summarizes selected results.

Selected Mean Absolute Organ Weights After 13 Weeks of Treatment							
Dose (ppm)	# Dogs	Thyroid (mg)	Heart (g)	Pancr. (g)	Liver (g)	Kidneys (g)	Spleen (g)
Males							
0	4	701	70.4	13.8	255	40.0	19.2
150	4	860	72.5	20.1[a]	253	41.4	19.1
300	4	875	80.1	17.7	269	43.7	21.0
1350/ 900	3	1045	72.3	20.2[a]	327	46.3	27.4
Females							
0	4	707	68.3	15.5	236	35.6	19.2
150	4	1020	68.5	19.4	224	36.0	20.3
300	4	798	66.4	17.3	236	36.6	20.6
900	4	844	65.4	21.5	281	39.8	30.3

Selected Mean Relative Organ Weights After 13 Weeks of Treatment							
Dose (ppm)	# Dogs	Thyroid	Heart	Pancr.	Liver	Kidneys	Spleen
Males							
0	4	0.0070	0.70	0.14	2.53	0.40	0.19
150	4	0.0084	0.71	0.20	2.47	0.40	0.19
300	4	0.0086	0.79	0.18	2.64	0.43	0.21
1350/ 900	3	0.0127[b]	0.90[b]	0.25[a]	4.05	0.58	0.35
Females							
0	4	0.0077	0.74	0.17	2.56	0.[illegible]	0.22
150	4	0.0118[b]	0.80	0.22	2.59	0.42	0.24
300	4	0.0091	0.75	0.20	2.68	0.41	0.24
900	4	0.0104[a]	0.80	0.27	3.49[a]	0.49	0.38

[a]Significant ($p<0.05$); [b]Significant ($p<0.01$)

11. Histopathology: The following table summarizes selected microscopic lesions that were observed either at study termination or in the 1 dog that was sacrificed in extremis. In high dose animals, cytoplasmic or vacuolated cell aggregation was observed in both sexes in a variety of tissues. Arteritis was also observed in a variety of tissues. The report stated that "nervous tissues immediately adjacent to the affected blood vessels demonstrated vacuolation consistent with focal edema secondary to the vascular inflammation. There was no evidence of nervous degeneration/necrosis, reactive gliosis or neuronophagia in these lesions." In the high dose group, atrophic gastric mucosa, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed. An aneurysm in the mediastinal region of the thoracic cavity was found in the animal that died prior to termination. In the mid-dose group, cytoplasmic vacuolation or vacuolated cell aggregation was also observed in a variety of tissues. In addition, atrophic gastric mucosa was found in 2 females.

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900[a]
Males					
Pericarditis of heart		1/4	0/4	0/4	2/4
Atrophy of thymus		0/4	0/4	0.4	2/4
Vacuolated cell aggregation in white pulp of spleen		0/4	0/4	1/4	4/4[b]
Atrophic white pulp of spleen		0/4	0/4	0/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of cervical lymph nodes		0/4	0/4	0/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of mesenteric lymph nodes		0/4	0/4	1/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of faucial tonsil		0/4	0/4	2/4	4/4[b]
Foamy cell aggregation of the lung		0/4	0/4	0/4	4/4[b]
Arteritis of the lung		0/4	0/4	0/4	1/4
Atrophic mucosa of the stomach		0/4	0/4	0/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of the ileum		0/4	0/4	1/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of the cecum		0/4	0/4	0/4	3/4

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900[a]
Vacuolated cell aggregation in lymph follicles of the colon		0/4	0/4	2/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of the rectum		0/4	0/4	2/4	3/4
Vacuolated hepatocytes		0/4	0/4	0/4	3/4
Kupffer cell proliferation		0/4	0/4	0/4	3/4
Vacuolated acinar cells of the pancreas		0/4	0/4	2/4	4/4[b]
Testis: Spermatid giant cells Arteritis Vacuolated seminiferous epithelial cells Decreased spermatogenesis		 0/4 0/4 0/4 0/4	 0/4 0/4 0/4 0/4	 1/4 0/4 0/4 0/4	 2/4 2/4 3/4 1/4
Arteritis of the epididymis		0/4	0/4	0/4	2/4
Vacuolated glandular cells of the parathyroid		0/4	0/4	0/4	4/4[b]
Brain (cerebrum) - arteritis in the meninx		0/4	0/4	0/4	1/4
Vacuolated nerve cells in the brain		0/4	0/4	0/4	3/4
Vacuolated nerve cells in the cervical spinal cord		0/4	0/4	0/4	4/4[b]
Spinal cord (thoracic) Vacuolated nerve cells Arteritis in nerve root/meninx		 0/4 0/4	 0/4 0/4	 0/4 0/4	 2/4 2/4

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900[a]
Vacuolated nerve cells in the lumbar spinal cord		0/4	0/4	0/4	3/4
Arteritis in optic nerve		0/4	0/4	0/4	2/4
Thoracic cavity: Arteritis/ruptured aneurysm Pleuritis		- -	- -	- -	2 1

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900[a]
Females					
Focal necrosis/cellular depletion of sternal bone marrow		0/4	0/4	0/4	2/4
Focal necrosis/cellular depletion of femoral bone marrow		0/4	0/4	0/4	3/4
Vacuolated cell aggregation in white pulp of spleen		0/4	0/4	1/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of cervical lymph nodes		0/4	0/4	2/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of mesenteric lymph nodes		0/4	0/4	2/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of faucial tonsils		0/4	0/4	3/4	4/4[b]
Foamy cell aggregation of lungs		0/4	0/4	1/4	4/4[b]
Atrophic mucosa of stomach		0/4	0/4	2/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of ileum		0/4	0/4	3/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of cecum		0/4	0/4	2/4	4/4[b]
Vacuolated cell aggregation in lymph follicles of colon		0/4	0/4	1/4	4/4[b]

Incidence of Selected Microscopic Lesions					
Site & Lesion	Dose (ppm)	0	150	300	1350/900[a]
Vacuolated cell aggregation in lymph follicles of rectum		0/4	0/4	0/4	4/4[b]
Liver: Vacuolated hepatocytes Kupffer cell proliferation		 0/4 0/4	 0/4 0/4	 0/4 0/4	 1/4 3/4
Vacuolated acinar cells of pancreas		0/4	0/4	0/4	3/4
Vacuolated C-cells of thyroid		0/4	0/4	0/4	3/4
Vacuolated glandular cells of parathyroid		0/4	0/4	0/4	4/4[b]
Vacuolated cortical cells of adrenal		1/4	1/4	1/4	2/4
Vacuolated nerve cells of cerebellum		0/4	0/4	0/4	1/4
Vacuolated nerve cells of pons		0/4	0/4	0/4	1/4
Vacuolated nerve cells of thoracic spinal cord		0/4	0/4	0/4	2/4
Vacuolated nerve cells of lumbar spinal cord		0/4	0/4	0/4	3/4
Thoracic cavity: arteritis		-	-	-	1

[a]1350/900 ppm in males dogs; only 900 ppm in female dogs.
[b]Significant at $p < 0.05$.
() = Number of tissues examined.

12. Quality Assurance Measures: Signed Good Laboratory Practice and Quality Assurance Statements were provided.

C. DISCUSSION: This was a well conducted study. The study is classified as Core Guideline and is acceptable for regulatory purposes. The NOEL is the lowest dose tested (150 ppm or 4.89 mg/kg/day for males and 5.38 mg/kg/day for females). The LEL is the mid-dose (300 ppm or 9.73 mg/kg/day for males and 10.47 mg/kg/day for females). Clinical signs of toxicity were observed in the high dose group. These included some neurological signs (decreased spontaneous motor activity and unsteady standing posture) which the authors stated were due to severe inanition. Decrease in body weights were especially observed in high dose males along with decreased food consumption in both sexes at the high dose. The hematological examinations indicated evidence of anemia and inanition. The clinical chemistry examinations indicated some evidence of liver injury, although slight. The microscopic examinations indicated chemically-related effects in a variety of tissues. Generally, these effects involved cytoplasmic or vacuolated cell aggregation and arteritis in a variety of tissues. In addition, atrophic gastric mucosa, Kupffer cell proliferation in the liver, atrophic white pulp in the spleen, focal necrosis/cellular depletion in the bone marrow and thymic atrophy were also observed.

Primary Review by: Roger Gardner 6/12/95 01159
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: 21-Day DermalStudy
Guideline §82-2
Species: Rabbit

EPA Identification No.s: EPA MRID No. 435575-03
EPA Pesticide Chemical Code: 110003
Submission No. S477588
Data Package No. D209722

Test Material: XDE-105

Synonyms: Spinosad (proposed common name for Factors A & D)

Sponsor: DowElanco

Study Number(s): DR-0323-1194-018

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

Title of Report: XDE-105: Probe and 21-Day Repeated Dose Dermal Toxicity Study in New Zealand White Rabbits

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

Report Issued: August 26, 1994

Executive Summary: Groups of 5 male and 5 female New Zealand White strain rabbits were given 15 dermal applications of XDE-105 at 0, 100, 500, or 1000 mg/kg/day for 21 days. Test animals were observed daily for clinical signs and mortality, and they were weighed weekly. Prior to sacrifice at 21 days, blood samples were collected for hematological, and biochemical observations. After sacrifice animals were subjected to macroscopic and limited microscopic examination, and selected organs were weighed.

Under the conditions of the test, dermal application of XDE -105 at doses up to 1000 mg/kg/day (a limit dose), there was no evidence of treatment-related toxicity. Therefore, **the NOEL for dermal and systemic toxicity in this study was 1000 mg/kg/day.**

Core Classification: This study satisfies §82-2 guideline requirements for a 21-day dermal toxicity study, and the study should be classified as Core Guideline (MRID 435575-03).

Materials and Methods

A. Test Animals: Male and female New Zealand White rabbits were used. They were acclimated for a period of 14 days. The animals weighed from 2.3 to 2.6 kg and were obtained from Hazleton Research Products, Inc., Kalamazoo, Michigan.

On the day before the study began, the trunks of animals selected for the study were clipped free of hair.

B. Test Substance: XDE-105 (88.0% a.i.; 76.1% Factor A, 11.9% Factor B) was supplied as a solid (Reference no. AGR293707 /ACD 13651).

C. Vehicle: Distilled water.

D. Dosage Preparation: Preparations for dosing were described in the report as follows:

> The neat test material was moistened with 0.6 ml distilled water per gram of test material and applied to the shaved backs of rabbits under a gauze patch. Probe animals were acclimated to an elastic jacket for six hours one day prior to the initiation of the probe study. Repeated-dose animals were acclimated to jackets for six hours a day for four days prior to the first application. Moistened test material was applied to a 10x15 cm clipped area (approximately 10% of the body surface area) on the back of each rabbit and covered with a porous gauze patch. The patch was backed by nonabsorbant cotton and was held in place with an elastic jacket. Jackets and patches were removed approximately six hours after application. The treated area was wiped with a water-dampened disposable towel to remove residual test material.

E. Study Design: The probe study was described in the report as follows:

> Four male...rabbits (2/dose) received a water-moistened topical application of 500 or 1000 mg XDE-150/kg/day, six hours/day, for four consecutive days...
>
> An ophthalmic examination was conducted on all animals prior to the start of the study. Animals were weighed prior to the first application and at termination of the study...Inlife observations for signs of toxicity were conducted on each day of the study...The dermal application site was subjectively evaluated on a daily basis for signs of dermal irritation...

The experimental design of the 21-day study was described in the report as follows:

> Groups of five male and five female...rabbits per dose level received water-moistened topical applications of 0 (water vehicle), 100, 500 or

1000 mg XDE-105/kg/day for six hours/day...All animals received a total of 15 applications during the 21-day interval...

F. Observations: The animals were observed daily for clinical signs, morbidity and mortality. Application sites were scored weekly for dermal irritation, and test animals were weighed weekly during the study. Food consumption was not determined during the study, but 4 oz. rations not consumed were noted in the report.

Ophthalmological examinations were conducted on all test animals prior to initiation of the study, at weekly intervals during the study and prior to termination.

A modified Draize scoring system was used to evaluate dermal application sites and was listed in the report as follows:

Erythema and Eschar	Grade
None	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to sever erythema	3
Severe erythema to slight eschar formation	4

Edema	Grade
None	0
Very slight (barely perceptible)	1
Well-defined (edges raised)	2
Moderate (raised approximately 1 millimeter)	3
Severe (raised more than 1 millimeter)	4

Scaling and Fissuring	Grade
None	0
Slight scaling	1
Moderate - severe scaling	2
Slight fissuring	3
Moderate - severe fissuring	4

Prior to termination of the study (one day for males and two days for females), blood samples were collected from the auricular artery. Hematological observations included the following:

Leukocyte count	Hemoglobin	Differential leukocyte counts
Erythrocyte count	Hematocrit	
	Platelets	

The following biochemistry parameters were also measured:

Sodium	Total bilirubin	Urea nitrogen
Potassium	Aspartase amino-transferase (AST)	Creatinine
Chloride	Alanine amino-transferase (ALT)	Total protein
Calcium		Albumin
Inorganic phosphorus		Globulin
Alkaline phosphatase		Glucose

After 21 days, the animals were sacrificed and subjected to necropsy. Macroscopic examinations of each animal were conducted and described in the report as follows:

> This necropsy included an *in situ* examination of the eyes by visual inspection of the cornea, lens and other internal components...Weights of the liver, kidneys and testes were recorded for each of the animals and the ratios of organ weights to terminal body weights were calculated for all animals...Special attention was given to the skin at the site of application of the test material. Samples of skin were obtained from 1) the clipped test material application site, 2) an unclipped site adjacent and caudal to the application site, and 3) a site on the ventral side of the animal distant from the application site.

Microscopic examinations were described in the report as follows:

> Histopathologic evaluation was undertaken on untreated and treated skin from all control and treated animals. In addition, histopathologic evaluation of the liver, kidneys and stomach was conducted on rabbits exposed to 0 (control) or 1000 mg/kg/day. Tissues from the 100 and 500 mg/kg/day were not examined because a treatment related effect was not present in the tissues examined from the rabbits exposed to 1000 mg/kg/day.

G. Statistical Analysis: The report describe statistical methods as follows:

> All parameters examined statistically were first tested for equality of variance using Bartlett's test. If the results from Bartlett's test were significant, then the data for the parameter were subjected to a transformation to obtain equality of the variances. The transformations examined were the common log, the inverse, and the square root, in that order with a Bartlett's test following each transformation. When Bartlett's test was satisfied no further transformations were applied, or if none of the transformations resulted in homogeneous variances the transformed data or the raw data with the lowest Bartlett's statistic was used. The selected form of the data was then subjected to the appropriate parametric analysis...
>
> Inlife body weights were evaluated using a three-way repeated-measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex and dose. In the three-way RM ANOVA, differences between the groups were primarily detected by the time-dose interaction.

Parameters analyzed by a three-way RM ANOVA involved several preliminary examinations. The first was an examination of the time-sex-dose interaction; if significant the analysis was repeated separately for each sex without examining the results of other factors. If the time-sex-dose interaction was not statistically identified then the sex-dose interaction was reviewed for significance. A significant finding was an indication that the parameter should be re-examined separately for each sex. After accounting for the influence of sex on the response to treatment, the time-dose interaction was examined. If the time-dose interaction was statistically identified, linear contrasts test the time-dose interaction for the comparisons of each dose group to he control group. A Bonferroni correction was used to compensate for the multiple comparisons with the control group. This was applied only when comparisons were made to the control group and was applied for the time-dose interaction.

Terminal body weight, organ weight (absolute and relative excluding the testes), hematologic parameters (excluding differential WBC), and clinical chemistry parameters were evaluaed using a two-way ANOVA with the factors of sex and dose; differences between the groups were primarily detected by the dose factor. For these parameters, the first examination was whether the sex-dose interaction was significant. If it was, a one-way ANOVA was done separately for each sex. Comparisons of individual dose groups to the control group were made with Dunnett's test only when statistically significant dose effect existed; tnis was subsequent to evaluation of the sex-dose interaction.The form of the ANOVA one-way or two-way, was determined by whether the analysis had been separated by sex or not.

Results for testes weights 9absolute and relative) were analyzed using a one-way ANOVA. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls suing Dunnett's test.

Reported Results

A. In-Life Observations:

1. Clinical Signs and Mortality: The report indicated that none of the test animal died during the study.

   The investigators also noted there were no effects of XDE-105 on the incidence of clinical signs, dermal irritation or ophthalmic changes in the 21-day study.

2. Body Weight and Food Consumption: There were no effects on body weight or food consumption according to the report. Food consumption was also unaffected by treatment with XDE-105.

3. Ophthalmology; There were no test substance related effects noted in test animals.

B. Clinical Pathology:

1. Hematology: Hematology results were described in the report as follows:

> ...(White blood cell count, red cell count and hematocrit) of the low and middle dose group were statistically different were statistically different from the controls. However, these differences were interpreted to be unrelated to treatment due to the lack of a dose-response relationship.

These results are summarized from the report as follows:

Observation	Dose (mg/kg/day) 0	100	500	1000
Males				
White cell count ($10^3/mm^3$)	8.81	6.92*	8.11	8.39
Red cell count ($10^6/mm^3$)	5.93	6.17	7.13*	6.19
Hematocrit (%)	35.6	37.0	41.0*	36.6
Females				
White cell count ($10^3/mm^3$)	8.02	6.73*	6.19	6.37
Red cell count ($10^6/mm^3$)	6.17	6.17	6.02	5.96
Hematocrit (%)	36.9	36.7	36.0	36.4

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

2. Clinical Chemistry: The report described these results as follows:

> Statistically identified differences in the urea nitrogen and chloride values between the low and/or middle-dose groups and the controls were interpreted to be unrelated to treatment due to the lack of a dose-response relationship at the high dose.

These results are summarized from the report as follows:

Observation	Dose (mg/kg/day) 0	100	500	1000
Males				
Urea nitrogen (mg/dl)	18	19	25*	18
Chloride (mmol/l)	111	112	110	109
Females				
Urea nitrogen (mg/dl)	18	20	18	19
Chloride (mmol/l)	113	114	115	115

* Statistically significantly different from controls, $p \leq 0.05$.

B. Necropsy:

1. Gross Observations: The only gross change noted by the investigators were considered to be spontaneously occurring and were not observed at incidences which were dose related.

2. Organ Weights: There were no effects on organ weight or organ to body weight ratios according to the report.

3. Microscopic Observations: The investigators noted no treatment related microscopic observations in the report. The incidence of any lesions were noted in one or two animals of each sex in both the control and 1000 mg/kg/day dose groups.

Discussion

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

> Repeated dermal application of XDE-105 resulted in no significant irritation at the dermal test site in rabbits administered 100, 500, or 1000 mg/kg body weight/day 5 days/week for 15 applications during a 21-day period. There was no evidence of systemic toxicity...Therefore, under the conditions of this study, the no-observed effect level for male and female New Zealand White rabbits was determined to be 1000 mg/kg/day, the accepted limit test level.

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

Primary Review by: Roger Gardner Roger Gardner 6/12/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

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 does not satisfy §82-7 guideline requirements for a subchronic mammalian neurotoxicity study and is classified as Core Supplementary (MRID 43557504). This study was an extension of a subchronic feeding study, and there were no positive control data from the testing laboratory presented in the report..

Spinosad (XDE-105) §82-7: Rat

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 Means (±
Body weight	4	100	1	400	53
Hindlimb grip	4	100	3	1200	43

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Spinosad (XDE-105) §82-7: Rat

	Test Periods	Number of Rats	Obs or Data Points/Rat	Total Obs or Data Points	Number Means (±
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 $SPF_{p \cdot q}$ 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 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 (α = 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 (α) per comparison was set at 0.02...The corrections for multiple statistical analyses were applied to α only, and probability values were reported without correction.

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

Spinosad (XDE-105) §82-7: Rat

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 .timulated 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:

Spinosad (XDE-105) §82-7: Rat

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.

Primary Review by: Roger Gardner Roger Gardner 6/12/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph.D. Karl P. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

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.

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).

These high dose group results may not be toxicologically significant because (1) the compared weight gains represent 1-2% of the body weights of the test animals, (2) the compensatory changes noted in food consumption occurred at the lowest dose tested as well as the 50 mg/kg/day dose level and they were not dose related, and (3) statistically significant differences were not consistently found for body weight or food consumption results. In addition,

there were no significant differences noted with respect to absolute and relative liver and kidney weights, and no animals were characterized in the report as thin in appearance. The incidence of animals with decreased fecal output could be interpreted coincidental. Although the incidence of aborted pregnancies was higher in the 50 mg/kg/day dose group than historical control values, the absence of similar observations at doses from 50 to 400 mg/kg/day in 28 animals from a range-finding study mentioned in the report dose not support the conclusion that XDE-105 causes abortions in rabbits. **The NOEL for maternal toxicity may be ≥50 mg/kg/day.**

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 does not satisfy §83-3 guideline requirements for a rabbit developmental toxicity study and should be classified as Core Supplementary. The highest dose tested (50 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.

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	α=0.01
Parametric ANOVA	α=0.10
Nonparametric ANOVA	α=0.10
Dunnett's Test	α0.05, two-sided
Wilcoxon Rank-Sum Test	α=0.05, two-sided with Bonferroni's correction
Fisher's Test	α=0.05, one-sided
Censored Wilcoxon Test	α=0.05, one-sided
Binomial Distribution Test	α=0.05, one-sided
Sequential Outliers Test	α=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:

	Dose level (mg/kg/day)			
Observation	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:

Spinosad 83-3: Rabbit

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

Spinosad 83-3: Rabbit

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 retroesophageal 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:

Spinosad 83-3: Rabbit

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 should be considered in determining the adequacy of the dose range tested in the main developmental study since the effects at the highest dose tested are not clearly associated with the administration of XDE-105.

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, but there was no

mention of similar findings in the description of the range-finding study at dose levels of 50, 100, 200, and 400 (a total of 28 pregnant animals). Again, the full report should be evaluated to verify this result since dose-related increases in the number of abortions would be expected to appear in a range-finding study where dose levels exceed the highest dose tested by as much as 8-fold.

011597

Primary Review by: Roger Gardner Roger Gardner 6/12/95
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
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 may be ≥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 satisfy §83-3 guideline requirements for a rat developmental toxicity study and should be classified as Core Supplementary. 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.

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	α=0.01
Parametric ANOVA	α=0.10
Nonparametric ANOVA	α=0.10
Dunnett's Test	α0.05, two-sided
Wilcoxon Rank-Sum Test	α=0.05, two-sided with Bonferroni's correction
Fisher's Test	α=0.05, one-sided
Censored Wilcoxon Test	α=0.05, one-sided
Binomial Distribution Test	α=0.05, one-sided
Sequential Outliers Test	α=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:

Observation	Dose level (mg/kg/day) 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:

	Dose level (mg/kg/day)			
Observation	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:

Spinosad 83-3: Rat

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
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.

Primary Review by: Roger Gardner Roger Gardner 6/12/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Reverse Mutation Assay (Ames Test)
Guideline 84-2
Species: *S. typhimurium* and *E. coli*

EPA Identification Nos.: EPA MRID No. 434145-22
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): 910820AMS3162, 910917AMS3162, and 910924AMS3162

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

Title of Report: The Effect of XDE-105 on the Induction of Reverse Mutations in *Salmonella typhimurium* and *Escherichia coli* Using the Ames Test

Author(s): Garriott, M.L., M.A. Rexroat, and D.W. Grothe

Report Issued: December 3, 1992

Executive Summary: The mutation rates observed after treatment of *Salmonella typhimurium* strains (TA1535, TA1537, TA98, and TA100) and one strain of *Escherichia. coli* (WP2/uvrA⁻) with XDE-105 increased in a dose-related manner when compared to the vehicle control. The colonies were shown in a replica plate assay to be predominately auxotrophs and not revertants. No growth of auxotrophs is expected in the Ames assay, but their presence in this assay suggests that XDE-105 supported their growth. The investigators noted that trace amounts of histidine and other amino acids were present in the test substance, which is a fermentation product. Therefore, an Ames assay with XDE-105 may not be appropriate, and this assay is considered to be unacceptable.

Core Classification: Unacceptable.

Materials and Methods

A. Test species: The bacteria used in the study were described in the report as follows:

> The *S. typhimurium* tester strains employed in this test were obtained from Dr. Bruce N. Ames of the University of California at Berkeley and the *E. coli* strain was obtained from Dr. M. H. L. Green of the MRC Cell Mutation Unit, Sussex, U.K. The test was conducted using four histidine auxotrophs of *S. typhimurium* LT-2 (TA1535, TA1537, TA98, and TA100) and one tryptophan auxotroph (WP2/uvrA) of *E. coli*.

The preparation of test cultures was described in the report as follows:

> Overnight cultures were prepared from frozen stocks by inoculating approximately 0.2 ml of the appropriate tester strain into 20 ml of 2.5% Oxoid Nutrient Broth #2...The cultures were incubated at approximately 37°C in a shaking water bath for approximately 16 hours.

B. Test substance: XDE-105 (88.0% a.i.) was supplied as a solid (Reference no. ACD13651).

C. Vehicle and positive control substances: The vehicle for the test substance and positive controls was reagent grade dimethyl sulfoxide (DMSO) which was used in a volume of 0.05 ml/plate in the study. N-ethyl-N'-amino-N-nitroso-guanidine (ENNG), 2-nitrofluorene (2-NF), 9-aminoacridine (9-AmAc), and 2-aminoanthracine (2-AA) were used as positive control substances.

D. Other test materials: The report described the preparation of the S9 mix as follows:

> ...The animals (200 to 220 g male Fischer 344 rats) were treated 5 days prior to sacrifice with a single 500-mg/kg intraperitoneal dose of Arochlor 1254. A sterile 25% homogenate was prepared in 0.15 M KCl at approximately 4°C and centrifuged for approximately 15 minutes at approximately 9000 x g. The supernatant fraction (S9) was stored at approximately -80°C...

In addition, the report noted:

> The S9 mixture was comprised of 10% S9 (v/v), 5 mM ß-nicotinamide adenine dinucleotide phosphate (NADP) and 4 mM glucose-6-phosphate, 33 mM KCl, 8 mM $MgCl_2$, and 0.25 M sodium phosphate buffer (pH 7.4)...

Preparation of the base medium was described in the report as follows:

> ...approximately 30-ml aliquots (of base agar) were dispensed into 100-mm petri dishes. The medium consisted of 1.5% Difco agar prepared in 1x Vogel-Bonner Salts and contained 2% glucose. The plates were inverted and maintained at room temperature for approximately 5 days after which they were inspected for contamination.

According to the report, the top agar medium was prepared as follows:

> This medium consisted of 0.54% Difco agar prepared in 0.45% NaCl and was maintained at approximately 45°C until used. Just prior to use, L-tryptophan, L-histidine and biotin were added to give a final concentration of 0.04 mM of each of these components.

The report further indicated the following:

> Prior to initiation of the mutation assays, 450 ml of top agar was prepared for both the nonactivated and activated assays. Each top agar was diluted by the addition of a 45-ml solution containing histidine, tryptophan and biotin (each 0.5 mM). Furthermore, 124 ml of "dilution salts" (50 mM KCl, 8 mM $MgCl_2$, and 0.5 M sodium phosphate buffer [pH 7.4]) were added to the top agar for the nonactivated assay.

E. Experimental design: The report described preliminary toxicity and precipitation tests as follows:

> Concentrations of 312.5, 625, 1250, 2500 and 5000 µg/plate of XDE-105 were tested with and without activation. The highest concentration of the test article in the mutagenicity assay was not expected to significantly decrease survival of TA100 or to result in appreciable precipitation formation in the top agar after incubation for approximately 48 hours.
>
> The toxicity test was conducted with and without metabolic activation exactly as described for the mutation assay except the top agar contained a 20-fold higher concentration of L-histidine (1 mM). In this test, an overnight culture of TA100 was diluted so that approximately 1000 to 1600 bacterial colonies would appear on control plates after incubation for approximately 48 hours. The presence of excess L-histidine in the top agar provided for the growth of both nonrevertant and revertant cells. Colonies on control and treated plates were counted after approximately 48 hours of incubation at approximately 37°C. Toxicity, evidenced by a decrease in colony number, was reported as a percentage of the control value.
>
> The precipitation test was conducted with and without metabolic activation as described for the toxicity assay, except that no

bacterial tester strain was included. The formation of a test article precipitate was detected by an automated colony counter.

Mutation assay procedures were described in the report as follows:

The nonactivated assays were conducted by combining 0.05 ml of the appropriate dilution of XDE-105, 0.1 ml of the appropriate bacterial tester strain, and 2.5 ml of the diluted top agar. For the activated assay, the appropriate dilution of the test article and tester strain was combined with 2 ml of the diluted top agar and 0.5 ml of the S9 mixture...Each tube was stirred and poured onto a plate containing the base agar medium. Each plate was swirled to distribute the top agar evenly and allowed to gel on a flat surface at room temperature. The plates were then inverted and incubated for approximately 48 hours at approximately 37°C.

Each control/test article concentration was tested in triplicate...The colony size discriminator (of the automated counter) was set at the lowest limit in order to maximize the identification of all colonies. Automated colony counting permitted approximately 86% of the plate to be enumerated. Therefore, the area counted and the total area of the plate were measured with the instrument and the counts corrected accordingly.

A replica plating procedure was used to confirm the genotypic characteristics of colonies that appeared on test plates so they could be identified as true revertants. These procedures were described in the report as follows:

Two 1-liter batches of agar were prepared and approximately 20-ml aliquots were dispensed into 100 x 15 mm petri dishes. One set of plates contained minimal agar supplemented with 0.04 mM biotin. The other set of plates contained minimal agar supplemented with 0.04 mM histidine, 0.04 mM tryptophan, and 0.04 mM biotin. Square plates were used in this procedure. The bottom of the plates were embossed with 36 quadrants that are referenced by numbers and letters. The embossed quadrants on these plates provided a convenient means for identifying the colonies inoculated onto the minimal agar plate and the corresponding amino acid supplemented plate.

Colonies were harvested from treated plates with a sterile wooden toothpick and were sequentially streaked onto the minimal plate and then onto the corresponding amino acid supplemented plate. If a colony grew on both plates, it was considered to be a true revertant from amino acid auxotrophy. If a colony did not grow on the minimal agar plate but did grow on the amino acid supplemented plate, then it was considered to be an auxotroph and not a revertant colony. Colonies were also selected from DMSO-treated and positive control treated plates in order to verify the selectivity of the system to genotypic differences.

The plates were scored by noting bacterial growth along the inoculated streak. For a given treatment, the results were presented as the number of colonies which showed growth on the biotin-supplemented plate versus the number of colonies which showed growth on the amino acid-supplemented plate.

F. interpretation of results: According to the report, a positive response was indicated when a concentration-related increase in the number of revertants was observed. The number of revertants should exceed the vehicle control value by at least two-fold for *S. typhimurium* strains TA98 and TA100 and *E. coli* strain WP2/uvrA⁻ and at least three-fold for *S. typhimurium* strains TA1535 and TA1537. These increases should also be noted at two successive concentrations.

Reported Results

A. Preliminary toxicity testing: There was no indication of excess toxicity in the tester strains when XDE-105 was evaluated at concentrations from 312.5 to 5000 µg/plate. Therefore, investigators selected this range of concentrations for the mutation assays. However, the investigators noted the following:

The presence of large and small "pinpoint colonies" was observed at all test concentrations and for all tester strains with and without metabolic activation. Pinpoint colonies have been described by Ames *et al.* (1975) and are believed to be auxotrophs that survive toxic effects of a compound and show limited growth in the small amounts of histidine and tryptophan present in the plates.

B. Mutation assay results: The mean values for triplicate counts from assays without metabolic activation were summarized in the report as follows:

		Mean Colony Counts (n=3)				
Treatment	µg/plate	TA1535	TA1537	TA98	TA100	WP2uvrA⁻
DMSO	0.05 ml	15	9	22	134	23
		14	8	28	119	21
XDE-105	312.5	124	31	92	222	140
	625	225	98	98	225	228
	1250	416	183	132	278	431
	2500	460	66	162	682	657
	5000	1050	70	377	563	912

Spinosad (XDE-105) §84-2: Ames Test

Treatment	µg/plate	Mean Colony Counts (n=3) TA1535	TA1537	TA98	TA100	WP2uvrA⁻
ENNC	5	125	-	-	578	820
	10	592	-	-	1217	1741
9-AmAc	50	-	286	-	-	-
	100	-	683	-	-	-
2-NF	0.5	-	-	175	-	-
	5	-	-	1359	-	-

The mean values for triplicate cou:ts from assays with metabolic acti ation were summarized in the eport as follows:

Treatment	µg/plate	Mean Colony Counts (n=3) TA1535	TA1537	TA98	TA100	WP2uvrA⁻
DMSO	0.05 ml	14	10	24	128	24
		11	8	31	120	21
XDE-105	312.5	232	148	141	336	97
	625	351	279	257	407	149
	1250	575	460	414	607	181
	2500	860	693	733	761	443
	5000	1238	1029	1027	1254	716
2AA	1.25	101	77	521	606	-
	2.5	280	212	1213	1316	-
	5	-	-	-	-	137
	10	-	-	-	-	316

Because these results suggested XDE-105 could induce reverse mutations in *S. typhimurium* and *E. coli* tester strains, a repeat assay was conducted to confirm the results from the first assay.

The mean values for triplicate counts from repeat assays without metabolic activation were summarized in the report as follows:

Treatment	µg/plate	Mean Colony Counts (n=3) TA1535	TA1537	TA98	TA100	WP2uvrA⁻
DMSO	0.05 ml	14	13	25	106	30
		11	9	17	115	32
XDE-105	312.5	127	53	65	178	188
	625	122	116	70	195	260
	1250	504	215	288	388	428
	2500	679	173	325	432	700
	5000	670	140	324	454	1149
ENNG	5	17	-	-	143	62
	10	22	-	-	190	109
9-AmAc	50	-	100	-	-	-
	100	-	1034	-	-	-

Spinosad (XDE-105) §84-2: Ames Test

Treatment	µg/plate	Mean Colony Counts (n=3) TA1535	TA1537	TA98	TA100	WP2uvrA⁻
2-NF	0.5	-	-	202	-	-
	5	-	-	1417	-	-

The mean values for triplicate counts from repeat assays with metabolic activation were summarized in the report as follows:

Treatment	µg/plate	Mean Colony Counts (n=3) TA1535	TA1537	TA98	TA100	WP2uvrA⁻
DMSO	0.05 ml	14	10	26	131	21
		11	9	32	127	29
XDE-105	312.5	163	232	211	354	139
	625	242	226	148	395	213
	1250	612	583	492	748	331
	2500	790	655	736	922	542
	5000	788	976	1085	1182	751
2AA	1.25	91	40	677	730	-
	2.5	160	97	1498	1439	-
	5	-	-	-	-	55
	10	-	-	-	-	480

C. Replica plating assay: The report's discussion of results from this assay is as follows:

> A representative number of small and large colonies were picked from the 1250 µg/plate test article concentration...This concentration was chosen to decrease the potential of picking spontaneous revertants...The results...show that for all strains, the majority of colonies observed were auxotrophs and not revertants.

Results of replica plating assays were summarized in the report as follows:

Test without metabolic activation

Tester Strain	Revertant colonies/Total colonies growing on his/tryp/bio plates 0 µg/plate	1250 µg/plate	Positive control
TA1535	6/6	2/24	6/6
TA1537	6/6	5/24	6/6
TA98	6/6	9/24	6/6
TA100	6/6	10/24	6/6
WP2uvrA⁻	6/6	4/24	6/6

Test with metabolic activation

Tester Strain	Revertant colonies/Total colonies growing on his/tryp/bio plates		
	0 µg/plate	1250 µg/plate	Positive control
TA1535	6/6	1/24	6/6
TA1537	5/6	1/24	6/6
TA98	5/6	3/24	6/6
TA100	6/6	10/24	6/6
WP2uvrA⁻	6/6	2/24	6/6

The report stated, "Based on these results, and the lack of test article toxicity, it is possible that the pinpoint colonies in this assay are spontaneous revertants occurring as a result of continued cell division by the auxotrophs in the presence of the amino acids contained in the test article."

Discussion

A. Authors' Conclusions: The authors' summarized the results as follows:

> ...tester strains utilized in the *Salmonella*/microsome test were sensitive to the appropriate positive controls. Although the number of colonies observed following treatment with XDE-105 increased in a concentration-related manner when compared to the vehicle control, the colonies were shown in a replica plate assay to be predominately auxotrophs and not revertants. Typically, no growth of auxotrophs is expected in the Ames assay. The fact that auxotrophs were present suggests that the test article was capable of supporting their growth. That this is probably occurring is substantiated by the discovery of trace amounts of histidine and other amino acids in the test article which is a fermentation product. Therefore, it was concluded that XDE-105 was not mutagenic in *S. typhimurium* or *E. coli* either with or without metabolic activation.

B. Reviewer's Discussion and Conclusions: There were repeated references to the presence of trace amounts of amino acids in the test article, but the report did not contain a report of test substance analyses to support those references. Since the presence of trace amounts of histidine and tryptophan are an important element in the conduct and interpretation of an Ames assay, a detailed analytical report on the test substance, specifically identifying the amino acids in its composition, is an essential part of the report that is missing.

011597

Primary Review by: Roger Gardner Roger Gardner 6/17/97
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl V. Baetcke 6/25/96
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Forward Mutation Assay
Guideline 84-2
Species: L5178Y Mouse Lymphoma Cells

EPA Identification Nos.: EPA MRID No. 434145-23
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): 910910MLA3162

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

Title of Report: The Effect of XDE-105 on the Induction of Forward Mutations at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells

Author(s): Garriott, M.L., K.C. Michaelis, and D.W. Grothe

Report Issued: July 17, 1992

Executive Summary: Spinosad did not induce forward mutations in mouse lymphoma L5178Y $Tk^{+/-}$ cells at concentrations of 0, 1, 5, 10, 15, 20 or 25 µg/ml without metabolic activation or at concentrations of 15 through 50 µg/ml with metabolic activation.

Core Classification: Acceptable.

Materials and Methods

A. Test species: The cells used in the study were described in the report as follows:

> The $Tk^{+/-}$ cell line (TK3.7.2C), a subline of the mouse lymphoma cell L5173Y heterozygous for thymidine kinase, was originally obtained from Dr. Donald Clive (Burroughs Wellcome, Research Triangle Park, NC).Suspension cultures were initiated from

stock cultures stored in liquid nitrogen and were maintained with growth medium in continuous logarithmic growth by periodic dilution of the cultures...Only viable cells were scored in this study with viability determined by trypan blue exclusion.

B. Test substance: XDE-105 (88.0% a.i.) was supplied as a solid (Reference no. ACD13651).

C. Vehicle and positive control substances: The vehicle for the test substance and positive controls was reagent grade dimethyl sulfoxide (DMSO) which was used in a concentration of 1% in the study. Ethylmethanesulfonate (EMS) and 3-methylcholanthrene (3MC) were used as positive control substances.

D. Other test materials: The report described the preparation of the S9 mix as follows:

...The animals (200 to 220 g male Fischer 344 rats) were treated 5 days prior to sacrifice with a single 500-mg/kg intraperitoneal dose of Arochlor 1254. A sterile 25% homogenate was prepared in 0.15 M KCl at approximately 4°C and centrifuged for approximately 15 minutes at approximately 9000 x g. The supernatant fraction (S9) was stored at approximately -80°C...

Preparation of the culture media was described in the report as follows:

Growth medium, designated $R_{10}P$, consisted of RPMI 1640 medium containing L-glutamine supplemented with 200 units/ml Penicilin, 200 µg/ml streptomycin, 100 µg/ml sodium pyruvate, 1 mg/ml Pluronic F68, and 10% horse serum. Medium containing no serum was designated R_0P. During exposure to the test article or the control agents cells were maintained in medium containing 3% serum. Nonselective cloning medium for the quantification of cell plating efficiency was $R_{20}P$ containing 220 µg/ml sodium pyruvate, 20% serum, and 0.4% (v/v) molten noble agar but without Pluronic F68. Selective cloning medium for the quantification of Tk+/- mutants was prepared by adding 3 µg/ml TFT (trifluorothymidine) to the nonselective cloning medium.

E. Experimental design: The report described preliminary toxicity and precipitation tests as follows:

Concentrations of XDE-105 used in the L5178Y Tk+/- were selected based on results of a preliminary toxicity test. The highest concentration tested was expected to exhibit 50% to 90% inhibition of growth unless precluded by solubility.

Mutation assay procedures were described in the report as follows:

Stock Tk+/- cells, which were harvested for approximately 10 minutes at approximately 1500 x g, were resuspended in 50% supernatant ($R_{10}P$) and 50% R_0P (final serum concentration 5%) to give 1 x 10E6 cells/ml. Six milliliters of this suspension were combined with 4 ml R_0P (without activation) or 4 ml of enzyme mixture (with activation) and 0.1 ml of a 100x concentration of the appropriate test article, positive control, or solvent control into a 50-ml disposable screw-cap centrifuge tube. XDE-105 concentrations of 1, 5, 10, 15, 20, 25, 30 and 35 µg/ml were chosen for the nonactivated assay and concentrations of 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml were chosen for the activated assay. The final serum concentration was 3%. The enzyme mixture was prepared in R0P in a final volume of 4 ml containing 45 mg sodium isocitrate, 24 mg ß-nicotinamide adenine dinucleotide phosphate (NADP) and 1 ml of S9. The S9 was diluted to 20% in R_0P prior to use. Incubation with the test article was for 4 hours at approximately 37°C in a roller drum. Following incubation the cells were washed twice by centrifugation in 10-ml portions of $R_{10}P$ and then resuspended in 20 ml $R_{10}P$.

To allow the expression of Tk+/- mutants, the cells were incubated in a roller drum at approximately 37°C for 48 hours. During this period of logarithmic growth, cell densities were determined every 24 hours, and the cells were diluted with $R_{10}P$ to 3 x 10E5 cells/ml daily. For cultures below these cell densities, dilution was not required. Cultures showing excessive toxicity (<10% suspension growth) after the 48-hour incubation period were discontinued.

Following this expression period, each culture was adjusted to 6 x 10E5 cells/ml with $R_{10}P$ (includes 20% excess in number of cells equivalent to the area loss with colony counter) and 1 ml of this suspension was serially diluted (1:9) in R_0P with a final 1:4 dilution yielding a suspension of 120 cells/ml in $R_{10}P$. One-milliliter aliquots of this suspension were combined with approximately 30 ml of cloning medium in petri plates...The plates were incubated for 12±2 days at approximately 37°C in a humidified 95%/5% air/CO_2 environment. Colonies of both Tk+/- and Tk-/- appeared on these plates and thus provided a measure of the number of viable L5178Y cells capable of growing in the soft agar medium.

For selection of the Tk-/- mutants, 2 ml of the 6 x 10E5 cells/ml suspension was combined with approximately 30 ml of the TFT-selection medium and dispensed into each of three petri plates and processed as described above. Only Tk-/- colonies appeared on these plates, thus providing a measure of the number of Tk-/- mutants capable of growing in soft-agar medium.

F. <u>interpretation of results</u>: The report described quantification of results as follows:

The number of viable colonies (growth in nonselective cloning medium) and the number of Tk-/- mutant colonies (growth in TFT-selection medium) were enumerated automatically..., and the arithmetic mean of the triplicate samples was determined. Colonies derived from the plates treated with the solvent

control were used to establish the size discrimination and sensitivity standards for counting colonies derived after chemical treatment. Both large and small colonies were included in the total count.

The criteria for interpretation of results were described in the report as follows:

Cultures treated with the solvent control or positive-control compounds must have yielded mutation data consistent with historical control data. The test article should have been tested to toxic levels, but only cultures with relative suspension growth >10% were considered.

A test article is judged to have induced a positive response for chemical-induced mammalian cell mutation when a concentration-dependent increase in $Tk^{+/-}$ mutation frequency is observed and when values for mutation index are at least twofold greater than control values at two successive treatment levels.

Reported Results

A. Preliminary toxicity testing: The report described the results of cytotoxicity assays as follows:

A concentration-dependent cytotoxic response was observed (for the nonactivated assays). Severe cytotoxicity resulted from the 25, 30, and 35 µg/ml treatments and these cultures could not be cloned. For the remaining treatments, values for suspension growth ranged from 13% (at the 20 µg/ml concentration) to 119% (at the 1 µg/ml level) of control values and showed a positive correlation with values for total survival that ranged from 13% to 119%...

A concentration-dependent cytotoxic response was observed (for the activated assays). Values for suspension growth ranged from 17% (at the 45 µg/ml concentration) to 106% (at the 15 µg/ml level) of control values and showed a positive correlation with values for total survival that ranged from 15% to 82%...

B. Mutation assay results: The mean values from triplicate counts from assays without metabolic activation were summarized in the report as follows:

Spinosad (XDE-105) §84-2: L5178Y $Tk^{+/-}$ Test

Treatment	Concentration (μg/ml)	Mean No. Colonies on Selective plates	Mean No. Colonies on Non-Selective Plates
		Nonactivated Test	
DMSO	(1%)	15	82
	(1%)	14	79
	(1%)	16	92
XDE-105	1	12	84
	5	15	105
	10	13	68
	15	13	108
	20	14	84
EMS	620	512	49
		Activated Test	
DMSO	(1%)	16	125
	(1%)	14	98
	(1%)	13	95
XDE-105	15	18	82
	20	18	101
	25	19	105
	30	14	88
	35	17	95
	40	12	109
	45	15	94
	50	19	116
3MC	2	212	78

Cloning efficiency, total survival, mutation frequency and mutation index results were summarized in the report as follows:

Spinosad (XDE-105) §84-2: L5178Y $Tk^{+/-}$ Test

Treatment	Concentration (μg/ml)	Percent Cloning Efficiency[a]	Percent Total Survival[b]	Mutation Frequency[c]	Mutation Index[d]
		Nonactivated Test			
DMSO	(1%)	100	100	1.8	
	(1%)	100	100	1.8	(1.0)
	(1%)	100	100	1.8	
XDE-105	1	100	119	1.4	0.8
	5	125	100	1.4	0.8
	10	81	61	1.9	1.1
	15	129	40	1.2	0.7
	20	100	13	1.7	0.9
EMS	620	58	14	104.5	38.1
		Activated Test			
DMSO	(1%)	100	100	1.3	
	(1%)	100	100	1.4	(1.0)
	(1%)	100	100	1.4	
XDE-105	15	77	82	2.2	1.6
	20	95	79	1.8	1.3
	25	99	56	1.8	1.3
	30	83	43	1.6	1.1
	35	90	18	1.8	1.3
	40	103	22	1.1	0.8
	45	89	15	1.1	1.1
	50	109	29	1.6	1.1
3MC	2	74	21	27.2	19.4

[a] Relative cloning efficiency of treated cultures; solvent RCE = 100%.
[b] (% suspension growth) x (% cloning efficiency).
[c] $Tk^{-/-}$ mutants per 1 x 10E5 colony forming cells.
[d] (mutation frequency of treated culture)/(mutation frequency of solvent control).

Discussion

A. Authors' Conclusions: The authors' summarized the results as follows:

> The L5178Y $Tk^{+/-}$ test was sensitive for detecting both ultimate and promutagens as evidenced by the positive responses observed with EMS and 3MC. Based on the results presented...XDE-105 was not mutagenic to L5178Y $Tk^{+/-}$ cells with or without metabolic activation.

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

Primary Review by: Roger Gardner Roger Gardner 6/12/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D.
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Chromosome Aberrations Assay
Guideline 84-2
Species: Chinese Hamster Ovary Cells

EPA Identification Nos.: EPA MRID No. 434145-24
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): 910918CAB3162, 911009CAB3162

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

Title of Report: The Effect of XDE-105 on the In Vitro Induction of Chromosome Aberrations in Chinese Hamster Ovary Cells

Author(s): Garriott, M.L., D.E.F. Kindig, and D.W. Grothe

Report Issued: July 17, 1992

Executive Summary: Spinosad did not increase the number of CHO cells with chromosome aberrations at concentrations of 20, 26, or 35 µg/ml without metabolic activation or at concentrations of 100, 250 or 500 µg/ml with metabolic activation.

Core Classification: Acceptable.

Materials and Methods

A. Test species: The cells used in the study were described in the report as follows:

> The stock CHO cells (subline WB_L) were originally obtained from Hazleton Laboratories America, Inc. (Kensington, MD) and were cryopreserved. Stock cells were screened and shown to be negative for mycoplasma contamination prior to use in studies by passage in aerobic and anaerobic broth followed by streaking on tryptic soy agar. Working cultures of the CHO cells were initiated from stock cells stored in liquid nitrogen and were maintained with McCoy's complete 5A medium. Cells were subcultured twice weekly.

Spinosad (XDE-105) §84-2: Chromosome Aberration in CHO Cells

B. Test substance: XDE-105 (88.0% a.i.) was supplied as a solid (Reference no. ACD13651).

C. Vehicle and positive control substances: The vehicle for the test substance and positive controls was reagent grade dimethyl sulfoxide (DMSO) which was used in a concentration of 1% in the study. Cyclophosphamide and mitomycin C were used as positive control substances.

D. Other test materials: The report described the preparation of the S9 mix as follows:

> ...The animals (200 to 220 g male Fischer 344 rats) were treated 5 days prior to sacrifice with a single 500-mg/kg intraperitoneal dose of Arochlor 1254. A sterile 25% homogenate was prepared in 0.15 M KCl at approximately 4°C and centrifuged for approximately 15 minutes at approximately 9000 x g. The supernatant fraction (S9) was stored at approximately -80°C...

Preparation of the culture media was described in the report as follows:

> Growth medium, designated McCoy's complete medium, consisted of McCoy's 5A medium containing L-glutamine supplemented with 25 mg/l gentamicin sulfate and 10% fetal calf serum. Growth medium containing no serum was designated Serum Free Medium (sfm).

E. Experimental design: The report described preliminary toxicity tests as follows:

> Concentrations of 50, 60, 70, 80, 90 and 100 µg/ml of XDE-105 without activation, and 10, 50, 100, 250, and 500 µg/ml of XDE-105 with activation were selected based on results of a preliminary toxicity test. Concentrations for the assay without activation were chosen to yield cultures for aberration scoring such that at least one treatment would have approximately 40% to 60% relative growth, and two to four treatments would have >40% to 60% relative growth. Concentrations for the assay with activation were chosen based on the presence of a precipitate at the highest concentration.

Assay procedures were described in the report as follows:

> For this study, exponentially growing cells were plated in 75 cm^2 plastic tissue culture flasks at an initial density of about 1 x 10^6 cells in 10 ml of McCoy's complete medium and incubated for approximately 24 hours. The medium was replaced with either McCoy's SFM (nonactivated assay) or McCoy's SFM plus the S9 metabolic activation system (activated assay). The metabolic activation system consisted of one part thawed rat liver homogenate (S9) added to three parts of cofactor mix. The sterile filtered cofactor mix contained 15 mg/ml isocitric acid and 8 mg/ml ß-nicotinamide adenine dinucleotide phosphate

> (NADP). Four mililiters of the S9 mix were added to each treatment flask. The cells were then exposed to XDE-105 for approximately 4 hours.
>
> Following exposure to the test article, cells were washed and the medium was changed back to McCoy's complete medium. An extended harvest time of 1½ cell cycles (18 to 24 hours) was used in this assay...
>
> Approximately 19 hours after exposure, 0.1 µg/ml Colcamide was added to two of the three cultures in each treatment group to arrest dividing cells in metaphase. The remaining culture in each treatment group was used to conduct a concurrent cytotoxicity test. From the results of the concurrent toxicity tests, three test article concentrations were chosen for evaluation for chromosome aberrations. In the assay without metabolic activation excessive toxicity was observed. Concentrations were adjusted to 20, 23, 26, 29, 32, and 35 µg/ml, and the assay was repeated.

Procedures to harvest cells and prepare slides for evaluation of chromosome aberrations were described in the report as follows:

> Metaphase cells were collected by mitotic shake-off approximately 2 hours after addition of Colcamide. The cells were centrifuged at about 120 x g for approximately 10 minutes...cells were resuspended in hypotonic solution (0.075 M KCl) for 10 to 12 minutes at 37°C. Cells were centrifuged again at about 180 x g for 10 minutes...The cell pellet was resuspended and approximately 4 ml of fixative (3:1 methanol:glacial acetic acid) were added slowly. The cells were washed two or three times with fixative and then were stored in fixative for 3 hours at 4°C. Following removal of the last fixative, the cell pellet was resuspended in a small amount of fresh fixative, and three to five drops of cell suspension were dropped onto a clean, cold, wet slide. At least two slides were prepared from each test culture. Slides...were allowed to air dry before staining in 4% Giemsa in a pH 6.8 phosphate buffer for 10 minutes.

The report described the procedures for evaluating metaphase cells as follows:

> Fifty metaphase figures from each treatment and solvent control culture (100/concentration) and 25 metaphase figures from each positive control culture were read. The chromosome number was determined and only cells with 19 to 23 (2n ± 2) chromosomes were scored. A standard form was used to record chromatid and chromosome breaks, gaps, deletions, exchanges, acentric fragments, rings, dicentrics, and other changes in chromosome morphology. The microscope stage coordinates of all scored cells with aberrations were recorded. One-hundred cells per slide were examined for ploidy increases and the percentage of cells with polyploidy was indicated on the score sheet...The number of aberrations per cell, percentage of cells with aberrations (TA), percentage of cells with aberrations including gaps (TAG), and percentage of cells with more than

one aberration were calculated. Each type of aberration was assigned a value of one, except for those cells that were pulverized or had greater than 10 aberrations. Each pulverized cell and any cell with 10 or more aberrations, was assigned a value of 10 for use when calculating the number of aberrations/cell.

F. interpretation of results: The criteria for interpretation of results were described in the report as follows:

Cultures treated with the solvent control or positive-control compounds must yield chromosome aberration frequencies consistent with historical data. The test article should be tested to toxic levels (i.e., decrease the mitotic cell count by approximately 50%) or to the limits of solubility.

A test article is identified as a clastogen when a dose-related increase in chromosomal aberrations is observed in which the number of aberrations is statistically ($p \leq 0.01$) greater than that of the concurrent control value, as determined by a trend test for Poisson distribution.

Reported Results

The report described the results of the assay as follows:

The assay without metabolic activation was conducted using XDE-105 concentrations of 20, 26, and 35 µg/ml, whereas concentrations of 100, 250, and 500 µg/ml were used in the assay with metabolic activation...The incidence of cells (in the assay without metabolic activation) with chromosome aberrations (TA) and cells with aberrations including gaps (TAG) ranged from 1% to 4% for the groups treated with XDE-105. The number of cells with aberrations did not significantly increase when compared to the number of cells with aberrations in cultures treated with the solvent control. The positive control (mitomycin C) produced 36% aberrant cells...

...The incidence of TA and TAG ranged from 1% to 4% in cultures treated with XDE-105 (with metabolic activation). As in the nonactivated assay, the number of cells with aberrations did not significantly increase compared to the number of cells with aberrations in cultures treated with the solvent alone. Cultures treated with the positive control (cyclophosphamide) showed 64% aberrant cells...

Statistical analyses indicated that treatment with XDE-105 had no effect on the number of cells with chromosomal aberrations...

Discussion

A. Authors' Conclusions: The authors' summarized their conclusions as follows:

> ...the in vitro chromosome aberration assay was sensitive to the positive controls, mitomycin C and cyclophosphamide, and that XDE-105 did not indicate an increase in the percentage of cells with chromosomal aberrations, with or without metabolic activation.

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

Primary Review by: Roger Gardner Roger Gardner 6/12/95 011597
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl W. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Micronucleus Test
Guideline 84-2
Species: Mice

EPA Identification Nos.: EPA MRID No. 434145-25
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): 910916MNT3162, 911007MNT3162

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

Title of Report: The Effect of XDE-105 on the In ViVo Induction of Micronuclei in Bone Marrow of ICR Mice.

Author(s): Garriott, M.L., j.d. Brunny, D.E.F. Kindig, and D.W. Grothe

Report Issued: July 17, 1992

Executive Summary: Spinosad did not increase the frequency of micronuclei in replicate assays with bone marrow cells from ICR mice treated with doses of 0, 500, 1000 or 2000 mg/kg/day for two consecutive days.

Core Classification: Acceptable.

Materials and Methods

A. Test species: The test animals used in the study were described in the report as follows:

> Male and female ICR (H3d:[ICR]) were supplied by Harlan sprague Dawley, Inc. (Indianapolis, IN). The animals were acclimated to the housing facilities for a minimum of 7 days before the test was initiated. Animals tested were approximately 8 weeks of age and weighed between 27.5 and 36.8 g (males) or between 24.1 and 31.9 g (females).

Spinosad (XDE-105) §84-2: Micronucleus Test in Mice

B. Test substance: XDE-105 (88.0% a.i.) was supplied as a solid (Reference no. ACD13651).

C. Vehicle and positive control substances: The vehicle for the test substance and positive control was 10% (w/v) acacia. Cyclophosphamide was used as the positive control substance.

D. Other test materials: The report described the preparation of doses as follows:

> Suspensions of XDE-105 were prepared on each day of dosing at concentrations of 22.7, 45.5, and 90.9 mg/ml in 10% (w/v) aqueous acacia, which also served as the vehicle control for this study. Cyclophosphamide was also suspended in 10% aqueous acacia.

E. Experimental design: The report described the selection of doses and route of administration as follows:

> XDE-105 was administered on 2 consecutive days by the oral route at doses of 0, 500, 1000, or 2000 mg/kg body weight. In a preliminary dose range-finding study, no signs of toxicity were observed in animals dosed with 2000 mg XDE-105/kg of body weight. In the absence of toxicity, 2000 mg/kg is the maximum dose recommended under current...guidelines. Furthermore, the high dose, 2000 mg/kg, represented the highest concentration of XDE-105 attainable in suspension.

Assay procedures were described in the report as follows:

> The present study was designed with five treatment groups, each with 5 mice/sex. XDE-105, cyclophosphamide, or the negative control were administered orally, in a volume equal to 25 ml/kg body weight, in two daily doses, delivered approximately 24 hours apart.
>
> Twenty-four hours after the second treatment, mice were killed...and the femurs were dissected free of muscle...bone marrow streaks were prepared on slides using the brush technique. Four streaks were applied to each slide, and two slides were prepared from each animal, one from each femur. The streaks of bone marrow were air-dried overnight. One bone marrow slide was then fixed and stained using a Midas™ II automated stainer, programmed to fix the slides in 100% methanol for 10 minutes, stain in Wright's stain for 6 minutes, stain in a Wright's-Giemsa mixture for 6 minutes, and rinse with deionized water for 2 minutes. the slides were then allowed to air-dry. The second slide was held in reserve.
>
> ...A combined total of 1000 polychromatic (PCE) and normochromatic erythrocytes (NCE) was counted from each animal using a differential counter. The ratio of the number of PCE to the number of NCE provides an index of toxicity. A total of 1000 PCE was examined for each animal and evaluated for the presence of micronuclei (MN).

F. interpretation of results: The criteria for interpretation of results were described in the report as follows:

> Animals treated with the vehicle control and positive control compound must yield MN frequencies consistent with historical data. The test article should be tested to toxic levels (i.e., the maximum tolerated dose [MTD], one-half the median lethal dose) or the limits of solubility.
>
> A trend test for Poisson distribution was performed on MPCE values from individual animals. The Mantel-Haenzsel test was used to pool the inference across sexes. A standard chi-square analysis was used to compare positive and negative control animals. Statistical analysis was applied to the PCE/NCE ratios.

Reported Results

The report described the results of the assay as follows:

> XDE-105 did not effect the PCE/NCE ratios of either sex. The mean MPCE ranged from 1.0 to 2.4 (males) and from 1.0 to 1.6 (females) for animals treated with XDE-105. The mean incidence of MPCE for the vehicle control was 0.6 for males and 1.0 for females. Animals treated with the positive control, cyclophosphamide, had mean MPCE incidences of 10.6 (males and 11.8 (females),...
>
> A trend test for Poisson distribution determined that the incidence of MPCE in males treated with XDE-105 increased significantly ($p \leq 0.01$) when compared to concurrent control values. There was no significant increase in the incidence of MPCE in females ($p=0.36$). When the incidence of MPCE for males and females was pooled, the p value ($p=0.011$) was also significant. However, all of the MCPE frequencies were within the range of the historical vehicle control values, and the positive results from the statistical analysis were attributed to an unusually low MPCE incidence in the vehicle control group males. Nevertheless, the assay was repeated to determine whether the positive findings ware reproducible.
>
> Again, XDE-105 did not effect the PCE/NCE ratios of either sex. The mean incidence of MPCE ranged from 1.4 to 2.0 and from 1.2 to 2.0 for treated males and females, respectively. The mean incidence of MPCE for the vehicle control was 1.6 for males and 1.2 for females. Animals treated with the positive control, cyclophosphamide, had mean MPCE incidences of 16.0 (males and 16.8 (females),...there was no statistically significant increase in the incidence of MPCE in males ($p=0.82$) or females ($p=0.25$).

Discussion

A. Authors' Conclusions: The authors' conclusions were presented as follows:

There was a statistically significant increase in the incidence of MPCE in males in the first assay. However, values for all animals were within the range for historical vehicle control. Furthermore, there was no significant increase in the incidence of MPCE in the replicate assay.

Based on the results of the two independent assays, it was concluded that the in vivo assay for micronuclei in ICR mice was sensitive to the positive control, cyclophosphamide, and that XDE-105 did not induce micronuclei in bone marrow PCE in either male or female ICR mice. Therefore, XDE-105 is not clastogenic and does not interact with the mitotic spindle.

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

Primary Review by: Roger Gardner Roger Gardner 6/2/95
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Karl Baetcke, Ph. D. Karl D. Baetcke 6/25/95
Toxicology Branch I/HED

DATA EVALUATION RECORD

Study Type: Unscheduled DNA Synthesis
Guideline 84-2
Species: Primary Rat Hepatocytes

EPA Identification Nos.: EPA MRID No. 434145-26
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): 910806UDS3162, 910827UDS3162

Testing Facility: Toxicology Research Laboratory, Lilly Research Laboratories, A Division of Eli Lilly & Co., Greenfield, IN.

Title of Report: The Effect of XDE-105 on the Induction of Unscheduled DNA Synthesis in Adult Rat Primary Hepatocytes.

Author(s): Garriott, M.L., D.J. Yount, and D.W. Grothe

Report Issued: June 10, 1992

Executive Summary: Spinosad did not induce unscheduled DNA synthesis (UDS) in adult rat hepatocytes *in vitro* at concentrations of 0.01 to 5 µg/ml. Concetrations from 10 to 1000 µg/ml of XDE-105 were cytotoxic.

Core Classification: Acceptable.

Materials and Methods

A. Test species: The cells used in the study were described in the report as follows:

> Primary cultures of adult rat hepatocytes used for the two studies were prepared...from the livers of two male Fischer 344 rats (Charles River Laboratories, Kingston, NY) each weighing approximately 220 g. The liver was perfused at ~37°C *in situ* via the hepatic portal vein for 1 to 2 minutes at a rate of 20 to 40 ml/min with Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution (HBSS) containing 0.5 mM ethyleneglycol-bis-(ß-aminoethylether) N,N'-tetraacetic acid (EGTA) and buffered with 0.025 M 2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) to pH 7.2

> to 7.4. The cells were disaggregated by continuous perfusion (20 to 40 ml/min) at-37°C for 10 to 14 minutes. The perfusion medium was Williams medium E that was buffered to pH 7.2 to 7.5 with 0.025 M HEPES and contained 100 units/ml of collagenase. Cells were detached by combing in fresh WEH-collagenase medium followed by filtration through 80-mesh nylon. The cells were washed twice with WEH containing 10% fetal bovine serum (FBS) and 50 mg/ml gentamicin. Cells were centrifuged at 20 x g for 4 minutes. The preparation used in these studies yielded $2.36x10^6$ and $1.87x10^6$ with 91% and 81% viability for Studies 910806UDS3162 AND 910817UDS3162, respectively.

B. Test substance: XDE-105 (88.0% a.i.) was supplied as a solid (Reference no. ACD13651).

C. Vehicle and positive control substances: The vehicle for the test substance and positive controls was reagent grade dimethyl sulfoxide (DMSO). N-Methyl-N,N'-nitro-N-nitrosoguanidine (MNNG) and 2-acetylaminofluorene (2-AAF) were used as positive control substances.

D. Other test materials: Preparation of the dosing solutions was described in the report as follows:

> Serial dilutions were made in DMSO followed by 1:100 dilutions in serum-free media to yield eight concentrations of XDE-105 ranging between 0.5 and 1000 µg/ml in Study 910806UDS3162 and 0.01 to 50 µg/ml in Study 910827UDS3162 and four concentrations each of MNNG and 2-AAF between 1 and 20 µg/ml and between 0.05 and 1 µg/ml, respectively....

E. Experimental design: Assay procedures were described in the report as follows:

> Freshly prepared hepatocytes were plated in WEH containing 10% FBS, 50 mg/ml gentamicin, and 100 units/ml each of penicillin and streptomycin at a density of $5x10^4$ cells/ml in 26 x 33 mm multiplates containing 10.5 x 22 mm plastic coverslips that were pretreated with 1 mg/ml collagen. The cultures were incubated at ~37°C in a 95/5%:air/CO_2 environment for ~2.5 hours. After incubation, the cells were washed once with WEH. Serum-free WEH containing 10 mCi/ml ^{3}H-TdR and the appropriate dilution of the test article was then applied to each culture. After ~20 hours of incubation, cells were washed by transferring the slips to a container of chilled D-PBS. The slips were then treated for 10 minutes with 1% sodium citrate, and fixed by three 1-hour washes with ethanol/acetic acid (3:1 v/v). All fixing and washing procedures were conducted at ~4°C. Slips were allowed to air dry and then attached to slides prior to staining with Aceto-Orcein stain...The stained slides were air-dried, individually dipped in undiluted NTB-2 liquid photographic emulsion, sealed in light-resistant, tightly sealed, desiccated boxes, incubated for 7 days at ~4C, developed with Kodak D-19 developer, and fixed with Kodak fixer...

The report described the procedures for evaluating hepatocytes as follows:

> The total number of silver grains over a particular cell (nuclear and cytoplasmic counts) was enumerated, and the nuclear and cytoplasmic areas were determined using a semi-automated...colony counter...These values were employed in an algorithm to determine the net nuclear grain count. In cases where the cytoplasmic count exceeded the nuclear count, a negative value was recorded.
>
> Nuclei of 20 morphologically unaltered cells, for each treatment were selected to quantify UDS responsiveness. A minimal criteria for selection of nucleus for quantification was that each nucleus had at least four grains over the nuclear area. Autoradiographic grain counts were conducted for the highest compound concentration that did not produce pronounced cytotoxicity and for all lower concentrations of the test article.

F. interpretation of results: The criteria for interpretation of results were described in the report as follows:

> Data for the Net Nuclear Count is analyzed by a covariate analysis of variance in which the replicate effect, slips within negative control effect, linear dose-response, and the linear response by replicate interaction are partitioned. The test article is considered to have induced a positive response for the induction of UDS if either the linear dose response or the linear response by replicate interaction terms are significant ($p \leq 0.01$). When a positive response is obtained, pair-wise comparisons using the unequal variance t test are run for each replicate to determine the cause of the significance...

Reported Results

The report described the results of the assay as follows:

> Primary cultures of adult rat hepatocytes were treated with 0.5, 1, 5, 10, 50, 100, 500, or 1000 µg/ml of XDE-105 in the first of two independent studies. Because of toxicity observed in the first experiment, lower concentrations were selected for the second assay and ranged from 0.01 to 50 µg/ml...Cultures treated with 1% DMSO showed no evidence of cytotoxicity or induction of UDS. Cytotoxicity resulted from treatment with XDE-105...at concentrations between 10 and 50 µg/ml...A slight toxic effect was noted at...1 and 5 µg/ml...No induction of UDS was observed in cultures treated with XDE-105...A positive autoradiographic response for UDS was noted in cultures treated with either the ultimate carcinogen, MNNG, or the procarcinogen, 2AAF.

The average number of net nuclear silver grains reported for the DMSO control group ranged from -5.6 to -3.7, and values for the XDE-105 groups ranged from -5.5 to -3.9. Values

reported for MNNG at 1, 5, and 10 µg/ml were -0.7, 9.5, and 45.6, respectively. For 2AAF at 0.05, 0.1, and 0.5 µg/ml were 6.4, 20.1, and 60.6, respectively. The highest concentrations of the two positive controls were cytotoxic and counts were not reported at those levels (20 and 1 µg/ml for MNNG and 2AAF, respectively.

Discussion

A. Authors' Conclusions: The authors summarized their conclusions as follows:

> The cultured adult rat hepatocytes were sensitive to the induction of UDS by both the ultimate carcinogen, MNNG, and the procarcinogen, 2AAF. No induction of UDS was observed in cultures treated with XDE-105. It was concluded that XDE-105 did not induce DNA repair synthesis in cultured rat hepatocytes.

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