6G4692

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

APR 24 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: 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 of Analytical Method and Residue Data.
(17 vol.)
MRID#s: 434503-01 through -06
and 437274-01 through -11.
DP Barcodes#: D219016, D224608, D223898, D223899.

From: G. Jeffrey Herndon, Chemist
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

Through: Michael Metzger, Acting Chief
Risk Characterization and Analysis Branch
Health Effects Division (7509C)

To: George LaRocca/Adam Heyward, PM Team 13
Insecticide-Rodenticide Branch
Registration Division (7505C)

The petitioner, DowElanco, is proposing a temporary tolerance of 0.02 ppm be established for the residues of the insecticide Spinosad (and designated by the company code XDE-105) from the proposed Section 5 (EUP) use on cotton.

Spinosad (the proposed common name for XDE-105) is a fermentation-derived product produced by Saccharopolyspora spinosa. The product consists of two related active ingredients: Factor A (CAS# 131929-60-7) or
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
and Factor D (CAS# 131929-63-0) or
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-methyl-

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1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione.

The two Factors differ by one methyl group.

The product designated by the company code NAF-144 is the killed microbial raw fermentation end-use product containing about 2.6% active ingredient. This product is not the subject of PP#6G04692; the company intends to register this product on crops other than cotton. 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.

This is the first tolerance request for this chemical.

RCAB defers the review of end-use products to Registration Division.

Conclusions

1. Data in this petition were not generated by Craven Laboratories.

2. The product chemistry data submitted with this petition are adequate to fulfill both the requirements for this EUP/temporary tolerance request, and a future Section 3/permanent tolerance request.

3. The proposed label satisfies the requirements of this EUP/temporary tolerance request.

4. For the purposes of this EUP/temporary tolerance request, the nature of the residue in cotton is adequately defined. The residue of concern is the parent compound only (Factor A + Factor D). The HED Metabolism Committee will determine which residues are of concern for a Section 3/permanent tolerance request.

5. For the purposes of this EUP/temporary tolerance request, the nature of the residue in animals (ruminants and poultry) is adequately defined. The residue of concern is the parent compound only (Factor A + Factor D). The HED Metabolism Committee will determine which residues are of concern to support the meat, milk, poultry, and egg tolerances associated with a Section 3/permanent tolerance request on cotton.

6a. The submitted analytical method and recoveries appears to be adequate. Therefore, RCAB will recommend that EPA lab validation be initiated.

6b. The results of the multi-residue testing will be sent to

the EPA ACB lab and FDA.

6c. The petitioner should submit Spinosad Factor A and Factor D standards, as well as the accompanying material safety data sheets (MSDS) to the EPA repository.

Attn: Terry Bundy
EPA Chemical Standards Repository (MD8)
2 Triangle Drive
Research Triangle Park, NC 27711

These standards, as well as a sample of the technical grade active ingredient, should also be sent to the EPA Beltsville laboratory.

Attn: Harvey Hundley, Lab Chief
Analytical Chemistry Laboratory
Building 306, BARC East
Beltsville, MD 20705

6d. 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.

7. The registrant has shown that spinosad Factors A and D are stable in frozen cottonseed for the durations that the field residue samples were stored (the two exaggerated studies that involved durations longer than 283 days were not used in the determination of RAC or processed commodity tolerances).

8a. The registrant has proposed a temporary tolerance on cotton seed at 0.02 ppm for the combined residues of spinosad (Factor A + Factor D). Based on the residue data provided, this temporary tolerance should be adequate to cover residues from the proposed use.

8b. No residue data were provided on cotton gin byproducts. As noted in Table II (September 1995), cotton gin byproducts are a cotton RAC that comprises up to 20% of the diet of beef and dairy cattle. For the purposes of this EUP/temporary tolerance request, a tolerance for cotton gin byproducts will not be necessary. As noted in Table II (September 1995), for a future Section 3/permanent tolerance request, at least 3 field trials for each type of harvesting (stripper and mechanical picker) will be needed, for a total of 6 field trials.

8c. The results of the processing study indicate that residues of spinosad do not concentrate in processed cottonseed commodities. Therefore, no temporary processed commodity tolerances are needed.

9a. The results of this confined rotational crop study support the results of the cotton metabolism study. 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.

9b. For the purposes of this EUP/temporary tolerance request, temporary rotational crop tolerances will not be established. Pending review of the results of the cotton metabolism and confined rotational crop studies by the HED Metabolism Committee, rotational crop field studies and permanent rotational crop tolerances will not need to be established to support a future Section 3/permanent tolerance request.

10a. For the purposes of this EUP and temporary tolerance petition, RCAB will accept the waiver of a hen and cow feeding study. Tolerances for residues of spinosad on meat, milk, poultry, and eggs will not be necessary for this Section 5/temporary tolerance petition.

10b. Provided that the maximum residues in cottonseed meal and other poultry feed items that may be treated with spinosad remain at or below 0.01 ppm, a poultry feeding study will not be required for a future Section 3/permanent tolerance request.

10c. Due to the higher dietary burden in cattle (more feed items), the need for including cotton gin byproducts into the theoretical diet of cattle, and the fact that the estimation of residues in cattle products involves an extrapolation across species (goat vs. cow), we do not consider a waiver of the cattle feeding study appropriate for a future Section 3/permanent tolerance request. Therefore, a feeding study using dairy cattle should be conducted as outlined in the Subdivision O Guidelines to determine if tolerances are needed for residues in livestock (excluding poultry) commodities.

11. No Codex, Canadian, or Mexican tolerances are established for spinosad. No compatibility problem exists between the proposed U.S. and Codex tolerances.

Recommendations

TOX considerations permitting, RCAB has no objections to the issuance of this EUP for use of spinosad (TRACER®) on cotton. In conjunction with this Section 5 (EUP) registration, a temporary tolerance should be established for the combined residues of spinosad (Factors A + D) on cottonseed (RAC) at 0.02 ppm.

For a future Section 3 registration and permanent tolerance request, the registrant will need to address the deficiencies outlined in Conclusions 6c, 8b, and 10c, in addition to any

potential issues raised by the HED Metabolism Committee. The analytical method will also need to be validated by the EPA laboratory (Conclusion 6a).

Detailed Considerations

Product Chemistry

The review of the submitted product chemistry data for the technical spinosad product is appended to this review as Attachment II and Attachment III (confidential appendix containing CBI)].

Comments

The product chemistry data submitted with this petition are adequate to fulfill both the requirements for this EUP/temporary tolerance request, and a future Section 3/permanent tolerance request.

Proposed Use

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

For control of tobacco budworm, cotton bollworm, cotton leafperforator, European corn borer, loopers, saltmarsh caterpillar, and armyworms in cotton, apply NAF-85 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. NAF-85 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). NAF-85 should not be applied to consecutive generations of tobacco budworm or cotton bollworm. However, multiple applications of NAF-85 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.

Comments

The proposed label satisfies the requirements of this EUP/temporary tolerance request.

Nature of the Residue

Metabolism in Plants

"^{14}C XDE-105 Cotton Nature of Residue Study", J.D. Magnussen, 8/8/94, Doc.# MET91063 (MRID# 437274-03)

and

"Characterization of the Residues in Seed from a 30-Day PHI ^{14}C XDE-105 (Factor A) Cotton Nature of Residue Study", J.D. Magnussen and S.A. Castetter, 4/27/95, Doc.# MET94033 (MRID# 437274-04)

Set-up

A plant metabolism study on cotton was submitted. Two different test substances were used: ^{14}C Factor A and ^{14}C Factor D. Both Factors were uniformly labeled in the macrolide portion of the molecule and were produced by fermentation using the actinomycete Saccharopolyspora spinosa. The specific activity of Factor A was 5.11 µCi/mg, Factor D was 5.12 µCi/mg.

The cotton plants (variety DPL-90) were field-grown at the DowElanco Research Farm in Wayside, MS. The crop was planted 6/7/91 and received the normal spectrum of chemicals and pesticides that are typically used on cotton grown in the Delta region. Due to adequate rainfall, no irrigation was necessary.

Application

Factor A

A total of 5 over-the-top spray applications were made to the Factor A plot at approximately 7 day intervals prior to the opening of any of the bolls on any of the plants. For each application, ^{14}C Factor A was applied at the rate equivalent of 0.345 lb./A. for a seasonal total of 1.725 lb./A.. This rate is 3.9X the proposed application rate and **4.75X the maximum proposed seasonal rate** (taking into account that the commercial product is 80% Factor A). The test substance was mixed with unlabeled Factor A (to give a specific activity of 4.0 µCi/mg), diluted with water, and applied using a CO_2 back pack sprayer.

Factor D

A total of 5 over-the-top spray applications were made to the Factor D plot at approximately 7 day intervals prior to the opening of any of the bolls on any of the plants. For each application, ^{14}C Factor D was applied at the rate equivalent of 0.178 lb./A. for a seasonal total of 0.890 lb./A.. This rate is 8X the proposed application rate and **10X the maximum proposed seasonal rate** (taking into account that the commercial product is 20% Factor D). The test substance was diluted with water and applied using a CO_2 back pack

sprayer.

Harvest

Samples of cotton leaves and bolls (seed + fiber) were harvested and the bolls ginned.

Storage

The samples were sent to the DowElanco Research Laboratories in Greenfield, ID (and later to Indianapolis, ID) and stored frozen until extraction/analysis.

Initial Analysis

Portions of the seed samples were fractured to separate the lint trash (portions of the hull with attached lint) from the coarse cottonseed meal. Total radioactivity in the prepared seed, leaf, and fiber samples both before and after extraction was determined by combustion and analyzed by liquid scintillation counting (LSC). The results are shown in Table 1.

Table 1

Results of Combustion Analyses on Treated Cotton Seed and Fiber

	ppm (parent equivalents)	
	Factor A	Factor D
cotton seed	0.289	0.113
cotton fiber	0.216	0.075

Extraction, Fractionation, and Analysis of Radioactive Residues

Cotton fiber

The cotton fiber samples were subjected to acid detergent, hydrolysis, and reacted with phenylhydrazine. The identities of the resulting glucozone derivatives were confirmed by MS and NMR. Nearly 100% of the total radioactivity from this fraction (from combustion) was accounted for in this cellulose fraction (a glucose polymer).

Cottonseed

Portions of the purified cottonseed (lint trash removed) extracted in methylene chloride. Various solvents (water, hexane, and ethanol), techniques (saponification, acid and base extractions, enzyme hydrolysis, acid and base hydrolysis, and

dialysis) , purification columns (silica gel, C_{18}, and cation exchange) were used to separate the radioactivity into oil, protein, ethanol soluble, water soluble, and lignin/cellulose fractions. Identification and confirmation work utilized HPLC, MS, and NMR. The results for the Factor A treated seed are shown in Table 2.

Table 2

Summary of Cotton Seed Characterization Results for **Factor A** Treated (4.75X) Seeds

component	% of total seed residue	ppm
oil fraction (#1)	31.6	0.091
water soluble proteins (#2)	4.5	0.013
storage proteins (#3)	10.6	0.031
acid detergent fiber from extracted meal (#4)	8.6	0.025
ethanol aqueous fraction (#5)	18.6	0.054
water soluble aqueous after protein precipitation (#6)	9.9	0.029
storage protein aqueous after protein precipitation (#7)	1.0	0.003
acid hydrolysate aqueous from extracted meal (#8)	10.2	0.029

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 components (refer to Attachment I for the names and structures of the compounds). Characterization work performed on the seed concluded that ^{14}C from the radiolabeled 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 acid in component #1.

Less definitive characterization work (using acid/base organic extraction, enzyme hydrolysis, and dialysis) also suggests that additional radioactivity had been incorporated into one of the primary protein fractions of cottonseed meal (components #2, 3, and 4). Acid detergent fiber by definition consists almost exclusively of cellulose and lignin. Therefore, the radioactivity found in component #4 should be due to incorporation into the glucose subunits that make up the cellulose molecules. This conclusion is substantiated by the MS and NMR work performed on the cotton fiber (see above).

In summary, the radioactivity associated with components #1, 2, 3, and 4 account 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 (radioactivity associated with seed components #5, 6, 7, and 8) 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.

Factor D treated seed were also subjected to a similar scheme, although less definitive identification techniques were employed (due to lower levels of radioactivity and similar results to those found in the Factor A treated seeds). The results are shown in Table 3.

Table 3

Summary of Cotton Seed Characterization Results for Factor D Treated (10X) Seeds

component	% of total seed residue	ppm
oil fraction	36.7	0.041
water soluble proteins	1.5	0.002
storage proteins	8.2	0.009
extracted meal	21.4	0.024
ethanol aqueous fraction	17.1	0.019
water soluble aqueous after protein precipitation	10.1	0.011
storage protein aqueous after protein precipitation	2.4	0.003

* - This component would be equivalent to the acid detergent fiber from extracted meal and acid hydrolysate aqueous from extracted meal fractions from the Factor A treated seeds

The extraction/characterization scheme employed with the Factor D cotton metabolism was similar to that used for Factor A. 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.

Overall

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.

Comments

For the purposes of this EUP/temporary tolerance request, the nature of the residue in cotton is adequately defined. The residue of concern is the parent compound only (Factor A + Factor D). The HED Metabolism Committee will determine which residues are of concern for a Section 3/permanent tolerance request.

Metabolism in Animals

Goat and hen metabolism studies were submitted with this petition.

Goat

"^{14}C XDE-105 (Factor A and D) Goat Metabolism Study: Tissues, Milk, Excreta", D.P. Rainey, ABC Laboratories and Dowelanco, 10/17/94, Doc.# MET93083 (MRID# 437274-06)

Set-up and Dosing

Two different test materials were used; one containing ^{14}C-XDE-105 Factor A (specific activity of 3.40 μCi/mg) and ^{14}C-XDE-105 Factor D (3.71 μCi/mg). In both cases, all the carbon molecules in the macrolide ring were radiolabeled. Enough of each of the solutions was added to gelatin capsules in order to have about 25 mg. of ^{14}C-XDE-105 (either Factor A or D) in each capsule.

Three total goats were used; one served as a control (fed placebos), one was fed the ^{14}C-XDE-105 Factor A, and one was fed ^{14}C-XDE-105 Factor D. The goats used for dosing weighed between 43 and 47 kg. each. The animals were fed a grain/alfalfa based diet at the rate of 3 kg./animal/day, and allowed to consume water ad libitum.

The capsules were administered orally at the rate of 1 capsule per day for 3 consecutive days. Based on the average animal weight of 45 kg., this total dose is equivalent to about 1.7 mg./kg.body weight. In terms of feeds, this dose is equivalent to about between 9 and 10 ppm in the feed.

Sample Collection

The animals were milked twice each day and the milk weighed after each milking. Total excretion of urine and feces was collected and weighed once a day.

Within 24 hours after the last dose, the animals were sacrificed and the following samples collected: muscle (longissimus dorsi, semimembranosus, and triceps), liver, kidney, fat (perirenal and omental), the entire rumen with contents, and the entire intestine with contents.

Initial Analysis

The various samples were homogenized and either counted directly by liquid scintillation counting (LSC) or combusted, with the resulting $^{14}CO_2$ counted by LSC. Table 4 lists the results of the combustion analyses of the milk samples in the goat studies. The results of the combustion analyses of the tissue samples in the goat studies are shown in the second lines of Tables 5 and 6.

Table 4

Concentrations of Radioactivity in Milk of Lactating Goats

	μg Equivalents ^{14}C-XDE-105/g	
Collection Time	**Factor A**	**Factor D**
Day 1 - PM	0.256	0.096
Day 1 - AM	0.367	0.100
Day 2 - PM	0.454	0.172
Day 2 - AM	0.535	0.138
Day 3 - PM	0.629	0.202
Day 3 - AM	0.623	0.120

Extraction, Cleanup, and Analysis of Radioactive Residues

Samples of fat, milk, muscle, liver, and kidney were initially homogenized with acetonitrile or acetonitrile/hexane. The organo-soluble extracts were purified on silica solid phase extraction tubes. The water-soluble extracts were purified on C_{18} solid phase extraction columns. The columns were eluted with various solvent mixtures and the eluted fractions analyzed by LSC. The various fractions were analyzed by HPLC using UV and LSC detectors and MS/EI and LC/MS/EI. The results of these analyses are found in Tables 5 and 6 (refer to Attachment I for the names and structures of the compounds).

Table 5

Metabolites Detected by HPLC in Samples from Goats Using ^{14}C-XDE-105 Factor A

	Matrix (Total Radioactive Residue)									
	Fat (TRR = 3.57 ppm)		Muscle (TRR = 0.30 ppm)		Kidney (TRR = 0.97 ppm)		Liver (TRR = 1.58 ppm)		Milk (TRR = 0.63 ppm)	
Component	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm
Factor A	86.0	3.07	50.0	0.15	35.1	0.34	29.7	0.47	71.4	0.45
MET A-Li-1	ND	ND	ND	ND	1.9	0.018	1.5	0.023	ND	ND
Factor B	0.7	0.026	8.3	0.025	10.2	0.099	2.9	0.046	1.9	0.012
MET A-Li-2	ND	ND	ND	ND	ND	ND	3.3	0.052	ND	ND
MET A-Li-3a	2.7	0.095	8.3	0.025	10.4	0.101	7.7	0.122	6.3	0.040
MET A-Li-3b	1.3	0.046	4.0	0.012	6.1	0.059	5.0	0.079	3.8	0.024
MET A-Li-4(5a)	ND	ND	13.3	0.040	15.5	0.15	3.0	0.047	1.6	0.010
MET A-Li-4(5b)	ND	ND	ND	ND	ND	ND	4.1	0.065	1.7	0.011
MET A-Li-4(5c)	ND	ND	ND	ND	ND	ND	4.1	0.064	1.4	0.009
Subtotal	90.7	3.24	84	0.252	79.0	0.767	61.2	0.968	88.2	0.556
Unidentified Extractable	7.5	0.0756	14.7	0.044	20.1	0.195	33.9	0.536	9.1	0.057
Nonextractable	1.6	0.057	1.2	0.004	1.9	0.018	5.7	0.090	2.1	0.013
TOTAL	100	3.56	100	0.30	100	0.97	100	1.58	100	0.63

ND : not detectable

Table 6

Metabolites Detected by HPLC in Samples from Goats Using ^{14}C-XDE-105 Factor D

	Matrix (Total Radioactive Residue)									
	Fat (TRR = 1.82 ppm)		Muscle (TRR = 0.11 ppm)		Kidney (TRR = 0.30 ppm)		Liver (TRR = 0.50 ppm)		Milk (TRR = 0.16 ppm)	
Component	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm
Factor D	84.6	1.54	57.3	0.063	40.0	0.12	20.4	0.102	81.3	0.13
Factor B of D	1.1	0.020	11.8	0.013	15.3	0.046	4.4	0.022	2.5	0.004
MET D-Li-1	2.5	0.046	2.7	0.003	ND	ND	5.6	0.028	ND	ND
MET D-Li-2	ND	ND	ND	ND	ND	ND	2.8	0.014	ND	ND
MET D-Li-3a	ND	ND	ND	ND	3.0	0.009	2.2	0.011	ND	ND
MET D-Li-3b	1.6	0.030	7.3	0.008	12.7	0.038	6.4	0.032	5.6	0.009
Subtotal	89.8	1.64	79	0.087	71	0.213	41.8	0.209	89.4	0.143
Unidentified Extractable	10.2	0.186	23	0.025	26.3	0.079	49	0.243	7.5	0.012
Nonextractable	0.4	0.007	1.3	0.001	2.7	0.008	8.2	0.041	1.7	0.003
TOTAL	100	1.83	103	0.113	100	0.30	99	0.493	99	0.158

ND : not detectable

Hen

"^{14}C XDE-105 (Factor A and D) Poultry Nature of Residue Study", J.D. Magnussen and S.A Castetter, ABC Laboratories and DowElanco, 10/10/94, Doc.# MET93018/MET93107 (MRID# 437274-05)

Set-up and Dosing

Two different test materials were used; one containing ^{14}C-XDE-105 Factor A (specific activity of 4.11 μCi/mg) and ^{14}C-XDE-105 Factor D (3.71 μCi/mg). In both cases, all the carbon molecules in the macrolide ring were radiolabeled. Enough of each of the solutions was added to gelatin capsules in order to have about 0.47 mg. of ^{14}C-XDE-105 (either Factor A or D) in each capsule.

A total of forty (40) laying hens were used; 20 served as controls (fed placebos), 10 were fed the ^{14}C-XDE-105 Factor A, and 10 were fed ^{14}C-XDE-105 Factor D. The animals were fed a laying mash based diet and water *ad libitum*. The quantity of food consumed was calculated on a daily basis (from the weight of feed refusals). The capsules were administered orally at the rate of 2 capsules per day

(1 in the morning, 1 in the evening) for 5 consecutive days. Based on the average animal weight of 1.5 kg., this total dose is equivalent to about 2.7 mg./kg.body weight. In terms of feeds, this dose is equivalent to about 9 ppm in the feed.

Sample Collection

Eggs were collected twice each day and composited. Excreta were collected once a day.

Within 21 hours after the last dose, the animals were sacrificed and the following samples were collected: composite muscle (breast and thigh), liver, kidney, and fat (abdominal).

Initial Analysis

The various samples were homogenized and combusted, with the resulting $^{14}CO_2$ counted by LSC. Table 7 lists the results of the combustion analyses of the egg samples in the hen studies. The results of the combustion analyses of the tissue samples in the hen studies are shown in Table 8.

Table 7

Concentrations of Radioactivity in Eggs of Laying Hens

	μg Equivalents ^{14}C-XDE-105/g	
Collection Time	Factor A	Factor D
Day 1 (first day of dosing)	< 0.007	< 0.006
Day 2	0.014	0.020
Day 3	0.084	0.077
Day 4	0.195	0.150
Day 5 (last day of dosing)	0.333	0.234
Day 6 (eggs laid between Day 5 collection and sacrifice)	0.391	0.336

Table 8

Concentrations of Radioactivity in Various Tissues of Laying Hens

	μg Equivalents ^{14}C-XDE-105/g	
Matrix	Factor A	Factor D
fat	2.19	1.03
muscle	0.124	0.131
kidney	0.607	0.781
liver	0.960	1.82

Extraction, Cleanup, and Analysis of Radioactive Residues

Samples of fat, eggs, muscle, and liver were initially homogenized with acetonitrile, acetonitrile/hexane, or methanol/water. The organo-soluble extracts were purified on silica solid phase extraction tubes. Analysis techniques used included acid hydrolysis, enzyme hydrolysis, and purification on C_{18} solid phase extraction columns. The various fractions were analyzed by TLC and/or HPLC using UV and LSC detectors. The registrant did isolation and identification work using excreta due to limited amounts of key tissue and egg samples. Any metabolites isolated in sufficient quantities were subjected to confirmation by MS. The results are shown in Tables 9 and 10.

Table 9

Metabolites Detected by TLC in Samples from Hens Using ^{14}C-XDE-105 Factor A

	Matrix (Total Radioactive Residue)							
	Fat (TRR = 2.19 ppm)		Muscle (TRR = 0.124 ppm)		Liver (TRR = 0.960 ppm)		Eggs (TRR = 0.391 ppm)	
Component	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm
Factor A	80.5	1.76	54.6	0.064	13.8	0.122	34.3	0.129
Factor B	2.0	0.044	12.0	0.014	11.3	0.100	11.0	0.041
Factor J/PA	1.5	0.033	2.3	0.003	5.5	0.048	3.3	0.012
Factor K/H	4.1	0.090	5.1	0.006	9.3	0.082	9.7	0.037
MET AP-1	ND	ND	ND	ND	8.3	0.073	ND	ND
MET AP-2	ND	ND	ND	ND	4.1	0.036	ND	ND
MET AP-3	ND	ND	ND	ND	2.7	0.024	1.4	0.005
MET AP-4	ND	ND	5.7	0.007	7.8	0.064	4.7	0.018
MET AP-5	ND	ND	ND	ND	2.4	0.021	1.6	0.006
MET AP-6	ND	ND	ND	ND	1.9	0.017	1.1	0.004
Subtotal	88.0	1.927	75.8	0.094	61.1	0.587	64.4	0.252
Unidentified extractable	11.6	0.254	18.5	0.023	34.3	0.329	25.1	0.098
Nonextractable	0.3	0.007	5.7	0.007	5.0	0.044	10.8	0.041
TOTAL	100	2.19	100	0.124	100	0.960	100	.391

ND : not detectable

Table 10

Metabolites Detected by TLC in Samples from Hens Using ^{14}C-XDE-105 Factor D

	Matrix (Total Radioactive Residue)							
	Fat (TRR = 1.03 ppm)		Muscle (TRR = 0.131 ppm)		Liver (TRR = 1.82 ppm)		Eggs (TRR = 0.336 ppm)	
Component	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm	% of ^{14}C	ppm
Factor D	78.9	0.806	39.1	0.048	3.3	0.058	21.5	0.069
Factor B of D	6.8	0.069	14.7	0.018	21.0	0.366	25.0	0.080
Factor J of D	2.4	0.025	ND	ND	ND	ND	ND	ND
Factor H/K of D	6.0	0.061	6.1	0.007	12.2	0.213	8.0	0.026
Pseudoaglycone	ND	ND	1.6	0.002	3.4	0.059	ND	ND
Metabolite DP-1	ND	ND	2.7	0.003	5.2	0.091	ND	ND
Metabolite DP-2	ND	ND	ND	ND	3.9	0.068	ND	ND
Metabolite DP-3	ND	ND	1.5	0.002	6.7	0.117	5.4	0.017
Metabolite DP-4	ND	ND	6.0	0.007	17.7	0.309	11.5	0.037
Metabolite DP-5	ND	ND	2.0	0.002	2.2	0.038	2.6	0.008
Metabolite DP-6	ND	ND	1.2	0.001	2.4	0.042	1.7	0.005
Metabolite DP-7	ND	ND	0.9	0.001	2.5	0.044	1.5	0.005
Metabolite DP-8	ND	ND	0.8	0.001	2.5	0.044	1.4	0.004
Subtotal	93.3	0.961	70.2	0.092	79.6	1.449	74.7	0.251
Uncharacterized Extractable	5.8	0.059	27.5	0.036	16.9	0.308	17.3	0.058
Nonextractable	0.1	0.001	2.2	0.003	3.6	0.063	8.4	0.027
TOTAL	99.1	1.02	100	0.131	100	1.82	100	0.336

ND : not detectable

Comments

Goat Metabolism

The results from the goat metabolism study show that residues of XDE-105 concentrate in tissues and milk. 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 concentrate in tissues and eggs. 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.

Overall

For the purposes of this EUP/temporary tolerance request, the nature of the residue in animals (ruminants and poultry) is adequately defined. The residue of concern is the parent compound only (Factor A + Factor D). The HED Metabolism Committee will determine which residues are of concern to support the meat, milk, poultry, and egg tolerances associated with a Section 3/permanent tolerance request on cotton.

For the purposes of this EUP, regulating animal commodities is not necessary (see section on Meat, Milk, Poultry, and Eggs).

Analytical Method

"Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection", S.D. West, 8/31/94, DowElanco, Doc.# RES94025 (MRID# 437274-07)

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), methylene chloride (soapstock). The extracts are purified by liquid-liquid partitioning and silica solid phase extraction. Factors 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).

The results of DowElanco's method trial recoveries are shown in Tables 11 and 12.

Table 11

Recoveries of XDE-105 **Factor A** from Various Cottonseed Matrices

Matrix	# of Samples	Fortification Range (ppm)	Average Recovery	Standard Deviation
cottonseed	18	0.01 - 0.10	99	14
meal	10	0.01 - 0.10	90	6
hulls	10	0.01 - 0.10	100	10
crude oil	18	0.01 - 0.10	96	7
refined oil	10	0.01 - 0.10	92	10
soapstock	18	0.01 - 0.10	99	4

Table 12

Recoveries of XDE-105 **Factor D** from Various Cottonseed Matrices

Matrix	# of Samples	Fortification Range (ppm)	Average Recovery	Standard Deviation
cottonseed	18	0.01 - 0.10	95	11
meal	10	0.01 - 0.10	85	8
hulls	10	0.01 - 0.10	100	10
crude oil	18	0.01 - 0.10	93	5
refined oil	10	0.01 - 0.10	86	11
soapstock	18	0.01 - 0.10	102	4

The method underwent independent lab validation at A and L Great Lakes Laboratories, Inc.:

"Determination of XDE-105 Insecticide in Cottonseed and Processed Commodities by High Performance Liquid Chromatography with Ultraviolet Detection", S.D. West, 3/27/95, A and L Great Lakes Laboratories, Inc., Doc.# RES95036 (MRID# 437274-08).

The results are shown in Table 13 below.

Table 13

Independent Lab Validation Recoveries of XDE-105 from Cottonseed

Matrix	Fortification	# of Samples	Fortification Range (ppm)	Average Recovery	Standard Deviation
cottonseed	Factor A	4	0.01 - 0.05	95	13
	Factor D	4	0.01 - 0.05	79	2

XDE-105 Factors A and D were subjected to FDA Multi-Residue Testing:

"Multi-Residue Methods Testing for Spinosyns A and D", L. Atkin and H.E. Dixon-White, 3/27/95, DowElanco, Doc.# RES95040 (MRID# 437274-09)

Factors A and D were not recovered from any of the Protocols. The results have been sent to FDA.

Comments

The submitted analytical method and recoveries appears to be adequate. Therefore, RCAB will recommend that EPA lab validation be initiated.

The results of the multi-residue testing will be sent to the EPA ACB lab and FDA.

The petitioner should submit Spinosad Factor A and Factor D standards, as well as the accompanying material safety data sheets (MSDS) to the EPA repository.

Attn: Terry Bundy
EPA Chemical Standards Repository (MD8)
2 Triangle Drive
Research Triangle Park, NC 27711

These standards, as well as a sample of the technical grade active ingredient, should also be sent to the EPA Beltsville laboratory.

> Attn: Harvey Hundley, Lab Chief
> Analytical Chemistry Laboratory
> Building 306, BARC East
> Beltsville, MD 20705

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.

Residue Data

Storage Stability

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 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%.

Comments

The registrant has shown that spinosad Factors A and D are stable in frozen cottonseed for the durations that the field residue samples were stored (the two exaggerated studies that involved durations longer than 283 days were not used in the determination or RAC or processed commodity tolerances).

Magnitude of the Residue

> "Magnitude of Residues of XDE-105 in Cottonseed After the Application of NAF-85 Insecticide", R.C. Gardner and S.D. West, 8/31/94, DowElanco, Doc.# RES93026R/RES92024R (MRID# 437274-10).

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. The results are presented in Tables 14 and 15.

Table 14

Residue Summary of XDE-105 Residues in/on Cotton from Unexaggerated (1X) Rate Trials

study site	average spray volume/application (gal./A.)	# applications	rate (g.ai./ha.)			PHI (days)	maximum total residues in ppm (uncorrected for method and storage recoveries)		
			average	final application	total		Factor A	Factor B	Total
Wayside, MS	18	5	103	140	513	28	0.003	ND	0.003
Winnsboro, LA	15	5	99	117	497	28	0.002	ND	0.002
Wilmont, AR	14	5	100	125	500	27	ND	ND	ND
Kelso, AR	14	5	101	126	505	27	ND	ND	ND
Fresno, CA	20	5	101	127	504	28	ND	ND	ND
							ND	ND	ND
Corcoran, CA	20	5	101	126	507	28	ND	ND	ND
							ND	ND	ND
Bakersfield, CA	20	5	100	124	501	28	ND	ND	ND
Somerton, AZ	15	5	98	124	492	28	0.001	ND	0.001
Mohawk, AZ	15	5	101	125	504	28	ND	ND	ND
Pattison, TX	18	5	100	126	502	28	ND	ND	ND
Orchard, TX	18	5	100	127	501	28	ND	ND	ND
Idalou, TX	20	5	100	125	502	28	ND	ND	ND
Lorenzo, TX	20	5	100	125	500	28	ND	ND	ND
Uvalde, TX	15	5	99	124	496	28	ND	ND	ND
Eakly, OK	15	5	100	125	500	28	ND	ND	ND
New Elm, GA	20	5	101	126	505	28	ND	ND	ND
Lucama, NC	19	4	94	100	374	28	ND	ND	ND

Table 15

Residue Summary of XDE-105 Residues in/on Cotton from Exaggerated Rate (2X - 6X) Trials

study site	average spray volume/application (gal./A.)	# applications	rate (g.ai./ha.) average	final application	total	PHI (days)	maximum total residues in ppm (uncorrected for method and storage recoveries) Factor A	Factor B	Total
Fresno, CA	30	5	201	201	1005 (2X)	14	0.003	ND	0.003
							ND	ND	ND
						28	ND	ND	ND
							ND	ND	ND
Burdette, MS	13	5	200	200	1000 (2X)	14	0.006	ND	0.006
							0.006	ND	0.006
							0.007	ND	0.007
			220	198	1099 (2X)	27	ND	ND	ND
							ND	ND	ND
							ND	ND	ND
Fresno, CA	20	5	606	758	3031 (6X)	28	0.010	ND	0.010
							0.007	ND	0.007
Wayside, MS	18	5	617	842	3085 (6X)	28	0.056	0.007	0.063
							0.056	0.005	0.061
							0.069	0.010	0.079

Processed Commodities

"Magnitude of Residues of XDE-105 in Processed Products from Cottonseed After the Application of NAF-85 Insecticide", R.C. Gardner and S.D. West, 10/25/94, DowElanco, Doc.# RES93026.01 (MRID# 437274-11)

The processing study was conducted using cotton seed grown harvested from the exaggerated trial conducted in Wayside, MS (from Table 15 above). Samples were generated from 5 applications of 454 and 842 g.ai./ha. (average of 617; final application of 842; and total of 3085 g.ai./ha.) and a 28 day PHI. The resulting total application of 3085 g.ai./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 DowElanco Analytical Method GRM 94.02. (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. The results are shown in Table 16.

Table 16

Summary of XDE-105 Residues in/on Cotton Processed Commodities

	Residues of Factor A + D (in ppm)*		
Cotton Matrix	range	average	Concentration Factor
cottonseed (RAC)	0.072 - 0.074	0.073	N/A
hulls	0.014 - 0.018	0.016	0.22
meal	< 0.003	< 0.003	< 0.041
crude oil	0.012	0.012	0.16
refined oil	0.015 - 0.016	0.0155	0.21
soapstock	< 0.003	< 0.003	< 0.041

* - Results are corrected for method recoveries of the different matrices.

Comments

The registrant has proposed a temporary tolerance on cotton seed at 0.02 ppm for the combined residues of spinosad (Factor A + Factor D). Based on the residue data provided, this temporary tolerance should be adequate to cover residues from the proposed use.

No residue data were provided on cotton gin byproducts. As noted in Table II (September 1995), cotton gin byproducts are a cotton RAC that comprises up to 20% of the diet of beef and dairy cattle. For the purposes of this EUP/temporary tolerance request, a tolerance for cotton gin byproducts will not be necessary. As noted in Table II (September 1995), for a future Section 3/permanent tolerance request, at least 3 field trials for each type of harvesting (stripper and mechanical picker) will be needed, for a total of 6 field trials.

The results of the processing study indicate that residues of spinosad do not concentrate in processed cottonseed commodities. Therefore, no temporary processed commodity tolerances are needed.

Rotational Crops

"A Confined Rotational Crop Study with ^{14}C 232105 (XDE-105; Factor A) Using Wheat, Radish, and Lettuce". D.P. Rainey, 9/22/94, Plant Sciences, Inc., Doc.# MET92047. (MRID# 437274-02).

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.

Plant samples were stored frozen (-20°C) until after harvest. Samples were ground and aliquots combusted to determine total radioactivity. The results are shown in Table 17.

Table 17

Concentrations of Radioactivity (ppm) in Crops from Various Intervals After Treatment of Soil with ^{14}C XDE-105 Factor A

sample	30-day aged soil	120-day aged soil	365-day aged soil
Wheat immature	0.023	0.048	0.010
grain	0.044	0.287	0.009
straw	0.135	0.504	0.027
Radish root	0.016	0.014	0.004
foliage	0.008	0.030	0.004
Lettuce	0.009	0.020	0.006

Crop samples containing radioactive residues greater than 0.01 ppm XDE-105 Factor A equivalents were subjected to a preliminary extraction/fractionation procedure involving a two-step extraction with Acetonitrile/water and acetonitrile followed by partitioning of the combined extracts with methylene chloride, resulting in a methylene chloride fraction, an aqueous fraction and an extracted tissue fraction. Aqueous and methylene chloride fractions containing greater than 0.01 ppm residue and extracted tissue fractions with 0.05 ppm residue or greater were subjected to further characterization. The results following analysis by LSC are shown in Table 18.

Table 18

Radioactivity in Plants Following Extraction (μg XDE-105 Factor A equivalents/g fresh weight)

		TRR	CH_2Cl_2 fraction		aqueous fraction		extracted tissue fraction	
ageing period (days)	crop	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
30	lettuce	0.009	N/A					
	radish root	0.016	21.1	0.003	47.3	0.008	31.6	0.005
	radish foliage	0.008	N/A					
	immature wheat	0.023	23.0	0.005	39.4	0.009	37.6	0.009
	wheat grain	0.044	4.5	0.002	1.7	0.001	93.9	0.041
	wheat straw	0.135	33.7	0.045	13.9	0.019	52.5	0.071
120	lettuce	0.020	20.9	0.004	32.0	0.006	47.2	0.009
	radish root	0.014	11.5	0.002	45.1	0.006	43.4	0.006
	radish foliage	0.030	23.1	0.007	35.7	0.011	41.3	0.012
	immature wheat	0.048	21.3	0.010	20.4	0.010	58.3	0.028
	wheat grain	0.287	8.7	0.025	1.2	0.003	90.1	0.259
	wheat straw	0.504	33.0	0.166	12.4	0.062	54.7	0.276
365	lettuce	0.006	N/A					
	radish root	0.004	N/A					
	radish foliage	0.004	N/A					
	immature wheat	0.010	N/A					
	wheat grain	0.009	N/A					
	wheat straw	0.027	20.0	0.005	24.2	0.007	55.8	0.015

N/A - not analyzed due to original combustion value of < 0.01 ppm

Plant matrix/solvent combinations that exhibited > 0.01 ppm radioactivity were subject to additional analyses. The starch was isolated from the 30 and 120-day wheat grain extracted tissue fraction, which was then subjected to acid hydrolysis and reaction with phenylhydrazine to form glucosazones. The identity of these ^{14}C glucosazones was confirmed by HPLC and MS. In addition, both the glucozone and starch were degraded to glucose, and the radioactivity analyzed.

The 30 and 120-day straw samples were subject to silica gel chromatography (CH_2Cl_2 fraction), partitioning with ethyl acetate under both acid and base conditions (aqueous fraction), and extraction with acidic buffer to form acid detergent fiber (extracted tissue fraction). Essentially the components were solvent extracted, partitioned, chromatographed, and/or further degraded (enzyme, acid, and/or base hydrolysis) until each characterized (but not identified) component accounted for < 0.01 ppm radioactivity.

Results

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.

In the 120-day wheat grain samples, the glucose subunits comprising the starch were shown to be radioactive. Enzyme work with the same grain samples suggested that radioactivity had been incorporated into the grain protein.

In the 120-day wheat straw samples, the lignin and cellulose were shown to be radioactive.

Comments

The results of this confined rotational crop study support the results of the cotton metabolism study. 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.

For the purposes of this EUP/temporary tolerance request, temporary rotational crop tolerances will not be established. Pending review of the results of the cotton metabolism and confined rotational crop studies by the HED Metabolism Committee, rotational crop field studies and permanent rotational crop tolerances will not need to be established to support a future Section 3/permanent tolerance request.

Meat, Milk, Poultry, and Eggs

The petitioner is requesting a waiver of ruminant and poultry feeding studies, and the meat, milk, poultry, and egg tolerances associated with them. The calculations used to determine the maximum residue levels that would be found in poultry and ruminant products are shown below.

Chicken

The poultry feed item associated with cotton is cottonseed meal. A chicken diet consisting of 20% cottonseed meal and a maximum residue value of 0.01 ppm (the LOQ of the method) was used, resulting in 0.002 ppm in the diet. Based on the hen metabolism study in which the highest residue value occurred in the fat at 2.19 ppm (based on feeding 9 ppm of XDE-105 in the diet), a reduction in residues is calculated as 2.19 ÷ 9 or 0.243. Therefore, the maximum expected residue level in chickens is 0.002 × 0.243 or 0.000486 ppm, which is below the method detection limit.

Cows/Goats

The cattle feed items associated with cotton are: undelinted seed, cotton gin byproducts, cottonseed meal, and cottonseed hulls. No residue data are currently available on cotton gin byproducts. Therefore, for the purposes of this exercise, we will assume a cow diet consisting of 100% cottonseed, cottonseed meal, and cottonseed hulls (total) and a maximum residue value of 0.01 ppm. Based on the goat metabolism study in which the highest residue value occurred in the fat at 3.57 ppm (based on feeding 9 ppm of XDE-105 in the diet), a reduction in residues is calculated as 3.57 ÷ 9 or 0.397. Therefore, the maximum expected residue level in cows is 0.01 x 0.397 or 0.00397, which above the limit of detection, but about 40% of the limit of quantitation.

Comments

For the purposes of this EUP and temporary tolerance petition, RCAB will accept the waiver of a hen and cow feeding study. Tolerances for residues of spinosad on meat, milk, poultry, and eggs will not be necessary for this Section 5/temporary tolerance petition.

Provided that the maximum residues in cottonseed meal and other poultry feed items that may be treated with spinosad remain at or below 0.01 ppm, a poultry feeding study will not be required for a future Section 3/permanent tolerance request.

Due to the higher dietary burden in cattle (more feed items), the need for including cotton gin byproducts into the theoretical diet of cattle, and the fact that the estimation of residues in cattle products involves an extrapolation across species (goat vs. cow), we do not consider a waiver of the cattle feeding study appropriate for a future Section 3/permanent tolerance request. Therefore, a feeding study using dairy cattle should be conducted as outlined in the Subdivision O Guidelines.

Other Considerations

No Codex, Canadian, or Mexican tolerances are established for spinosad. No compatibility problem exists between the proposed U.S. and Codex tolerances.

Attachment I : Chemical Names and Structures of Cited Compounds (3 pages)

Attachment II : Product Chemistry Review of Spinosad (2 pages)

Attachment III : Confidential Appendix portion of the Product Chemistry Review of Spinosad (1 page)

cc (without attachment): circu., PIRAT.

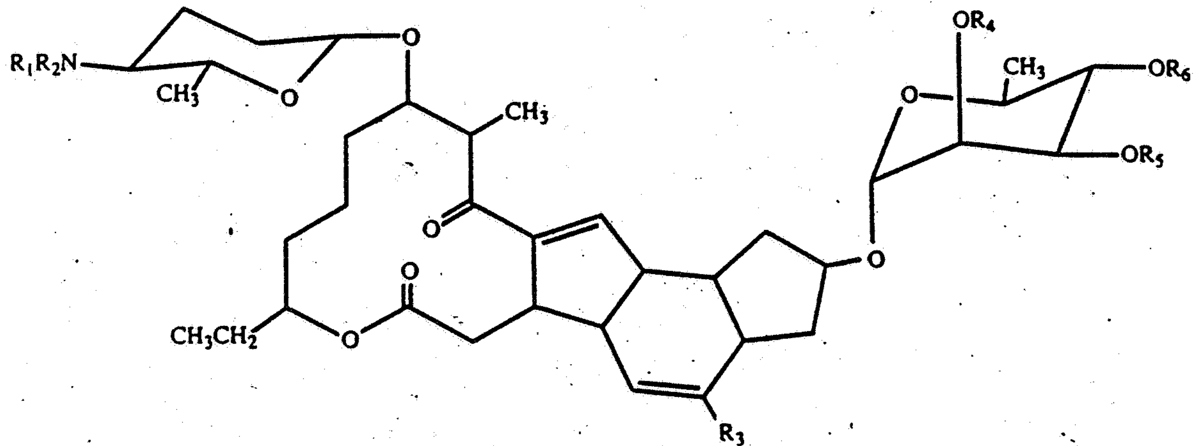
cc (with attachments): PP#6G04692, Chemistry RF, G.J. Herndon.

RDI: Chemistry Branch Senior Scientist: R.A. Loranger: 4/17/96, Acting Branch Chief: M. Metzger: 4/24/96.

H7509C: RCAB: G.J. Herndon: 305-6362: CM#2, Rm. 804C: 4/16/96.

Attachment I

Structures of Identified Factor A Metabolites

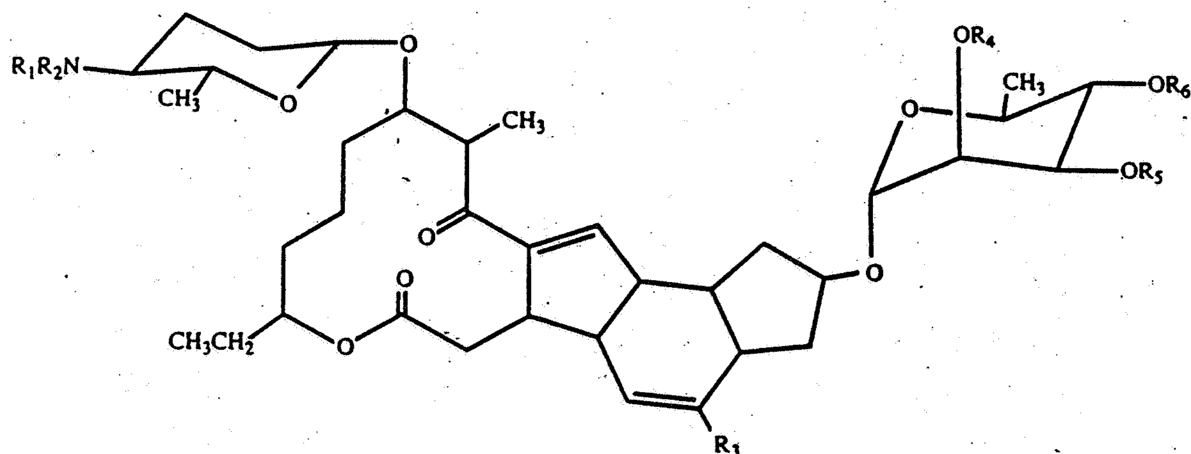


Metabolite ID	R_1	R_2	R_3	R_4	R_5	R_6
A	CH_3	CH_3	H	CH_3	CH_3	CH_3
B	CH_3	H	H	CH_3	CH_3	CH_3
H	CH_3	CH_3	H	H	CH_3	CH_3
J	CH_3	CH_3	H	CH_3	H	CH_3
K	CH_3	CH_3	H	CH_3	CH_3	H
PA[a]	--	--	H	CH_3	CH_3	CH_3
AP-2[a,b]	--	--	H	H	CH_3	CH_3
AP-3/AP-4[b]	CH_3	H	H	H	CH_3	H

[a] Both the pseudoaglycone (PA) and AP-2 were formed as a result of the loss of the forosamine sugar. Thus neither has an R_1 or R_2 moiety. (See structure in Figure 38.)

[b] The structure shown is one of the three possible isomers for each of these metabolites.

Structures of Identified Factor D Metabolites

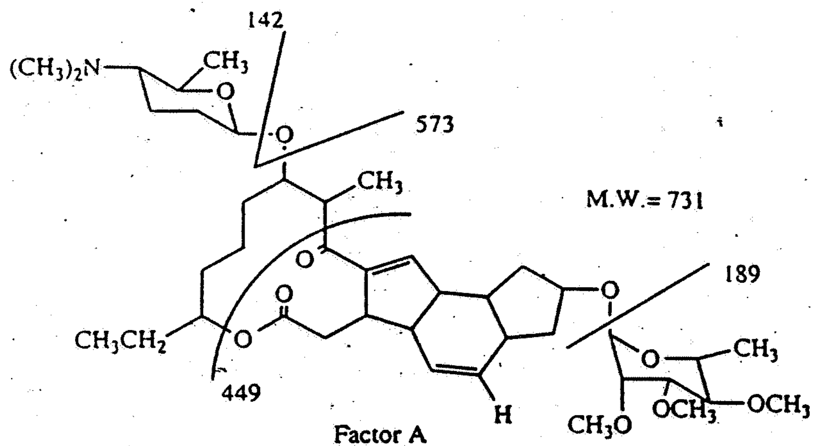


Metabolite ID	R_1	R_2	R_3	R_4	R_5	R_6
D	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3
B of D	CH_3	H	CH_3	CH_3	CH_3	CH_3
J of D	CH_3	CH_3	CH_3	CH_3	H	CH_3
H/K of D	CH_3	CH_3	CH_3	CH_3	CH_3	H
PA of D[a]	--	--	CH_3	CH_3	CH_3	CH_3
DP-3/DP-4[b]	CH_3	H	CH_3	CH_3	CH_3	H
DP-5[b]	CH_3	CH_3	CH_3	H	CH_3	H
DP-6, DP-7 and DP-8[b]	CH_3	H	CH_3	H	CH_3	H

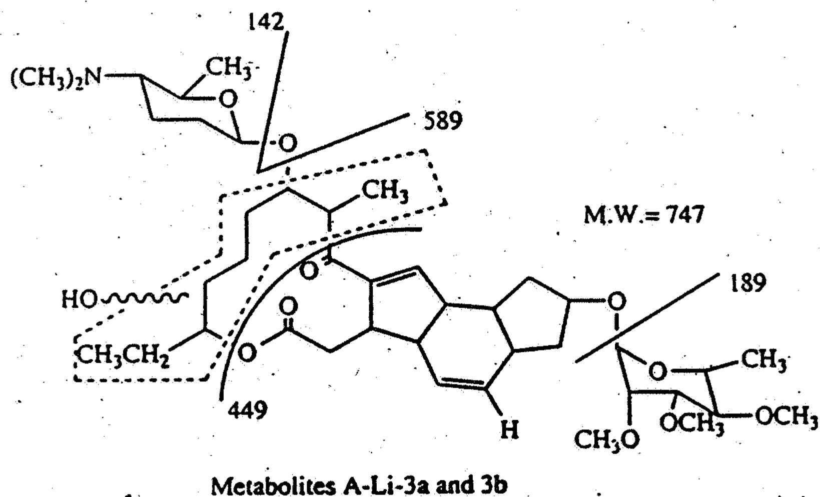
[a] The pseudoaglycone of D (PA of D) was formed as a result of the loss of the forosamine sugar. Thus, it does not have an R_1 or R_2 moiety. (See structure in Figure 39.)

[b] The structure shown is one of the three possible isomers for each of these metabolites.

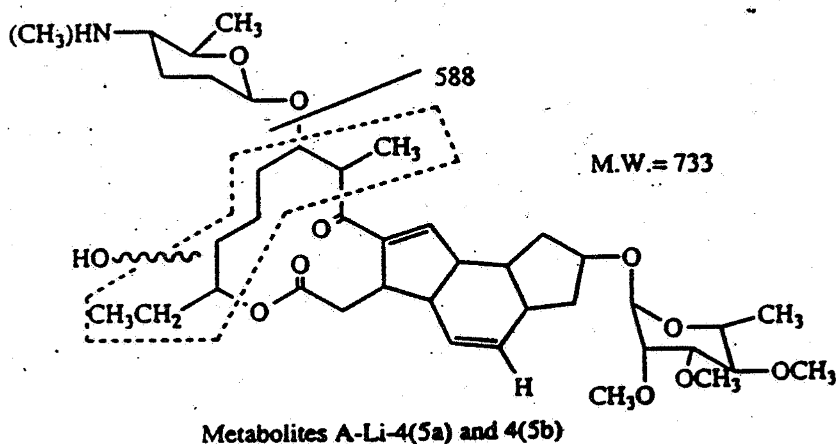
Proposed Structures for Metabolites A-Li-3a and 3b and A-Li-4 (5a) and 4 (5b) and A-Li-5c



Factor A



Metabolites A-Li-3a and 3b



Metabolites A-Li-4(5a) and 4(5b)

Attachment II

REVIEW OF PRODUCT CHEMISTRY (SUBDIVISION D), GLN'S 61 TO 63

Table 1: Manufacturing and Impurity Data for TECHNICAL SPINOSAD[1].

GLN	MRID	Status[2]	Deficiency[3]
61-1: Product Identity & Disclosure of Ingredients	434503-04	A	
61-2: Starting Materials & Manufacturing Process	434503-04	A	
61-3: Discussion of Impurities	434503-04	A	
62-1: Preliminary Analysis	434503-05	A	
62-2: Certification of Limits	434503-05	A	
62-3: Analytical Methods	434503-05	A	

[1] For example, test substance might be PAI and product might by 95% technical MP.
[2] A = Acceptable. N = Unacceptable (see Deficiency).
[3] Refer to CBI Appendix A for details.

Table 2: Physical and Chemical Properties for TECHNICAL SPINOSAD			
GLN	MRID	Status[1]	Result[2] or Deficiency
63-2: Color	434503-06	A	light grey to white
63-3: Physical State	434503-06	A	solid
63-4: Odor	434503-06	A	slightly stale water
63-5: Melting Point	434503-06	A	Factor A : 84 - 99.5C Factor B : 161.5 - 170C
63-6: Boiling Point	434503-06	N/A	
63-7: Density, Bulk Density, or Specific Gravity	434503-06	A	0.512 at 20C
63-8: Solubility (at 20C)	434503-06	A	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
63-9: Vapor Pressure (at 25C)	434503-06	A	Factor A : 3.0×10^{-11} KPa Factor B : 2.0×10^{-11} KPa
63-10: Dissociation Constant	434503-06	A	Factor A : 8.10 pKa Factor B : 7.87 pKa
63-11: Octanol/Water Partition Coefficient	434503-06	A	Factor A : $\log K_{ow} = 3.9$ Factor B : $\log K_{ow} = 4.4$
63-12: pH	434503-06	A	7.74 for a 10% slurry of XDE-105 in water
63-13: Stability	434503-06	A	XDE-105 was stable after 28 days: ambient, 122F, and in contact with stainless steel, brass, and ferric chloride
63-14: Oxidizing or Reducing Action		N/A	
63-15: Flammability		N/A	
63-16: Explodability		N/A	
63-17: Storage Stability		N/A	
63-18: Viscosity		N/A	
63-19: Miscibility		N/A	
63-20: Corrosion Characteristics		N/A	

[1] A = Acceptable; N = Unacceptable (see Deficiency); N/A = Not applicable.
[2] For example, "brown" for 63-1; "155° C" for 63-4.

Page 36 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

✓ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

_____ FIFRA registration data.

_____ The document is a duplicate of page(s) _________.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.