1-9-97

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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

JAN 9 1997

MEMORANDUM

SUBJECT: **Spinosad - 110003.** Health Effects Division Risk Characterization Document for Use of Spinosad (Tracer®) on Cotton (PP#6F04735)

FROM: Catherine Eiden, Chemist
Registration Section
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

THROUGH: Mike Metzger, Acting Chief
Risk Characterization Branch
Health Effects Division (7509C)

and

for Margaret Stasikowski, Director
Health Effects Division (7509C)

1/9/97

TO: George LaRocca/Adam Heyward
Project Manager Team 13
Registration Division (7505C)

EXECUTIVE SUMMARY

The Health Effects Division (HED) has reviewed toxicology and residue chemistry data submitted by the registrant in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and 40 CFR §158 to support the use of Spinosad (Factors A and D) on cotton, only. Toxicology data requirements for a food-use registration although mostly complete have not been fully satisfied. Residue chemistry data requirements also remain outstanding. However, HED can recommend for a time-limited tolerance/conditional registration of Spinosad for use on cotton, only. HED recommends a time-limited tolerance of 0.02 ppm for Spinosad residues on cottonseed provided the tolerance is issued with an expiration date to allow the registrant time to resolve the toxicology and residue chemistry deficiencies.

The registrant must conduct six field trials providing data on Spinosad residues on cotton gin byproducts. The residue field trials should include at least 3 field trials for each type of harvesting (stripper and picker) for a total of at least 6 field trials. The registrant has indicated their intention to provide these to the Agency data by June 30, 1997.

The requirement for a ruminant feeding study is reserved pending the results of the cotton gin byproduct field data. Note: the registrant has already conducted this study and intends to submit it in the future with other pending registration requests.

A final Section B should be submitted that incorporates the proposed maximum seasonal rate of 0.45 lbs. a.i./A and a 28-day pre-harvest interval (PHI).

Additional data are required to upgrade the chronic feeding study in rats (83-1(a)). Histopathology data from the high dose female animals that either died or were exterminated during the chronic feeding study in rats are required. The oncogenicity study in mice is considered supplemental and does not satisfy guideline 83-2(b), but may be upgraded when additional details on the incidence of hemangiomas in female mice, including a second mouse study, have been reviewed by HED. A review of the additional information is needed to clarify the oncogenic potential of Spinosad.

Risk Characterization

Dietary Risk: The chronic dietary risk estimate for the general U.S. population is $<1\%$ of the RfD and represents the chronic dietary risk estimated for all of the 22 population subgroups. This dietary risk will be reevaluated with respect to secondary residues in ruminant tissues and milk upon receipt of the outstanding field trial data for cotton gin byproducts. Even considering additional exposure through secondary residues in meat and milk, the chronic dietary risk from the use of Spinosad on cotton appears to be minimal. An acute dietary risk estimate is not required.

Water Risk: Chronic water risks are conservatively estimated to be 10% of the RfD for the general U.S. population. Spinosad is not expected to be mobile in a soil and water environment and poses relatively little threat to drinking water. The risk estimate of 10% of the RfD is very conservative and can be refined as more data on the environmental fate of Spinosad become available.

Non-Occupational (residential) Risks: No chronic or acute residential exposure scenarios exist for uses of Spinosad on cotton; therefore, there is no chronic or acute residential risks expected from the use of Spinosad on cotton. Future registrations for Spinosad that include residential uses will require a risk estimate for those residential uses.

Aggregate Exposure/Risk: Based on the risk estimates above, aggregate chronic risks are expected to be approximately $\leq 10\%$ of the RfD for all population subgroups. Available data do not indicate any increased pre- or postnatal sensitivity; no additional uncertainty factor for increased sensitivity in infants and children is appropriate. There is no risk concern considering aggregate exposure to Spinosad.

I. BACKGROUND

Spinosad is the proposed common name for the end-use product (containing the technical grade active ingredient known as XDE-105 Technical. XDE-105 Technical consists primarily of 2 closely related factors (Factors A and D) whose chemical structures differ by a single methyl group. Factor A is 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)-tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione. Factor D is 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)-tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione. Factors A + D are fermentation-based products produced by the bacterium Saccharopolyspora spinosa. Spinosad is an insecticide.

The product designated by the company code NAF-144 is the killed microbial raw fermentation end-use product containing about 2.6% active ingredient. The product designated by the company code NAF-85 (Tracer®) is the purified fermentation end-use product for use on cotton. This product contains about 44.2% active ingredient. The product designated by the company code XDE-105 is also a purified fermentation product and is designated as the technical for NAF-85. This product contains about 90.4% active ingredient.

DowElanco has requested a Section 3 registration for use of Spinosad (Tracer®) on cotton. In conjunction with the Section 3, the registrant has proposed that a permanent tolerance be established for residues of Spinosad (Factors A + D) on cottonseed at 0.02 ppm. This is the first tolerance request for this chemical. RCAB defers the product chemistry review of the end-use products to the Registration Division.

II. USE PATTERN

Tracer® (NAF-85) is a suspension concentrate formulation containing 44.2% active ingredient (Spinosad), or 4 pounds of actiye ingredient per gallon.

Tracer® is used for the control of tobacco budworm, cotton bollworm, cotton leaf perforator, European corn borer, loopers, saltmarsh caterpillar, and armyworms in cotton at the rate of 1.4 to 2.8 fl.oz. (up to 3.6 fl.oz for control of armyworms) of formulation/A (0.044 to 0.11 lb.ai./A) depending on the size of the individual insects, the insect population, or the density of the cotton canopy. Tracer® should be mixed with water prior to application using either ground equipment (minimum of 5 gallons of spray volume) or aerially (minimum of 2 gallons per acre). Tracer® should not be applied to consecutive generations of tobacco budworm or cotton bollworm. However, multiple applications of Tracer® can be used to reduce a single insect generation below the economic threshold. Do not exceed 0.45 lb.ai./A/season (14.4 fl.oz. of formulation/A/season). Do not apply within 28 days of harvest.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

The product chemistry data submitted in accordance with 40 CFR 158.155 through 158.190 for technical Spinosad has been reviewed and are acceptable.

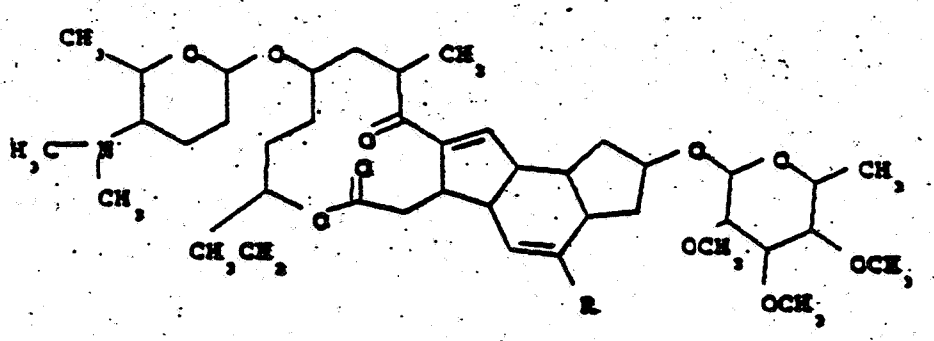
Chemical Name: Common Name = Spinosad

Factor A: 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)-tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione.

Factor D: 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)-tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione.

Structure

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



Physical/Chemical Properties for technical Spinosad

Color	light grey to white
Physical State	solid
Odor	slightly stale water
Melting Point	Factor A: 84-99.5C Factor B: 161.5-170C
Boiling Point	N/A
Density, Bulk Density, or Specific Gravity	0.512 at 20C

Solubility (at 20C)	Factor A	Factor B
Water	89.4 ppm	0.495 ppm
Acetone	16.8 g/.1L	1.01 g/.1L

Dichloromethane	52.5 g/.1L	44.8 g/.1L
Hexane	0.448 g/.1L	743 g/.1L

Vapor Pressure (at 25C) Factor A: 3.0 X 10^{-11} KPa
Factor B: 2.0 X 10^{-11} KPa

Dissociation Constant Factor A: 8.10 pKa
Factor B: 7.87 pKa

Octanol/Water Partition Coefficient Factor A: log K_{ow} = 3.9
Factor B: log K_{ow} = 4.4

pH 7.74 for a 10% slurry of XDE-105 in water
Stability XDE-105 was stable after 28 days: ambient, 122F, and in contact with stainless steel, brass, and ferric chloride

Oxidizing or Reducing Action	N/A
Flammability	N/A
Explodability	N/A
Storage Stability	N/A
Viscosity	N/A
Miscibility	N/A
Corrosion Characteristics	N/A

B. Human Risk Assessment

1. Hazard Assessment

The status of Spinosad with respect to the toxicology data requirements for a terrestrial food use for the technical grade active ingredient (TGAI) is given in Table 1.

Table 1. Data Requirements (40 CFR 158.340) for Terrestrial Food Use: Technical (TGAI)

Guideline #	Study Type	Required	Satisfied
81-1	Acute oral toxicity - rat	Yes	Yes
81-2	Acute dermal toxicity - rat	Yes	Yes
81-3	Acute inhalation toxicity - rat	Yes	Yes
81-4	Primary eye irritation - rabbit	Yes	Yes
81-5	Primary dermal irritation - rabbit	Yes	Yes
81-6	Dermal sensitization - guinea pig	Yes	Yes
81-7	Acute Neurotoxicity - rodent		Yes

Table 1. Data Requirements (40 CFR 158.340) for Terrestrial Food Use: Technical (TGAI)			
Guideline #	Study Type	Required	Satisfied
82-1	90-Day feeding - rodent	Yes	Yes
82-1	90-Day Feeding -dog	Yes	Yes
82-2	21-Day Dermal Toxicity		Yes
82-5	90-Day Neurotoxicity - rodent		Yes
83-1(a)	Chronic toxicity - rodent	Yes	No
83-1(b)	Chronic toxicity -dog	Yes	Yes
83-2(a)	Oncogenicity - rat	Yes	Yes
83-2(b)	Oncogenicity - mouse	Yes	No
83-3(a)	Developmental toxicity - rodent	Yes	Yes
83-3(b)	Developmental toxicity - rabbit	Yes	Yes
83-4	2-Generation reproduction (rat)	Yes	Yes
84-2	Gene mutation	Yes	Yes
84-2	Structural chromosomal aberration	Yes	Yes
84-2	Other genotoxic effects	Yes	Yes
85-1	General metabolism	Yes	Yes

The status of Spinosad with respect to the toxicology data requirements for a terrestrial food use for the end-use product (Tracer®) is given in Table 2.

Table 2. Data Requirements (40 CFR 158.340) for Terrestrial Food Use: End Use Product			
Guideline #	Study Type	Required	Satisfied
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

a. Acute Toxicity

The Table 3 below is a summary of the acute toxicity of the technical grade active ingredient (88 - 90.4%) product for Spinosad.

Table 3

TEST	RESULTS	TOXICITY CATEGORY
Oral LD50 - rat MRID#: 43414515	LD50 = 3738 mg/kg (M) LD50 > 5000 mg/kg (F) LD50 > 5000 mg/kg (M + F)	IV
Dermal LD50 - rat MRID#: 43414516	LD50 > 2000 mg/kg (F) LD50 > 2000 mg/kg (M) LD50 > 2800 mg/kg (M + F)	III
Inhalation - rat MRID#: 43414517	LC50 > 5.18 mg/L LC50 > 5.18 mg/L LC50 > 5.18 mg/L	IV
Primary Eye Irritation - rabbit MRID#: 43414518	Slight conjunctival irritation	IV
Primary Dermal Irritation - rabbit MRID#: 43414519	No erythema and edema	IV
Dermal Sensitizer - Guinea pig MRID#: 43414520	Nonsensitizer	N/A

The Table 4 below is a summary of the acute toxicity of the end-use (44% formulation) product for Spinosad.

Table 4

TEST	RESULTS	TOXICITY CATEGORY
Oral LD50 - rat MRID#: 43414509	LD50 > 5000 mg/kg (M + F) LD50 > 5000 mg/kg (F) LD50 > 5000 mg/kg (M)	IV
Dermal LD50 - rat MRID#: 43414510	LD50 > 2000 mg/kg (F) LD50 > 2000 mg/kg (M) LD50 > 2800 mg/kg (M + F)	III
Inhalation - rat MRID#: 43414511	LC50 > 5 mg/L LC50 > 5 mg/L LC50 > 5 mg/L	IV
Primary Eye Irritation - rabbit MRID#: 43414512	Slight conjunctival irritation	IV
Primary Dermal Irritation - rabbit MRID#: 43414513	Slight transient erythema and edema	IV
Dermal Sensitizer - Guinea pig MRID#: 43414514	Nonsensitizer	N/A

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

b. Subchronic Toxicity

i. Subchronic Oral Toxicity in Dogs

XDE-105 (Spinosad, 88.0% pure) was tested in a 13-week oral feeding study in male and female Beagle dogs (MRID 43444102). The dosing levels used 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 high dose was reduced to from 33.4 to 22.5 mg/kg/day in males on day 38.

The LOEL is 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 hematological and biochemical evidence of anemia and possible liver damage. **The NOEL is 4.89 mg/kg/day in males or 5.38 mg/kg/day in females and the LOEL is 9.73 mg/kg/day in males or 10.47 mg/kg/day in females.**

ii. Subchronic Oral Toxicity in Mice

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 (MRID no. 43566602). Mortalities in 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.

The NOEL was established at 7.5 mg/kg/day. The LOEL was 22.5 mg/kg/day in mice, based on cytoplasmic vacuolation of cells from the lymphoid organs, liver, kidney, stomach, ovary, female reproductive tract, and epididymis. 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 also showed minimal and/or slight lymphoid vacuolar change compared with none in the control group.

iii. 21-Day Dermal Study

In a 21-day dermal study in rabbits (MRID no. 43557503) the NOEL for dermal and systemic toxicity was 1000 mg/kg/day (limit dose). 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 (MRID 43557503). 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.**

iv. Subchronic Oral Neurotoxicity Study

In a 13-week feeding neurotoxicity study, 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, or 42.7 mg XDE-105/kg body weight for males and 0, 2.6, 5.2, 10.4 or 52.1 mg/kg/day for females, MRID 43557504). 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 satisfy §82-7 guideline requirements for a subchronic mammalian neurotoxicity study and is classified as Acceptable.

c. Chronic Toxicity and Carcinogenicity

i. Chronic Toxicity in Dogs

In a chronic toxicity study (MRID No.: 43701504), XDE-105 (Spinosad, 87.2% ai) was administered to four beagle dogs/sex/dose in the diet at dose levels of 50/60, 100/120, or 300/360 ppm (1.44, 2.68, or 8.46 mg/kg/day, respectively, ♂; 1.33, 2.72, or 8.22 mg/kg/day, respectively, ♀) for 52 weeks.

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

ii. Chronic Toxicity in Rats

A chronic feeding study using rats is under review and indicates that the rat is a less sensitive species than the dog with respect to Spinosad. The rat feeding study data support the NOEL selected from the dog feeding study as the basis of the RfD. The rat feeding study review indicates that additional histopathology data on the animals that died during the study are required to upgrade the study to an acceptable status. NOELs and LOELs cannot be established for this study until the data required to upgrade the study are provided. This data requirement will not impinge on the conditional registration of Spinosad for use on cotton, but is a condition of registration and will impinge on a continuing registration for Spinosad on cotton, and any additional uses of Spinosad.

iii. Oncogenicity in Mice

Spinosad (XDE-105) was administered to CD-1 male and female mice (50/sex/group) for up to 18 months at 0, 25, 80, and 360 ppm in the diet (equivalent to 0, 3.4, 11.4, and 50.9 mg/kg/day in males or 4.2, 13.8, and 67.0 mg/kg/day in females at the low-, mid-, and high-dose groups, respectively: MRID 43701505). Two satellite groups of 10 mice/sex/group were included for sacrifice at 3 and 12 months. Females in the high dose group were terminated at 15 months because of excessive mortality and weight loss.

Based on the decreased weight gains, increased mortality, the hematologic effects, and the gross finding of increased thickening of the gastric mucosa in females and the histologic changes in the stomach of males, the systemic LOEL was established as 360 ppm equivalent to 50.9 mg/kg/day in male mice and 67 mg/kg/day in female mice. The NOEL is 80 ppm equivalent to 11.4 mg/kg/day for males and 13.8 mg/kg/day for females.

This study is classified as supplemental, but may be upgraded when review of additional details on the incidence of hemangiomas in female mice and a second mouse long-term feeding study are completed.

d. Developmental Toxicity

i. Developmental Toxicity in Rabbits

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 on gestation day 7 through 19 (MRID 43414521).

The report concluded that maternal toxicity was observed at the highest dose tested (50 mg/kg/day) and was indicated by decreased defecation (in 6/20 animals compared with 2/10 in the control group), decreased body weight gain (28% less than that for the control group during gestation), and reduced food consumption (the high dose group consumed an average amount that was 74% of the control group value). However, there was only a 1-2 % difference in the mean body weights between the control and 50 mg/kg/day dose groups. These high dose group results, along with results from a range-finding study (MRID 4370703), suggested that **the NOEL for maternal toxicity is $\geq$ 50 mg/kg/day.** Although the incidence of aborted pregnancies was higher in the 50 mg/kg/day dose group than historical control values, the treatment and observation periods in the range-finding study were not adequate to confirm the investigators' conclusion (the study was terminated at gestation day 20 when abortions were noted at gestation days 22 and 27 in the main study). There were no developmental effects that could be attributed to administration of XDE-105. **The NOEL for developmental toxicity is $\geq$ 50 mg/kg/day (highest dose tested).**

This study along with the range-finding study (MRID 43770703) satisfies guideline 83-3 requirements for a rabbit developmental toxicity study and should be classified as acceptable. The highest dose tested (50 mg/kg/day) approached an adequate level as indicated by the range-finding study.

ii. Developmental Toxicity in Rats

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 on gestation day 6 through 16 (MRID 43557505). Marginal maternal toxicity was reported at the highest dose tested and was indicated by decreased body weight gain and lightly reduced body weight for one day in the high dose animals. No animals exhibited dose-related clinical signs. **The NOEL for maternal toxicity is $\geq$200 mg/kg/day (highest dose tested).**

There were no developmental effects that could be attributed to administration of XDE-105. **The NOEL for developmental toxicity is $\geq$200 mg/kg/day (highest dose tested).**

e. Reproductive Toxicity

i. Reproductive Toxicity in Rats

In a 2-generation reproduction study (MRID 43701506) XDE-105 (88.0% Spinosad a.i.) was administered to 30 Sprague Dawley rats/sex/dose in diet at target dose levels 0, 0.005, 0.02,

and 0.2% w/w (equivalent to 0, 3, 10 and 100 mg/kg/day). **The LOEL for systemic toxicity is 100 mg/kg/day based on increases in heart, kidney, liver, spleen, and thyroid weights (both sexes), corroborative histopathology in the spleen and thyroid (both sexes), heart and kidney (males only), and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. The NOEL for systemic toxicity is 10 mg/kg/day.**

The LOEL for reproductive toxicity is 100 mg/kg/day based on both maternal and reproductive effects including decreases in litter size, survival (F_2 litters only), and body weights in the offspring, and increased incidence of dystocia and/or vaginal bleeding after parturition with associated increases in mortality in the dams. The NOEL for reproductive (offspring) and systemic (parental) toxicity is the same and is 10 mg/kg/day.

f. Mutagenicity

A series of mutagenicity studies including an *in vitro* forward mutation assay (mouse lymphoma cells), *in vitro* chromosome aberration assay (Chinese hamster ovary 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.

g. Metabolism

There were no major differences between the bioavailability, routes of excretion, or metabolism of ^{14}C-XDE-105 (Factor A) and ^{14}C-XDE-105 (Factor D) in Fischer 344 rats following oral administration as a suspension of 100 mg/kg body weight. The major elimination route was fecal excretion for both Factors. Approximately 80% (Factor A) and ~66% (Factor D) of the dose was absorbed with ~20% (Factor A) and ~34% (Factor D) of the dose eliminated unabsorbed in the feces. By 48 hr post-dosing, >60% (Factor A) and >80% (Factor D) of the administered dose had been recovered in the urine and the feces. Based on the terminal half-lives for fecal and urinary excretion the elimination half-life for Factor A ranged from 25 to 42 hr and the half-life for Factor D ranged from 29 to 33 hr. The tissues and carcass contained very low levels of radioactivity at 168 hr post-dosing, <0.1% of the administered dose per gram of tissue. The primary fecal, urinary and the biliary metabolites were identified as the glutathione conjugates of the parent and N- and O-demethylated XDE-105. The absorption, distribution, metabolism and elimination of ^{14}C-XDE-105 were similar for Factors A and D, and demonstrated no appreciable differences based on dose or repeated dosing (MRIDs 43701508, -09, -10, and -11).

2. Dose Response Assessment

The toxicological database for Spinosad was evaluated by the Reference Dose (RfD) Peer Review Committee and the Toxicology Endpoint Selection Committee (TESC) within HED. The RfD Peer Review Committee comprehensively evaluates the toxicological database for a pesticide chemical and establishes the RfD for the chemical. It also operates as the HED

quality assurance unit with respect to the acceptance or rejection of toxicological data for regulatory purposes, and determines whether a chemical has been sufficiently tested to evaluate its carcinogenic potential and its effects on developmental and reproductive parameters. The RfD Peer Review Committee classifies the "negative" chemicals with respect to carcinogenicity and/or developmental and reproductive effects, and refers suspect chemicals either to the Cancer or Development and Reproductive Effects Peer Review Committees.

The TESC considers the available toxicology data for a pesticide chemical and identifies what toxicological endpoint (if any) and dose level of concern should be used for: 1) an acute dietary risk assessment, 2) a short-term occupational or residential exposure (1 to 7 days) risk assessment, 3) an intermediate-term occupational or residential exposure (1 week to several months) risk assessment, and 4) a chronic (non-cancer) occupational or residential exposure risk assessment.

a. Reference Dose

The HED RfD Committee met on August 1, 1996 to assess the toxicological database for Spinosad. As a result of that meeting the RfD was set as 0.0268 mg/kg/day based on a NOEL of 2.68 mg/kg/day and a 100-fold uncertainty factor (based on uncertainties assoeiated with inter- and intraspecies extrapolations). This NOEL was chosen from the toxicological studies summarized earlier in this document, and is the NOEL established from the 2-year chronic feeding study using dogs as test animals.

As of the date of this assessment, Spinosad has not been reviewed by the World Health Organization (WHO).

b. Carcinogenicity Classification

Additional data need to be evaluated to clarify the classification of the carcinogenic potenital of Spinosad.

c. Other Toxicological Endpoints

The TESC met on August 6, 1996 to consider the available toxicology data for Spinosad. During that meeting toxicology endpoints and dose levels of concern for use in risk assessments were identified and are summarized below.

Limited dermal absorption is expected to occur based on the chemical structure of the active ingredient and the lack of dermal or systemic toxicity at the limit-dose tested (1000 mg/kg/day) in a 21-day dermal study. If there is a need for a chronic risk assessment, a factor of no greater than 10% should be used for dermal absorption.

No appropriate acute dietary endpoint was selected. The NOEL from an acute neurotoxicity study conducted on rats was $\geq$ 2000 mg/kg/day. No maternal or developmental toxicity was seen at $\geq$ 50 mg/kg/day in a developmental toxicity study in rabbits.

No appropriate short-term or intermediate-term occupational or residential endpoints were identified. The combination of the molecular structure and size, and the lack of dermal or systemic toxicity at 1000 mg/kg/day in a dermal toxicity study indicate lack of dermal absorption for Spinosad.

The NOEL from the one year chronic feeding study (2.68 mg/kg/day) was selected as the toxicity endpoint for chronic occupational or residential risk assessment. The NOEL was based on decreases in body weight gain, alterations in clinical chemistry parameters and histopathological lesions of the thyroid gland observed at 8.22 mg/kg/day (LOEL). However, this assessment was not done since there are no chronic occupational or residential exposure scenarios.

Exposure via inhalation is not a concern. The LC_{50} is > 5.18 mg/L. This places Spinosad in toxicity category IV.

3. Dietary Exposure and Risk Characterization

a. Dietary Exposure

i. Plant Metabolism

A plant metabolism study on cotton was submitted by the petitioner. Two different substances were used: Factor A and Factor D. Both were uniformly radio-labelled in the macrolide ring with ^{14}C. The test substances were applied to cotton plants. Factor A was applied at 4.75X the maximum seasonal rate, and Factor D was applied at 10X the maximum seasonal rate. Cotton leaves and bolls were collected and the boll samples were ginned. Total radioactive residues (TRR) were determined in separated seed, leaf, and fiber (hulls and attached lint) by combustion and analyzed with liquid scintillation counting (LSC).

Cottonseeds (lint and trash removed) treated with Factor A were extracted with various solvents, acid and base extractions, subjected to enzymolysis, and acid and base hydrolysis, purified and analyzed by HPLC, MS, and NMR. The extensive extraction procedures used on the seed resulted in eight (8) fractions containing radioactivity for analysis. In summary, no parent material (Factor A or D) or any closely related metabolites (standards were available for Factors B, H, J, K, and pseudoaglycone) were found in any of the major seed fractions. Characterization work performed on the seed concluded that ^{14}C from the radiolabelled test material had become incorporated into the fatty acids comprising cottonseed oil; HPLC analysis showed ^{14}C was associated with the bromophenacyl derivatives of linoleic and oleic/palmitic acids present in the one of the fractions.

The radioactivity associated with various fractions (extracts) accounts for 55% of the TRR. This radioactivity has been shown to be incorporated into or associated with natural products. The remaining 45% of the radioactivity associated with seed fractions was aqueous soluble and shown to be highly polar. The characterization work done suggests that the residues were natural product related but could not definitively distinguish between the possibility of highly degraded parent metabolites (resulting from cleavage of the macrolide portion of the

molecule) and minor natural product constituents. No metabolites containing the intact macrolide ring were found.

Cottonseeds treated with Factor D were extracted similarly and the collected fractions analyzed with the same techniques; the results were the same. The results from the distribution of the radioactivity in the various components between Factors A and D were very similar. No parent compound or metabolites containing the intact macrolide ring were found. Characterization work revealed that ^{14}C from the radiolabeled test material had become incorporated into the fatty acids comprising cottonseed oil.

Other metabolism studies (ruminant and poultry) reveal that the macrolide portion of the XDE-105 molecule is relatively resistant to cleavage. However, photolysis studies have shown that XDE-105 is susceptible to breakdown ($t_{½}$ on leaf surfaces is about 3.4 hours). Therefore, the registrant proposes that the initial metabolism of XDE-105 on the cotton plant occurs first through photochemical degradation of the macrolide ring (by ring cleavage or reduction of the double bonds). It may then be further metabolized by the plant itself or by microorganisms present on the leaf surfaces. The registrant believes that the metabolism progresses to a point where small radiolabeled carbon fragments are produced which pass into the carbon pool and then into various natural plant constituents.

HED concludes that for the purposes of this Section 3 registration on cottonseed, only, the nature of the residue is understood and that the residue of concern in plants are the parent compound (Factors A and D).

ii. Animal Metabolism

Goat Metabolism: The results from the goat metabolism study show that residues of XDE-105 were detectable in tissues and milk from animals fed 9 to 10 ppm of Spinosad (Factors A and D) in their diet. The transfer of XDE-105 residues tend to be higher in fattier tissues (fat and liver). Most of the radioactivity was readily extractable (was not extensively conjugated). The parent compound was the major metabolite found in tissues (fat, muscle, kidney, and liver) and milk from goats fed either Factor A or Factor D.

In the metabolism of both Factor A and Factor D, the proposed pathways involved either the loss of a single methyl group from the N-methyl moiety on the foroamine sugar and/or the hydroxylation of the macrolide at several different positions.

Hen Metabolism: The identification work performed on the hen was not as thorough as with the goat. The results from the hen metabolism study show that residues of XDE-105 were detectable in tissues and eggs from animals fed 9 ppm Spinosad (Factors A and D) in their diet. The transfer of XDE-105 residues tend to be higher in fattier tissues (fat and liver). Most of the radioactivity was readily extractable (was not extensively conjugated). The parent compound was the major metabolite found in tissues (fat, muscle, and liver) and eggs from hens fed Factor A. The parent compound was the major metabolite found in fat and muscle from hens fed Factor D (the parent compound was a secondary residue in liver and eggs).

In the metabolism of both Factor A and Factor D, the two primary pathways involved either the loss of a single methyl group from the N-methyl moiety on the foroamine sugar and/or the loss of one or two methyl groups from the O-methyl moieties on the trimethyl rhamnose sugar. A third pathway which was relatively minor in comparison to the other two involved the loss of the forosamine sugar.

For the purposes of this conditional, time-limited Section 3 tolerance request on cottonseed only, the nature of the residue in animals (ruminants and poultry) is adequately defined. The residue of concern is the parent compound only (Factors A and D).

iii. Analytical Methods-Plants

In the proposed enforcement method for residues of Factors A and D in cottonseed, crop samples (cottonseed, meal, hulls, crude oil, refined oil, and soapstock) are ground prior to extraction. Samples are extracted with either 60% hexane/40% acetone (cottonseed, meal, or hulls), hexane (cottonseed oil), or methylene chloride (soapstock). The extracts are purified by liquid-liquid partitioning and silica solid phase extraction. Factors A and D are determined simultaneously by HPLC using a reverse phase column (ODS-AQ) with a UV detector at 250nm. To confirm the residue, the sample is injected into the HPLC using a different column (C_8 cation), solvent system, and/or wavelength (235, 250, or 275 nm).

Method recoveries from cottonseed, meal, hulls, crude and refined oil, and soapstock fortified at 0.01 to 0.10 ppm with Factors A and D ranged from 85 to 102%. The method was successfully validated by an independent laboratory whose fortified cottonseed samples showed recoveries of 79 to 95% after fortification at 0.01 to 0.05 ppm with Factors A and D. The proposed enforcement method has also undergone a successful petition method validation (PMV) at the EPA Beltsville Laboratory. The proposed enforcement method meets the requirements of OPPTS Test Guidelines Series 860 for Residue Analytical Methods (860.1340) for the determination of residues of Factors A and D on cottonseed.

iv. Analytical Methods-Animals

HED has determined that for the purposes of this conditional, time-limited Section 3 tolerance on cottonseed, tolerances for animal commodities are unwarranted. No analytical method for animals has been presented as a proposed enforcement method. If the results of a potential, future ruminant or poultry feeding study indicate the need for meat, milk, poultry, or egg tolerances, independent lab validation of the analytical method for analyzing these products will be required.

v. Storage Stability

The registrant has provided storage stability data showing recoveries of Spinosad (Factors A and D) for durations up to 283 days. Factor A recoveries ranged from 79 to 110%, Factor D recoveries ranged from 62 to 113%. With the exception of the exaggerated rate trials from Fresno, CA and Burdette, MS, the field trial residue samples were stored frozen for a maximum of 58 days. Field samples from the 2 exaggerated trials mentioned were stored

frozen 331 and 333 days (respectively) from harvest to analysis. The samples from the exaggerated rate trials were not used to establish raw agricultural commodities or processed commodity tolerances.

vi. Magnitude of the Residue: Crop Field Trials/Processed Commodities

The registrant conducted residue trials at 19 sites in 9 states in 1992 and 1993. The trials were conducted using tractor-mounted or backpack compressed gas sprayers and spray volumes of 11 to 30 gallons per acre. Application rates varied from 75 and 200 g.ai./ha., with 5 applications at 6 to 16 day intervals between applications, 14 to 28 day PHIs. The formulations used to generate the field trial residue data were the same suspension concentrate formulations that are proposed on the label (about 44% XDE-105). Sample analyses were performed by DowElanco Laboratories in Indianapolis, Indiana. Residues of Factors A and D were mostly non-detectable in cottonseed from the field trials conducted at a 1X rate. Samples from three field trials had 0.001, 0.002, and 0.003 ppm of Factors A and D. HED proposes a time-limited, conditional tolerance of 0.02 ppm for residues of Spinosad (Factors A and D) on cottonseed. A residue field trial study for Spinosad residues on cotton gin byproducts is required as a condition of registration.

A processing study was conducted using cotton seed grown and harvested from the exaggerated field trial conducted in Wayside, MS (from Table 15 above). Samples were generated from 5 applications of 454 and 842 g. a.i./ha. (average of 617; final application of 842; and total of 3085 g. a.i./ha.) and a 28-day PHI. The resulting total application of 3085 g. a.i./ha. is about 6X the proposed maximum label rate. Cottonseed samples were obtained by simple ginning of the combine-harvested cotton bolls. Samples were shipped frozen from DowElanco to the Food Protein Research and Development Center at Texas A&M University. Cottonseed was delinted, then dehulled; the resulting kernels were heat-expanded and flaked, then hexane-solvent extracted, and the flakes (meal) desolventized; the crude oil was refined with sodium hydroxide and the solvent evaporated to recover the oil and the soapstock. Cottonseed and processed samples (hulls, meal, crude oil, refined oil, and soapstock) were analyzed using the proposed enforcement analytical method (as discussed under the Analytical Method section). Residues of Factors A and D were determined by HPLC/UV, with a LOD of 0.003 ppm and LOQ of 0.01 ppm. Residues of Spinosad (Factors A and D) do not concentrate in commodities processed from cottonseed.

vii. Magnitude of the Residue: Meat, Milk, Poultry and Eggs

Requirements for a poultry feeding study have been waived based on the minimal impact of Spinosad residues on cotton on a typical poultry diet, and an extrapolation of the Spinosad residues present in tissues and eggs from the poultry metabolism study to a 1X feeding level in the poultry diet. Extrapolated residues are expected to be below the limit of detection (0.01 ppm) for the analytical method used in the metabolism studies for the determination of Spinosad residues in animal tissues. The requirement for a ruminant feeding study is reserved pending the results of the field trial for cotton gin byproducts. Residues of Spinosad on cottonseed, meal, and hulls in the ruminant diets used in the animal feeding studies were calculated to be >700X of the actual residues expected in the diets. The total

dietary burden for ruminants from the use of Spinosad on cotton cannot be determined until the residue trial data for cotton gin byproducts are reviewed. However, based on an extrapolation of residues of Spinosad observed in tissues in the ruminant metabolism study to a 1X feeding level, secondary residues in ruminant tissues and milk are expected to be negligible. The total dietary burden of Spinosad residues from cotton uses on the ruminant diet will be recalculated upon receipt of the cotton gin byproducts field trial residue data.

viii. Rotational Crops: Confined and Limited Field Studies

A confined rotational crop study was conducted using wheat, lettuce and radish sown 30, 120, and 365 days post application and grown to maturity. The study was conducted using ^{14}C XDE-105 Factor A, which was uniformly labeled in the macrolide portion of the molecule, similar to the plant and animal metabolism studies. ^{14}C XDE-105 Factor A was sprayed over sandy loam soil in boxes measuring 2.5'X 3'X 2' at the rate of 1100g./ha., or about **2.2X the proposed maximum seasonal label rate**. The boxes were located outdoors and were aged for periods of 30, 120, and 365 days before being moved indoors (greenhouse) prior the rotational crops being planted. No crops were grown in the soil during the ageing period (any weeds that sprouted during the ageing process were pulled and discarded). The rotational crops were planted in separate pots containing either treated or non-treated soil. One control plot (containing all three crops) and two treated plots (one containing wheat only, and the other containing ½ lettuce and ½ radish) were planted at each interval. Plants were fertilized, watered, and fungicides/insecticides applied as needed. Growth rates and harvest schedules for the crops in this confined rotational crop study appeared to follow typical field growth rates.

Crop samples containing radioactive residues greater than 0.01 ppm Factor A equivalents were subjected to a preliminary extraction/fractionation procedure. Plant matrix/solvent combinations that exhibited > 0.01 ppm radioactivity were subject to additional analyses. No detectable Factor A was observed in any of the rotational crop matrices. The only Factor A related residues observed in any of the rotational crop matrices were two radiolabeled components that were detected in the methylene chloride fraction from the 120 DAT straw. The XDE-105 molecule is metabolized to the point where it enters the general carbon pool and is incorporated into various natural plant constituents. The parent compound does not appear to be taken up and/or translocated within the plants tested. Neither rotational crop tolerances nor limited field trials are necessary for this conditional, time-limited tolerance for cottonseed.

b. Dietary Exposure from Drinking Water

HED does not have available data to perform a quantitative risk assessment for Spinosad at this time. Although data indicate little potential for soil mobility or leaching, Spinosad is persistent in the environment. Therefore, water risks will be assumed to account for 10% of the total allowable chronic and acute risk until further data are provided. Based on analysis of water monitoring data for a large number of pesticides with varying toxicities, soil mobility characteristics, environmental stabilities, physical/chemical properties, and toxicities, the assumption of 10% of the total acute and chronic risk allocated to drinking

very for Spinosad

water is considered conservative and protective of the public health.

c. Dietary Risk Assessment and Characterization

The tolerance proposed in the permanent petition 6G04692, 0.02 ppm, to cover residues of Spinosad on cottonseed resulting from its use on cotton was used to perform a chronic dietary analysis. The analysis also assumed that 100 percent of the cotton crop was treated with Spinosad. Specifically, two commodities processed from cottonseed: cotton seed oil and cottonseed meal were included in the dietary risk assessment. Tolerance level residues on the oil and meal were assumed; however, HED notes that Spinosad residues do not concentrate in processed commodities, and therefore, this risk estimate is very conservative. The resultant dietary exposure estimated the Theoretical Maximum Residue Contribution (TMRC) for the general population and 22 subgroups.

The chronic dietary exposure analysis did not include tolerances for secondary residues of Spinosad in ruminant and poultry tissues, milk, and eggs. With respect to residues of Spinosad in poultry tissues and eggs from cotton uses, HED considers this to be a 180.6(a)3 situation. That is, there is no reasonable expectation of finite residues of Spinosad in poultry tissues and eggs from cotton uses. Additional registrations for Spinosad on other poultry feedstuffs may impact this categorization. Residue data from field trials for cotton gin byproducts and ruminant feeding studies with diets based on the resultant Spinosad residues on all cotton feedstuffs are not available at this time. However, extrapolation from existing ruminant metabolism studies indicate that secondary residues of Spinosad in ruminant commodities are expected to be negligible. The chronic dietary analysis for Spinosad will be reevaluated when the required cotton gin byproducts field trial data are received and reviewed and a total dietary burden for ruminants from Spinosad residues on cotton feedstuffs can be calculated.

Chronic Exposure Analysis - Exposure from Proposed Tolerances

Subgroup	Exposure (mg/kg/day)	%RfD
U.S. Population	0.0000	<1%

The chronic dietary risk estimated above for the general U.S. population represents the chronic dietary risk estimated for all of the 22 population subgroups. This dietary risk will be reevaluated with respect to secondary residues in ruminant tissues and milk upon receipt of the outstanding field trial data for cotton gin byproducts. Even considering additional exposure through secondary residues in meat and milk, the chronic dietary risk from the use of Spinosad on cotton appears to be minimal.

4. Occupational/Residential Exposure and Risk Characterization

Estimating risk is based on two components-hazard and exposure. HED does anticipate there will be occupational exposure resulting from the use of Spinosad on cotton. However, as discussed, the TESC selected no toxicity endpoint of concern for short-term and

intermediate-term occupational or residential exposure. Therefore, short-term and intermediate-term occupational or residential risk assessments are not required.

The TESC did select a toxicity endpoint for chronic (non-cancer) exposure to be used in a risk assessment only if there is concern for chronic exposure. HED has determined that Spinosad use on cotton does not pose a chronic exposure scenario for workers. There are no registered residential uses for Spinosad. Homeowners are not expected to be exposed to Spinosad residues.

5. Aggregate Exposure/Risk

Based on the available data and worst-case assumptions used for dietary/water/residential exposure and risk estimates, the general U.S. population group is representative of the most exposed population, with a risk estimate from combined sources of approximately 10% of the RfD (specifically, food: <1%, water: 10%).

6. Determination of Safety for Infants and Children

The toxicological database for evaluating pre- and postnatal toxicity for Spinosad is mostly complete. Available data indicate that no developmental toxicity was observed in the rabbit study at the highest dose tested (≥50 mg/kg/day). Slight maternal toxicity was observed in the rabbit at the highest dose tested and consisted of marginal reductions in body weight gain, defecation, and food consumption. In the rat developmental study, a slight one-day reduction in maternal body weight gain and body weight was observed at the highest dose tested, but otherwise no developmental or maternal toxicity was observed at a high dose level (≥200 mg/kg/day). 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).

Reproductive toxicity appears to be related to systemic maternal toxicity, and was characterized by decreases in mean litter size and body weight throughout lactation. The NOEL for reproductive and systemic (parental) toxicity is 10 mg/kg/day. These data taken together suggest minimal concern for developmental or reproductive toxicity and do not indicate any increased pre- or postnatal sensitivity in the offspring; no additional uncertainty factor for increased sensitivity in infants and children is appropriate.

7. Common Mode of Toxicity

Spinosad is a unique insecticide structurally unrelated to other registered pesticides. The Agency has not made a determination whether Spinosad and any other pesticide have a common mode of toxicity and require cumulative risk assessment. For the purposes of this tolerance and registration application, the Agency has considered only risks from Spinosad. If required, cumulative risks will be assessed as part of Reregistration and tolerance reassessment, and when methodologies for determining common mode of toxicity and for performing cumulative risk assessment are finalized.

DATA REQUIREMENTS - WHICH MUST BE SATISFIED PRIOR TO A SECTION 3 REGISTRATION

A. Toxicology

Additional data are required with respect to a conditional registration for Spinosad use on cotton, and a time-limited tolerance for residues of Spinosad on cottonseed. The chronic feeding study in rats is considered unacceptable, but may be upgraded to an acceptable status with additional histopathology data from the high dose and control group female animals that either died or were sacrificed during the study (MRID 43701507). The oncogenicity study in mice is considered supplemental and does not satisfy guideline 83-2, but may be upgraded pending review of additional details on the incidence of hemangiomas in female mice and a second mouse oncogenicity study.

B. Residue Chemistry

Residue data from a field trial for Spinosad residues on cotton gin byproducts are required. The residue field trials should include at least 3 field trials for each type of harvesting (stripper and picker) for a total of at least 6 field trials. The registrant has indicated their intention to provide these to the Agency data by June 30, 1997.

The requirement for a ruminant feeding study is reserved pending the results of the cotton gin byproduct field data. Note: the registrant has already conducted this study and intends to submit it in the future with other pending registration requests.

LABELING REQUIREMENTS

A final Section B should be submitted that incorporates the proposed maximum seasonal rate of 0.45 lbs. a.i./A and a 28-day pre-harvest interval (PHI).

REFERENCES

Gardner, R., 1995. Preliminary Review of mammalian Toxicology Data Submitted by DowElanco to Support an EUP for Use of Spinosad on Cotton as an Insecticide. May 31, 1995.

Gardner, R., 1996. Toxicology Endpoint Selection Document - Spinosad. September 13, 1996.

Herndon, G. J., 1996. PP# 6G04692. Section 5 registration (62719-EUP-GE) and Temporary Tolerance Petition for Use of Spinosad or XDE-105 (End-Use Product named TRACER®) on Cotton; Evaluation or Analytical Method and Residue Data. Memo to George LaRocca/Adam Heyward, PM 13. April 24, 1996.

Herndon, G. J., 1996. PP# 6F04735: Section 3 Registration and Permanent Tolerance Petition for Use of Spinosad or XDE-105 (End-Use Product Named TRACER®) on Cotton; Registrant's Response to Deficiencies Outlined in memo of G.J. Herndon Dated 4/24/96 Concerning PP#6G04692. Memo to George LaRocca/Adam Heyward, PM 13. June 17, 1996.

Herndon, G. J., 1996. PP#6G04692 and PP#6F04735. Spinosad (XDE-105) on Cotton Commodities. Results of the Petition Method Validation Request. Memo to George LaRocca/Adam Heyward, PM 13. August 13, 1996.

Griffin, R. 1996. Dietary Exposure Analysis for Spinosad in Cottonseed Meal and Oil. Memo to George LaRocca/Adam Heyward, PM 13. December 31, 1996.