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

January 23, 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP No. 6F4761/6H5754. Spinosad (110003) on Apples, Brassica/Cole Crops. Review of Residue Data and Analytical Methodology. D228434, D228510. CB Nos. 17404, 17670. MRID Nos. 44058810, 44058811, 44058812, 44058813, 44058814, 44058815, 44058816, 44058817, 44058818, 44058819, 44058820, 44058821, 44058822, 44058823, 44058824, 44058825, 44058826, 44058827, 44058828, 44058829. Case Nos. S509051, S509217.

FROM: Stephanie H. Willett, Chemist
Tolerance Petition Section 2
Chemistry Branch I-Tolerance Support
Health Effects Division (7509C)

THRU: Elizabeth Haeberer, Acting Chief
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TO: Debbie McCall, Acting Section Head
Registration Section
Risk Characterization and Analysis Branch (7509C)

Attached is the review of a petition from DowElanco requesting the establishment of permanent tolerances for residues of the insecticide spinosad and apples, apple pomace, Brassica (cole) leafy vegetables, meat, meat byproducts and milk at levels ranging from 0.20 to 15 ppm. The review was performed by Dynamac Corporation under the supervision of CBTS, HED. The data assessment has undergone secondary review within the branch and has been revised to reflect current HED and OPP policy.

If any additional input is needed, please advise.

cc: RF, 6F4761, S. Willett, E. Haeberer, Circ., PM 13 (G. LaRocca/A. Heyward)

CM2:305-6380:RM 804C:7509C:SHWillett:shw-12/17/96

RDI: R. Loranger, 1/2/97; E. Haeberer, 1/15/97

SPINOSAD
PP#6F04761/FAP#6H05754
(CBTS NO. 17404; DP BARCODES D228434 AND D228510)

Permanent Tolerance Petitions for Use of Spinosad on Apples and Brassica Leafy Vegetables

December 2, 1996

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
The Dynamac Building
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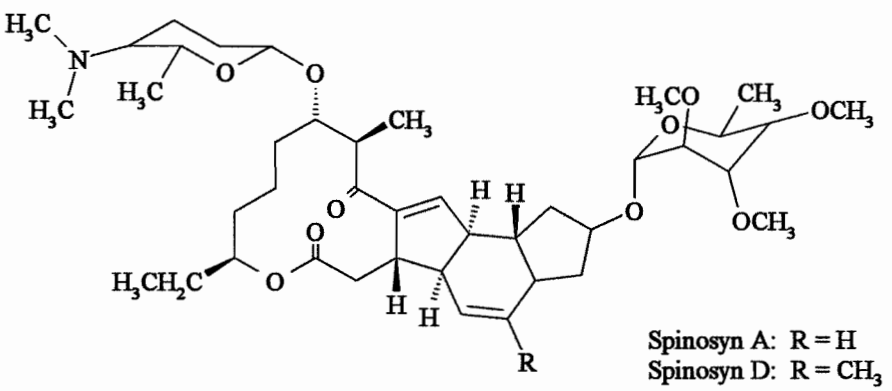
SPINOSAD



PERMANENT TOLERANCE PETITIONS (PP#6F04761/FAP#6H05754) FOR USE OF SPINOSAD ON APPLES AND BRASSICA LEAFY VEGETABLES

(CBTS NO. 17404; DP BARCODES D228434 AND D228510)

INTRODUCTION

DowElanco has submitted a petition for the establishment of permanent tolerances for residues of the insecticide spinosad (designated by the company code XDE-105) in conjunction with a request for a Section 3 registration of an 80% water dispersible granular formulation (Product name = NAF-127) for use of spinosad on apples and Brassica (cole) leafy vegetables. The petitioner is proposing the establishment of permanent tolerances for residues of spinosad (total of spinosyn A and spinosyn D) as follows:

Apples	0.20 ppm
Brassica (cole) leafy vegetables, head and stem subgroup	2.0 ppm
Brassica (cole) leafy vegetables, greens subgroup	15.0 ppm
Apple pomace, wet	0.5 ppm

The petitioner is additionally proposing the establishment of permanent tolerances for the combined residues of spinosyn A, spinosyn D, spinosyn B, and N-demethyl spinosyn D as follows:

Cattle, fat	1.0 ppm
Cattle, meat	0.05 ppm
Cattle, meat byproducts	0.2 ppm
Goats, fat	1.0 ppm
Goats, meat	0.05 ppm

Goats, meat byproducts	0.2 ppm
Hogs, fat	1.0 ppm
Hogs, meat	0.05 ppm
Hogs, meat byproducts	0.2 ppm
Horses, meat, fat, and meat byproducts	1.0 ppm
Milk, fat	0.5 ppm
Milk, whole	0.02 ppm
Sheep, fat	1.0 ppm
Sheep, meat	0.05 ppm
Sheep, meat byproducts	0.2 ppm

Spinosad is a fermentation product of *Saccharopolyspora spinosa*. The product consists of two related active ingredients: **Spinosyn A** (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 **Spinosyn D** (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-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione. The two active ingredients are typically present at an 85:15 ratio. Spinosad is intended for use to control lepidopterous and other insect pests. A petition for a permanent tolerance for residues of spinosad (spinosyns A + D) in/on cottonseed at 0.02 ppm is currently in reject status pending submission of a cattle feeding study; RCAB had recommended in favor of the issuance of an EUP/temporary tolerance (at 0.02 ppm) for use of spinosad on cotton (PP#6F04735, DP Barcodes D226824, D227014, D228791, G. J. Herndon, 6/17/96 and 8/13/96; and PP#6G04692, DP Barcodes D219016, D224608, D223898, D223899, G. J. Herndon, 4/24/96).

Associated with this petition are 20 volumes of residue chemistry submissions which are evaluated in this document.

CONCLUSIONS

OPPTS 830 Series GLNs: Product Properties

1. Adequate product chemistry data for the spinosad technical product have been provided. No impurities are expected to cause residues of concern.

OPPTS GLN 860.1200: Proposed Uses

2. The proposed Section B is marginally adequate. Although the maximum use rates (single and seasonal), application equipment types, and preharvest intervals were

appropriately described, additional information concerning specific timing of applications (i.e., crop growth stage at application) and/or retreatment intervals needs to be included in the proposed label.

OPPTS GLN 860.1300: Nature of the Residue - Plants

3a. The qualitative nature of the residue in plants is adequately understood based on metabolism studies conducted on apples, cabbage, tomatoes, and turnips. The parents, spinosyns A and D, were the major ^{14}C-residues identified in early-harvest samples (e.g., 0 to 3 days after treatment, DAT) of apple fruits/leaves, cabbage leaves, tomatoes, and turnip tops/roots. Minor metabolites identified include spinosyn B, N-demethyl spinosyn D, spinosyn K, and N-formyl spinosyn B. [*The chemical names and molecular structures of spinosyns A and D and other identified metabolites are depicted in Figure 2*]. In samples collected at subsequent intervals, the residue levels of spinosyns A and D declined significantly. The decline in residue levels of the parents was accompanied by incremental increases in nonextractable and polar ^{14}C-residues. Extensive fractionation and characterization of nonextractable and polar ^{14}C-residues in selected RAC samples indicates that most of the radioactivity was degraded to multicomponent residues of low molecular weight which are subsequently incorporated into natural plant constituents.

3b. The metabolism studies conducted on apples, cabbage, tomatoes, and turnips demonstrated a rapid dissipation of spinosyns A and D. The petitioner has provided evidence to indicate that photolysis plays a role on the initial degradation of the parent compounds. The petitioner contends that the initial step in the metabolism of spinosyns A and D is the conversion of the parents to metabolites resulting from modifications to the forosamine portion of the molecule, such as spinosyn B and N-demethyl spinosyn D. The rhamnose and macrolide portions are subsequently modified to form polar and nonextractable residues.

3c. The results of these plant metabolism studies will be presented to the HED Metabolism Committee which will determine the residues of concern in plant commodities.

OPPTS GLN 860.1300: Nature of the Residue - Livestock

4a. The qualitative nature of the residue in ruminants is adequately understood based on previously submitted/evaluated goat metabolism studies (PP#6G04692, G. J. Herndon, 4/24/96). The results of the studies indicate that residues of spinosyns A and D concentrate in tissues and milk, with greater transfer in fattier tissues (fat and liver). Radioactivity was readily extractable indicating that ^{14}C-residues were not extensively conjugated. The parent compounds, spinosyns A and D were the major ^{14}C-residues identified in tissues (fat, muscle, kidney, and liver) and milk.

4b. The results of the ruminant metabolism studies will be presented to the HED Metabolism Committee which will determine the residues of concern in animal commodities.

4c. There are no poultry feed items associated with the proposed uses on apples and Brassica (cole) leafy vegetables. Therefore, data pertaining to the metabolism of spinosad in poultry are not required to support this petition.

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

5a. HPLC methods GRM 95.05 and GRM 94.22 are adequate for the purposes of tolerance enforcement and collection of residue data for spinosyns A, D, K, and B and N-demethyl spinosyn D in/on apple and Brassica (cole) leafy vegetable commodities. Adequate independent method validation and concurrent method recovery data have been submitted. HPLC methods GRM 95.05 and GRM 94.22 are similar to the method proposed for cottonseed which has undergone successful petition method validation (DP Barcode D228791, G. J. Herndon, 8/13/96).

5b. Although data concerning the radiovalidation of the proposed enforcement methods were not provided, such data will not be required because samples from the plant metabolism studies were subjected to extraction and characterization procedures similar to the proposed enforcement methods.

OPPTS GLN 860.1340: Residue Analytical Methods - Animal Commodities

6a. HPLC method GRM 95.03 is adequate for the purposes of collection of residue data for spinosyns A, D, and B and N-demethyl spinosyn D in animal commodities; adequate independent method validation and concurrent method recovery data have been submitted. The method has also been adequately radiovalidated. CBTS will forward HPLC method GRM 95.03 to EPA ACL for a method trial.

6b. The petitioner submitted a description of and validation data for immunoassay method GRM 95.14, a method for the determination of total spinosyn-related residues in milk and cattle muscle, liver, and kidney. Adequate validation data for spinosyn A were submitted.

OPPTS GLN 860.1360: Multiresidue Method

7. Data pertaining to multiresidue methods testing of spinosyns B and K and N-demethyl spinosyn D were submitted. These data will be forwarded to FDA for review. The petitioner had previously submitted data pertaining to the multiresidue methods testing of spinosyns A and D in conjunction with PP#6G04692 which were forwarded to FDA (G. J. Herndon, 4/24/96).

OPPTS GLN 860.1380: Storage Stability Data

8a. Plant commodities: The storage stability data for apples, apple juice, and Brassica leafy vegetables are adequate. The data indicate that fortified residues of spinosyns A, D, B, and K, and N-demethyl spinosyn D are relatively stable under frozen storage conditions for at least 12 months in/on cabbage, 6 months in/on apples, and 3 months in/on apple juice. These data support the storage conditions and intervals of samples from the submitted field trial and processing studies.

8b. Animal commodities: The storage stability data for animal commodities are adequate. Residues of spinosyns A, D, and B and N-demethyl spinosyn D are stable in milk during frozen storage for up to 136 days (4.5 months). Radioactive residues of spinosyns A, D, and B and N-demethyl spinosyn D were also found to be stable in milk, fat, kidney, liver, and muscle for at least 1.6 years of frozen storage. These data support the storage conditions and intervals of samples from the submitted cattle feeding study.

OPPTS GLN 860.1500: Crop Field Trials

9a. Apples: The submitted apple field trial data are adequate. The data indicate that the combined residues of spinosyns A and D will not exceed the proposed 0.20-ppm tolerance in/on apples harvested 7 days following the last of five sequential foliar broadcast applications, with 6- to 74-day retreatment intervals, of the 80% WDG formulation at ~0.045 + 0.062 + 0.089 + 0.089 + 0.161 lb ai/A (1x the proposed maximum seasonal rate). The combined residues in/on apples, treated as described above, were <0.006-0.105 ppm (64 samples). No significant difference was observed between the concentrated (~50 gal/A) and dilute spray (~200 gal/A) applications.

9b. Data from residue decline studies indicate that spinosad residues decline at 3-day and 7-day posttreatment intervals (PTIs). Residue levels at 10-day and 14-day PTIs were similar to or less than those for the 7-day PTI samples.

10a. Brassica (cole) Leafy Vegetables Group: The submitted field trial data on Brassica (cole) leafy vegetables are adequate. They indicate that residues of spinosyns A and D will not exceed the proposed subgroup tolerances for the head and stem subgroup (2.0 ppm) and greens subgroup (15.0 ppm) of the Brassica (cole) leafy vegetables in/on samples harvested one day following the last of four sequential foliar broadcast applications, with 3- to 5-day retreatment intervals, of the 80% WDG formulation at ~0.089 + 0.089 + 0.134 + 0.134 lb ai/A (1x the proposed maximum seasonal rate). The combined residues in/on representative commodities, treated as described above, were 0.105-0.599 ppm in/on broccoli (16 samples), <0.014-1.198 ppm in/on cabbage with wrapper leaves (16 samples), and <0.043-6.764 ppm in/on mustard greens (16 samples).

10b. Data from the residue decline studies indicate that spinosad residues decline rapidly in broccoli and mustard greens harvested at 3, 5, 7, and 10 days following treatment. The petitioner determined that residues of spinosad had a half-life of approximately 4-5 days in broccoli and 2 days in mustard greens.

10c. Based on the highest residue value obtained from samples harvested at the proposed 1-day PHI and treated at the proposed maximum use rates, the proposed tolerance level of 2.0 ppm for the head and stem subgroup is appropriate. However, the petitioner should submit a revised section F to reduce the proposed tolerance for the greens subgroup of Brassica (cole) leafy vegetables from 15 to 10 ppm.

OPPTS GLN 860.1520: Processed Food/Feed

11a. The submitted apple processing data are adequate. The data indicate that total residues of spinosyns A and D concentrated 5.3x in wet pomace processed from apples bearing detectable residues. No concentration of residues was observed in juice processed from treated apples.

11b. Based on the available field trial data, the HAFT (Total A+D) for apples harvested 7 days following treatment at the maximum proposed seasonal application rate (0.45 lb ai/A) is 0.089 ppm. Therefore, the maximum total spinosyns A and D residues expected in apple wet pomace would be 0.48 ppm. The available data support the proposed tolerance of 0.5 ppm for residues of spinosyns A and D in apple wet pomace.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

12a. The submitted dairy cattle feeding data are adequate. They indicate that tolerances for residues of spinosad are required for milk and the fat, meat, and meat byproducts of cattle, goat, hogs, horses, and sheep. Detectable residues of spinosad were observed in the milk, fat, kidney, and liver of cattle fed spinosad at ~2x the maximum theoretical dietary burden (the lowest dosing level) for 28 days.

12b. The available data do not support all the proposed tolerances for animal tissues (0.05 ppm for meat, 0.2 ppm for meat byproducts, and 1.0 ppm for fat), milk (0.02 ppm) and for milk fat (0.5 ppm). While the meat byproduct and milk fat tolerances are appropriate, the meat and milk tolerances should be revised to 0.04 ppm (i.e., the sum of the LOQs for the four moieties. Milk residues from the maximum expected dietary burden are estimated to be as high as 0.03 ppm by extrapolation of the feeding study results. The fat tissue tolerance should be lowered to 0.4 ppm. The petitioner must submit a revised Section F proposing these tolerances. In addition, the petitioner should modify the proposed tolerance levels for horse fat, meat, and meat byproducts to coincide with the tolerance levels for cattle, goat, hogs, and sheep.

12c. There are no poultry feed items associated with this petition. Therefore, data pertaining to the magnitude of spinosyn residues in poultry commodities are not required.

OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops

13. The qualitative nature of the residue in rotational crops is adequately understood based on an acceptable confined rotational crop study submitted and reviewed in conjunction with PP#6G04692. Extensive/limited rotational crop field studies need not be conducted and tolerances for rotational crops need not be established to support this permanent tolerance request.

Codex, Canadian, or Mexican Tolerance Issues

14. No Codex, Canadian, or Mexican tolerances are established for spinosad. No compatibility problems exist between the proposed U.S. and Codex tolerances.

RECOMMENDATIONS

CBTS will present the results of the studies pertaining to plant and animal metabolism to the HED Metabolism Committee. The Committee will determine the spinosad residues of concern in plants and animals.

Assuming that the residues of concern are determined to be spinosyns A and D for plants, and spinosyns A, D, and B, and N-demethyl spinosyn D for animals, CBTS will recommend in favor of the proposed permanent tolerances provided the petitioner submits revised Sections B (see Conclusion 2) and F (see Conclusions 10c and 12b).

DETAILED CONSIDERATIONS

OPPTS 830 Series GLNs: Product Properties

Product chemistry data for the end-use product, NAF-127, were submitted with this petition (MRIDs 44058801-44058803). Review of these data is deferred to Registration Division. Product chemistry data for the spinosad technical product were reviewed in conjunction with PP#6G04692 (G. J. Herndon, 4/24/96). It was concluded that the available product chemistry data were adequate to fulfill the requirements for a Section 3/permanent tolerance request. No additional product chemistry data are required for the purposes of this permanent tolerance petition.

OPPTS GLN 860.1200: Proposed Uses

The petitioner provided a specimen label for an 80% water dispersible granular (WDG) formulation (Product name = NAF-127) proposed for use on apples and cole crops. The proposed use patterns are described below.

On apples, the 80% WDG formulation is proposed for multiple foliar applications to apple orchards at 0.06-0.16 lb ai/A/application or 0.015-0.04 lb ai/100 gal as a dilute spray. The label states that lb ai/A rates are based on a standard of 400 gallons of dilute spray per acre. Concentrated spray applications may be made at 0.06-0.16 lb ai/A. Applications are to be made using ground or aerial equipment; aerial applications are to be made in a minimum of 10 gal/A. A maximum single application rate of 0.16 lb ai/A (regardless of tree row volume) and a maximum seasonal application rate of 0.45 lb ai/A are proposed. A PHI of 7 days is proposed.

On cole crops [including but not limited to: broccoli, Chinese broccoli, broccoli raab, Brussels sprouts, cabbage, Chinese cabbage (bok choy and napa), cauliflower, cavalo, collards, kale, kohlrabi, mizuna, mustard greens, mustard spinach, Chinese mustard cabbage (gai choy), and rape greens], the 80% WDG formulation is proposed for multiple foliar applications at 0.023-0.13 lb ai/A/application. A maximum seasonal rate of 0.45 lb ai/A and a PHI of 1 day are proposed.

For insecticide resistance management, it is proposed that the product not be used on consecutive generations of insects, and that more than three consecutive applications or continuous use for more than 30 days be prohibited. No rotational crop restrictions or plantback intervals are proposed.

Conclusions: The proposed Section B is marginally adequate. Although the maximum use rates (single and seasonal), application equipment types, and preharvest intervals were appropriately described, additional information concerning specific timing of applications (i.e., crop growth stage at application) and/or retreatment intervals needs to be included in the proposed label.

OPPTS GLN 860.1300: Nature of the Residue - Plants

An acceptable cotton metabolism study (1994, 1995; MRIDs 43727403 and 43727404) was evaluated (DP Barcodes D219016, D224608, D223898, and D223899; G. J. Herndon; 4/24/96) in conjunction with a temporary tolerance petition (PP#6G04692) for the establishment of a temporary tolerance for residues of spinosad on cotton. It was concluded that for the purposes of the temporary tolerance request, the nature of the residue in cotton is adequately defined. The residues of concern are the parent compounds only, spinosyns A and D. The review also stated that the HED Metabolism Committee will be consulted in order to determine which residues are of concern for a Section 3/permanent tolerance request.

In support of the current petition and permanent tolerance request, the petitioner has submitted metabolism studies on apples, cabbage, tomatoes, and turnips. These metabolism studies are evaluated in this petition review. All studies utilized the test substances [^{14}C]spinosyn A (specific activity 3.40-4.11 μCi/mg, radiochemical purity 98.0-98.6%) and [^{14}C]spinosyn D (specific activity 3.71-4.27 μCi/mg, radiochemical purity 97.9-98.7%) uniformly labeled in the macrolide portion (see Figure 1) of the molecule which were produced by fermentation using the actinomycete *Saccharopolyspora spinosa*.

Apples

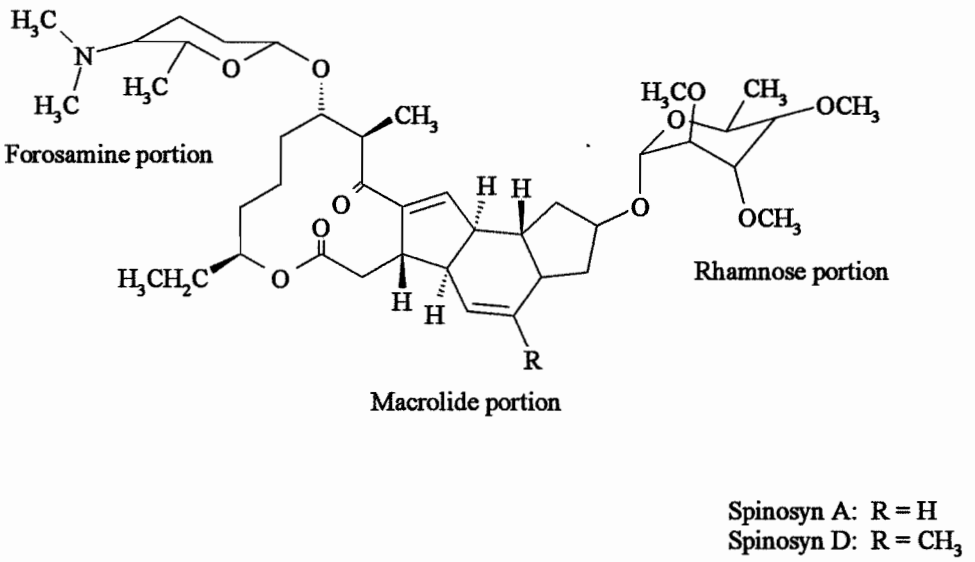
DowElanco has submitted data (citations listed below) from an apple metabolism study conducted to investigate the nature of the residue on apple fruits and leaves, the influence of photolysis on metabolism of the parent compounds, and the translocation of ^{14}C-residues on a tree treated with [^{14}C]spinosyn A. The metabolism data for apple leaves were submitted to provide surrogate data for tea which will be the subject of a future registration application. The field portion of the study was conducted by Plant Sciences, Inc. (Watsonville, CA). The residue characterization portion was conducted by the Residue Chemistry Group of DowElanco Research Laboratories (Indianapolis, IN).

> MRID 44058811. Berard, D. and Satonin, D. (1995) Nature of Residue Study in Apple Leaves Using (carbon-14) XDE-105: Factor A and D: Lab Project Number: MET93041. Unpublished Study Prepared By DowElanco. 194 p.

MRID 44058813. Graper, L. (1996) Nature of Residue Study in Apples Using (carbon-14) XDE-105: Factor A and D: Lab Project Number: MET93040. Unpublished Study Prepared By DowElanco and Plant Sciences, Inc. 230 p.

Set-up: Three mature Dwarf Red Delicious variety of apple trees were used in the study. One tree was treated with the test substance [^{14}C]spinosyn A, another tree with [^{14}C]spinosyn D, and the third apple tree served as a control. For the evaluation of the influence of photolysis, certain apple fruits and leaves were covered with a vinyl light-blocking material shortly after test substance application. Apple fruits and leaves intended for the evaluation of translocation of ^{14}C-residues were situated on a tree branch that was protected from spray during the application of [^{14}C]spinosyn A; the branch was uncovered after treatment.

Figure 1. Designations and structures of test substances.



Application: [^{14}C]Spinosyn A and [^{14}C]spinosyn D were applied as a spray treatment at 885 and 349 ppm, respectively. The treatment was made to apple trees approximately one month prior to fruit maturity using a 0.5-gallon sprayer adjusted to deliver a controlled, even, spray droplet pattern. According to the petitioner, the application rates were 1.5x and 2.3x the proposed maximum seasonal rate on apples for spinosyns A and D, respectively. The petitioner's calculation of application rates assumes a maximum of five applications per growing season, an active ingredient concentration of 150 ppm per application, and a spinosad commercial product containing ~80% spinosyn A [885 ÷ (5 x 150 x 0.8)] and ~20% spinosyn D [349 ÷ (5 x 150 x 0.2)].

Sample collection and shipping: Treated and untreated fruit and leaf samples were collected 0, 3, 7 or 10, 14, 28, and 42 days after treatment (DAT). Samples that had been protected from light were collected 3 and 7 DAT. Apple fruits intended for translocation investigation were collected 0 and 42 DAT and apple leaves intended for translocation investigation were collected 0, 3, 7, 10, and 28 DAT. All harvested samples were placed in plastic bags, sealed, and then transported on ice to the analytical laboratory, DowElanco. Upon receipt at DowElanco, the fruits and leaves were sequentially rinsed with acetonitrile:methanol:water (4:4:1, v:v:v), hexane:dichloromethane (1:1, v:v), and methanol (MeOH); the rinses were then pooled. The rinsed fruits were peeled, and the peel was frozen with liquid nitrogen and homogenized. The peeled apple (pulp) was chopped and homogenized with water. All rinsates, peel, and pulp samples were stored frozen (-20 C) prior to analysis.

Total radioactive residues (TRR): The total radioactivity in peel, pulp, and leaf samples, both before and after extraction, was determined by combustion and liquid scintillation spectrometry (LSS). Radioactivity in rinses, sample extracts, and other liquid samples was determined either by direct LSS analysis or by drying sample aliquots into quartz boats and combusting the samples. The LSS limit of detection (LOD) was not reported; however, the petitioner used a limit of 0.001 ppm when reporting results. The results of the TRR determination are presented in Table 1.

Extraction of ^{14}C-residues: Radioactive residues in peel and leaves were initially extracted with acetonitrile (ACN), and the ACN extract was concentrated for chromatographic analysis. Radioactive residues in pulp were initially extracted with ACN:water (80:20, v:v) and ACN. The filtrates were combined and partitioned with dichloromethane (DCM); the resulting aqueous phase was further partitioned with ethyl acetate (EtOAc). The DCM and EtOAc phases were combined to yield an organic phase for chromatographic analysis. Selected fractions were subjected to solid-phase extraction (SPE) procedures using C-18 Sep-Pak cartridge columns and various elution solvents. The eluate(s) containing the highest radioactivity was reserved for chromatographic analysis. Radioactivity in the extracts was determined by LSS; the radioactivity in the nonextractable residues was determined by LSS following combustion.

Table 1. Total radioactive residues in/on apples harvested at various intervals following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A (885 ppm) or D (349 ppm).

DAT [a]	TRR (ppm [^{14}C]spinosyn A or D equivalents)					
	Whole Fruit			Leaves		
	Metabolism Investigation	Photolysis [b] Investigation	Translocation [c] Investigation	Metabolism Investigation	Photolysis [b] Investigation	Translocation [c] Investigation
Spinosyn A Treatment						
0	2.694	--	0.002	216.9	216.9	0.023
3	3.226	1.768	--	135.1	153.8	0.125
7	2.229	1.873	--	205.8	169.3	0.310
10	--	--	--	175.4	--	0.564
14	1.845	--	--	--	--	--
28	1.570	--	--	128.3	--	0.844
42	1.251	--	0.017	--	--	--
Spinosyn D Treatment						
0	0.981	--	--	88.7	88.7	--
3	1.158	0.840	--	96.6	90.4	--
7	1.209	0.828	--	70.6	125.9	--
10	--	--	--	71.8	--	--
14	0.842	--	--	--	--	--
28	0.741	--	--	43.1	--	--
42	0.513	--	--	--	--	--

[a] DAT = days after treatment.

[b] Samples collected from the photolysis investigation were obtained from areas of the trees covered with a vinyl material to exclude light.

[c] Samples collected from the translocation investigation were obtained from a portion of the tree that was covered before the application of [^{14}C]spinosyn A.

Characterization and identification of ^{14}C-residues: The fruit and leaf rinses, and various pulp, peel, and leaf extracts were analyzed by one-dimensional TLC and/or HPLC. Initial identification of ^{14}C-residues was confirmed by cochromatography with non-labeled reference standards.

TLC separations were conducted on precoated silica gel plates (F-254) developed with toluene:isopropanol:diethylamine (84:7:7, v:v:v:, System A), n-butanol:water:acetic acid (60:25:15, v:v:v:, System B) or toluene:isopropanol (84:7, v:v, System B for leaves), and chloroform:MeOH (85:15, v:v, System C). Radioactive areas were quantitated by radioscan imaging and visualized by exposure of the plates to X-ray film or phosphorous screen; reference compounds were detected under UV light. HPLC separations were accomplished

using a YMC ODS or ODS C-18 column and an isocratic mobile phase of ACN:MeOH:2% ammonium acetate (4:4:1, v:v:v) or a gradient mobile phase of 2% ammonium acetate (A) and ACN:MeOH:ammonium acetate (B; 4:4:1, v:v:v) changing from 85:15 (A:B) to 100% B over 30 minutes. The HPLC system was equipped with a variable wavelength absorbance detector (250 nm) and radiochemical detectors; identification of ^{14}C-residues was achieved by comparison of retention times with those of non-labeled reference standards.

Certain peaks from TLC/HPLC analyses were pooled and subjected to mass spectrometry (MS) analyses. MS analysis confirmed the identification of the parent compounds and multiple polar components; it also indicated the presence of characteristic fragments associated with the rhamnose and forosamine portions of the molecule. The petitioner provided sample calculations related to the biological and analytical phases of the study and representative TLC and HPLC chromatograms and MS spectra.

The 14- and 42-DAT fruit rinse samples from trees treated with [^{14}C]spinosyn A, and all DAT ^{14}C-treated leaf rinse samples were acid hydrolyzed (1 N HCl) following initial extraction to characterize residues related to spinosyns A and D. The forosamine portion of spinosyn-related residues is hydrolyzed by acid to form pseudoaglycones. TLC analyses of the rinses before and after acid hydrolysis demonstrated the conversion of spinosyns A and B to pseudoaglycone A (psA), and spinosyn D to a pseudoaglycone typical compound (a standard for psD was not available).

Tables 2-A and 2-B present the distribution of radioactive residues following routine solvent extraction and chromatographic analysis of samples collected for metabolism investigation. Summaries of metabolites identified in apple matrices are presented in Tables 3-A and 3-B. The chemical names and molecular structures of these metabolites are depicted in Figure 2.

The data presented in Tables 2-A and 2-B indicate that the majority of the total radioactivity remained on the apple fruit/leaf surface and was soluble in organic solvents. The radioactivity identified in 0-DAT fruit rinses consisted mainly of the parent compounds, spinosyns A (~86% of TRR) and D (~84% of TRR); the metabolites spinosyn B and N-demethyl spinosyn D were identified as minor components. However, in 3-DAT fruit rinses the radioactivity associated with spinosyns A and B declined to ~33% and 10% of TRR, respectively. These data suggest that spinosyns A and D are rapidly metabolized. In peel and pulp samples that were collected from early sampling intervals, the parent compounds were only qualitatively identified in the organic fractions; the majority of the radioactivity in peel and pulp samples that were collected from later intervals did not identify the parents but resolved polar multicomponent residues.

Further characterization of polar residues: Exhaustive attempts were made to elucidate the nature of the polar ^{14}C-residues. For these investigations, the 14- and 42-DAT fruit samples were utilized. A brief summary of the procedures and results are described below.

14- and 42-DAT fruit rinse: An aliquot of the fruit rinse was subjected to medium bore column chromatography and the eluate fractions were characterized/identified by a combination of TLC, SPE/HPLC, and MS analyses. The extensive analyses indicated that most of the radioactivity was composed of polar, multicomponent residues. Analysis of the rinse of 14-DAT fruit treated with [^{14}C]spinosyn A by MS confirmed the identity of spinosyns A and B, and provided partial structural information about an unidentified metabolite which appeared to contain a structure similar to spinosyn A but with the addition of two oxygen atoms to the rhamnose and/or macrolide portions of the molecule. Spinosyns A and B, and the unknown metabolite comprised 0.179 ppm (9.7% TRR), 0.011 ppm (0.6% TRR), and 0.017 ppm (0.9% TRR), respectively, of the total radioactivity in the pooled 14-DAT fruit rinse ([^{14}C]spinosyn A treatment). No unidentified metabolite comprised more than 0.031 ppm (1.7% of TRR). Similar multicomponent profiles were found in the 42-DAT (spinosyn A treatment) and 14- and 42-DAT (spinosyn D treatment) fruit rinses.

Radioactive residues in the rinse of 14-DAT fruit treated with spinosyn A were also separated into fractions by TLC, and the fractions were examined using an spinosyn immunoassay test kit. The spinosyn immunoassay showed high sensitivity to radioactive residues from fractions containing spinosyns A and B, and low sensitivity to radioactive residues in other fractions. This low sensitivity to radioactive residues in these other fractions suggests that the radioactive residues do not resemble factors which the spinosyn immunoassay detects such as spinosyns A, B, C, E, F, and K, or psA.

14- and 42-DAT fruit peel and pulp: Subsamples of fruit peel and pulp were repeatedly extracted with ACN and subjected to several techniques [i.e., acid hydrolysis (0.1 N HCl), base hydrolysis (0.1 N NaOH), enzyme hydrolysis (cellulase and pectinase), and purification columns (small and medium bore silica gel column chromatography, silica Sep-Pak chromatography)]. Chromatographic analyses (TLC and HPLC) of extracts or fractions suggested that most of the radioactivity was polar, highly water soluble, or nonextractable. Only very small amounts of extractable radioactivity appeared to cochromatograph with spinosyns A or D. Enzyme hydrolysis of nonextractable residues were ineffective in releasing bound residues. Mild acid and base hydrolyses of nonextractable residues were successful in solubilizing significant amounts of residues. However, the solubilized residues were generally polar and highly water soluble.

Quantitative data from the photolysis portion of the study are not presented herein; however, the data demonstrated that radioactive residues in/on photolysis samples were not metabolized to the extent observed in the metabolism study samples. Because much less metabolism occurred in the photolysis study samples than in the "normal" metabolism samples, the petitioner concluded that photolysis was the predominant mechanism in the metabolism of

spinosyns A and D. CBTS notes that in the previously evaluated cotton metabolism study (DP Barcodes D219016, D224608, D223898, and D223899; G. J. Herndon; 4/24/96), it was demonstrated that spinosyns A and D are susceptible to breakdowns ($t_{½}$ on leaf surfaces is ~3.4 hours) based on photolysis studies. The results of the translocation portion of the study demonstrated that only low levels of radioactive residues were translocated from a treated branch into the fruit; some degree of translocation was observed from a treated branch into the untreated leaves.

Table 2-A. Distribution of residues in/on apple matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A at 885 ppm.

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Fruit (TRR = 2.694 ppm)			
Rinse	94.8	2.555	TLC: Spinosyn A 85.9% TRR 2.314 ppm Spinosyn B 3.4% TRR 0.092 ppm Origin 1.6% TRR 0.043 ppm Other 3.9% TRR 0.105 ppm HPLC: Spinosyn A 83.6% TRR 2.252 ppm Spinosyn B 1.5% TRR 0.040 ppm Precipitate 2.0% TRR 0.054 ppm Other 7.7% TRR 0.207 ppm
Peel	4.3	0.115	Extracted with ACN.
ACN	3.2	0.086	TLC resolved the parent as the major ^{14}C-residue identified; however, no quantitative data were provided.
Nonextractable	1.0	0.027	Not further analyzed (N/A).
Pulp	0.9	0.024	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	0.6	0.016	TLC resolved the parent as the major ^{14}C-residue identified; however, no quantitative data were provided.
Aqueous	0.1	0.003	N/A.
Nonextractable	0.0	0.000	N/A.
3-DAT Fruit (TRR = 3.226 ppm)			
Rinse	86.0	2.775	TLC: Spinosyn A 33.4% TRR 1.077 ppm Spinosyn B 11.3% TRR 0.365 ppm Origin 19.3% TRR 0.623 ppm Other 22.0% TRR 0.710 ppm HPLC: Spinosyn A 34.2% TRR 1.103 ppm Spinosyn B 3.4% TRR 0.110 ppm Precipitate 2.5% TRR 0.081 ppm Other 45.8% TRR 1.478 ppm
Peel	13.2	0.425	Extracted with ACN.
ACN	6.5	0.210	TLC identified the parent and a significant amount of radioactivity at the origin; however, no quantitative data were provided.
Nonextractable	5.7	0.184	N/A.

(continued; footnotes follow)

Table 2-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Pulp	0.8	0.026	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	0.3	0.010	TLC identified the parent and a significant amount of radioactivity at the origin; however, no quantitative data were provided.
Aqueous	0.3	0.010	N/A.
Nonextractable	0.1	0.003	N/A.
7-DAT Fruit (TRR = 2.290 ppm)			
Rinse	81.2	1.858	TLC: Spinosyn A 17.7% TRR 0.405 ppm Spinosyn B 9.8% TRR 0.224 ppm Origin 32.6% TRR 0.747 ppm Other 21.1% TRR 0.483 ppm HPLC: Spinosyn A 16.6% TRR 0.380 ppm Spinosyn B 2.5% TRR 0.057 ppm Precipitate 6.0% TRR 0.137 ppm Other 56.1% TRR 1.285 ppm
Peel	17.3	0.397	N/A.
Pulp	1.5	0.035	N/A.
14-DAT Fruit (TRR = 1.845 ppm)			
Rinse	79.8	1.471	TLC: Spinosyn A 11.3% TRR 0.208 ppm Spinosyn B 6.8% TRR 0.125 ppm Origin 37.5% TRR 0.692 ppm Other 24.3% TRR 0.448 ppm HPLC: Spinosyn A 10.2% TRR 0.188 ppm Spinosyn B 3.0% TRR 0.055 ppm Precipitate 6.6% TRR 0.122 ppm Other 60.0% TRR 1.107 ppm
Peel	17.2	0.318	Extracted with ACN.
ACN	8.1	0.149	Initial TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided. SPE/TLC Spinosyn A 0.011% TRR < 0.001 ppm Spinosyn B 0.004% TRR < 0.001 ppm Origin 0.009% TRR < 0.001 ppm

(continued; footnotes follow)

Table 2-A *(continued)*)

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Nonextractable	9.3	0.172	Sequentially extracted with ACN:water, refluxed with mild acid (0.1 N HCl) and base (0.1 N NaOH), and strong base (1 N NaOH), and partitioned with DCM and EtOAc. Separately, enzyme hydrolyzed with cellulase and pectinase; little radioactivity (0.6% TRR, 0.011 ppm) was released by enzyme hydrolysis.
Final organic	0.4	0.007	N/A.
Final aqueous	1.4	0.026	N/A.
Final nonextractable	0.1	0.002	N/A.
Pulp	3.0	0.056	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	0.6	0.011	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Aqueous	1.9	0.035	N/A.
Nonextractable	0.4	0.007	N/A.
28-DAT Fruit (TRR = 1.570 ppm)			
Rinse	54.2	0.851	TLC: Spinosyn A 5.0% TRR 0.079 ppm Spinosyn B 0.9% TRR 0.014 ppm Origin 35.5% TRR 0.557 ppm Other 12.9% TRR 0.203 ppm
Peel	25.6	0.402	N/A.
Pulp	20.2	0.317	N/A.
42-DAT Fruit (TRR = 1.251 ppm)			
Rinse	63.9	0.801	TLC: Spinosyn A 1.9% TRR 0.024 ppm Spinosyn B Not resolved Not resolved Origin 46.3% TRR 0.579 ppm Other 15.7% TRR 0.196 ppm
Peel	26.4	0.331	Extracted with ACN.
ACN	10.7	0.134	SPE/TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Nonextractable	11.1	0.139	Sequentially extracted with ACN:water, refluxed with mild acid (0.1 N HCl), and partitioned with DCM and EtOAc.

(continued; footnotes follow)

Table 2-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Final organic	1.1	0.014	TLC identified spinosyn A (~0.1% TRR, 0.001 ppm); however, no quantitative data were provided.
Final aqueous	1.4	0.018	N/A.
Final nonextractable	4.1	0.051	N/A.
Pulp	9.5	0.119	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	1.7	0.021	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Aqueous	6.5	0.081	Treated with phenylhydrazine to precipitate sugars as osazone derivatives; MS confirmed osazone derivatives.
Nonextractable	0.6	0.008	N/A.
0-DAT Leaves (TRR = 216.9 ppm)			
Rinse	98.7	214.080	TLC: Spinosyn A 84.5% TRR 183.281 ppm Other 12.8% TRR 27.763 ppm SPE/TLC Spinosyn A 91.1% TRR 197.596 ppm Remaining radioactivity characterized as polar and/or multicomponent. HPLC confirmed spinosyn A and identified spinosyn B.
ACN	1.0	2.169	SPE/TLC: Spinosyn A 0.8% TRR 1.735 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	0.4	0.868	N/A.
3-DAT Leaves (TRR = 135.1 ppm)			
Rinse	85.7	115.781	TLC: Spinosyn A 15.9% TRR 21.481 ppm Other 65.9% TRR 89.031 ppm SPE/TLC: Spinosyn A 14.2% TRR 19.184 ppm Remaining radioactivity characterized as polar and/or multicomponent. HPLC confirmed spinosyn A.

(continued; footnotes follow)

Table 2-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
ACN	4.3	5.809	SPE/TLC: Spinosyn A 0.7% TRR 0.946 ppm Remaining radioactivity characterized as polar and/or multicomponent. HPLC/MS confirmed spinosyn A and identified spinosyn B.
Nonextractable	9.9	13.375	A mild acidic extraction was initiated but was terminated because of accident (i.e., equipment breakage).
7-DAT Leaves (TRR = 205.8 ppm)			
Rinse	85.1	175.136	TLC: Spinosyn A 9.8% TRR 20.168 ppm Other 73.1% TRR 150.440 ppm SPE/TLC: Spinosyn A 10.7% TRR 22.021 ppm Remaining radioactivity characterized as polar and/or multicomponent.
ACN	3.8	7.820	SPE/TLC: Spinosyn A 0.5% TRR 1.029 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	11.0	22.638	N/A.
10-DAT Leaves (TRR = 175.4 ppm)			
Rinse	71.8	125.937	TLC: Spinosyn A 4.9% TRR 8.595 ppm Other 64.9% TRR 113.835 ppm SPE/TLC: Spinosyn A 2.9% TRR 5.087 ppm Remaining radioactivity characterized as polar and/or multicomponent.
ACN	6.2	10.875	SPE/TLC: Spinosyn A 0.1% TRR 0.175 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	22.0	38.588	N/A.

(continued; footnotes follow)

Table 2-A *(continued)*)

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
28-DAT Leaves (TRR = 128.3 ppm)			
Rinse	61.0	78.263	TLC: Spinosyn A Not resolved Not resolved Other 48.9% TRR 62.739 ppm SPE/TLC: Spinosyn A 1.3% TRR 1.668 ppm Remaining radioactivity characterized as polar and/or multicomponent. HPLC confirmed radioactivity was multicomponent in nature.
ACN	6.8	8.724	SPE/TLC: Spinosyn A 0.2% TRR 0.257 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	32.2	41.313	Sequentially hydrolyzed with 0.1 N HCl and MeOH, and the fractions combined and partitioned with EtOAc.
EtOAc	3.5	4.491	TLC Other 3.08% TRR 3.952 ppm HPLC confirmed residues were multicomponent.
Aqueous	13.7	17.577	HPLC Other 13.61% TRR 17.462 ppm SPE fractionation confirmed residues are highly polar.
Nonextractable	15.0	19.245	Hydrolyzed with H_2SO_4 and the hydrolysate reacted with phenylhydrazine to form glucosazone.
Glucosazone	2.3	3.014	MS characterized radioactivity as being incorporated into glucose sub-units of cellulose and other natural plant constituents.
Nonextractable	5.6	7.122	N/A.

[a] The % TRR was normalized to 100% by the petitioner.

[b] Ppm values are expressed in terms of [^{14}C]spinosyn A equivalents and were calculated by the study reviewer.

[c] The "other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin. The "other" radioactivity quantified from HPLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or precipitate. Precipitate is the material resulting from concentration and preparation of samples for HPLC analyses.

Table 2-B. Distribution of residues in/on apple matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn D at 349 ppm.

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Fruit (TRR = 0.981 ppm)			
Rinse	92.5	0.908	TLC: Spinosyn D 83.8% TRR 0.822 ppm N-Demethyl Spinosyn D 3.7% TRR 0.036 ppm Origin 0.0% TRR 0.000 ppm Other 5.0% TRR 0.049 ppm HPLC: Spinosyn D 73.2% TRR 0.718 ppm N-Demethyl Spinosyn D 3.5% TRR 0.034 ppm Precipitate 2.4% TRR 0.024 ppm Other 13.4% TRR 0.131 ppm
Peel	3.2	0.031	Extracted with ACN.
ACN	2.1	0.021	TLC resolved the parent as the major ^{14}C-residue identified; however, no quantitative data were provided.
Nonextractable	0.5	0.005	Not further analyzed (N/A).
Pulp	4.3	0.042	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	3.5	0.034	TLC resolved the parent as the major ^{14}C-residue identified; however, no quantitative data were provided.
Aqueous	0.0	0.000	N/A.
Nonextractable	0.0	0.000	N/A.
3-DAT Fruit (TRR = 1.158 ppm)			
Rinse	76.3	0.883	TLC: Spinosyn D 10.2% TRR 0.118 ppm N-Demethyl Spinosyn D 11.2% TRR 0.130 ppm Origin 23.5% TRR 0.272 ppm Other 31.3% TRR 0.362 ppm HPLC: Spinosyn D 9.3% TRR 0.108 ppm N-Demethyl Spinosyn D 2.0% TRR 0.023 ppm Precipitate 4.4% TRR 0.051 ppm Other 60.6% TRR 0.702 ppm
Peel	21.4	0.248	Extracted with ACN.
ACN	11.2	0.130	TLC identified the parent and a significant amount of radioactivity at the origin; however, no quantitative data were provided.
Nonextractable	7.8	0.090	N/A.

(continued; footnotes follow)

Table 2-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Pulp	2.4	0.027	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	2.5	0.029	TLC identified the parent and a significant amount of radioactivity at the origin; however, no quantitative data were provided.
Aqueous	0.4	0.005	N/A.
Nonextractable	0.3	0.003	N/A.
7-DAT Fruit (TRR = 1.209 ppm)			
Rinse	73.9	0.894	TLC: Spinosyn D 5.8% TRR 0.070 ppm N-Demethyl Spinosyn D 4.4% TRR 0.053 ppm Origin 36.6% TRR 0.442 ppm Other 27.0% TRR 0.326 ppm HPLC: Spinosyn D 4.5% TRR 0.054 ppm N-Demethyl Spinosyn D 1.1% TRR 0.013 ppm Precipitate 7.3% TRR 0.088 ppm Other 61.0% TRR 0.737 ppm
Peel	15.7	0.190	N/A.
Pulp	10.4	0.125	N/A.
14-DAT Fruit (TRR = 0.842 ppm)			
Rinse	70.3	0.592	TLC: Spinosyn D Not resolved Not resolved N-Demethyl Spinosyn D 1.8% TRR 0.015 ppm Origin 41.9% TRR 0.353 ppm Other 26.5% TRR 0.223 ppm
Peel	25.8	0.217	Extracted with ACN.
ACN	9.5	0.080	Initial TLC and SPE/TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Nonextractable	12.4	0.104	Sequentially extracted with ACN:water, refluxed with mild acid (0.1 N HCl), and partitioned with DCM and EtOAc.
Final organic	1.6	0.013	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Final aqueous	1.1	0.009	N/A.
Final nonextractable	4.6	0.039	N/A.
Pulp	3.9	0.033	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	1.6	0.013	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.

(continued; footnotes follow)

Table 2-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Aqueous	2.6	0.022	N/A.
Nonextractable	0.6	0.005	N/A.
28-DAT Fruit (TRR = 0.741 ppm)			
Rinse	59.1	0.438	TLC: Spinosyn D 2.5% TRR 0.019 ppm N-Demethyl Spinosyn D 2.0% TRR 0.015 ppm Origin 43.9% TRR 0.325 ppm Other 10.7% TRR 0.079 ppm
Peel	34.3	0.254	N/A.
Pulp	6.6	0.049	N/A.
42-DAT Fruit (TRR = 0.513 ppm)			
Rinse	58.6	0.301	TLC: Spinosyn D Not resolved Not resolved N-Demethyl Spinosyn D Not resolved Not resolved Origin 42.8% TRR 0.220 ppm Other 15.8% TRR 0.081 ppm
Peel	32.8	0.168	Extracted with ACN.
ACN	14.1	0.072	Initial TLC and SPE/TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Nonextractable	12.8	0.066	Sequentially extracted with ACN:water, refluxed with mild acid (0.1 N HCl), and partitioned with DCM and EtOAc.
Final organic	1.7	0.009	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Final aqueous	1.3	0.007	N/A.
Final nonextractable	4.7	0.024	N/A.
Pulp	8.6	0.044	Extracted with ACN:water and ACN, and the filtrates partitioned with DCM and EtOAc.
Organic	2.4	0.012	TLC showed significant amount of radioactivity at the origin; however, no quantitative data were provided.
Aqueous	6.5	0.033	Treated with phenylhydrazine to precipitate sugars as osazone derivatives; residues are not associated with sugars as low radioactivity was recovered in the osazone precipitate (1.2% TRR, 0.006 ppm); MS confirmed osazone derivatives.
Nonextractable	0.7	0.004	N/A.

(continued; footnotes follow)

Table 2-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Leaves (TRR = 88.7 ppm)			
Rinse	98.1	87.015	TLC: Spinosyn D 88.7% TRR 78.677 ppm Other 8.1% TRR 7.185 ppm SPE/TLC: Spinosyn D 90.2% TRR 80.007 ppm Remaining radioactivity was characterized as polar and/or multicomponent. HPLC confirmed spinosyn D and identified N-demethyl spinosyn D.
ACN	1.5	1.331	SPE/TLC: Spinosyn D 1.2% TRR 1.064 ppm Remaining radioactivity was characterized as polar and/or multicomponent.
Nonextractable	0.4	0.355	N/A.
3-DAT Leaves (TRR = 96.6 ppm)			
Rinse	82.2	79.405	TLC: Spinosyn D 2.8% TRR 2.705 ppm Other 77.9% TRR 75.251 ppm SPE/TLC: Spinosyn D 7.2% TRR 6.955 ppm Remaining radioactivity was characterized as polar and/or multicomponent. HPLC confirmed spinosyn D.
ACN	6.7	6.472	SPE/TLC: Spinosyn D 0.3% TRR 0.290 ppm Remaining radioactivity was characterized as polar and/or multicomponent. HPLC/MS confirmed spinosyn D.
Nonextractable	11.2	10.819	Sequentially hydrolyzed with 0.1 N HCl and MeOH, and the fractions combined and then partitioned with EtOAc.
EtOAc	2.4	2.318	TLC Other 2.25% TRR 2.174 ppm HPLC confirmed residues were multiple polar components.

(continued; footnotes follow)

Table 2-B *(continued)*)

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Aqueous	4.1	3.961	HPLC Other 4.06% TRR 3.922 ppm SPE fractionation confirmed residues were highly polar.
Nonextractable	4.7	4.540	Hydrolyzed with H_2SO_4 and the hydrolysate reacted with phenylhydrazine to form glucosazone.
Glucosazone	0.8	0.729	MS characterized radioactivity as being incorporated into glucose sub-units of cellulose and other natural plant constituents.
Nonextractable	2.0	1.958	N/A.
7-DAT Leaves (TRR = 70.6 ppm)			
Rinse	77.6	54.786	TLC: Spinosyn D Not resolved Not resolved Other 72.6% TRR 51.256 ppm SPE/TLC: Spinosyn D 2.5% TRR 1.765 ppm Remaining radioactivity characterized as polar and/or multicomponent.
ACN	7.2	5.083	SPE/TLC: Spinosyn D 0.3% TRR 0.212 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	15.1	10.661	N/A.
10-DAT Leaves (TRR = 71.8 ppm)			
Rinse	69.4	49.829	TLC: Spinosyn D Not resolved Not resolved Other 63.8% TRR 45.808 ppm SPE/TLC: The parent was not resolved. Radioactivity was characterized as polar and/or multicomponent.
ACN	8.3	5.959	SPE/TLC: Spinosyn D 0.2% TRR 0.144 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	22.3	16.011	N/A.

(continued; footnotes follow)

Table 2-B *(continued)*)

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
28-DAT Leaves (TRR = 43.1 ppm)			
Rinse	57.5	24.783	TLC: Spinosyn D Not resolved Not resolved 13 Other regions 52.7% TRR 22.714 ppm SPE/TLC: The parent was not resolved. Radioactivity was characterized as polar multicomponent. HPLC confirmed residues were multicomponent.
ACN	11.1	4.784	SPE/TLC: Spinosyn D 0.5% TRR 0.216 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	31.5	13.577	Sequentially hydrolyzed with 0.1 N HCl and MeOH, and the fractions combined and then partitioned with EtOAc.
EtOAc	3.2	1.379	TLC Other 2.73% TRR 1.177 ppm HPLC confirmed residues were multiple polar components.
Aqueous	13.4	5.775	HPLC Other 13.31% TRR 5.737 ppm SPE fractionation confirmed residues were highly polar.
Nonextractable	14.9	6.422	Hydrolyzed with H_2SO_4 and the hydrolysate reacted with phenylhydrazine to form glucosazone.
Glucosazone	2.2	0.952	MS characterized radioactivity as being incorporated into glucose sub-units of cellulose and other natural plant constituents.
Nonextractable	7.2	3.089	N/A.

[a] The % TRR was normalized to 100% by the petitioner.

[b] Ppm values are expressed in terms of [^{14}C]spinosyn A equivalents and were calculated by the study reviewer.

[c] The "other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin. The "other" radioactivity quantified from HPLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or precipitate. Precipitate is the material resulting from concentration and preparation of samples for HPLC analyses.

Table 3-A. Summary of radioactive residues initially identified in/on apple matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A at 885 ppm.

Fraction/Metabolite		0-DAT Fruit (TRR = 2.694 ppm)		3-DAT Fruit (TRR = 3.226 ppm)		14-DAT Fruit [b] (TRR = 1.845 ppm)		42-DAT Fruit [b] (TRR = 1.251 ppm)	
		% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]
Identified by TLC (Confirmed by HPLC)									
Spinosyn A		85.9	2.314	33.4	1.077	11.3	0.208	1.9	0.024
Spinosyn B		3.4	0.092	11.3	0.365	6.8	0.125	--	--
Total Identified		**89.3**	**2.406**	**44.7**	**1.442**	**18.1**	**0.333**	**1.9**	**0.024**
Characterized									
Rinse:	Origin	1.6	0.043	19.3	0.623	37.5	0.692	46.3	0.579
	Other [c]	3.9	0.105	22.0	0.710	24.3	0.448	15.7	0.196
Peel:	ACN [d]	3.2	0.086	6.5	0.210	8.1	0.149	10.7	0.134
Pulp:	Organic [d]	0.6	0.016	0.3	0.010	0.6	0.011	1.7	0.021
	Aqueous	0.1	0.003	0.3	0.010	1.9	0.035	6.5	0.081
Total Identified/ Characterized		**98.7**	**2.659**	**93.1**	**3.005**	**90.5**	**1.668**	**82.8**	**1.035**
Nonextractable		1.0	0.027	5.8	0.187	9.7	0.179	11.7	0.147

[a] Ppm values are expressed in terms of [^{14}C]spinosyn A equivalents and were calculated by the study reviewer.

[b] Exhaustive fractionation and characterization of radioactive residues in 14- and 42-DAT rinse, peel, and pulp was conducted. These procedures showed that the majority of the radioactivity was composed of polar and/or multicomponent residues present at low levels.

[c] The "other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

[d] TLC analyses of the fraction resolved the parent from samples collected at early posttreatment intervals. However, no quantitative data were provided.

Table 3-B. Summary of radioactive residues initially identified in/on apple matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn D at 349 ppm.

Fraction/Metabolite	0-DAT Fruit (TRR = 0.981 ppm)		3-DAT Fruit (TRR = 1.158 ppm)		14-DAT Fruit [b] (TRR = 0.842 ppm)		42-DAT Fruit [b] (TRR = 0.513 ppm)	
	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]
Identified by TLC (Confirmed by HPLC)								
Spinosyn D	83.8	0.822	10.2	0.118	--	--	--	--
N-demethyl spinosyn D	3.7	0.036	11.2	0.130	1.8	0.015	--	--
Total Identified	**87.5**	**0.858**	**21.4**	**0.248**	**1.8**	**0.015**	--	--
Characterized								
Rinse: Origin	0.0	0.000	23.5	0.272	41.9	0.353	42.8	0.220
Other [c]	5.0	0.049	31.3	0.362	26.5	0.223	15.8	0.081
Peel: ACN [d]	2.1	0.021	11.2	0.130	9.5	0.080	14.1	0.072
Pulp: Organic [d]	3.5	0.034	2.5	0.029	1.6	0.013	2.4	0.012
Aqueous	0.0	0.000	0.4	0.005	2.6	0.022	6.5	0.033
Total Identified/ Characterized	**98.1**	**0.962**	**90.3**	**1.046**	**83.9**	**0.706**	**81.6**	**0.418**
Nonextractable	0.5	0.005	8.1	0.093	13.0	0.109	13.5	0.070

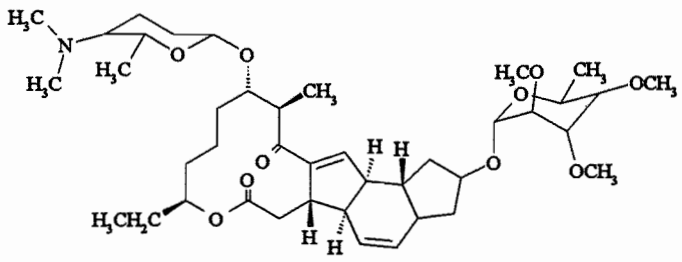
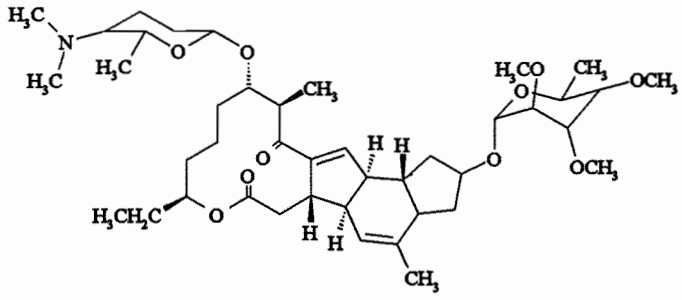
[a] Ppm values are expressed in terms of [^{14}C]spinosyn D equivalents and were calculated by the study reviewer.

[b] Exhaustive fractionation and characterization of radioactive residues in 14- and 42-DAT rinse, peel, and pulp was conducted. These procedures showed that the majority of the radioactivity was composed of polar and/or multicomponent residues present at low levels.

[c] The "other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

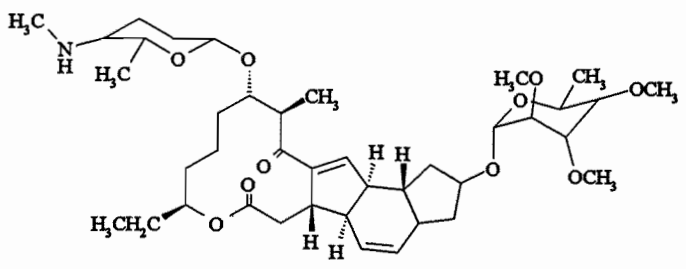
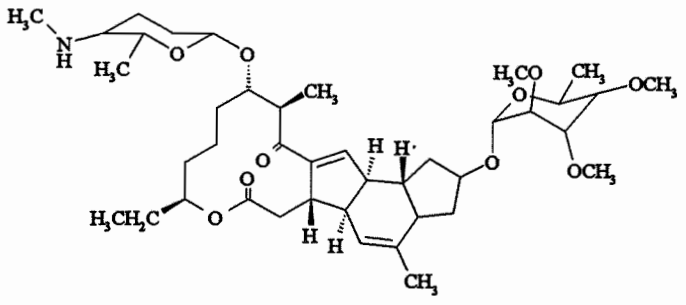
[d] TLC analyses of the fraction resolved the parent from samples collected at early posttreatment intervals. However, no quantitative data were provided.

Figure 2. Chemical structures of spinosad and its identified metabolites in plants.

Common Name Chemical Name	Structure	Crops - Commodities/Substrates
Spinosyn A **(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		**Apples** - Fruits (rinse, peel, and pulp) and leaves. **Cabbage** - Leaves. **Tomato** - Fruit, juice, and wet pomace. **Turnip** - Tops and roots.
Spinosyn D **(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-methyl-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione		**Apples** - Fruits (rinse, peel, and pulp) and leaves. **Cabbage** - Leaves. **Turnip** - Tops and roots.

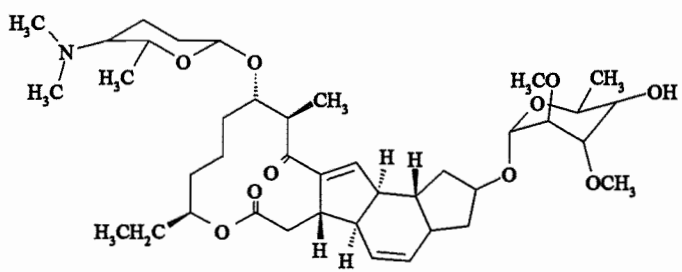
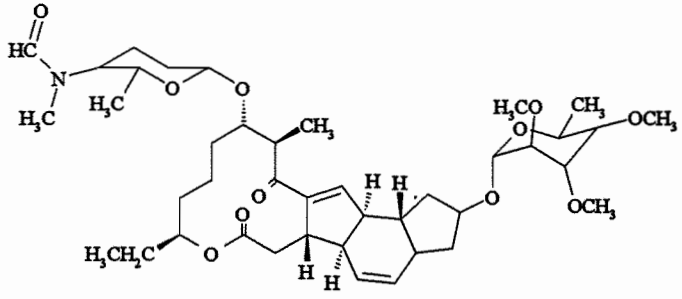
(continued; footnote follows)

Figure 2. *(continued.)*

Common Name Chemical Name	Structure	**Crops** - Commodities/Substrates
Spinosyn B **(Factor B)** 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,-10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-13-[[tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl]oxy]-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione		**Apples** - Fruits and leaves. **Cabbage** - Leaves. **Tomato** - Fruit, juice, and wet pomace. **Turnip** - Tops and roots.
N-Demethyl Spinosyn D **(Factor B of D)** 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,-10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-13-[[tetrahydro-6-methyl-5-(methylamino)-2H-pyran-2-yl]oxy]-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione		**Apples** - Fruits and leaves. **Cabbage** - Leaves. **Turnip** - Turnip tops and roots.

(continued; footnote follows)

Figure 2. *(continued.)*

Common Name Chemical Name	Structure	**Crops** - Commodities/Substrates
Spinosyn K **(Factor K)** 2-[(6-deoxy-2,3-di-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		**Turnip** - Tops and roots. [a]
N-Formyl Spinosyn B **(X323,450)** 2-[(6-deoxy-2,3,4-tri-O-methyl-α-L-manno-pyranosyl)oxy]-9-ethyl-2,3,3a,5a,5b,6,9,-10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-13-[[tetrahydro-6-methyl-5-(N-formyl-N-methylamino)-2H-pyran-2-yl]oxy]-1H-as-Indaceno[3,2-d]oxacyclododecin-7,15-dione		**Turnip** - Tops. [a]

[a] Identified but not quantified.

Storage stability: All rinsates, peel, and pulp samples were stored frozen (-20 C) prior to analysis. The determination of total radioactivity and the initial characterization of radioactive residues in rinses, pulp, and peel were completed in <3 months. The portion of the study pertaining to exhaustive characterization of radioactive residues in rinses, pulp, and peel was completed within 11 months. To demonstrate that radioactive residues had not declined during the study period, the petitioner provided data comparing the residue profiles of 0-DAT rinses that were fractionated and characterized by the procedures described earlier at the study initiation and before termination. The results indicate that radioactive residues of spinosyns A, B, and D, and N-demethyl spinosyn D did not degrade as a result of sample shipping and storage.

Conclusions: The apple metabolism studies are acceptable. The majority of the total radioactivity remained on apple fruit and leaf surfaces and was soluble in organic solvents. Initial chromatographic analyses of apple fruit and leaf rinses and the organic extracts of pulp, peel, and leaf suggest that spinosyns A and D are rapidly metabolized. The parents comprised the majority of the total radioactivity in 0-DAT fruit and leaf rinses; spinosyn B and N-demethyl spinosyn D were identified as minor metabolites. The residue levels of spinosyns A and D declined significantly in fruit/leaf rinses collected/analyzed at later sampling intervals; the decrease in residue levels of the parents was accompanied by incremental increases in polar components that remained at the origin of TLC plates. Extensive fractionation and characterization of polar ^{14}C-residues in selected fruit and leaf rinses and in peel and pulp samples indicated that most of the radioactivity was degraded to multicomponent residues of low molecular weights which are subsequently incorporated into natural plant constituents.

Cabbage

DowElanco has submitted data from a single study (citation listed below) depicting the metabolism of [^{14}C]spinosyn A and [^{14}C]spinosyn D on cabbage following a single application. The field portion of this study was conducted by DowElanco Research Station (Wayside, MS). The residue characterization portion was conducted by the Residue Chemistry Group of DowElanco Research Laboratories (Indianapolis, IN).

> MRID 44058812. Berard, D. (1995) Nature of (carbon-14) XDE-105: Residues Resulting from a Single Application of Compound to Cabbage: Lab Project Number: MET92019. Unpublished Study Prepared by DowElanco. 145 p.

Set-up: Cabbage plants (Wakamine variety) that had been grown from seed were transplanted to three plots. Each plot was 16 feet in length with two rows of cabbage plants each separated by 2 feet. One plot was treated with the test substance [^{14}C]spinosyn A, another plot with [^{14}C]spinosyn D, and the third plot served as a control. The plots were separately enclosed with wire fencing and the soil was covered with polyethylene plastic to prevent contamination.

Application: [^{14}C]Spinosyn A and [^{14}C]spinosyn D were applied as a spray treatment at 1444 and 1384 ppm, respectively; the intended application concentration was 1349 ppm. The treatment was made to immature cabbage plants approximately one month prior to maturity using a pressurized hand sprayer. According to the petitioner, the application rates were 3.1x and 12.3x, respectively, the proposed maximum seasonal rate on cabbage for spinosyns A and D. The petitioner's calculations assume that each plant was treated with 34.2 mg of spinosyn A or D, that each plant could be treated with 13.9 mg of spinosyn A or D over the growing season, and that the spinosad commercial products contain ~80% spinosyn A [34.2 mg/plant ÷ (13.9 mg/plant x 80%)] and ~20% spinosyn D [34.2 mg/plant ÷ (13.9 mg/plant x 0.80%)].

Sample collection and shipping: Cabbage plants were harvested, by cutting the stem just above the soil, at 0, 3, 10, 19, and 34 days after treatment (DAT). Two plants were harvested at each interval except at the final harvest when all of the remaining treated plants were collected. The roots were dug from the soil with a small shovel. Samples were packaged with an ice substitute and shipped by air freight to the analytical laboratory, DowElanco. Upon receipt at DowElanco, samples were temporarily refrigerated or larger samples were frozen. Certain leaf samples (i.e., 10-DAT samples) were separated into "upper leaves" (those containing the cabbage head and leaves formed after the application of the test substances) and the "lower leaves" (those which were actually treated with the test substances). While the "lower leaves" would typically not be harvested commercially, these samples were collected and analyzed because they were expected to contain the highest level of radioactivity. Root samples were washed under a stream of water to remove soil. All leaf and root samples were frozen with liquid nitrogen, homogenized, and stored frozen (-20 C) prior to analysis.

Total radioactive residues (TRR): The total radioactivity in leaf and root samples, both before and after extraction, was determined by direct LSS, or combustion and LSS. The LSS LOD was not reported. The results of the TRR determinations are presented in Table 4.

Table 4. Total radioactive residues in/on cabbage matrices harvested at various intervals following a single foliar spray application of uniformly ring-labeled [^{14}C]spinosyn A (1444 ppm) or D (1384 ppm).

DAT [a]	Replicate	TRR (ppm [^{14}C]spinosyn A or D equivalents)			
		Leaves			Roots
		Upper [b]	Lower [c]	Total [d]	
Spinosyn A Treatment					
0	1	29.413	--	29.413	0.026
	2	74.356	--	74.356 [e]	0.045
3	1	19.204	--	19.204 [e]	0.241
	2	17.149	--	17.149	0.147
10	1	1.070	4.884	3.776	0.198
	2	4.338	--	4.338 [e]	0.285
19	1	0.187	4.334	2.182	0.162
	2	0.085	3.933 [e]	1.923	0.148
34	1	0.030	2.445 [e]	0.778	0.444
	2	0.037	2.099	0.727	0.384
Spinosyn D Treatment					
0	1	89.143	--	89.143 [e]	0.089
	2	52.317	--	52.317	0.204
3	1	25.419	--	25.419 [e]	0.399
	2	17.495	--	17.945	0.166
10	1	6.097	--	6.097	0.763
	2	6.519	--	6.519 [e]	0.309
19	1	0.054	2.683	1.409	0.103
	2	0.126	5.278 [e]	2.945	0.268
34	1	0.017	2.481 [e]	0.891	0.267
	2	0.020	2.039	0.717	0.247

[a] DAT = Days after treatment.

[b] Upper refers to the upper portion of the plant or the new growth such as the head if the sample was divided into two portions. If not, "upper" refers to the entire aerial portion of the plant.

[c] Lower leaves include the treated leaves; lower leaves are typically not harvested commercially.

[d] Total leaf residues were calculated by the petitioner from the sum of the total dpm in each of the upper and lower leaf samples divided by the total weight of the aerial portion of the plant.

[e] Samples extracted for residue analysis.

Extraction of ^{14}C-residues: Radioactive residues in leaves and roots were initially extracted with ACN:water (80:20, v:v) and ACN. The initial extracts were combined and partitioned with DCM; the resulting aqueous phase was further partitioned with EtOAc. The DCM and EtOAc phases were combined to yield an organic phase which was evaporated to dryness and

reserved for chromatographic analysis. Selected fractions/extracts were further subjected to SPE procedures using C-18 Sep-Pak cartridge and various elution solvents. The eluate(s) containing the highest radioactivity was reserved for chromatographic analysis. Radioactivity in the extracts was determined by LSS; the radioactivity in the nonextractable residues was determined by LSS following combustion.

Characterization and identification of ^{14}C-residues: The various cabbage leaf and root extracts were analyzed by one-dimensional TLC and/or reversed-phase HPLC. TLC separations were conducted as described for the apple metabolism study except for use of the solvent system, toluene:isopropanol:diethylamine (84:7:7, v:v:v; System A) and EtOAc:diethylamine (99:1, v:v; System B). Prior to TLC quantitation, the 19-DAT and 34-DAT leaf samples required clean-up on silica SPE columns using the following elution solvents: toluene, DCM:ACN, MeOH, and MeOH + acetic acid. The MeOH fractions (containing the highest radioactivity) were combined for TLC analysis. HPLC separations were accomplished using the system and mobile phases described for the apple metabolism study. Initial identification of ^{14}C-residues was confirmed by cochromatography with non-labeled reference standards and/or by MS as described for the apple metabolism study. The petitioner provided sample calculations related to the biological and analytical phases of the study and representative TLC and HPLC chromatograms and MS spectra.

Mild acidic hydrolysis of the organic extracts of leaves was performed to demonstrate that the amino sugar or forosamine portion of spinosyns A and D may be selectively cleaved to produce compounds like those designated as pseudoaglycones (ps). This procedure was also employed to confirm the identification of the parent compounds. Briefly, aliquots were evaporated to dryness and redissolved in MeOH then heated to 65 C for 30 minutes with 1 N HCl, and extracted repeatedly with EtOAc. The EtOAc extracts were combined, concentrated, and reserved for chromatographic analysis. HPLC analysis identified individual components of pseudoaglycones of Spinosyns H, J, and K; identification of psA and psK was confirmed by MS.

The distribution of radioactive residues in/on cabbage matrices following routine solvent extraction and chromatographic analysis is presented in Tables 5-A and 5-B. Summaries of metabolites identified in cabbage are presented in Tables 6-A and 6-B. The chemical names and molecular structures of these metabolites are depicted in Figure 2.

The data presented in Tables 5-A and 5-B suggest that spinosyns A and D are rapidly metabolized. The parent compounds, spinosyns A and D, were the major radioactive residues identified in 0-DAT extracts; the metabolites spinosyn B and N-demethyl spinosyn D were identified as minor metabolites. However, less than 11% and 14% of the total radioactivity remained as spinosyns A and D, respectively, on 3-DAT extracts. The majority of the radioactivity in the organic fractions of cabbage leaf and root samples collected from later intervals remained at the TLC plate origin.

Further characterization of polar and nonextractable ^{14}C-residues: Exhaustive attempts were made to elucidate the nature of the polar and nonextractable ^{14}C-residues. For these investigations, the 3- and 34-DAT samples were utilized. A brief summary of the procedures and results are described below.

Subsamples of the aqueous fraction of the 3-DAT and 34-DAT leaf samples following the initial extraction were subjected to various characterization techniques [i.e., column chromatography (C-18 SPE chromatography and cation exchange resin chromatography), solvent partitioning at acidic and alkaline pH, enzyme treatment (β-glucosidase), and/or strong acid hydrolysis]. Chromatographic analyses of extracts/fractions suggested that most of the radioactivity was polar, highly water soluble, or nonextractable. A relatively small amount of radioactivity in the 34-DAT cabbage sample (spinosyn A treatment) cochromatographed with the spinosyn A reference compound following enzyme hydrolysis; however, more than half of the TRR was not extractable into organic solvent following enzyme or strong acid hydrolysis. Ion exchange chromatography of the 3-DAT cabbage (spinosyn A and D treatment) and 34-DAT cabbage (spinosyn A treatment) samples suggested that residues were "unlike" the parent compounds because spinosyns A and D should remain bound to the cation exchange resin and approximately two-thirds of radioactivity from these samples was eluted from the ion exchange column with water.

The nonextractable radioactive residues of the 3-DAT and 34-DAT leaf samples were subjected to a more rigorous sequential extraction following the initial extraction procedure. Subsamples were repeatedly extracted with ACN and subjected to several characterization techniques [i.e., acid hydrolysis (0.1 N HCl), preparation of acid detergent fiber and osazone derivatives of glucose, and C-18 SPE chromatography]. Chromatographic analyses of extracts/fractions identified the parent compounds spinosyns A and D, and the metabolites spinosyn B and N-demethyl spinosyn D; however, the majority of the radioactivity remained at the plate origin suggesting that most of the radioactivity was polar, highly water soluble, or nonextractable. Mild acid hydrolyses of nonextractable residues were successful in solubilizing significant amounts of residues. A material with similar mobility to that of aglycone of spinosyn A was present in both samples treated with spinosyn A and samples treated with spinosyn D. The identification of glucosazone products was confirmed by MS thus supporting that the remaining radioactivity was incorporated into natural plant constituents.

Table 5-A. Distribution of residues in/on cabbage matrices following a single foliar application of uniformly ring-labeled [^{14}C]spinosyn A at 1444 ppm.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
O-DAT Cabbage Leaves (TRR = 74.356 ppm)			
Organic	94.1	69.936	TLC: Spinosyn A 40.6% TRR 30.157 ppm Spinosyn B 19.9% TRR 14.833 ppm Origin 6.7% TRR 4.968 ppm Other 26.5% TRR 19.591 ppm SPE/TLC: Spinosyn A 40.4% TRR 30.039 ppm
Aqueous	2.0	1.470	Not further analyzed (N/A).
Nonextractable	4.0	2.950	N/A.
3-DAT Cabbage Leaves (TRR = 19.204 ppm)			
Organic	70.6	13.550	TLC: Spinosyn A 10.2% TRR 1.952 ppm Spinosyn B 15.2% TRR 2.915 ppm Origin 21.9% TRR 4.203 ppm Other 22.7% TRR 4.325 ppm
Aqueous	14.2	2.733	Subjected to a series of partitioning procedures using various organic solvents. TLC and SPE/HPLC analyses of the phases characterized the majority of the radioactivity as polar (remaining at the origin).
Nonextractable	15.2	2.921	Nonextractable residues from a later extraction (14.5% TRR, 2.781 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	4.6	0.876	TLC: Spinosyn A 0.19% TRR 0.037 ppm Spinosyn B 0.45% TRR 0.087 ppm Origin 1.26% TRR 0.241 ppm Other 2.49% TRR 0.479 ppm
Aqueous	1.3	0.241	N/A.
Nonextractable	8.5	1.631	Hydrolyzed with 0.1 N HCl and the hydrolysate partitioned with EtOAc.
Organic	0.9	0.182	TLC: Origin 0.43% TRR 0.082 ppm Other 0.38% TRR 0.071 ppm HPLC and/or MS: Spinosyns A, B, and K, psA, and psK confirmed.
Aqueous	1.5	0.293	N/A.
Nonextractable	5.2	1.008	Subjected to acid detergent fiber and glucosazone preparation and MS; radiolabeled glucose identified.
10-DAT Cabbage Leaves (TRR = 4.338 ppm)			

(continued; footnotes follow)

Table 5-A *(continued)*.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
Organic	48.8	2.119	TLC: Spinosyn A 2.3% TRR 0.102 ppm Spinosyn B 10.4% TRR 0.453 ppm Origin 13.5% TRR 0.586 ppm Other 22.2% TRR 0.969 ppm
Aqueous	18.3	0.795	N/A.
Nonextractable	32.8	1.425	N/A.
19-DAT Cabbage Leaves (TRR = 3.933 ppm)			
Organic	45.8	1.801	SPE/TLC: Spinosyn A 1.1% TRR 0.042 ppm Spinosyn B 6.0% TRR 0.236 ppm Origin 11.8% TRR 0.464 ppm Other 24.0% TRR 0.753 ppm
Aqueous	12.8	0.503	N/A.
Nonextractable	41.4	1.629	N/A.
34-DAT Cabbage Leaves (TRR = 2.445 ppm)			
Organic	38.8	0.950	SPE/TLC: Spinosyn A 0.6% TRR 0.015 ppm Spinosyn B 1.2% TRR 0.028 ppm Origin 6.2% TRR 0.152 ppm Other 29.1% TRR 0.716 ppm
Aqueous	15.9	0.389	SPE/HPLC: The majority of residues eluted with more polar solvents; parent and degradates of similar polarity not resolved. Subjected to enzyme hydrolysis with β-glucosidase and the hydrolysate partitioned with EtOAc and analyzed chromatographically. TLC: Spinosyn A 1.0% TRR 0.024 ppm Origin 2.6% TRR 0.064 ppm Subjected to acid hydrolysis with 2 N HCl.
Nonextractable	45.3	1.107	Nonextractable residues from a later extraction (41.7% TRR, 1.019 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	7.4	0.181	N/A.
Aqueous	4.5	0.110	N/A.
Nonextractable	8.5	1.631	Hydrolyzed with 0.1 N HCl and the hydrolysate partitioned with EtOAc.
Organic	2.2	0.054	TLC resolved two predominant zones each containing <1% TRR; not further analyzed.

(continued; footnotes follow)

Table 5-A *(continued)*.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
Aqueous	7.8	0.191	N/A.
Nonextractable	16.9	0.413	Subjected to acid detergent fiber and glucosazone preparation and MS; radiolabeled glucose identified.
34-DAT Cabbage Roots (TRR = 0.444 ppm)			
Organic	26.8	0.119	Partitioned on small silica gel columns.
Aqueous	30.9	0.137	N/A.
Nonextractable	42.3	0.188	N/A.

[a] Expressed in terms of [^{14}C]spinosyn A equivalents.

[b] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, D, H, J, or K, N-demethyl spinosyn D, or the corresponding pseudoaglycones.

Table 5-B. Distribution of residues in/on cabbage matrices following a single foliar application of uniformly ring-labeled [^{14}C]spinosyn D at 1384 ppm.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
0-DAT Cabbage Leaves (TRR = 89.143 ppm)			
Organic	95.7	85.334	TLC: Spinosyn D 48.0% TRR 42.826 ppm N-Demethyl Spinosyn D 19.1% TRR 17.062 ppm Origin 3.0% TRR 2.645 ppm Other 25.6% TRR 22.801 ppm SPE/TLC: Spinosyn D 42.6% TRR 37.975 ppm
Aqueous	1.7	1.504	Not further analyzed (N/A).
Nonextractable	2.6	2.305	N/A.
3-DAT Cabbage Leaves (TRR = 25.419 ppm)			
Organic	74.7	18.998	TLC: Spinosyn D 13.4% TRR 3.418 ppm Spinosyn B/D 12.5% TRR 3.190 ppm Origin 21.4% TRR 5.450 ppm Other 26.2% TRR 6.665 ppm
Aqueous	11.2	2.837	Subjected to a series of partitioning procedures using various organic solvents. TLC and SPE/HPLC analyses of the phases characterized the majority of the radioactivity as polar (remaining at origin).
Nonextractable	14.1	3.584	Nonextractable residues from a later extraction (11.1% TRR, 2.811 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	3.4	0.875	TLC: Spinosyn D 0.1% TRR 0.026 ppm Spinosyn B/D 0.4% TRR 0.098 ppm Origin 0.9% TRR 0.237 ppm Other 1.5% TRR 0.390 ppm
Aqueous	1.2	0.305	N/A.
Nonextractable	7.0	1.769	Hydrolyzed with 0.1 N HCl and the hydrolysate partitioned with EtOAc.
Organic	1.0	0.260	TLC: Origin 0.2% TRR 0.047 ppm Other 0.6% TRR 0.138 ppm
Aqueous	1.2	0.299	N/A.
Nonextractable	4.3	1.082	Subjected to acid detergent fiber and glucosazone preparation and MS; radiolabeled glucose identified.

(continued; footnotes follow)

Table 5-B *(continued)*.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
10-DAT Cabbage Leaves (TRR = 6.519 ppm)			
Organic	57.3	3.732	TLC: Spinosyn D 5.3% TRR 0.347 ppm Spinosyn B/D 6.2% TRR 0.407 ppm Origin 14.0% TRR 0.911 ppm Other 31.2% TRR 1.699 ppm
Aqueous	16.3	1.063	N/A.
Nonextractable	26.4	1.724	N/A.
19-DAT Cabbage Leaves (TRR = 5.278 ppm)			
Organic	50.0	2.637	SPE/TLC: Spinosyn D 4.3% TRR 0.229 ppm Spinosyn B/D 4.6% TRR 0.241 ppm Origin 8.1% TRR 0.426 ppm Other 28.6% TRR 1.510 ppm
Aqueous	15.9	0.838	N/A.
Nonextractable	34.2	1.803	N/A.
34-DAT Cabbage Leaves (TRR = 2.481 ppm)			
Organic	43.5	1.079	SPE/TLC: Spinosyn D 4.5% TRR 0.112 ppm N-Demethyl Spinosyn D 4.1% TRR 0.103 ppm Origin 5.3% TRR 0.131 ppm Other 28.2% TRR 0.696 ppm
Aqueous	15.2	0.378	SPE/HPLC: Residues eluted with more polar solvents; parent and degradates of similar polarity not resolved.
Nonextractable	41.3	1.024	Subjected to acid detergent fiber and glucosazone preparation and MS; radiolabeled glucose identified.
34-DAT Cabbage Roots (TRR = 0.267 ppm)			
Organic	38.0	0.102	Partitioned on small silica gel columns.
Aqueous	21.9	0.058	N/A.
Nonextractable	40.1	0.107	N/A.

[a] Expressed in terms of [^{14}C]spinosyn A equivalents.

[b] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, D, H, J, or K, N-demethyl spinosyn D, or the corresponding pseudoaglycones.

Table 6-A. Summary of radioactive residues identified in/on cabbage matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A at 1444 ppm.

Fraction/Metabolite	0-DAT Leaves (TRR = 74.356 ppm)		3-DAT Leaves (TRR = 19.204 ppm)		34-DAT Leaves (TRR = 2.445 ppm)	
	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]
Identified by TLC (Confirmed by HPLC and/or MS)						
Spinosyn A	40.6	30.157	10.39	1.989	1.6	0.039
Spinosyn B	19.9	14.833	15.65	3.002	1.2	0.028
Total Identified	**60.5**	**44.990**	**26.04**	**4.991**	**2.8**	**0.067**
Characterized						
Origin	6.7	4.968	23.59	4.526	8.8	0.216
Other [b,c]	26.5	19.591	25.57	4.875	29.1	0.716
Aqueous	2.0	1.470	17.0	3.267	12.3	0.301
Total Identified/Characterized	**95.7**	**71.019**	**92.2**	**17.659**	**53.0**	**1.300**
Nonextractable	4.0	2.950	5.2	1.008	16.9	0.413

[a] Ppm values are expressed in terms of [^{14}C]spinosyn A equivalents.

[b] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, D, H, J, or K, N-demethyl spinosyn D, or the corresponding pseudoaglycones.

[c] Exhaustive fractionation and characterization of radioactive residues in 3-DAT and 34-DAT leaves was conducted. These procedures showed that the majority of the radioactivity was composed of polar, multicomponent residues present at low levels.

Table 6-B. Summary of radioactive residues identified in/on cabbage matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn D at 1384 ppm.

Fraction/Metabolite	0-DAT Leaves (TRR = 89.143 ppm)		3-DAT Leaves (TRR = 25.419 ppm)		34-DAT Leaves (TRR = 2.481 ppm)	
	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]
Identified by TLC						
Spinosyn D	48.0	42.826	13.5	3.444	4.5	0.112
Spinosyn B/D [b]	19.1	17.062	12.9	3.288	4.1	0.103
Total Identified	**67.1**	**59.888**	**26.4**	**6.732**	**8.6**	**0.215**
Characterized						
Origin	3.0	2.645	22.5	5.734	5.3	0.131
Other [c,d]	25.6	22.801	28.3	7.193	28.2	0.696
Aqueous	1.7	1.504	13.6	3.441	15.2	0.378
Total Identified/Characterized	**97.4**	**86.838**	**90.8**	**23.100**	**57.3**	**1.420**
Nonextractable	2.6	2.305	4.3	1.082	26.4 [e]	1.724 [e]

[a] Ppm values are expressed in terms of [^{14}C]spinosyn D equivalents.

[b] Zones which co-eluted with spinosyn B may be attributed to spinosyn B with some contribution from spinosyn D possible.

[c] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, D, H, J, or K, N-demethyl spinosyn D, or the corresponding pseudoaglycones.

[d] Exhaustive fractionation and characterization of radioactive residues in 3-DAT and 34-DAT leaves was conducted. These procedures showed that the majority of the radioactivity was composed of polar, multicomponent residues present at low levels.

[e] Further fractionation of these nonextractable residues suggested the incorporation into natural plant constituents.

Storage stability: All homogenized cabbage leaf and root samples were stored frozen (-20 C) for a maximum interval of 31 months prior to analysis. To demonstrate that radioactive residues had not declined during storage, the petitioner provided concurrent storage stability data. Subsamples of 0-DAT control cabbage leaves were separately fortified with [^{14}C]spinosyn A (specific activity 0.67 μCi/mg) and [^{14}C]spinosyn D (specific activity 0.64 μCi/mg) and stored frozen past the duration of the study. The total radioactive residues in the fortified samples remained consistent at the initiation/end of the storage stability investigation. The distribution of radioactive residues into organic, aqueous, and nonextractable fractions of fortified samples was also comparable with the distribution presented for treated samples.

Conclusions: The cabbage metabolism study is acceptable. The study indicates that the total radioactive residues declined at each subsequent sampling interval. The majority of the total radioactivity remained on the cabbage leaves and was soluble in organic solvents. Initial chromatographic analyses of cabbage leaves suggest that spinosyns A and D are rapidly metabolized. The parents were the major radioactive residues identified in 0-DAT leaf samples; metabolites B and N-demethyl spinosyn D were identified as minor metabolites. The residue levels of spinosyns A and D declined significantly in leaf samples collected/analyzed at later sampling intervals; the decrease in residue levels of the parents was accompanied by an incremental increase in unidentified polar ^{14}C-residues. Extensive fractionation and characterization of polar ^{14}C-residues in selected leaf samples indicated that most of the radioactivity was composed of polar, multicomponent residues present at low levels.

Cabbage and Tomato

DowElanco has submitted data from a single study (citation listed below) depicting the metabolism of [^{14}C]spinosyn A following repeat applications to cabbage and tomato. The field portion of this study was conducted by DowElanco Research Farm (Greenfield, IN). The residue characterization portion was conducted by the Residue Chemistry Group of DowElanco Research Laboratories (Indianapolis, IN).

MRID 44058815. Satonin, D.; Collins, R. (1996) Characterization of Residues in Cabbage and Tomatoes Receiving Repeat Applications of (Carbon-14) XDE-105: Lab Project Number: MET94032. Unpublished Study Prepared by DowElanco. 114 p.

Set-up/application: One or four foliar applications of [^{14}C]spinosyn A were made to cabbage (Copenhagen Market variety) and tomatoes (Early Girl hybrid variety) at the following rates: 0.089 + 0.089 + 0.134 + 0.134 lb ai/A. For plants which received multiple applications, treatments were made with 7- to 8-day retreatment intervals. The maximum applied rate was 0.446 lb ai/A which is ~1x the proposed maximum seasonal application rate for cole crops. The treated cabbage and tomato plants were transplanted into containers (21-25 gallon size) with pea gravel and a plastic pipe for drainage. The tomatoes were planted one to a container while the cabbages were planted three plants per container; a total of two tomato and six

cabbage plants were treated. For the control plot (9 x 16 ft), four tomato plants and six cabbage plants were transplanted directly into the soil. The treated and untreated plots were separately enclosed with wire fencing and the soil covered with polyethylene plastic.

Sample collection and shipping: Cabbage leaf samples were collected three days after the first and final (fourth) spray treatment. Tomato fruit samples were collected 3 days after the first treatment, and 0 and 3 days after the final treatment. Tomato leaves and vines were harvested three 3 days following the final treatment. Two plants were collected at each sampling interval except at the final harvest when all of the remaining plants were collected. Samples were packaged with an ice substitute and shipped by air freight to the analytical laboratory, DowElanco. Upon receipt at DowElanco, the 0-DAT samples were frozen (-20 C), the 3-DAT single-application samples were stored in a refrigerator for 2 days prior to homogenization with liquid nitrogen, and the 3-DAT multiple-application samples were ground to medium-sized particles, frozen at -20 C, and homogenized to finer particles two days later. All samples were stored frozen (-20 C) after preparation until analysis.

A subsample of the 3-DAT multiple-application tomatoes were processed into juice and seeds/peels (wet pomace) fractions. These tomatoes were cut into small pieces and processed through a tomato press to separate the juice from the seeds and peels (wet pomace). Wet pomace was blended with liquid nitrogen, and both the juice and wet pomace were stored frozen (-20 C) until analysis.

Total radioactive residues (TRR): The total radioactivity in cabbage and tomato samples, both before and after extraction, was determined by direct LSS, or combustion and LSS. The LSS LOD was not reported. The results of the TRR determinations are presented in Table 7.

Table 7. Total radioactive residues found in/on cabbage and tomato matrices harvested at various intervals following single or multiple foliar spray application of [^{14}C]spinosyn A at 0.089-0.134 lb ai/A/application.

Matrix	Number of Applications	DAT [a] (days)	TRR (ppm [^{14}C]spinosyn A equivalents)
Cabbage leaves	1	3	2.376
	4	3	5.626
Tomatoes	1	3	0.037
	4	0	0.127
		3	0.080
Tomato juice	4	3	0.048
Tomato wet pomace	4	3	0.281

[a] DAT = Days after treatment.
[b] Average of five analyses.

Extraction of ^{14}C-residues: Radioactive residues in leaves and roots were initially extracted with ACN:water (80:20, v:v) and ACN. The filtrates were combined and partitioned with DCM; the resulting aqueous phase was further partitioned with EtOAc. The DCM and EtOAc phases were combined to yield an organic phase which was concentrated and reserved for chromatographic analysis. Selected fractions/extracts were further subjected to SPE procedures using C-18 Sep-Pak cartridge and various elution solvents. The eluate(s) containing the highest radioactivity was reserved for chromatographic analysis. Radioactivity in the extracts was determined by LSS; the radioactivity in the nonextractable residues was determined by LSS following combustion.

Hydrolysis of ^{14}C-residues: The nonextractable radioactive residues following the initial extraction procedure were further extracted with ACN or ACN:water, and then subjected to several characterization techniques [i.e., acid hydrolysis (0.1 N HCl), cellulose hydrolysis, and preparation of glucosazone derivatives]. The hydrolysates were reserved for chromatographic analyses.

Characterization and identification of ^{14}C-residues: The various cabbage leaf and tomato extracts were initially analyzed by one-dimensional TLC and/or reversed-phase HPLC. TLC separations were conducted as described above for the single treatment cabbage metabolism study. Prior to chromatographic analysis, tomato extracts required clean-up on silica SPE columns using a combination of the following elution solvents: DCM, ACN, DCM:diethylamine, ACN:diethylamine, and MeOH:diethylamine. Only the ACN:diethylamine and MeOH:diethylamine fractions contained >0.005 ppm and were thus subjected to TLC analyses. HPLC separations were accomplished using the same system and mobile phases described for the apple metabolism study. Selective peaks of radioactive residues from HPLC analyses were subjected to MS analyses. The petitioner provided sample

calculations related to the biological and analytical phases of the study and representative TLC and HPLC chromatograms and MS spectra.

Mild acid hydrolysis of the organic extracts as described for the apple metabolism study was conducted to confirm the identification of spinosyns A, B, and K, and to determine whether unknowns contained the rhamnose and macrolide portions of the molecule intact.. TLC analyses of the organic extracts before and after acid hydrolysis demonstrated the conversion of spinosyns A and K to psA and psK.

Medium bore silica gel column chromatography was conducted to separate fractions in the organic extract of the 3-DAT (multiple application) cabbage sample. Fractions containing radioactivity >0.01 ppm were analyzed by a combination of TLC, HPLC, and MS. These procedures indicated that majority of the radioactive residues were multicomponent in nature; spinosyns A, B, and K were identified/confirmed as minor metabolites.

Subsamples of the aqueous fraction of the tomato fruit and juice samples were partitioned by C-18 SPE chromatography. No eluting fraction contained >10% TRR and no further analyses were done. However, the fractionation pattern indicated that extracts were multicomponent in nature.

The distribution of radioactive residues in/on cabbage and tomato matrices following routine solvent extraction and chromatographic analysis is presented in Table 8. Summaries of metabolites identified in cabbage or tomato are presented in Table 9. The chemical names and molecular structures of these metabolites are depicted in Figure 2.

Storage stability: All homogenized cabbage and tomato samples were stored frozen (-20 C) prior to analysis. Analysis dates were not provided by the petitioner; however, samples were extracted within 12 months of harvest, except for the 3-DAT (multiple application) cabbage sample which was extracted ~14 months after harvest. No supporting storage stability data were submitted with this study. The storage stability data submitted for cabbage leaves treated with [^{14}C]spinosyn A or D will be adequate to support this cabbage and tomato metabolism study.

Conclusions: The submitted data, depicting the metabolism of [^{14}C]spinosyn A following repeat applications to cabbage and tomato, are adequate. The results are consistent with the submitted data on the metabolism of [^{14}C]spinosyn A and [^{14}C]spinosyn D in cabbage following a single application.

Table 8. Distribution of residues in/on cabbage and tomato matrices following one or multiple foliar applications of uniformly ring-labeled [^{14}C]spinosyn A at 0.089-0.134 lb ai/A/application.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
3-DAT Cabbage Leaves (1 application; TRR = 2.376 ppm)			
DCM	67.5	1.604	TLC: Spinosyn A 15.4% TRR 0.367 ppm Spinosyns B/K 8.6% TRR 0.204 ppm Origin 24.7% TRR 0.586 ppm Other 12.2% TRR 0.291 ppm
Aqueous	12.4	0.296	Not further analyzed (N/A).
Nonextractable	20.1	0.476	N/A.
3-DAT Cabbage Leaves (4 applications; TRR = 5.626 ppm)			
DCM	65.8	3.701	TLC: Spinosyn A 11.7% TRR 0.657 ppm Spinosyns B/K 13.3% TRR 0.747 ppm Origin 21.0% TRR 1.182 ppm Other 13.0% TRR 0.731 ppm HPLC: Spinosyn A 10.4% TRR 0.585 ppm Spinosyn B 2.3% TRR 0.129 ppm Spinosyn K 7.7% TRR 0.433 ppm Origin 20.2% TRR 1.136 ppm Other 24.7% TRR 1.390 ppm SPE/TLC: Resolved majority of radioactivity at origin. Identified spinosyns A, B, and K. HPLC and LC/MS: Confirmed identifications of spinosyns A, B, and K; LC/MS also identified protonated spinosyns A and B. Medium-bore HPLC/TLC: Demonstrated majority of radioactive residues were multicomponent in nature. Confirmed identifications of spinosyns A, B, and K.
Aqueous	10.7	0.604	HPLC: Resolved many polar components; not further analyzed.
Nonextractable	23.5	1.321	Refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	7.2	0.406	TLC: Origin 4.4% TRR 0.248 ppm
Aqueous	3.9	0.221	N/A.
Nonextractable	12.4	0.696	Hydrolyzed with 0.1 N HCl and the hydrolysate partitioned with EtOAc.

(continued; footnotes follow)

Table 8 *(continued)*.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
Organic	2.0	0.113	TLC: Aglycone of Spinosyn A 0.4% TRR 0.023 ppm Origin 1.1% TRR 0.062 ppm
Aqueous	4.5	0.253	N/A.
Nonextractable	5.9	0.332	Cellulose hydrolyzed; treated for glucosazone derivatives: MS of the isolated derivative demonstrated a portion of the radioactivity was incorporated into glucose.
3-DAT Tomatoes (1 application; TRR = 0.037 ppm)			
DCM	66.6	0.025	SPE/TLC: Spinosyn A 20.2% TRR 0.007 ppm Spinosyn B 2.3% TRR 0.001 ppm Origin 2.4% TRR 0.001 ppm Other 3.9% TRR 0.002 ppm
Aqueous	12.4	0.005	N/A.
Nonextractable	21.0	0.008	N/A.
0-DAT Tomatoes (4 applications; TRR = 0.127 ppm)			
DCM	82.4	0.105	SPE/TLC: Spinosyn A 65.0% TRR 0.082 ppm Spinosyn B 4.4% TRR 0.006 ppm Origin 6.5% TRR 0.008 ppm Other 1.1% TRR 0.002 ppm HPLC: Spinosyn A confirmed.
Aqueous	8.3	0.011	N/A.
Nonextractable	9.2	0.012	N/A.
3-DAT Tomatoes (4 applications; TRR = 0.080 ppm)			
Organic	59.3	0.047	SPE/TLC: Spinosyn A 23.7% TRR 0.019 ppm Spinosyn B 3.1% TRR 0.003 ppm Origin 15.5% TRR 0.012 ppm Other 8.8% TRR 0.008 ppm HPLC: Spinosyn A confirmed.
Aqueous	20.5	0.016	SPE: No eluting fraction contained >10% TRR.
Nonextractable	20.2	0.016	Refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	5.7	0.005	TLC: Origin 2.4% TRR 0.002 ppm Other 2.3% TRR 0.002 ppm

(continued; footnotes follow)

Table 8 *(continued)*.

Matrix Extracts	%TRR	ppm [a]	Characterization/Identification [b]
Aqueous	2.7	0.002	N/A.
Nonextractable	11.8	0.009	N/A.
Tomato Juice (TRR = 0.048 ppm)			
DCM	62.9	0.030	TLC: Spinosyn A 19.5% TRR 0.009 ppm Spinosyn B 2.1% TRR 0.001 ppm Origin 0.5% TRR 0.0003 ppm Other 30.3% TRR 0.0157 ppm HPLC: Spinosyn A confirmed.
Aqueous	28.2	0.014	SPE: No eluting fraction contained >10% TRR.
Nonextractable	8.9	0.004	N/A.
Tomato Wet Pomace (TRR = 0.281 ppm)			
DCM	64.8	0.182	TLC: Spinosyn A 32.6% TRR 0.092 ppm Spinosyn B 1.4% TRR 0.004 ppm Origin 4.5% TRR 0.013 ppm Other 5.5% TRR 0.015 ppm HPLC: Spinosyn A confirmed.
Aqueous	7.6	0.021	N/A.
Nonextractable	28.7	0.081	Refluxed with ACN:water, then partitioned with DCM and EtOAc.
Organic	10.5	0.030	TLC: Spinosyn A 2.7% TRR 0.008 ppm Spinosyn B 1.4% TRR 0.004 ppm Origin 3.1% TRR 0.009 ppm Other 2.4% TRR 0.007 ppm HPLC: Spinosyns A and B confirmed.
Aqueous	2.5	0.007	N/A.
Nonextractable	15.7	0.044	Hydrolyzed with 0.1 N HCl and the hydrolysate partitioned with EtOAc.
Organic	2.2	0.006	N/A.
Aqueous	5.2	0.014	N/A.
Nonextractable	8.3	0.023	N/A.

[a] Expressed in terms of [^{14}C]spinosyn A equivalents.

[b] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, C, H, J, or K, psA (pseudoaglycone of spinosyn A), rpsA (reverse pseudoaglycone of spinosyn A), aglycone of spinosyn A, or psK (pseudoaglycone of spinosyn K).

Table 9. Summary of radioactive residues characterized/identified in/on cabbage and tomato matrices following single or multiple foliar applications of uniformly ring-labeled [^{14}C]Spinosyn A at 0.089-0.134 lb ai/A/application.

Metabolite/ Fraction	Cabbage Leaves				Tomato					
	3-DAT/ 1 application (TRR = 2.376 ppm)		3-DAT/ 4 applications (TRR = 5.626 ppm)		3-DAT/ 1 application (TRR = 0.037 ppm)		0-day DAT/ 4 applications (TRR = 0.127 ppm)		3-DAT/ 4 applications (TRR = 0.080 ppm)	
	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]	% TRR	ppm [a]
Identified by TLC (Confirmed by HPLC and/or MS)										
Spinosyn A	15.4	0.367	11.7	0.657	20.2	0.007	65.0	0.082	23.7	0.019
Spinosyn B/K [b]	8.6	0.204	13.3	0.747	--	--	--	--	--	--
Spinosyn B	--	--	--	--	2.3	0.001	4.4	0.006	3.1	0.003
Spinosyn K	--	--	--	--	--	--	--	--	--	--
Total Identified	**24.0**	**0.571**	**25.0**	**1.404**	**22.5**	**0.008**	**69.4**	**0.088**	**26.8**	**0.022**
Characterized										
Origin	24.7	0.586	26.5	1.492	2.4	0.001	6.5	0.008	17.9	0.014
Other [c]	12.2	0.291	13.0	0.731	3.9	0.002	1.1	0.002	11.1	0.010
Aqueous	12.4	0.296	19.1	1.078	12.4	0.005	8.3	0.011	23.2	0.018
Total Identified/ Characterized	**73.3**	**1.744**	**83.6**	**4.705**	**41.2**	**0.016**	**85.3**	**0.109**	**79.0**	**0.064**
Nonextractable	20.1	0.476	5.9	0.332	21.0	0.008	9.2	0.012	11.8	0.009

[a] Expressed in terms of [^{14}C]spinosyn A equivalents.

[b] Zones which co-eluted with spinosyn B may be attributed to spinosyn B with some contribution from spinosyn K possible.

[c] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, C, H, J, or K, psA (pseudoaglycone of spinosyn A), rpsA (reverse pseudoaglycone of spinosyn A), aglycone of spinosyn A, or psK (pseudoaglycone of spinosyn K).

Turnips

DowElanco has submitted data from a single study (citation listed below) investigating the metabolism of [^{14}C]spinosad in turnips. The field portion of this study was conducted by Plant Sciences, Inc. (Watsonville, CA). The residue characterization portion was conducted by the Residue Chemistry Group of DowElanco Research Laboratories (Indianapolis, IN).

> MRID 44058814 Satonin, D.; Berard, D. (1995) Nature of Residue Study in Turnips Using (carbon-14) XDE-105: Factor A and D: Lab Project Number: MET92069. Unpublished study prepared by DowElanco and Plant Sciences, Inc. 291 p.

Set-up: Turnips (Seven Top variety) were grown in three polyethylene-lined boxes in screenhouses, and were watered by overhead sprinkler irrigation prior to treatment and by sprinkler irrigation to the soil following treatment. The crop received fertilizer and maintenance pesticides as needed. Two of the boxes received treatments and the third was reserved for a control.

Application: Turnips in each of two treatment boxes received a single foliar spray application of [^{14}C]spinosyn A (at 977 ppm) or D (at 511 ppm) 53 days after planting. Applications were made using a 0.5-L hand trigger sprayer adjusted to deliver an even droplet spray. According to the petitioner, the application rates were 1.6x and 3.4x the proposed maximum seasonal rate for spinosyns A and D, respectively. The petitioner's application rate calculations assume a maximum of five applications per growing season, an active ingredient concentration of 150 ppm/application, and a ratio in the commercial product of 80% spinosyn A [977 ÷ (5 x 150 x 0.8)] and 20% spinosyn D [511 ÷ (5 x 150 x 0.2)]. The test substances were not diluted with unlabeled spinosyn A or D prior to application.

Sample collection and shipping: Samples of treated turnip tops and roots were collected 0, 10, 24, and 48 days after treatment (DAT). Two 0-DAT samples were collected from each plot: the first was collected immediately after treatment, when foliage was still dripping, and the second was collected about 25 minutes after treatment. The 0-DAT "wet leaf" samples were reserved for evaluation of the effects of photolysis on spinosyns A and D. Turnip plants were harvested by cutting the foliage from the roots at about 1 inch above the crown and gently pulling the roots from the soil. After excess soil had been removed by light brushing, the roots were rinsed in water and patted dry. The samples were then sealed in plastic bags and transported on dry ice to the field laboratory, where they were stored at <0 C until shipment. Within 0-7 days after collection, samples were shipped on dry ice to DowElanco, where they were transferred to freezers maintained at ~-20 C.

Total radioactive residues (TRR): On the day of sampling, triplicate aliquots of turnip tops and roots were analyzed at Plant Sciences for TRR by LSS following combustion. The reported LOD and limit of quantitation (LOQ) were 0.001 ppm and 0.005 ppm, respectively. The TRR in samples of turnip tops and roots are presented in Table 10. The data indicate that

the majority of the total radioactivity remained on turnip tops, and that TRR levels in tops and roots declined steadily as the harvest interval increased. The petitioner concluded that the steady decline in TRR in roots confirmed that translocation was limited.

Table 10. Total radioactive residues in/on turnip tops and roots harvested following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A (977 ppm) or D (511 ppm).

	TRR (ppm [^{14}C]spinosyn A or D equivalents)			
	Spinosyn A Treatment		Spinosyn D Treatment	
DAT [a]	Tops	Roots	Tops	Roots
0	38.939	3.527	20.341	1.691
10	21.638	1.382	12.594	0.431
24	5.752	0.384	4.682	0.212
48	0.333	0.177	0.298	0.094

[a] DAT = days after treatment.

Extraction of ^{14}C-residues: Samples of turnip tops and roots were subjected to extraction and hydrolysis procedures for residue characterization and identification. During the fractionation procedures, aliquots of extracts, hydrolysates, and nonextractable residues were analyzed for radioactivity by LSS or combustion/LSS.

Homogenized samples of turnip tops and roots were extracted as described above for the apple metabolism study (pulp), and the combined DCM and EtOAc phases were reserved for chromatographic analysis. DCM extracts of 0-, 10- and 24-DAT matrices were also subjected to silica gel or C-18 SPE as described for the apple metabolism study; eluates containing the highest levels of radioactivity were reserved for chromatographic analysis.

Characterization and identification of ^{14}C-residues: Extracts of turnip tops and roots were analyzed by one-dimensional TLC and/or reversed-phase HPLC. The petitioner reported that 10-DAT samples were subjected to the most exhaustive analysis because the harvest interval most closely matched the projected use pattern, and that more characterization work was conducted on 10-DAT samples treated with spinosyn A because the levels of this ingredient would be significantly higher in the end-use product.

TLC separations were conducted as described for the apple metabolism study except for use of the solvent system, toluene:isopropanol:diethylamine (84:7:7, v:v:v:, System A) or chloroform:MeOH:diethylamine (90:3:7, v:v:v, System B). A small silica column cleanup was required for samples of 48-DAT tops and roots prior to TLC analysis. HPLC separations were accomplished using the system and mobile phases described for the apple metabolism study except that for Method A the gradient changed from 100% A to 100% B over 15

minutes and for method B the gradient changed from 85:15 (A:B) to 100% B over 30 minutes. Initial identification of ^{14}C-residues was confirmed by cochromatography with non-labeled reference standards and/or by MS as described for the apple metabolism study. The petitioner provided sample calculations related to the biological and analytical phases of the study and representative TLC and HPLC chromatograms and MS spectra.

Medium bore silica gel column chromatography was used to separate fractions in the initial organic extracts of 10- and 24-DAT leaves from the spinosad A treatment. The separated fractions were then analyzed by TLC. Identification of metabolites was confirmed by HPLC and/or MS. Fractions containing significant amounts of unidentified radioactivity were subjected to mild acid hydrolysis to characterize residues.

Mild acid hydrolysis (1 N HCl) of the initial organic extracts of 0-DAT, 10-DAT, and 24-DAT leaves and roots as described for the apple metabolism study was used to confirm the identification of spinosyns A, B, and K, and to determine whether unknowns contained the rhamnose and macrolide portions of the molecule intact. Although standards were only available for the hydrolysis products of pseudoaglycone A (psA) and psK, chromatographic regions for spinosyn D samples which corresponded to psA and psK regions were assumed to be due to the analogous pseudoaglycones.

Radioactive residues in selected fractions of the initial organic extracts of 24-DAT tops following SPE/TLC, were examined using a spinosyn immunoassay test kit. Results indicated that components remaining at the origin in TLC analysis were not similar to spinosyn A or D, and confirmed the identification of spinosyns A and D in specific fractions.

The petitioner reported that spinosyn K cochromatographed with spinosyn B under the TLC conditions used for this study, and noted that zones which co-eluted with spinosyn B would be attributed to spinosyn B with the understanding that some contribution from spinosyn K could be present. Subsequent MS analysis confirmed the presence of spinosyn K in 10-DAT tops.

The distribution of radioactive residues in/on turnip matrices treated with spinosyns A and D is presented in Tables 11-A and 11-B, respectively. Summaries of metabolites identified in turnips are presented in Tables 12-A and 12-B. The chemical names and molecular structures of these identified metabolites are depicted in Figure 2.

The data presented in Tables 11-A, 11-B, 12-A, and 12-B indicate that spinosyns A and D are rapidly metabolized in turnip tops and roots, and that photolysis is a major factor in the rate of metabolism. The parent compounds and their N-demethylated metabolites (spinosyn B and N-demethyl spinosyn D) were identified in 0-DAT tops at 88.7% and 84.5% of the TRR for plants treated with spinosyn A and D, respectively, and had declined to 11.3% and 6.1% of the TRR in 10-DAT tops. When plants were harvested at 48 DAT, levels of the parent compounds were 0.2% of the TRR for both plants treated with spinosyn A and plants treated with spinosyn D. In turnip roots, levels of the parent compounds and their N-demethylated

metabolites declined more slowly or not at all over the sampling intervals, from 91.7% and 88.5% of the TRR in 0-DAT plants treated with spinosyn A and D, respectively, to 31.3% and 32.3% of the TRR in 10-DAT plants and 33.8% and 26.0% of the TRR in 48-DAT plants. The parents were the major radioactive residues identified in 0-DAT samples, but by the 10-DAT sampling interval, levels of the N-demethylated metabolites had increased with respect to the levels of the parent compounds.

Further characterization of polar and nonextractable ^{14}C-residues: Exhaustive attempts were made to elucidate the nature of the polar and nonextractable ^{14}C-residues. For these investigations, the 10-, 24-, and 48-DAT samples were utilized. A brief summary of the procedures and results are described below.

The resulting aqueous fractions for 10-, 24-, and 48-DAT tops and roots underwent a series of treatments involving acid/base partitioning into ACN and DCM, enzyme hydrolysis (ß-glucosidase), and additional acid/base partitioning and strong acid hydrolysis (2 N HCl). The resulting organic phases were evaporated to dryness by rotary evaporation and reserved for chromatographic analysis.

Nonextractable residues of 10- and 48-DAT tops and roots remaining from the first extraction series were further extracted with ACN:water (70:30, v:v) and partitioned with DCM and EtOAC. The resulting nonextractable residues were subjected to mild acid hydrolysis (0.1 N HCl), and the remaining nonextractable residues were rinsed with MeOH. The acid layer and MeOH filtrate were combined, and the solution partitioned with EtOAc. The organic phases were reserved for chromatographic analysis.

Acid-detergent fiber preparation was conducted on nonextractable residues of selected matrices following additional extraction and hydrolysis steps to identify natural structural components such as cellulose and lignin. The nonextractable residues of 10-DAT tops were then hydrolyzed with 72% H_2SO_4 to prepare glucosazone derivatives which indicated that the test substance had been incorporated into glucose.

The unidentified radioactivity in organic and aqueous extracts was characterized as polar and/or multicomponent. In nonextractable residues, unidentified radioactivity was shown to have been incorporated into natural plant constituents.

The results of the photolysis study on "wet leaves" indicated that spinosyn A is less susceptible to photolysis than spinosyn D. Residues of spinosyn A in wet leaves (78.7% TRR) were comparable to those in 0-DAT leaves (81.4% TRR), whereas residues of spinosyn D in wet leaves were significantly higher (77.9% TRR) than those in 0-DAT leaves (68.2% TRR). The petitioner reported that spinosyn B and N-demethyl spinosyn D were also detected in wet leaf samples. The petitioner noted that the more rapid disappearance of spinosyns A and D from turnip tops than from turnip roots corroborated the importance of photolysis in the metabolism of spinosad.

Table 11-A. Distribution of residues in/on turnip tops and roots following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A at 977 ppm.

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Tops (TRR = 38.939 ppm)			
Organic	99.0	38.550	TLC: Spinosyn A 81.4% TRR 31.707 ppm Spinosyn B/K 7.3% TRR 2.841 ppm Origin 4.8% TRR 1.862 ppm Other 2.7% TRR 1.049 ppm HPLC: Confirmed TLC results; no quantitative data were provided. SPE/TLC: Spinosyn A 81.9% TRR 31.903 ppm Spinosyn B/K 7.2% TRR 2.817 ppm Remaining radioactivity characterized as polar and/or multicomponent. MS identified N-formyl spinosyn B.
Aqueous	0.1	0.039	Not further analyzed (N/A).
Nonextractable	0.8	0.312	N/A.
10-DAT Tops (TRR = 21.638 ppm)			
Organic	69.9	15.125	TLC: Spinosyn A 2.1% TRR 0.448 ppm Spinosyn B/K 9.2% TRR 1.998 ppm Origin 28.0% TRR 6.067 ppm Other 23.5% TRR 5.079 ppm HPLC: Many unresolved peaks confirming multicomponent residues. Medium bore/TLC: Spinosyn A 2.0% TRR 0.431 ppm Spinosyn B/K 2.6% TRR 0.562 ppm Spinosyn K 2.0% TRR 0.442 ppm Remaining radioactivity characterized as polar and/or multicomponent. HPLC confirmed spinosyns A and K; HPLC and MS confirmed spinosyn B. SPE/TLC: Spinosyn A 2.2% TRR 0.478 ppm Spinosyn B/K 3.2% TRR 0.684 ppm Remaining radioactivity characterized as polar and/or multicomponent.

(continued; footnotes follow)

Table 11-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Aqueous	13.6	2.943	The aqueous fraction from a later extraction (11.3% TRR, 2.439 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	8.4	1.803	TLC: The majority of radioactivity remained at the origin for each organic phase.
Aqueous	2.9	0.636	N/A.
Nonextractable	16.5	3.570	Nonextractable residues from a later extraction (17.9% TRR, 3.865 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 6.5% TRR 1.411 ppm; Aqueous 1.8% TRR 0.387 ppm Resulting nonextractable residues were subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	1.9	0.410	TLC: Some radioactivity co-eluted with spinosyn B in initial organic phase; no parent or related molecules identified in final organic phase except aglycone of spinosyn A identified (0.6% TRR, 0.123 ppm) as a result of acid hydrolysis.
Aqueous	4.9	1.068	SPE: No fraction >1.0% TRR.
Nonextractable	2.7	0.590	Subjected to acid-detergent fiber and glucosazone preparation; MS identified radiolabeled glucose.
24-DAT Tops (TRR = 5.752 ppm)			
Organic	50.6	2.911	TLC: Spinosyn A 1.3% TRR 0.075 ppm Spinosyn B/K 1.1% TRR 0.066 ppm Origin 21.0% TRR 1.207 ppm Other 18.5% TRR 1.064 ppm HPLC: Confirmed spinosyns A and B. Medium bore/TLC: Radioactivity characterized as polar and/or multicomponent. HPLC demonstrated residues were fractionated into many components. SPE/TLC: Spinosyn A 0.6% TRR 0.032 ppm Spinosyn B/K 1.6% TRR 0.093 ppm Remaining radioactivity characterized as polar and/or multicomponent.

(continued; footnotes follow)

Table 11-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Aqueous	20.5	1.179	Sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	13.4	0.769	TLC: The majority of radioactivity remained at origin for each organic phase.
Aqueous	7.1	0.408	N/A.
Nonextractable	28.9	1.662	N/A.
48-DAT Tops (TRR = 0.333 ppm)			
Organic	22.5	0.075	Small silica column cleanup/TLC: Spinosyn A 0.2% TRR 0.001 ppm Spinosyn B/K 0.9% TRR 0.003 ppm Origin 9.7% TRR 0.032 ppm Other 5.1% TRR 0.017 ppm
Aqueous	27.9	0.093	The aqueous fraction from a later extraction (22.0% TRR, 0.073 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	12.0	0.040	TLC: The majority of radioactivity remained at origin for combined organic phases.
Aqueous	10.0	0.033	N/A.
Nonextractable	49.6	0.165	Refluxed with ACN:water, then partitioned with DCM and EtOAc. Organic 6.7% TRR 0.022 ppm Aqueous 6.9% TRR 0.023 ppm Resulting nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	2.3	0.008	N/A.
Aqueous	16.4	0.055	N/A.
Nonextractable	11.4	0.038	Subjected to acid-detergent fiber preparation; radiolabeled cellulose and lignin suggested.

(continued; footnotes follow)

Table 11-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Roots (TRR = 3.527 ppm)			
Organic	96.5	3.404	TLC: Spinosyn A 87.0% TRR 3.070 ppm Spinosyn B/K 4.7% TRR 0.166 ppm Origin 1.7% TRR 0.060 ppm Other 0.9% TRR 0.031 ppm HPLC: Confirmed TLC results; no quantitative data were provided. SPE/TLC: Spinosyn A 84.3% TRR 2.974 ppm Spinosyn B/K 5.6% TRR 0.198 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	0.2	0.007	N/A.
Nonextractable	3.3	0.116	N/A.
10-DAT Roots (TRR = 1.382 ppm)			
Organic	62.3	0.861	TLC: Spinosyn A 21.0% TRR 0.290 ppm Spinosyn B/K 10.3% TRR 0.142 ppm Origin 12.7% TRR 0.176 ppm Other 14.3% TRR 0.198 ppm HPLC: Confirmed spinosyns A and B. SPE/TLC: Spinosyn A 16.9% TRR 0.234 ppm Spinosyn B/K 8.9% TRR 0.123 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	6.3	0.087	The aqueous fraction from a later extraction (6.9% TRR, 0.095 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	3.7	0.050	TLC: (initial pH 2 fraction only): majority of radioactivity remained at origin.
Aqueous	3.1	0.043	N/A.

(continued; footnotes follow)

Table 11-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Nonextractable	31.4	0.434	Nonextractable residues from a later extraction (29.4% TRR, 0.407 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 7.4% TRR 0.102 ppm Aqueous 1.8% TRR 0.025 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	3.4	0.047	TLC zones with high activity were scraped and analyzed by reverse-phase HPLC: Spinosyn B/K 1.3% TRR 0.018 ppm PsA was identified (1.7% TRR, 0.024 ppm) following mild acid hydrolysis.
Aqueous	5.3	0.073	SPE: The majority of radioactivity (1.8% TRR, 0.025 ppm) eluted through the column; remaining radioactivity characterized as polar and/or multicomponent.
Nonextractable	11.6	0.160	Subjected to acid-detergent fiber preparation; radiolabeled cellulose and lignin suggested.
24-DAT Roots (TRR = 0.384 ppm)			
Organic	52.1	0.200	TLC: Spinosyn A 21.8% TRR 0.084 ppm Spinosyn B/K 6.7% TRR 0.026 ppm Origin 6.8% TRR 0.026 ppm Other 8.5% TRR 0.033 ppm SPE/TLC: Spinosyn A 22.6% TRR 0.087 ppm Spinosyn B/K 6.4% TRR 0.025 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	13.8	0.053	Sequentially adjusted to pH 2 and partitioned with ACN and DCM, and hydrolyzed with ß-glucosidase.
Organic (Total phases)	2.5	0.010	TLC: The majority of radioactivity remained at origin for combined phases.
Aqueous	11.3	0.043	N/A.
Nonextractable	34.1	0.131	N/A.

(continued; footnotes follow)

Table 11-A *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
48-DAT Roots (TRR = 0.177 ppm)			
Organic	52.5	0.093	TLC: Spinosyn A 26.4% TRR 0.047 ppm Spinosyn B/K 7.4% TRR 0.013 ppm Origin 5.9% TRR 0.010 ppm Other 3.2% TRR 0.006 ppm HPLC: Confirmed spinosyns A and B; no quantitative data were provided.
Aqueous	10.8	0.019	The aqueous fraction from a later extraction (12.5% TRR, 0.022 ppm) was sequentially adjusted to pH 2 and partitioned with ACN and DCM; and hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned.
Organic (Total phases)	3.7	0.005	TLC: The majority of radioactivity remained at origin for combined organic phases.
Aqueous	9.7	0.017	Not analyzed further.
Nonextractable	36.7	0.065	Refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 7.0% TRR 0.012 ppm Aqueous 3.4% TRR 0.006 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	3.3	0.006	TLC: The majority of radioactivity remained at origin for initial organic phase.
Aqueous	10.1	0.018	N/A.
Nonextractable	9.5	0.017	Subjected to acid-detergent fiber preparation; radiolabeled cellulose and lignin suggested.

[a] Results of initial DCM extractions normalized to 100% by the petitioner.

[b] Expressed in terms of [^{14}C]spinosyn A equivalents; calculated by the study reviewer.

[c] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

Table 11-B. Distribution of residues in/on turnip tops and roots following a single spray application of uniformly ring-labeled [^{14}C]spinosyn D at 511 ppm.

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
0-DAT Tops (TRR = 20.341 ppm)			
Organic	98.6	20.056	TLC: Spinosyn D 68.2% TRR 13.865 ppm N-Demethyl Spinosyn D 16.3% TRR 3.315 ppm Origin 3.8% TRR 0.764 ppm Other 7.1% TRR 1.446 ppm HPLC: Confirmed TLC results; no quantitative data were provided. SPE/TLC: Spinosyn D 67.7% TRR 13.769 ppm N-Demethyl Spinosyn D 11.9% TRR 2.427 ppm Remaining radioactivity characterized as polar and/or multicomponent. MS suggested a metabolite analogous to N-formyl spinosyn B.
Aqueous	0.6	0.122	Not further analyzed (N/A).
Nonextractable	0.8	0.163	N/A.
10-DAT Tops (TRR = 12.594 ppm)			
Organic	75.4	9.496	TLC: Spinosyn D 0.6% TRR 0.076 ppm N-Demethyl Spinosyn D 5.5% TRR 0.695 ppm Origin 28.4% TRR 3.578 ppm Other 28.3% TRR 3.558 ppm HPLC: Many unresolved peaks confirming multicomponent residues. SPE/TLC: Spinosyn D 0.7% TRR 0.086 ppm N-Demethyl Spinosyn D 3.2% TRR 0.404 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	15.4	1.939	The aqueous fraction from a later extraction (11.6% TRR, 1.455 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	8.0	1.005	TLC: majority of radioactivity remained at origin for each organic phase.
Aqueous	3.6	0.452	N/A.

(continued; footnotes follow)

Table 11-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Nonextractable	9.2	1.159	The nonextractable residues from a later extraction (13.1% TRR, 1.652 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 5.4% TRR 0.681 ppm Aqueous 1.4% TRR 0.180 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	1.4	0.181	TLC: The majority of radioactivity at plate origin; some radioactivity co-eluted with N-demethyl spinosyn D in initial organic phase; no parent or related molecules identified in final organic phase.
Aqueous	3.0	0.373	SPE: No fraction >1.0% TRR.
Nonextractable	1.9	0.237	Subjected to acid-detergent fiber and glucosazone preparation; MS identified radiolabeled glucose.
24-DAT Tops (TRR = 4.682 ppm)			
Organic	58.0	2.716	TLC: Spinosyn D 0.3% TRR 0.016 ppm Origin 27.2% TRR 1.273 ppm Other 16.1% TRR 0.755 ppm HPLC: Confirmed TLC results; no quantitative data were provided. SPE/TLC: Results similar to those for samples treated with spinosyn A; no quantitative data were provided.
Aqueous	22.6	1.058	Sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	15.4	0.723	TLC: The majority of radioactivity remained at origin; HPLC confirmed multicomponent residues.
Aqueous	7.2	0.336	N/A.
Nonextractable	19.4	0.908	N/A.
48-DAT Tops (TRR = 0.298 ppm)			
Organic	35.7	0.106	Small silica column cleanup/TLC: Spinosyn D 0.2% TRR 0.001 ppm Origin 16.9% TRR 0.050 ppm Other 9.7% TRR 0.029 ppm

(continued; footnotes follow)

Table 11-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Aqueous	26.1	0.078	The aqueous fraction from a later extraction (22.3% TRR, 0.066 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	13.0	0.038	TLC: The majority of radioactivity remained at origin for combined organic phases.
Aqueous	9.3	0.028	N/A.
Nonextractable	38.2	0.114	Refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 8.5% TRR 0.025 ppm Aqueous 6.3% TRR 0.019 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.
Organic	3.1	0.009	N/A.
Aqueous	8.4	0.025	N/A.
Nonextractable	8.4	0.025	Subjected to acid-detergent fiber preparation; radiolabeled lignin and cellulose suggested.
0-DAT Roots (TRR = 1.691 ppm)			
Organic	97.8	1.654	TLC: Spinosyn D 79.6% TRR 1.346 ppm N-Demethyl Spinosyn D 8.9% TRR 0.151 ppm Origin 0.7% TRR 0.011 ppm Other 0.9% TRR 0.015 ppm HPLC: Confirmed TLC results; no quantitative data were provided. SPE/TLC: Spinosyn D 77.1% TRR 1.305 ppm N-Demethyl Spinosyn D 6.8% TRR 0.115 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	0.3	0.005	N/A.
Nonextractable	1.9	0.032	N/A.

(continued; footnotes follow)

Table 11-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
10-DAT Roots (TRR = 0.431 ppm)			
Organic	63.2	0.272	TLC: Spinosyn D 22.3% TRR 0.096 ppm N-Demethyl Spinosyn D 10.0% TRR 0.043 ppm Origin 6.7% TRR 0.029 ppm Other 17.0% TRR 0.073 ppm HPLC: Confirmed spinosyn D and N-demethyl spinosyn D. SPE/TLC: Spinosyn D 19.0% TRR 0.082 ppm N-Demethyl Spinosyn D 4.2% TRR 0.018 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	7.1	0.031	The aqueous fraction from a later extraction (7.0% TRR, 0.030 ppm) was sequentially adjusted to pH 2 and pH 10 and partitioned with ACN and DCM; hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned; adjusted to pH 10 and partitioned; and hydrolyzed with 2 N HCl and partitioned.
Organic (Total phases)	3.4	0.014	N/A.
Aqueous	3.6	0.016	N/A.
Nonextractable	29.7	0.128	Nonextractable residues from a later extraction (27.5% TRR, 0.118 ppm) were refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 7.0% TRR 0.030 ppm Aqueous 1.7% TRR 0.007 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl), and partitioned with organic.
Organic	4.2	0.018	TLC: No fraction >0.01 ppm.
Aqueous	6.0	0.026	N/A.
Nonextractable	8.7	0.037	Subjected to acid-detergent fiber preparation; radiolabeled cellulose and lignin suggested.

(continued; footnotes follow)

Table 11-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
24-DAT Roots (TRR = 0.212 ppm)			
Organic	50.9	0.108	TLC: Spinosyn D 17.2% TRR 0.036 ppm N-Demethyl Spinosyn D 4.6% TRR 0.010 ppm Origin 6.3% TRR 0.013 ppm Other 16.0% TRR 0.034 ppm SPE/TLC: Spinosyn D 13.3% TRR 0.028 ppm N-Demethyl Spinosyn D 4.9% TRR 0.010 ppm Remaining radioactivity characterized as polar and/or multicomponent.
Aqueous	11.3	0.024	Sequentially adjusted to pH 2 and partitioned with ACN and DCM, and hydrolyzed with ß-glucosidase.
Organic (Total phases)	2.8	0.006	TLC: The majority of radioactivity remained at origin.
Aqueous	8.5	0.018	N/A.
Nonextractable	37.8	0.080	N/A.
48-DAT Roots (TRR = 0.094 ppm)			
Organic	49.8	0.047	TLC: Spinosyn D 19.2% TRR 0.018 ppm N-Demethyl Spinosyn D 6.8% TRR 0.006 ppm Origin 4.6% TRR 0.004 ppm Other 18.2% TRR 0.017 ppm HPLC: Confirmed spinosyn D and N-demethyl spinosyn D; no quantitative data were provided.
Aqueous	12.4	0.012	The aqueous fraction from a later extraction (16.6% TRR, 0.016 ppm) was sequentially adjusted to pH 2 and partitioned with ACN and DCM; and hydrolyzed with ß-glucosidase, adjusted to pH 2 and partitioned.
Organic	3.7	0.003	TLC: The majority of radioactivity remained at origin for combined organic phases.
Aqueous	12.9	0.012	N/A.
Nonextractable	37.9	0.036	Refluxed with ACN:water, then partitioned with DCM and EtOAc: Organic 5.8% TRR 0.005 ppm Aqueous 3.5% TRR 0.003 ppm Remaining nonextractable residues subjected to acid hydrolysis (0.1 N HCl) and partitioned with organic.

(continued; footnotes follow)

Table 11-B *(continued)*

Matrix Extracts	% TRR [a]	ppm [b]	Characterization/Identification [c]
Organic	2.8	0.003	TLC: The majority of radioactivity remained at origin for initial organic phase.
Aqueous	11.7	0.011	N/A.
Nonextractable	10.8	0.010	Subjected to acid-detergent fiber preparation; radiolabeled cellulose and lignin suggested.

[a] Results of initial DCM extractions normalized to 100% by the petitioner.

[b] Expressed in terms of [^{14}C]spinosyn D equivalents; calculated by the study reviewer.

[c] "Other" radioactivity quantified from TLC analysis does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

Table 12-A. Summary of radioactive residues initially identified in/on turnip matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn A at 977 ppm.

Fraction/ Metabolite	0-DAT Tops (TRR = 38.939 ppm)		10-DAT Tops (TRR = 21.638 ppm)		48-DAT Tops (TRR = 0.333 ppm)		0-DAT Roots (TRR = 3.527 ppm)		10-DAT Roots (TRR = 1.382)		48-DAT Roots (TRR = 0.177 ppm)	
	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]
Identified by TLC (Confirmed by HPLC and/or MS)												
Spinosyn A	81.4	31.707	2.1	0.448	0.2	0.001	87.0	3.070	21.0	0.290	26.4	0.047
Spinosyn B/K [c]	7.3	2.841	9.2	1.998	0.9	0.003	4.7	0.166	10.3	0.142	7.4	0.013
Total Identified	**88.7**	**34.548**	**11.3**	**2.446**	**1.1**	**0.004**	**91.7**	**3.236**	**31.3**	**0.432**	**33.8**	**0.060**
Characterized [d]												
Origin	4.8	1.862	28.0	6.067	9.7	0.032	1.7	0.060	12.7	0.176	5.9	0.010
Other [e]	2.7	1.049	23.5	5.079	5.1	0.017	0.9	0.031	14.3	0.198	3.2	0.006
Organic	--	--	16.8	3.624	21.0	0.070	--	--	14.5	0.199	14.0	0.023
Aqueous	0.1	0.039	9.6	2.091	33.3	0.111	0.2	0.007	10.2	0.141	23.2	0.041
Total Identified/ Characterized	**96.3**	**37.498**	**89.2**	**19.307**	**70.2**	**0.234**	**94.5**	**3.334**	**83.0**	**1.146**	**80.1**	**0.140**
Nonextractable [f]	0.8	0.312	2.7	0.590	11.4	0.038	3.3	0.116	11.6	0.160	9.5	0.017

[a] Results of initial DCM extractions normalized to 100% by the petitioner.

[b] Expressed in terms of [^{14}C]spinosyn A equivalents; calculated by the study reviewer.

[c] Zones which co-eluted with spinosyn B may be attributed to spinosyn B with some contribution from spinosyn K possible.

[d] "Other" and origin radioactivity from DCM extract; "organic" and "aqueous" from further characterization of aqueous phase and nonextractable residues. Further characterization confirmed that radioactive residues were polar and/or multicomponent.

[e] Does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

[f] Further characterization confirmed that radioactive residues were incorporated into natural plant constituents.

Table 12-B. Summary of radioactive residues initially identified in/on turnip matrices following a single spray application of uniformly ring-labeled [^{14}C]spinosyn D at 511 ppm.

Fraction/ Metabolite	0-DAT Tops (TRR = 20.341 ppm)		10-DAT Tops (TRR = 12.594 ppm)		48-DAT Tops (TRR = 0.298 ppm)		0-DAT Roots (TRR = 1.691 ppm)		10-DAT Roots (TRR = 0.431)		48-DAT Roots (TRR = 0.094 ppm)	
	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]	% TRR [a]	ppm [b]
Identified by TLC (Confirmed by HPLC and/or MS)												
Spinosyn D	68.2	13.865	0.6	0.076	0.2	0.001	79.6	1.346	22.3	0.096	19.2	0.018
N-Demethyl Spinosyn D	16.3	3.315	5.5	0.695	--	--	8.9	0.151	10.0	0.043	6.8	0.006
Total Identified	**84.5**	**17.180**	**6.1**	**0.771**	**0.2**	**0.001**	**88.5**	**1.497**	**32.3**	**0.139**	**26.0**	**0.024**
Characterized [c]												
Origin	3.8	0.764	28.4	3.578	16.9	0.050	0.7	0.011	6.7	0.029	4.6	0.004
Other [d]	7.1	1.446	28.3	3.558	9.7	0.029	0.9	0.015	17.0	0.073	18.2	0.017
Organic	--	--	14.8	1.867	24.6	0.072	--	--	14.6	0.062	12.3	0.011
Aqueous	0.6	0.122	8.0	1.005	24.0	0.072	0.3	0.005	11.3	0.049	28.1	0.026
Total Identified/ Characterized	**96.0**	**19.512**	**85.6**	**10.779**	**75.4**	**0.224**	**90.4**	**1.528**	**81.9**	**0.352**	**89.2**	**0.082**
Nonextractable [e]	0.8	0.163	1.9	0.237	8.4	0.025	1.9	0.032	8.7	0.037	10.8	0.010

[a] Results of initial DCM extractions normalized to 100% by the petitioner.

[b] Expressed in terms of [^{14}C]spinosyn D equivalents; calculated by the study reviewer.

[c] "Other" and origin radioactivity from DCM extract; "organic" and "aqueous" from further characterization of aqueous phase and nonextractable residues. Further characterization confirmed that radioactive residues were polar and/or multicomponent.

[d] Does not include spinosyn A, B, or D, N-demethyl spinosyn D, or origin.

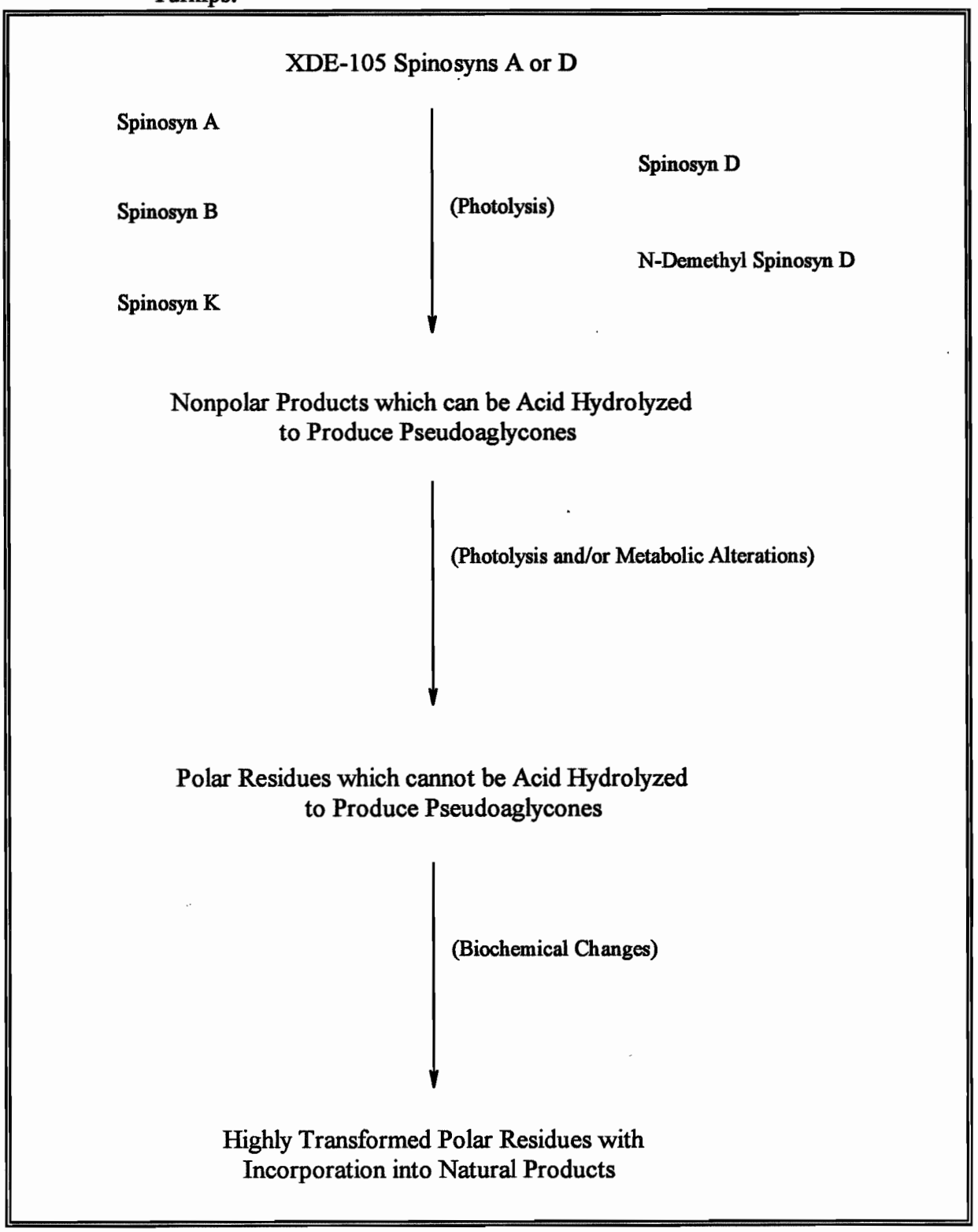
[e] Further characterization confirmed that radioactive residues were incorporated into natural plant constituents.

Storage stability: All turnip top and root samples were stored frozen (~-20 C) prior to analysis. The determination of total radioactivity was completed on the day of harvest, and extraction and characterization procedures were initiated within 13 days of harvest. Final analysis was completed within 4-32 months. To demonstrate that radioactive residues had not declined during the study period, the petitioner provided data comparing the residue profiles of untreated 0-DAT tops and roots fortified with spinosyn A or D and analyzed 9 days after harvest with the profiles of the fortified samples following storage at -20 C for 28 months. TLC analysis following initial extraction procedures was conducted as described above and showed no decline in residues of spinosyn A or D.

Conclusions: The turnip metabolism study is acceptable. The study indicates that the total radioactive residues declined steadily in turnip tops and roots as the harvest interval increased. Although the majority of the total radioactivity from plants harvested at the 0- and 10-DAT sampling intervals was soluble in organic solvents, by the 48-DAT sampling interval, the proportion of residues soluble in organic solvents versus those soluble in aqueous solvents or remaining in nonextractable residues had greatly decreased. The data indicate that spinosyns A and D are rapidly metabolized in turnip tops and roots, and that photolysis is a major factor in the rate of metabolism; residues of spinosyns A and D declined more rapidly in turnip tops across the sampling intervals than they did in turnip roots. The parent compounds were the major radioactive residues identified in 0-DAT samples, but by the 10-DAT sampling interval, levels of the N-demethylated metabolites (spinosyn B and N-demethyl spinosyn D) had increased with respect to the levels of the parent compounds. A new metabolite, N-formyl spinosyn B was identified, but not quantitated in samples of 0-DAT turnip tops treated with spinosyn A. Extensive fractionation and characterization of polar ^{14}C-residues in selected samples of turnip roots and tops indicated that most of the radioactivity was composed of polar and/or multicomponent residues present at low levels. Radioactivity remaining in nonextractable residues following extensive characterization attempts was shown to have been incorporated into natural plant constituents.

Proposed metabolic pathway for plants: The metabolism studies conducted on apples, cabbage, tomatoes, and turnips demonstrated a rapid dissipation of spinosyns A and D. The petitioner has provided evidence to indicate that photolysis plays a role on the initial degradation of the parent compounds. The petitioner contends that the initial step in the metabolism of spinosyns A and D is the conversion of the parents to metabolites resulting from modifications to the forosamine portion of the molecule, such as spinosyn B and N-demethyl spinosyn D. The rhamnose and macrolide portions are subsequently modified to form polar and nonextractable residues. Figure 3 depicts a schematic diagram of the proposed metabolic pathway for plants.

Figure 3. Proposed Metabolic Pathway for Spinosad in Apples, Cabbage, Tomatoes, and Turnips.



OPPTS GLN 860.1300: Nature of the Residue - Livestock

No data pertaining to the metabolism of spinosad in livestock were submitted with this petition. Goat metabolism studies, reflecting separate dosing with spinosyns A and D at 9-10 ppm in the diet, were submitted and reviewed in conjunction with PP#6G04692 (G. J. Herndon, 4/24/96). These dosing levels represent 18-20x and 36-40x the maximum theoretical dietary burden of spinosad to beef and dairy cattle, respectively, based on the proposed use (see "Meat, Milk, Poultry, and Eggs"). The results of the studies indicate that residues of spinosad concentrate in tissues and milk, with greater transfer in fattier tissues (fat and liver). Radioactivity was readily extractable indicating that ^{14}C-residues were not extensively conjugated. The parent compounds, spinosyns A and D, were the major ^{14}C-residues identified in tissues (fat, muscle, kidney, and liver) and milk. The proposed metabolic pathways involve either the loss of a single methyl group from the N-methyl moiety and/or the hydroxylation of the macrolide at several different positions.

The results of these metabolism studies will be presented to the HED Metabolism Committee, which will determine the residues of concern in animal commodities.

There are no poultry feed items associated with the proposed uses on apples and cole crops. Therefore, data pertaining to the metabolism of spinosad in poultry are not required to support this petition.

OPPTS GLN 860.1340: Residue Analytical Method - Plant Commodities

Data collection methods: Samples of apple commodities from the submitted apple field trial and processing studies were analyzed for residues of spinosyns A, D, K, and B and N-demethyl spinosyn D using HPLC method GRM 95.05. Samples of Brassica leafy vegetables from the submitted field trials were analyzed for residues of spinosyns A, D, K, and B and N-demethyl spinosyn D using HPLC method GRM 94.22. The petitioner submitted the following method validation data for methods GRM 94.22 and GRM 95.05:

MRID 44058816 Yeh, L. (1996) Residue Method Validation Report for the Determination of Spinosyns A, D, K, and B, and N-Demethyl Spinosyn D in Cabbage, Mustard Greens, and Broccoli: Lab Project Number: RES94110: GRM 94.22. Unpublished Study Prepared by DowElanco. 62 p.

MRID 44058817 Yeh, L. (1996) Residue Method Validation Report for the Determination of Spinosyns A, D, K, and B, and N-Demethyl Spinosyn D in Apple and Apple Processed Fractions: Lab Project Number: RES95061: GRM 95.05. Unpublished Study Prepared by DowElanco. 51 p.

GRM 94.22: Samples of Brassica leafy vegetable commodities are frozen with liquid nitrogen, ground, and then homogenized with ACN:water (80:20, v:v). The filtered extract is partitioned with DCM, and the DCM phase is removed. Methanol and 1 N sodium hydroxide are added to the remaining aqueous phase and it is partitioned again with DCM; the combined DCM phases are rotary evaporated to dryness. The extract is redissolved in hexane and cleaned up on a silica SPE column; residues are eluted with triethylamine:ACN (1:99, v:v). The eluate is evaporated to dryness under a stream of nitrogen and redissolved in MeOH:ACN:water (20:20:60, v:v:v) for cleanup on a cyclohexyl SPE column. After the sample is applied to the cyclohexyl SPE column, the column is washed with acetone and residues are eluted with triethylamine:ACN (2:98, v:v); for mustard green samples, a portion of the acetone wash is also collected. The eluate is evaporated to dryness under a stream of nitrogen and redissolved in MeOH:ACN:2% ammonium acetate (1:1:1, v:v:v) for HPLC analysis using a UV detector (250 nm), an ODS-AQ column, a guard column, and a mobile phase of MeOH:ACN:2% ammonium acetate (42:42:16, v:v:v). Confirmation of residues can be accomplished using a C-18/cation mixed mode column. The stated LODs and LOQs are 0.003 ppm and 0.01 ppm, respectively, for each analyte. The petitioner reported that a set of 15-20 samples could be prepared for analysis in one day with overnight injection of the samples onto the HPLC using an autosampler. Sample calculations and representative chromatograms were provided.

GRM 95.05: Samples of apple commodities (apples, apple wet pomace, and apple juice) are frozen with liquid nitrogen and ground (except juice) and then analyzed as described above for GRM 94.22 with the following exceptions: (i) after the first DCM partition, sufficient 1 N sodium hydroxide is added to the aqueous phase to adjust the pH to >9; (ii) residues are eluted from the silica SPE column using DCM:MeOH (75:25, v:v); (iii) an ODS-AM column may be used in place of the ODS-AQ column; and (iv) confirmation of residues is accomplished by analyzing final solutions by LC/MS [ODS-AQ column, ACN:MeOH:2% ammonium acetate (40:40:20, v:v:v) mobile phase, and selective ion monitoring]. The stated LODs are 0.003 ppm for each analyte in apples and apple juice, and 0.006 ppm for each analyte in apple pomace. The stated LOQs are 0.01 ppm for each analyte in apples and apple juice, and 0.02 ppm for each analyte in apple pomace. Sample calculations and representative chromatograms were provided.

The petitioner conducted method validation recovery analyses for methods GRM 94.22 and GRM 95.05 using commercially purchased samples of apples, apple juice, broccoli, cabbage, and mustard greens and an untreated sample of wet apple pomace from the apple processing study. Samples were fortified with each of the five analytes on the day of analysis. The results of the method validation study are presented in Table 13. Concurrent method recovery analyses that were conducted in conjunction with the apple and Brassica leafy vegetable field trial and apple processing studies are also presented in Table 13.

Table 13. Method validation and concurrent method recoveries of spinosyns A, D, B, and K, and N-demethyl spinosyn D from fortified samples of apple and Brassica leafy vegetable commodities analyzed using HPLC methods GRM 95.05 (apple commodities) or GRM 94.22 (Brassica leafy vegetable commodities).

Commodity	Fortification Level, ppm	% Recovery [a]				
		Spinosyn A	Spinosyn D	Spinosyn B	Spinosyn K	N-Demethyl Spinosyn D
Method Validation						
Apples	0.010-1.0	86-101 (20)	85-95 (20)	84-95 (20)	88-95 (20)	82-93 (20)
Apple juice	0.010-1.0	91-98 (20)	89-99 (20)	82-95 (20)	89-98 (20)	81-94 (20)
Apple wet pomace	0.020-2.0	86-95 (20)	81-93 (20)	81-92 (20)	81-93 (20)	79-97 (20)
Broccoli	0.010-2.0	90-98 (23)	85-98 (23)	64, 68, 68; 71-80 (23)	86-96 (23)	64, 68, 68; 71-84 (23)
Cabbage	0.010-2.0	76-91 (23)	64, 67, 67, 69; 71-90 (23)	70-79 (23)	69, 69; 71-90 (23)	68, 68, 69, 69; 70-79 (23)
Mustard greens	0.010-2.0	88-102 (23)	83-95 (23)	78-89 (23)	82-91 (23)	75-86 (23)
Concurrent Method Recovery						
Apples	0.005-0.3	53, 69; 73-108 (55)	60, 66, 68; 70-104 (55)	58, 63, 65, 65, 69; 71-100 (57)	58, 64; 72-113; 121 (53)	47, 59, 66, 68; 70-99 (54)
Apples (processing)	0.01-0.3	85-92 (6)	82-93 (6)	80-96 (6)	76-90 (6)	84-96 (6)
Apple juice	0.01-0.3	79-95 (10)	81-98 (10)	66; 72-86 (10)	37 [b]; 78-96 (5)	63; 72-99 (6)
Apple wet pomace	0.02-3.0	88-105; 122 (4)	81-101 (3)	83-101 (4)	93-98 (4)	86-101 (4)
Broccoli	0.01-0.10	49, 55; 78-95 (14)	69; 73-106 (13)	55, 59, 61; 70-97 (14)	72-99 (14)	60, 61, 65, 65; 71-104 (14)
Cabbage	0.01-0.10 [c]	72-107 (33)	67; 70-103 (30)	46-69 (13); 70-85 (18)	70-99 (31)	49-69 (14); 70-86 (17)
Mustard greens	0.01-7.0	63, 69; 70-105 (22)	62, 68, 69; 70-106; 124 (22)	54, 61, 67; 71-92 (25)	67, 68; 71-109 (25)	52, 62, 66, 67, 68; 71-85 (25)

[a] Number of recovery analyses in parentheses. Recovery values outside the acceptable 70-120% range are listed separately.

[b] Interference was attributed to contamination during sample preparation; value was determined by the petitioner to be an outlier at the 96% confidence level using the Q-test.

[c] For spinosyn A only an additional two samples were fortified at 4.0 ppm.

Interference study - HPLC methods GRM 94.22 and GRM 95.05: The petitioner additionally conducted an interference study with GRM 94.22 using 13 pesticides commonly used on

Brassica vegetables, and with GRM 95.05 using 61 pesticides commonly used on Brassica vegetables, tomatoes, peppers, and apples. Of the compounds tested, paraquat dichloride resulted in a broad peak outside the retention time window of spinosyn analytes when directly injected onto the HPLC (GRM 94.22), and six pesticides (chlorpyrifos, ethion, fenvalerate, pendimethalin, PCNB, and trifluralin) were found to interfere when directly injected on the HPLC (GRM 95.05), but were not found to interfere when carried through the SPE column cleanup procedures. The following compounds were tested:

GRM 94.22:

Azinphos-methyl	Endosulfan	Methyl parathion
Benomyl	Fenamiphos	Napropamide
Carbaryl	Fonofos	Oxyfluorfen
Chlorothalonil	Iprodione	Paraquat dichloride
DCPA		

GRM 95.05:

Aldicarb	Fenvalerate	Oxamyl
Azinphos-methyl	Fluazifop-butyl	Oxydemeton-methyl
Benomyl	Fonofos	Oxyfluorfen
Bensulide	Gibberellic acid	Paraquat dichloride
Carbaryl	Glyphosate	PCNB
Chlorothalonil	Imidacloprid	Pebulate
Chlorpyrifos	Imidan	Pendimethalin
Clofentazine	Iprodione	Permethrin
DCNA	Isoproturon	Phorate
DCPA	Mancozeb	Phosphamidon
Diazinon	Metalaxyl	Pronamide
Dicofol	Methamidophos	Propargite
Dimethoate	Methidathion	Sethoxydim
Disulfoton	Methomyl	Simazine
Diuron	Methyl parathion	Terbacil
Endosulfan	Metribuzin	Thiophanate
EPTC	Myclobutanil	Triadimefon
Ethion	Napropamide	Trifluralin
Ethoprop	Norflurazon	Triforine
Fenamiphos	Oryzalin	Ziram
Fenarimol		

Independent laboratory validation - HPLC method GRM 94.22: The petitioner submitted the following data pertaining to the independent laboratory validation of HPLC method GRM 94.22:

MRID 44058818 Yeh, L.; Taylor, M. (1996) Independent Laboratory Validation of Method GRM 94.22--Determination of Spinosyns A, D, K, and B, and N-Demethyl Spinosyn D in Cabbage, Mustard Greens, and Broccoli: Lab Project Number: 43009: RES95158. Unpublished Study Prepared by ABC Labs., Inc. 59 p.

The validation study was conducted by ABC Laboratories (Columbia, MO) using untreated samples of cabbage from the cabbage field trials. Samples were fortified with spinosyns A, D, K, and B and N-demethyl spinosyn D at 0.01 ppm and 0.05 ppm and analyzed by the test laboratory using GRM 94.22. The fortification levels used represent the LOQ and five times the LOQ. The results of the independent validation study are presented in Table 14. Sample calculations and representative chromatograms were included in the submission. The test laboratory had problems with interference and peak separation for spinosyn K and N-demethyl spinosyn D which were solved by the use of a different HPLC column (ODS-AM) and a new lot of cyclohexyl SPE columns following consultation with the petitioner. The test laboratory stated that analysis of a set of 7 samples required approximately 8 person-hours, with an additional half day required for data analysis.

Independent laboratory validation - HPLC method GRM 95.05: The petitioner submitted the following data pertaining to the independent laboratory validation of HPLC method GRM 95.05:

MRID 44058819 Yeh, L.; Kendall, T.; Markley, B. (1996) Independent Laboratory Validation of Method GRM 95.05--Determination of Spinosyns A, D, K, and B, and N-Demethyl Spinosyn D in Apple and Apple Processed Fractions: Lab Project Number: RES95160. Unpublished Study Prepared by Wildlife International Ltd. 31 p.

The validation study was conducted by Wildlife International (Easton, MD) using untreated samples of apples from the field trials. Samples were fortified with spinosyns A, D, K, and B and N-demethyl spinosyn D at 0.01 ppm and 0.05 ppm and analyzed by the test method. The fortification levels used represent the LOQ and five times the LOQ. The results of the independent validation study are presented in Table 14. The only modifications to the method made by the test laboratory were to substitute rotary evaporation for evaporation under a stream of nitrogen. Sample calculations and representative chromatograms were included in the submission. The test laboratory stated that analysis of a one sample set required 8.5 person-hours or one calendar day.

Table 14. Independent laboratory validation of HPLC methods GRM 94.22 and 95.05 using samples of apples (GRM 95.05) and cabbage (GRM 94.22).

Commodity	Fortification Level, ppm	Recovery, %				
		Spinosyn A	Spinosyn D	Spinosyn K	Spinosyn B	N-Demethyl Spinosyn D
Apple	0.01	77, 94	84, 91	75, 103	61, 80	93, 107
	0.05	89, 91	85, 92	84, 86	74, 80	75, 80
Cabbage	0.01	98, 98	96, 99	102, 129	75, 75	71, 72
	0.05	93, 94	92, 93	93, 107	77, 80	76, 79

Enforcement methods - plant commodities: The petitioner is proposing HPLC methods GRM 95.05 and GRM 94.22 for the enforcement of tolerances for residues of spinosyns A and D in apple and Brassica leafy vegetable commodities, respectively.

Conclusions: HPLC methods GRM 95.05 and GRM 94.22 are adequate for the purposes of tolerance enforcement and collection of residue data for spinosyns A, D, K, and B and N-demethyl spinosyn D in/on apple and Brassica leafy vegetable commodities. Adequate independent method validation and concurrent method recovery data have been submitted. HPLC methods GRM 95.05 and GRM 94.22 are similar to the method proposed for cottonseed which has undergone successful petition method validation (DP Barcode D228791, G. J. Herndon, 8/13/96).

Although data concerning the radiovalidation of the proposed enforcement methods were not provided, such data will not be required because samples from the plant metabolism studies were subjected to extraction and characterization procedures similar to the proposed enforcement methods.

OPPTS GLN 860.1340: Residue Analytical Methods - Animal Commodities

Data collection methods: Samples of cattle matrices from the submitted cattle feeding study were analyzed for residues of spinosyns A, D, and B and N-demethyl spinosyn D using HPLC method GRM 95.03, and for residues of spinosyn-related compounds using immunoassay method GRM 95.14. The petitioner submitted the following method validation data for these HPLC and immunoassay methods:

MRID 44058822 West, S.; Turner, L. (1995) Residue Method Validation Report for the Determination of Spinosad and Metabolites in Beef Tissues, Milk, and Cream by High Performance Liquid Chromatography with Ultraviolet Detection: Lab Project Number: RES95094: GRM 95.03. Unpublished Study Prepared by DowElanco. 95 p.

MRID 44058823 Young, D.; Mihaliak, C. (1996) Residue Method Validation Report for the Determination of Residues of Spinosad in Bovine Tissues and Milk by Immunoassay: Lab Project Number: RES95144: GRM 95.14. Unpublished Study Prepared by DowElanco. 71 p.

HPLC method GRM 95.03: For analysis using HPLC method GRM 95.03, samples of tissue are frozen with liquid nitrogen, ground, and homogenized with ACN:water (80:20, v:v). The homogenate is then heated to reflux for 1 hour and an aliquot is removed and filtered. The extract is partitioned with hexane and the hexane phase discarded. The extract is partitioned with DCM and the DCM phase is rotary evaporated to dryness and redissolved in hexane. The extract is then cleaned up on a silica SPE column; residues are eluted with DCM:MeOH (75:25, v:v). The eluate is evaporated to dryness under a stream of nitrogen and redissolved

in MeOH:ACN:water (20:20:60, v:v:v) for cleanup on a cyclohexyl SPE column; residues are eluted with triethylamine:ACN (2:98, v:v). The eluate is evaporated to dryness under a stream of nitrogen and redissolved in MeOH:ACN:2% ammonium acetate (1:1:1, v:v:v) for HPLC analysis using a UV detector (250 nm), an ODS-AQ column, and a gradient mobile phase of MeOH, ACN, and 2% ammonium acetate. Confirmation of residues can be accomplished using a C-18/cation mixed mode column and, if necessary, analysis at different wavelengths (235 or 275 nm). For milk and cream, a sample is homogenized with ACN, centrifuged, and the supernatant is isolated. Analysis then proceeds as described above for tissues beginning with DCM partitioning.

For fat samples, the sample is homogenized with hexane:DCM (60:40, v:v) and the homogenate is heated to reflux for 1 hour. An aliquot is removed and mixed with hexane and ACN. The ACN:DCM phase is removed and the remaining phase is partitioned with ACN. The ACN phase is combined with the ACN:DCM phase and the extract is rotary evaporated to dryness (with the addition of MeOH to ensure removal of all traces of water). The residue is redissolved in hexane and cleaned up on silica and cyclohexyl SPE columns, as described above for tissues, prior to HPLC analysis. The stated LOD is 0.003 ppm for all analytes in all matrices, and the LOQ is 0.01 ppm for all analytes in all matrices. The petitioner reported that a set of 12-16 samples could be prepared for analysis in one day with overnight injection of the samples onto the HPLC using an autosampler. Sample calculations and representative chromatograms were provided.

The petitioner conducted method validation recovery analyses for the HPLC method using commercially purchased samples of beef tissues (lean muscle, liver, kidney, and fat) and whole milk samples from untreated cattle (dairy barn at Eli Lilly, Greenfield, IN); cream samples were obtained from the whole milk samples. Tissue samples were chopped, frozen with liquid nitrogen, and then ground. Samples were fortified with each of the four analytes on the day of analysis. The results of the method validation study are presented in Table 15. Concurrent method recovery analyses that were conducted in conjunction with the dairy cattle feeding study are also presented in Table 15.

Interference studies - HPLC method GRM 95.03: The petitioner additionally conducted interference studies using 70 pesticides (commonly used on cotton and vegetables) and 10 therapeutic compounds (commonly used for weight gain and/or disease control). Of the compounds tested, five pesticides (avermectin, dicofol, propargite, thiodicarb, and tralomethrin) were found to interfere when directly injected on the HPLC, but were not found to interfere when carried through the entire analytical procedure. The following compounds were tested:

Pesticides:

Acephate	Fenamiphos	Oryzalin
Aldicarb	Fenvalerate	Oxamyl
Avermectin	Fluazifop-butyl	Oxyfluorfen
Azinphos-methyl	Flumetsulam	Paraquat dichloride
Benomyl	Fluometuron	PCNB
Bensulide	Fonofos	Pendamethalin
Bifenthrin	Glyphosate	Permethrin
Bloc	Imidan	Profenofos
Botran	Iprodione	Prometryn
Butifos	Isoproturon	Pronamide
Carbaryl	Karmex (DCMU)	Propargite
Chlorothalonil	Kelthane	Sethoxydim
Chlorpyrifos	Malathion	Simazine
Cyanazine	Mancozeb	Sulprofos
Cyhalothrin	Metalaxyl	Terbacil
Cypermethrin	Mepiquat chloride	Terbufos
DCPA	Methidathion	Thiodicarb
Diazinon	Methomyl	Tillam
Dicofol	Methyl parathion	Tralomethrin
Dimethoate	Metribuzin	Triadimefon
Disulfoton	MSMA	Trifluralin
Endosulfan	Napropamide	Triforine
EPTC	Norflurazon	Ziram
Ethephon		

Therapeutic Compounds:

Bacitracin zinc	Penicillin G potassium	Sulfathiazole
Chlorotetracycline hydrochloride	Propylene glycol	Tilmicosin
Monensin sodium	Ractopamine hydrochloride	Tylosin
Oxytetracycline hydrochloride		

Immunoassay method GRM 95.14: Immunoassay method GRM 95.14 determines combined residues of spinosyn A and related spinosyns, including major metabolites and degradates, in tissues and milk using the Spinosad RaPID Assay Kit manufactured by Ohmicron Environmental Diagnostics Corporation. Samples of tissue are frozen with liquid nitrogen, ground, and homogenized with ACN:water (80:20, v:v). The homogenate is then heated to reflux for 1 hour and filtered. An aliquot is removed, evaporated to dryness under a stream of nitrogen, and redissolved in Spinosad Sample Diluent (obtained from Ohmicron). Milk samples are extracted with ACN and an aliquot of the extract is evaporated to dryness and redissolved in Spinosad Sample Diluent. The extracts are then analyzed using the assay kit. The analysis involves sequential addition of enzyme-conjugated spinosad and paramagnetic particles coated with antibodies specific to spinosad. The mixture is incubated to allow the spinosad residues in the sample and enzyme-conjugated spinosad to compete for antibody sites on the paramagnetic particles. Following incubation, a magnetic field is applied to the particles and unbound reagents are decanted; the particles are then washed to remove any unbound enzyme conjugate. The extract is incubated with an enzyme substrate (hydrogen

peroxide) and a chromogen (3,3',5,5'-tetramethylbenzidine) which react with enzyme conjugate to form a colored product, the absorbance of which is determined at 450 nm. Because the added enzyme-conjugated spinosad and spinosad residues in the sample compete for antibody sites, the concentration of spinosad in the sample is inversely proportional to the level of color development. Quantitation of residue levels is based linear regression analysis of a calibration curve. The stated LOD is 0.003 ppm and the LOQ is 0.01 ppm.

The petitioner conducted method validation recovery analyses for the immunoassay method using commercially purchased samples of beef tissues (lean muscle, liver, and kidney) and whole milk samples from untreated cattle (dairy barn at Eli Lilly, Greenfield, IN). Tissue samples were chopped, frozen with liquid nitrogen, and then ground. Samples were fortified with spinosyn A because this compound is the major component of spinosad. In addition, samples of tissue were fortified with spinosad TGAI (technical grade active ingredient), which contained 76.1% spinosyn A and 11.9% spinosyn D. The results of the method validation study are presented in Table 15. Concurrent method recovery analyses that were conducted in conjunction with the dairy cattle feeding study are also presented in Table 15; these analyses also reflect fortification of samples with spinosyn A.

Interference study - Immunoassay method GRM 95.14: The petitioner reported that several spinosyn analogs, metabolites, and degradates were tested to determine the ability of the immunoassay method to detect these residues. It was reported that spinosyns A, D, K, and B and N-demethyl spinosyn D were detected by the method, although the sensitivity was not the same for all compounds. No data representing fortification of samples with compounds other than spinosyn A and spinosad TGAI were presented in the submission. Several compounds were tested for potential interference in the immunoassay determination of spinosyns using the test kit. Of the tested compounds, carbendazim was found to have an I_{50} less than 10 mg/mL (5.56 mg/mL); I_{50} is the concentration which results in a 50% inhibition of conjugate binding. The following compounds were tested:

Pesticides:	Metribuzin	Inorganic Compounds:
Alachlor	Parathion	Calcium (chloride dihydrate)
Aldicarb	Parathion methyl	Copper (chloride)
Azinphos-methyl	Phosmet	Iron (chloride hexahydrate)
Carbaryl	Picloram	Magnesium (chloride hexahydrate)
Carbendazim	Procymidone	Manganese (chloride)
Carbofuran	Propachlor	Mercuric chloride
Chlorothalonil	Thiabendazole	Nickel (sulfate hexahydrate)
Chlorpyrifos	Triclopyr	Nitrate (sodium)
Chlorpyrifos-methyl	Vinclozolin	Peroxide (hydrogen)
Cyanazine		Phosphate (sodium, heptahydrate)
2,4-D	Organic Compounds:	Silicates (sodium meta-)
Dicamba	N-Acetylglucosamine	Sodium chloride
Dinoseb	Aflatoxin B1	Sulfate (sodium)
EPN	Aflatoxin G1	Sulfite (sodium)
Iprodione	Humic acid	Thiosulfate (sodium, pentahydrate)
Malathion	β-Lactose	Zinc (chloride)
Metalaxyl	Methyl oleate	
Methamidophos	Polyoxin D	
Methiocarb	L(+) Rhamnose	
Methomyl		

The petitioner performed statistical comparisons of the HPLC method and the immunoassay method using the results of the cattle feeding study (see "Meat, Milk, Poultry, Eggs"). When the results from the whole milk, muscle, and kidney samples were compared, a correlation coefficient of approximately 0.95 was obtained. Comparison of the liver analyses indicates a positive bias in immunoassay analyses which the petitioner attributed to the presence of hydroxylated metabolites of spinosad; the HPLC method does not determine the hydroxylated metabolites.

The petitioner also conducted an extraction efficiency study using samples from the goat metabolism study (MRID 43727406; see PP#6G04692, DP Barcodes D219016, D224608, D223898, and D223899, G. J. Herndon, 4/24/96). Goat tissue and whole milk samples were reanalyzed for total radioactive residues (TRR) and analyzed using the immunoassay method. The results of the immunoassay analyses were compared to the results of the TRR determinations. The immunoassay recovered 101.1% of TRR for kidney, 83.8% of TRR for liver, 77.4% of TRR for muscle, and 92.5% of TRR for whole milk. These results indicate that the immunoassay method can adequately recover spinosad-related residues from samples of animal tissue and milk. The petitioner additionally isolated metabolites from the goat liver sample and subjected the isolated metabolites to immunoassay analysis. Spinosyns A and B were isolated as well as the metabolites determined to be hydroxylated [METs A-Li-1, A-Li-2, A-Li-3a, A-Li-3b, A-Li-4(5a), A-Li-4(5b), and A-Li-4(5c)]. The reactivities of these compounds (defined as the ratio of the concentration determined by immunoassay and the concentration determined by radioassay) ranged from 60% (MET A-Li-4) to 89% (spinosyn A).

Table 15. Method validation and concurrent method recoveries of spinosad-related residues from fortified samples of cattle commodities analyzed using HPLC method GRM 95.03 or immunoassay method GRM 95.14.

Commodity	Fortification Level, ppm	Recovery, % [a]			
		Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D
HPLC Method GRM 95.03 - Method Validation					
Milk	0.010-1.0	95-116 (20)	90-114 (20)	94-109 (20)	92-112 (20)
Cream	0.010, 10.0	96-114 (11)	87-115 (11)	96-116 (11)	97-113 (11)
Fat	0.010, 10.0	81-108 (11)	84-107 (11)	85-102 (11)	56; 72-93 (11)
Kidney	0.010, 1.0	76-97 (11)	76-99 (11)	94-119; 122, 124, 129, 136 (11)	86-107 (11)
Liver	0.010, 1.0	97-120 (11)	76-110 (11)	88-117; 122, 125 (11)	71-109 (11)
Muscle	0.010, 1.0	81-107 (11)	82-93 (11)	91-107 (11)	92-107 (11)
HPLC Method GRM 95.03 - Concurrent Method Recovery					
Milk	0.010-1.0	95-120; 122 (29)	90-115; 122, 123, 125, 126 (29)	86-109 (29)	84-107 (29)
Cream	0.010-7.0	107-116 (6)	104-116 (6)	94-101 (6)	92-99 (6)
Skim milk	0.010-1.0	109-115 (6)	108-117 (6)	90-103 (6)	89-103 (6)
Fat	0.010-4.0 [b]	80-101 (11)	78-96 (10)	56, 57, 66; 72-84 (10)	59, 60; 74-84 (10)
Kidney	0.010-1.0	73-104 (12)	68, 69; 70-105 (12)	83-107 (12)	89-107 (12)
Liver	0.010-2.0	82-109 (12)	79-116 (12)	73-99 (12)	66; 70-108 (12)
Muscle	0.010, 0.50	61, 67; 79-102 (12)	61, 64; 76-102 (12)	74-108 (12)	74-107 (12)
Immunoassay Method GRM 95.14 - Method Validation [c]					
Milk	0.010-0.500	67; 73-100 (32)			
Kidney	0.010-0.500 0.100 [d]	65, 68; 70-84 (14) 68			
Liver	0.010-5.00 0.100 [d]	64, 65, 67; 70-91 (19) 61			
Muscle	0.010-0.500 0.100 [d]	68, 69, 69; 71-86 (22) 73			
Immunoassay Method GRM 95.14 - Concurrent Method Recovery [c]					
Milk	0.010-1.0	77-119; 124, 125, 127 (87)			
Kidney	0.010	68; 70-83 (7)			
Liver	0.010-5.0	67; 70-91 (12)			
Muscle	0.010-0.50	69, 69; 71-85 (15)			

[a] Each recovery value represents one sample unless otherwise indicated in parentheses; recovery values outside the acceptable 70-120% range are listed separately. [b] An additional fortification with spinosyn A at 10.0 ppm was also conducted. [c] Samples were fortified with spinosyn A unless otherwise indicated. [d] Fortified with spinosad TGAI (technical grade active ingredient) containing 76.1% spinosyn A and 11.9% spinosyn D.

Independent laboratory validation - HPLC method GRM 95.03: The petitioner submitted the following data pertaining to the independent laboratory validation of HPLC method GRM 95.03:

MRID 44058820 West, S.; Larese, J. (1996) Independent Laboratory Validation of Method GRM 95.03--Determination of Spinosad and Metabolites in Beef Tissues, Milk, and Cream by High Performance Liquid Chromatography with Ultraviolet Detection: Lab Project Number: DE-03-96: RES95155: GRM 95.03. Unpublished Study Prepared by Enviro-Bio-Tech Ltd. 74 p.

MRID 44058821 West, S.; Kendall, T.; Markley, B. (1996) Independent Laboratory Validation of Method GRM 95.03--Determination of Spinosad and Metabolites in Beef Tissues, Milk, and Cream by High Performance Liquid Chromatography with Ultraviolet Detection: Lab Project Number: RES95155B: GRM 95.03. Unpublished Study Prepared by Wildlife International Ltd. 72p.

The validation studies were conducted by Enviro-Bio-Tech, Ltd (Bernville, PA) using cattle muscle and fat and by Wildlife International Ltd. (Easton, MD) using milk. Samples of control cattle milk, muscle, and fat from the cattle feeding study (see "Meat, Milk, Poultry, Eggs") were fortified with spinosyns A, D, and B, and N-demethyl spinosyn D at 0.01 ppm and 0.05 ppm and analyzed by the test laboratories using GRM 95.03. The results of the independent validation studies are presented in Table 16. Slight modifications to the method were made by each laboratory (e.g., use of rotary evaporation instead of evaporation under a stream of nitrogen); no problems with the analytical procedures were reported by either laboratory. The independent laboratory validation data indicate that HPLC method GRM 95.03 can adequately recover residues of spinosyns A, D, and B and N-demethyl spinosyn D from cattle muscle and milk. The fortification levels used in the studies represent the LOQ and five times the LOQ for each analyte.

Table 16. Independent laboratory validation of HPLC method GRM 95.03 using samples of cattle muscle, fat, and milk.

Commodity	Fortification Level, ppm	Recovery, %			
		Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D
Muscle	0.01	73.2, 77.5	89.2, 116	95.0, 96.3	106, 110
	0.05	82.0, 92.6	82.4, 94.0	82.2, 93.2	83.4, 95.2
Fat	0.01	76.8, 81.2	73.4, 75.4	90.1, 92.6	76.6, 79.6
	0.05	88.8, 91.0	89.8, 93.2	89.4, 93.0	89.2, 97.6
Milk	0.01	107, 120	102, 117	111, 119	104, 110
	0.05	105, 119	108, 119	101, 110	102, 114

Enforcement method - animal commodities: For enforcement of tolerances for residues of spinosyns A, D, and B, and N-demethyl spinosyn D, the petitioner proposes HPLC method GRM 95.03.

Radiovalidation of the proposed enforcement method(s): The petitioner submitted (included in MRID 44058822) radiovalidation data for HPLC method GRM 95.03 using samples from the goat metabolism study (MRID 43727406; see PP#6G04692, DP Barcodes D219016, D224608, D223898, and D223899, G. J. Herndon, 4/24/96). Samples of milk, lean muscle, liver, kidney, and fat were re-extracted and analyzed using the procedures described in the metabolism study and samples were analyzed using HPLC method GRM 95.03. The results of the radiovalidation study are presented in Table 17. The radiovalidation data indicate that HPLC method GRM 95.03 can adequately recover residues of spinosyns A, D, and B, and N-demethyl spinosyn D from milk and animal tissues. No additional radiovalidation data are required for animal commodities.

Table 17. Comparison of residues of spinosyns A, D, and B, and N-demethyl spinosyn D in milk and animal tissues as determined in the metabolism study and by analysis using HPLC method GRM 95.03.

Commodity	Method	Residues, ppm				
		Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D	Total
Milk	Radiochemical	0.41	0.11	0.01	0.004	0.53
	GRM 95.03	0.463	0.131	0.017	0.007	0.618
Fat	Radiochemical	2.98	1.48	0.05	0.03	4.54
	GRM 95.03	2.38	1.44	0.145	0.112	4.08
Kidney	Radiochemical	0.39	0.10	0.08	0.03	0.60
	GRM 95.03	0.321	0.098	0.065	0.041	0.525
Liver	Radiochemical	0.50	0.13	0.11	0.05	0.79
	GRM 95.03	0.456	0.114	0.093	0.043	0.706
Muscle	Radiochemical	0.16	0.06	0.025	0.009	0.25
	GRM 95.03	0.140	0.060	0.021	0.010	0.231

Conclusions: HPLC method GRM 95.03 is adequate for the purposes of collection of residue data for spinosyns A, D, and B and N-demethyl spinosyn D in animal commodities; adequate independent method validation and concurrent method recovery data have been submitted. The method has also been adequately radiovalidated. CBTS will forward HPLC method GRM 95.03 to EPA ACL for a method trial.

The petitioner submitted a description of and validation data for immunoassay method GRM 95.14, a method for the determination of total spinosyn-related residues in milk and cattle muscle, liver, and kidney. Adequate validation data for spinosyn A were submitted.

OPPTS GLN 860.1360: Multiresidue Method

The petitioner submitted the following data pertaining to multiresidue methods testing of spinosyns B and K and N-demethyl spinosyn D:

> MRID 44058810 Satonin, D. (1996) Multi-Residue Methods Testing for Spinosyns B, K, and N-Demethyl Spinosyn D: Lab Project Number: RES96068. Unpublished Study Prepared by DowElanco. 35 p.

These data will be forwarded to FDA for review. The petitioner had previously submitted data pertaining to the multiresidue methods testing of spinosyns A and D in conjunction with PP#6G04692 which were forwarded to FDA (G. J. Herndon, 4/24/96).

OPPTS GLN 860.1380: Storage Stability Data

Storage stability data - plant commodities: The RAC samples from the apple and Brassica leafy vegetable field trials were promptly frozen after harvest, and shipped via freezer truck or overnight delivery in insulated boxes with dry ice to DowElanco Analytical Services (Indianapolis, IN). The 7-day PHI apple samples from the IN test site were delivered directly to the laboratory in coolers on ice within 3.5 hours of collection. The 0-day PHI apple samples from one of the CA test sites were shipped on the day of collection by overnight delivery on blue ice as "fresh fruit." A 1-day PHI apple sample was harvested from the CA test and shipped frozen for comparison to the "fresh fruit" shipped samples. All samples were received at DowElanco in good conditions, except for one control apple sample from one of the CA test sites; the control sample had thawed and the fruits were soft. At DowElanco all samples were stored frozen (-20 C) until preparation for analysis. The total storage intervals between harvest and analysis were 19-124 days (~1-4 months) for apples, 46-193 days (~2-6 months) for broccoli, 35-145 days (~1-5 months) for cabbage, and 40-271 days (~1-9 months) for mustard greens.

RAC samples from the apple processing study were shipped frozen on blue ice by Federal Express to DowElanco Global Environmental Chemistry Laboratory (Indianapolis, IN). Samples for processing were shipped under ambient conditions to Wm. J. Englar and Associates, Inc. (Moses Lake, WA) for processing. Samples were stored frozen (-22 C) at the processing facility. Processed samples were shipped overnight on dry ice by Federal Express to DowElanco for analysis. The RAC and processed samples were stored frozen (-20 C) at DowElanco until preparation for analysis. Samples were analyzed within 84 days (~3 months) of processing. Total storage intervals from harvest to analysis were up to 91 days (~3 months) for apples washed and unwashed, 89 days (~3 months) for juice, and 33 days (~1 month) for wet pomace.

The petitioner has submitted the following storage stability data for cabbage, apples, and apple juice in support of the Brassica leafy vegetable and apple field trial and apple processing studies:

MRID 44058824 Phillips, A.; Rutherford, B. (1996) Frozen Storage Stability of Spinosyns A, D, B, K and N-Demethyl Spinosyn D in Cabbage: Lab Project Number: RES94098: 94.22. Unpublished Study Prepared by DowElanco. 82 p.

MRID 44058825 Robb, C.; Bormett, G. (1996) Frozen Storage Stability of Spinosyns A, D, B, K and N-Demethyl Spinosyn D in Apples and Apple Juice: Lab Project Number: RES95100: GRM 95.05. Unpublished Study Prepared by DowElanco. 124 p.

Field trial control samples (apples and apple juice) or grocery samples (cabbage) were placed in HDPE (cabbage and apple samples) or LDPE (apple juice) containers and fortified with spinosyns A, D, B, and K, and N-demethyl spinosyn D at 0.10 ppm. The fortified and unfortified samples were stored frozen at approximately -20 C. Replicate samples were removed from storage and analyzed after 0, 1, 3, 4, 6, and 12 months of frozen storage for cabbage samples, after 0, 1, 3, and 6 months of frozen storage for apple samples, and after 0, 1, 2, and 3 months of frozen storage for apple juice samples. Additional apple samples were removed from frozen storage after 6 months and stored overnight at room temperature prior to analysis to simulate the storage conditions of apple samples received warm from overnight shipping. Apple and apple juice samples were analyzed using DowElanco method GRM 95.05, and cabbage samples were analyzed using DowElanco method GRM 94.22. The LOQ for all matrices was 0.01 ppm.

Apparent residues of spinosyns A, D, B, and K, and N-demethyl spinosyn D each were less than the LOD (<0.003 ppm) in/on six unfortified samples of cabbage, and in/on four unfortified samples each of apples and apple juice, except that detectable residues of spinosyn A were observed in one sample of unfortified cabbage at 0.006 ppm (12-month interval). The results of the storage stability study are presented in Table 18.

Table 18. Storage stability and concurrent method recoveries (fresh fortification recovery) of carbaryl residues of concern from samples of apples and apple juice fortified individually with spinosyns A, D, B, and K, and N-demethyl spinosyn D at 0.10 ppm and stored frozen at -20 C.

Commodity	Storage Period (Months)	Spinosyn Residue Fortified	Fresh Fortification Recovery (%)	Storage Stability Recovery (%)	Corrected Storage Stability Recovery (%) [a]
Apples	0	A	89, 90, 90, 93, 93	--	--
		D	87, 89, 89, 90, 92	--	--
		B	78, 79, 80, 82, 85	--	--
		K	83, 86, 86, 88, 88	--	--
		N-demethyl D	78, 79, 80, 81, 82	--	--
	1	A	87, 90	82, 85, 87	93, 95, 98
		D	86, 89	81, 85, 86	93, 97, 98
		B	87, 87	75, 75, 78	86, 86, 90
		K	87, 90	82, 85, 88	93, 96, 99
		N-demethyl D	84, 84	71, 73, 75	85, 87, 89
	3	A	87, 88	86, 87, 89	98, 99, 102
		D	92, 93	85, 86, 88	92, 93, 95
		B	80, 84	76, 81	93, 99
		K	85, 92	84, 85, 86	95, 97, 97
		N-demethyl D	78, 82	72, 78	91, 98
	6	A	90, 92	88, 91, 95	96, 99, 104
		D	86, 88	83, 87, 91	95, 100, 104
		B	77, 82	84, 87, 87	105, 108, 109
		K	92, 93	86, 89, 92	93, 97, 100
		N-demethyl D	78, 83	82, 84, 84	101, 105, 105
	6 + 1 day at RT [b]	A	(90, 92)	90	99
		D	(86, 88)	86	99
		B	(77, 82)	85	106
		K	(92, 93)	80	86
		N-demethyl D	(78, 83)	81	101

(continued; footnotes follow)

Table 18 *(continued)*.

Commodity	Storage Period (Months)	Spinosyn Residue Fortified	Fresh Fortification Recovery (%)	Storage Stability Recovery (%)	Corrected Storage Stability Recovery (%) [a]
Apple juice	0	A	78, 82, 83, 85, 87	--	--
		D	77, 81, 81, 84, 85	--	--
		B	66, 67, 68, 72	--	--
		K	81, 81, 82, 86	--	--
		N-demethyl D	70, 71, 72, 75	--	--
	1	A	87, 89	83, 87, 87	95, 99, 99
		D	84, 87	80, 84, 84	94, 98, 99
		B	82, 84	80, 80, 84	96, 97, 101
		K	86, 88	84, 85, 86	96, 97, 100
		N-demethyl D	80, 83	77, 79, 82	95, 97, 101
	2	A	82, 93	79, 81, 83	91, 93, 96
		D	80, 91	77, 79, 81	90, 92, 95
		B	69, 77	73, 73, 75	100, 100, 103
		K	77, 88	77, 79, 81	93, 95, 98
		N-demethyl D	69, 76	71, 71, 74	98, 99, 102
	3	A	85, 90	90, 90, 96	103, 103, 109
		D	82, 88	86, 87, 92	101, 102, 108
		B	78, 79	80, 82, 82	101, 104, 104
		K	89, 89	87, 87, 87	98, 98, 98
		N-demethyl D	78, 80	78, 80, 80	99, 102, 102
Cabbage	0	A	78, 81, 83, 89	--	--
		D	80, 82, 83, 89	--	--
		B	59, 66, 68, 68, 68	--	--
		K	65, 66, 67, 69, 78	--	--
		N-demethyl D	59, 66, 67, 68, 68	--	--

(continued; footnotes follow)

Table 18 *(continued)*.

Commodity	Storage Period (Months)	Spinosyn Residue Fortified	Fresh Fortification Recovery (%)	Storage Stability Recovery (%)	Corrected Storage Stability Recovery (%) [a]
Cabbage (continued)	1	A	88, 95	80, 81, 83	87, 89, 91
		D	88, 94	80, 82, 84	87, 90, 92
		B	73, 80	70, 76, 81	91, 99, 106
		K	90, 94	89, 90, 93	96, 97, 101
		N-demethyl D	72, 79	68, 75, 79	90, 98, 105
	3	A	66, 76	64, 68, 70	91, 95, 99
		D	64, 74	63, 65, 69	91, 94, 99
		B	68, 72	60, 62, 70	86, 88, 100
		K	70, 82	65, 66, 70	85, 87, 91
		N-demethyl D	66, 70	58, 59, 67	84, 87, 98
	4	A	97, 98	89, 90	91, 93
		D	92, 94	83, 88	89, 95
		B	82, 85	71, 77, 77	84, 92, 93
		K	95	84, 84, 85	88, 89, 90
		N-demethyl D	81, 81	67, 76, 77	83, 93, 95
	6	A	91, 94	87, 92, 93	94, 100, 100
		D	86, 87	82, 87, 89	94, 100, 103
		B	78, 79	81, 82, 83	103, 104, 106
		K	82, 88	86, 87, 88	102, 103, 103
		N-demethyl D	74, 76	77, 79, 79	103, 105, 106
	12	A	69, 78	64, 73, 77	88, 100, 105
		D	64, 76	67, 69, 73	97, 99, 105
		B	60, 65	66, 67, 69	105, 108, 111
		K	79, 91	69, 70, 72	81, 82, 84
		N-demethyl D	56, 58	61, 62, 65	106, 108, 114

[a] Calculated by dividing the storage stability recovery by the average fresh fortification recovery.

[b] Samples were removed from frozen storage after 6 months and stored 1 day at room temperature to simulate the storage conditions of samples which were shipped overnight as "fresh fruits."

Conclusions: The storage stability data for apples, apple juice, and Brassica leafy vegetables are adequate. The data indicate that fortified residues of spinosyns A, D, B, and K, and N-demethyl spinosyn D are relatively stable under frozen storage conditions for at least 12 months in/on cabbage, 6 months in/on apples, and 3 months in/on apple juice.

Samples from the submitted field trial and processing studies were stored frozen for a maximum interval of 10 months for broccoli, 9 months for mustard, 5 months for cabbage, 4 months for apples, 3 months for apple juice, and 1 month for apple wet pomace prior to analysis. The available frozen storage stability data support the storage intervals of the submitted field trials and processing study. No storage stability data are required for apple wet pomace since these samples were analyzed for residues within 30 days of harvest.

Storage stability data - animal commodities: Samples of animal commodities from the submitted cattle feeding study were frozen (-20 C) after collection and stored frozen until shipment to DowElanco for analysis. At DowElanco, samples of milk, cream, and skim milk were stored frozen (-20 C) for 20-135 days (~1-4.5 months) prior to HPLC analysis and for 3-148 days (up to ~5 months) prior to immunoassay analysis. Samples of kidney, liver, and muscle were stored frozen (-20 C) for 6-21 days prior to HPLC and immunoassay analysis and fat samples were stored frozen for 6-14 days (36- to 85-day sampling intervals) or 70 days (28-day sampling interval) prior to analysis.

The petitioner conducted a concurrent storage stability study with milk by fortifying control samples of milk with spinosyns A, D, and B and N-demethyl spinosyn D at 0.10 ppm, storing samples frozen for 41 and 136 days, and analyzing samples using HPLC method GRM 95.03. The results of the milk storage stability study are presented in Table 19-A. These data indicate that residues of spinosyns A, D, and B and N-demethyl spinosyn D are stable in milk during frozen storage for up to 136 days (4.5 months).

Supporting storage stability data for animal tissues other than fat are not required because samples were analyzed within 21 days of collection. To support the 70-day storage interval for fat samples, the petitioner referred to the radiovalidation data presented in MRID 44058822. The petitioner stated that samples of goat milk and tissue from the metabolism study (MRID 43727406) were reanalyzed, using the procedures reported in the metabolism study, prior to analyzing the samples using the proposed enforcement method. These reanalyses were reportedly conducted 1.6 years following the initial metabolism analyses. The results for spinosyns A, D, and B and N-demethyl spinosyn D that were reported in the metabolism study (see PP#6G04692, DP Barcodes D219016, D224608, D223898, and D223899, G. J. Herndon, 4/24/96) as well the results from the reanalyses (as reported in MRID 44058822) are presented in Table 19-B. These data indicate that radioactive residues of spinosyns A, D, and B and N-demethyl spinosyn D are stable in milk, fat, kidney, liver, and muscle during 1.6 years of frozen storage.

Table 19-A. Storage stability of residues of spinosyns A, D, and B and N-demethyl spinosyn D in milk samples fortified with each analyte at 0.10 ppm, stored frozen for up to 136 days, and analyzed using HPLC method GRM 95.03.

Analyte	Storage Interval (days)	Fresh Fortification Recovery (%)	Apparent Recovery in Stored Samples (%)	Corrected Recovery in Stored Samples (%) [a]
Spinosyn A	0	90, 95, 104, 104, 105	--	--
	41	97, 100	79, 87, 102	80, 89, 104
	136	97, 101	77, 81, 81	78, 82, 82
Spinosyn D	0	88, 92, 100, 100, 101	--	--
	41	93, 97	77, 85, 97	81, 89, 102
	136	94, 96	75, 77, 77	78, 81, 81
Spinosyn B	0	99, 101, 102, 103, 110	--	--
	41	90, 93	89, 94, 95	97, 102, 104
	136	90, 97	80, 81, 85	85, 86, 91
N-Demethyl Spinosyn D	0	95, 98, 99, 101, 107	--	--
	41	89, 92	84, 91, 92	93, 100, 102
	136	90, 95	74, 75, 80	80, 81, 86

[a] Corrected for average fresh fortification recovery.

Table 19-B. Storage stability of [^{14}C]residues of spinosyns A, D, and B and N-demethyl spinosyn D in animal tissues during frozen storage for 1.6 years.

Commodity	Analysis [a]	[^{14}C]Residues, ppm				
		Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D	Total
Milk	Initial	0.45	0.13	0.012	0.004	0.60
	Final	0.41	0.11	0.01	0.004	0.53
Fat	Initial	3.07	1.54	0.026	0.020	4.66
	Final	2.98	1.48	0.05	0.03	4.54
Kidney	Initial	0.34	0.12	0.099	0.046	0.61
	Final	0.39	0.10	0.08	0.03	0.60
Liver	Initial	0.47	0.102	0.046	0.022	0.64
	Final	0.50	0.13	0.11	0.05	0.79
Muscle	Initial	0.15	0.063	0.025	0.013	0.25
	Final	0.16	0.06	0.025	0.009	0.25

[a] Initial = Reported in MRID 43727406 with goat metabolism study. Final = Reported in MRID 44058822 with radiovalidation data.

OPPTS GLN 860.1500: Crop Field Trials

Apples

DowElanco submitted the following data from 16 tests depicting residues of spinosad in/on apples:

> MRID 44058827 Bargar, E.; Bolles, H.; Robb, C. (1996) Magnitude of the Residue of Spinosad: DE-105 in Apples: Lab Project Number: RES95014: GRM 95.05. Unpublished Study Prepared by DowElanco. 236 p.

Trials were conducted in CA(2), ID, IL, IN, MI(2), NC, NY(2), OR(2), PA, VA, and WA(2). Mature fruits were harvested 0, 7, and 14 days following the last of five sequential applications, at 6- to 74-day retreatment intervals, of the 80% WDG formulation at ~0.045, 0.062, 0.089, 0.089, and 0.161 lb ai/A using ground equipment. Total seasonal application rates were 0.442-0.515 lb ai/A. The first application was made to apples at the pink stage, the second and third applications were made with approximately 4-week retreatment intervals, the fourth application was made approximately 6 weeks following the third application, and the final application was made at maturity, approximately 1 to 10 weeks following the fourth application. At each test site, two trials were performed separately using concentrate and dilute spray applications. Concentrate applications were made in ~47-72 gal/A of water and dilute spray applications were made in ~188-285 gal/A of water. Mature fruit samples were also harvested at 3- and 10-day posttreatment intervals (PTIs) in one of the NY and WA trials to provide additional harvest points for a residue decline study.

One control and duplicate treated samples were collected from each test. Samples were analyzed for residues of spinosyns A, D, B, and K and N-demethyl spinosyn D using DowElanco method GRM 95.05. The petitioner noted that the 14-day PTI fruit samples were analyzed in only six of the tests because residue levels in the 14-day PTI samples were similar to or lower than the 7-day PTI samples, and the petitioner wishes to support a 7-day PTI.

Residues of spinosyns A, D, B, and K and N-demethyl spinosyn D were each less than the LOQ (<0.01 ppm) in/on 42 samples of untreated apples. Residues of spinosyns B and K and N-demethyl spinosyn D were each less than the LOQ (<0.01 ppm) in all of the treated apple samples; spinosyn B residues ranged <0.003-0.0068 ppm, spinosyn K residues were <0.004 ppm (LOD), and N-demethyl spinosyn D residues ranged <0.003-0.0059 ppm. Residues of spinosyns A and D in/on treated samples are presented in Table 20.

Table 20. Residues of spinosyns A and D in/on apple fruits harvested 0-14 days following five sequential applications of the 80% WDG formulation at ~0.045, 0.062, 0.089, 0.089, and 0.161 lb ai/A (~0.45 lb ai/A/season).

Test Location		PTI	Residues (ppm) from Concentrate Spray Applications [a]			Residues (ppm) from Dilute Spray Applications [a]		
Region	County, State	(days)	A	D	Total [b]	A	D	Total [b]
1	Yates, NY	0	0.041, 0.049	(0.006), (0.004)	0.048, 0.053	0.051, 0.076	(0.006), 0.010	0.056, 0.085
	Lehigh, PA		0.055, 0.072	(0.006), (0.009)	0.062, 0.081	0.092, 0.098	0.010, 0.012	0.102, 0.110
	Frederick, VA		0.045, 0.053	(0.005), (0.006)	0.050, 0.059	0.064, 0.086	(0.007), 0.010	0.072, 0.096
	Yates, NY	7	<0.003, <0.003	<0.003, <0.003	<0.006, <0.006	(0.003), (0.004)	<0.003, <0.003	<0.006, <0.007
	Lehigh, PA		<0.003, <0.003	<0.003, <0.003	<0.006, <0.006	<0.003, <0.003	<0.003, <0.003	<0.006, <0.006
	Frederick, VA		(0.009), 0.011	<0.003, <0.003	<0.012, <0.014	0.010, 0.012	<0.003, <0.003	<0.013, <0.015
	Wayne, NY	0	0.099, 0.102	0.013, 0.012	0.112, 0.114	0.066, 0.091	(0.009), 0.012	0.075, 0.103
		3	0.018, 0.021	(0.003), <0.003	0.021, <0.024	0.023, 0.025	(0.003), (0.004)	0.026, 0.029
		7	0.015, 0.016	<0.003, <0.003	<0.018, <0.019	(0.007), 0.010	<0.003, <0.003	<0.010, <0.013
		10	(0.003), (0.004)	<0.003, <0.003	<0.006, <0.007	(0.003), (0.006)	<0.003, <0.003	<0.006, <0.009
		14	<0.003, <0.003	<0.003, <0.003	<0.006, <0.006	<0.003, <0.003	<0.003, <0.003	<0.006, <0.006
2	Durham, NC	0	0.121, 0.135	0.013, 0.015	0.134, 0.150	0.064, 0.095	(0.007), 0.011	0.071, 0.106
		7	0.011, 0.022	<0.003, <0.003	<0.014, <0.025	0.025, 0.039	(0.004), (0.005)	0.029, 0.045
		14	0.014, 0.018	<0.003, <0.003	<0.017, <0.021	0.011, 0.017	<0.003, <0.003	<0.014, <0.020
5	Marion, IL	0	0.162, 0.217	0.022, 0.029	0.184, 0.246	0.090, 0.114	0.014, 0.016	0.104, 0.130
	Hancock, IN		0.053, 0.062	(0.006), (0.007)	0.060, 0.069	0.055, 0.092	(0.008), 0.013	0.063, 0.104
	Oceana, MI		0.099, 0.106	0.012, 0.013	0.111, 0.118	0.067, 0.068	(0.008), (0.008)	0.075, 0.076
	Ottawa, MI		0.136, 0.160	0.017, 0.021	0.153, 0.181	0.190, 0.220	0.025, 0.027	0.215, 0.246
	Marion, IL	7	0.022, 0.025	<0.003, <0.003	<0.025, <0.028	0.015, 0.017	<0.003, <0.003	<0.018, <0.020
	Hancock, IN		<0.003, <0.003	<0.003, <0.003	<0.006, <0.006	(0.003), (0.005)	<0.003, <0.003	<0.006, <0.008
	Oceana, MI		(0.004), (0.004)	<0.003, (0.006)	<0.007, 0.010	0.014, 0.016	<0.003, <0.003	<0.017, <0.019
	Ottawa, MI		(0.006), 0.011	<0.003, <0.003	<0.009, <0.014	0.024, 0.029	(0.003), (0.005)	0.028, 0.033
10	Fresno, CA	0	0.122, 0.181	0.014, 0.020	0.137, 0.201	0.068, 0.074	0.010, (0.009)	0.078, 0.083
	Santa Cruz, CA		0.053, 0.058	(0.004), (0.006)	0.057, 0.065	0.074, 0.097	(0.009), 0.011	0.083, 0.108
	Santa Cruz, CA	1	0.040, 0.048	(0.005), (0.005)	0.044, 0.053	0.059, 0.068	(0.007), 0.010	0.067, 0.078
10 (cont'd)	Fresno, CA	7	0.041, 0.048	(0.006), (0.006)	0.047, 0.054	0.040, 0.041	(0.005), (0.006)	0.045, 0.047

(continued; footnotes follow)

Table 20 (*continued*).

Test Location		PTI	Residues (ppm) from Concentrate Spray Applications [a]			Residues (ppm) from Dilute Spray Applications [a]		
Region	County, State	(days)	A	D	Total [b]	A	D	Total [b]
	Santa Cruz, CA		(0.007), (0.008)	<0.003, <0.003	<0.010, <0.011	0.015, 0.031	<0.003, (0.003)	<0.018, 0.034
	Fresno, CA	14	0.046, 0.064	(0.006), (0.008)	0.053, 0.072	0.035, 0.039	(0.005), (0.005)	0.040, 0.044
	Santa Cruz, CA		(0.005), (0.005)	<0.003, <0.003	<0.008, <0.008	0.010, 0.010	<0.003, <0.003	<0.013, <0.013
11	Payette, ID	0	0.117, 0.145	0.014, 0.019	0.131, 0.163	0.097, 0.130	0.013, 0.015	0.110, 0.144
	Hood River, OR		0.056, 0.066	(0.006), (0.008)	0.062, 0.074	0.074, 0.093	(0.009), 0.011	0.083, 0.104
	Wasco, OR		0.051, 0.052	(0.007), (0.007)	0.059, 0.059	0.062, 0.065	(0.008), (0.008)	0.070, 0.073
	Franklin, WA		0.045. 0.047	(0.005), (0.005)	0.049, 0.052	0.061, 0.065	(0.007), (0.008)	0.068, 0.073
	Payette, ID	7	0.075, 0.083	(0.009), 0.013	0.084, 0.096	0.062, 0.092	0.010, 0.013	0.072, 0.105
	Hood River, OR		0.021, 0.029	(0.003), (0.004)	0.024, 0.033	0.026, 0.030	(0.003), (0.004)	0.029, 0.033
	Wasco, OR		0.013, 0.020	<0.003, <0.003	<0.016, <0.023	0.019, 0.022	<0.003, <0.003	<0.021, 0.025
	Franklin, WA		0.037, 0.044	(0.004), (0.004)	0.041, 0.048	0.035, 0.049	(0.004), (0.006)	0.039, 0.055
	Grant, WA	0	0.082, 0.091	(0.009), 0.013	0.091, 0.104	0.074, 0.080	0.010, (0.008)	0.084, 0.088
		3	0.056, 0.059	(0.006), (0.007)	0.061, 0.066	0.062, 0.075	(0.007), 0.011	0.070, 0.086
		7	0.026, 0.040	(0.004), (0.004)	0.030, 0.045	0.032, 0.040	(0.003), (0.006)	0.034, 0.045
		10	0.027, 0.037	(0.004), (0.005)	0.032, 0.042	0.011, 0.014	<0.003, (0.003)	<0.014, 0.016
		14	0.028, 0.036	(0.003), (0.005)	0.031, 0.041	0.013, 0.023	<0.003, (0.003)	<0.016, 0.026

[a] Residues in treated samples were not corrected for concurrent method recovery. Residue values in parentheses are greater than the LOD but less than the LOQ.

[b] Totals were calculated using more significant figures than presented.

A total of 16 field trials were conducted in Regions 1 (4 trials), 2 (1 trial), 5 (4 trials), 10 (2 trials), and 11 (5 trials). The number and location of field trials is adequate to support the proposed use on apples.

Conclusions: The submitted apple field trial data are adequate. The data indicate that the combined residues of spinosyns A and D will not exceed the proposed 0.20-ppm tolerance in/on apples harvested 7 days following the last of five sequential foliar broadcast applications, with 6- to 74-day retreatment intervals, of the 80% WDG formulation at ~0.045 + 0.062 + 0.089 + 0.089 + 0.161 lb ai/A (1x the proposed maximum seasonal rate). The combined residues in/on apples, treated as described above, were <0.006-0.105 ppm (64 samples). No significant difference was observed between the concentrated (~50 gal/A) and dilute spray (~200 gal/A) applications.

Data from residue decline studies indicate that spinosad residues decline at 3-day and 7-day PTIs. Residue levels at 10-day and 14-day PTIs were similar to or less than those for the 7-day PTI samples.

Brassica (cole) Leafy Vegetables Group

DowElanco submitted the following data from 24 tests depicting residues of spinosad in/on Brassica leafy vegetables:

> MRID 44058828 Rutherford, B.; Bormett, G. (1996) Magnitude of the Residue of Spinosad: DE-105 in Brassica Vegetables: Lab Project Number: RES95001: 94.22. Unpublished Study Prepared by DowElanco. 259 p.

Eight trials with broccoli were conducted in AZ, CA(5), OR, and TX; eight trials with cabbage were conducted in CA, FL, IN(2), PA(2), TX, and VA; and eight trials with mustard greens were conducted in AZ, CA, IN, MS, TX(2), and VA(2). Mature broccoli with stalks, cabbage with and without wrapper leaves, and mustard greens were harvested 1, 3, and 5 days following the last of four sequential applications, at 3- to 5-day retreatment intervals, of the 80% WDG formulation at ~0.089, 0.089, 0.134, and 0.134 lb ai/A using ground equipment. Applications were made as broadcast sprays at all test sites except those in PA and TX; applications in PA and TX were made as directed sprays. Total seasonal application rates were 0.441-0.469 lb ai/A. Applications were made in ~28-36 gal/A of water. Mature broccoli and mustard green samples were also harvested at 0-, 7-, and 10-day PTIs in one of the broccoli (CA) and one of the mustard green (IN) trials to provide additional harvest points for a residue decline study.

One control and duplicate treated samples were collected from each test; samples of cabbage with and without wrapper leaves were collected in the cabbage trials. Samples were analyzed for residues of spinosyns A, D, B, and K and N-demethyl spinosyn D using DowElanco

method GRM 94.22. The petitioner noted that the 5-day PTI samples were analyzed in only two mustard green tests, two cabbage tests, and the two residue decline studies because residue levels were acceptable at the shorter PTIs.

Residues of spinosyns A, D, B, and K and N-demethyl spinosyn D were each less than the calculated LOQ (<0.014, <0.013, <0.012, <0.010, and <0.012 ppm, respectively) in/on 20 samples of untreated broccoli, 18 samples each of untreated cabbage with and without wrapper leaves, and 22 samples of untreated mustard greens, except that detectable residues of spinosyn A were observed in one sample of untreated mustard greens at 0.014 ppm and detectable residues of spinosyn D were observed in one sample of untreated mustard greens at 0.014 ppm. The results of the field trials are presented in Table 21.

Table 21. Residues of spinosyns A, D, B and K and N-demethyl spinosyn D in/on broccoli, cabbage, and mustard greens harvested 0-14 days following four sequential applications of the 80% WDG formulation at ~0.089, 0.089, 0.134, and 0.134 lb ai/A (~0.45 lb ai/A/season).

Test Location		PTI	Spinosyn Residues (ppm) [a]					
Region	County, State	(days)	A	D	B	K	N-Demethyl D	Total A + D
Broccoli								
6	Wharton, TX	1	0.094, 0.125	(0.011), 0.014	(0.011), (0.011)	<0.003, <0.003	(0.004), <0.003	0.105, 0.139
		3	0.022, 0.023	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.026, <0.027
10	Yuma, AZ	1	0.174, 0.175	0.024, 0.024	0.020, 0.020	<0.003, <0.003	(0.004), (0.003)	0.198, 0.199
	Imperial, CA		0.298, 0.348	0.028, 0.036	0.014, 0.017	<0.003, <0.003	<0.003, <0.003	0.326, 0.384
	Monterey, CA		0.309, 0.392	0.033, 0.045	(0.011), (0.011)	<0.003, <0.003	(0.005), (0.003)	0.342, 0.437
			0.130, 0.159	(0.012), 0.019	(0.011), 0.012	(0.003), <0.003	<0.003, <0.003	0.142, 0.178
	San Joaquin, CA		0.138, 0.502	0.015, 0.065	(0.011), 0.039	<0.003, (0.003)	<0.003, (0.003)	0.153, 0.567
	Yuma, AZ	3	0.072, 0.122	(0.010), 0.016	(0.008), 0.013	<0.003, <0.003	<0.003, <0.003	0.082, 0.138
	Imperial, CA		0.171, 0.201	0.020, 0.022	(0.009), (0.010)	<0.003, <0.003	<0.003, <0.003	0.191, 0.223
	Monterey, CA		0.155, 0.221	0.018, 0.028	(0.009), 0.013	<0.003, <0.003	<0.003, (0.004)	0.173, 0.249
			0.081, 0.112	(0.008), 0.013	(0.007), (0.009)	<0.003, <0.003	<0.003, (0.003)	0.089, 0.125
	San Joaquin, CA		0.120, 0.281	0.013, 0.031	(0.010), 0.027	<0.003, (0.004)	<0.003, (0.003)	0.133, 0.312
	Fresno, CA	0	0.455, 0.488	0.055, 0.059	0.029, 0.031	<0.003, <0.003	<0.003, <0.003	0.510, 0.547
		1	0.308, 0.530	0.036, 0.069	0.016, 0.032	<0.003, <0.003	<0.003, (0.003)	0.344, 0.599
		3	0.239, 0.320	0.031, 0.039	0.018, 0.024	<0.003, <0.003	<0.003, (0.005)	0.270, 0.359
		5	0.175, 0.237	0.023, 0.033	0.012, 0.016	<0.003, <0.003	<0.003, <0.003	0.198, 0.270
		7	0.128, 0.151	0.014, 0.019	0.012, 0.012	<0.003, <0.003	<0.003, <0.003	0.142, 0.170
		10	0.078, 0.099	(0.010), 0.017	(0.007), (0.010)	<0.003, <0.003	<0.003, <0.003	0.088, 0.116
12	Washington, OR	1	0.381, 0.403	0.051, 0.053	0.039, 0.038	(0.009), (0.008)	(0.004), (0.004)	0.432, 0.456
		3	0.317, 0.384	0.044, 0.054	0.024, 0.027	(0.007), (0.009)	(0.003), (0.003)	0.361, 0.438
Cabbage with wrapper leaves								
1	Berks, PA	1	0.049, 0.091	(0.005), (0.011)	(0.007), (0.010)	<0.003, <0.003	<0.003, <0.003	0.054, 0.102
			0.023, 0.023	<0.004, (0.005)	<0.004, (0.004)	<0.003, <0.003	<0.003, <0.003	<0.027, 0.028
		3	(0.004), 0.015	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.008, <0.019
			0.019, 0.023	<0.004, <0.004	(0.004), (0.004)	(0.003), (0.004)	<0.003, (0.006)	<0.023, <0.027

(continued; footnotes follow)

Table 21 *(continued)*.

Test Location		PTI	Spinosyn Residues (ppm) [a]					
Region	County, State	(days)	A	D	B	K	N-Demethyl D	Total A + D
Cabbage with wrapper leaves (continued)								
2	Greensville, VA	1	0.189, 0.463	0.026, 0.061	0.014, 0.038	<0.003, <0.003	<0.003, (0.005)	0.215, 0.524
		3	0.124, 0.131	0.018, 0.016	(0.009), (0.009)	(0.004), <0.003	(0.003), (0.005)	0.142, 0.147
3	Seminole, FL	1	0.867, 1.009	0.134, 0.189	0.029, 0.035	<0.003, <0.003	(0.004), (0.005)	1.001, 1.198
		3	0.035, 0.049	(0.006), (0.008)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	0.041, 0.057
		5	0.029, 0.039	(0.008), (0.006)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	0.037, 0.045
5	Hancock, IN	1	(0.010), (0.011)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	(0.003), <0.003	<0.014, <0.015
			0.045, 0.062	(0.006), (0.007)	<0.004, (0.004)	<0.003, <0.003	<0.003, <0.003	0.051, 0.069
		3	(0.004), (0.009)	<0.004, <0.004	<0.004, <0.004	(0.004), <0.003	<0.003, (0.003)	<0.008, <0.013
			(0.012), (0.013)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.016, <0.017
6	Wharton, TX	1	0.792, 0.810	0.121, 0.169	0.060, 0.094	(0.008), (0.010)	(0.011), 0.016	0.913, 0.979
		3	0.214, 0.418	0.031, 0.058	0.030, 0.044	(0.003), 0.011	(0.005), (0.007)	0.245, 0.476
		5	0.111, 0.181	0.017, 0.028	0.018, 0.027	<0.004, (0.004)	(0.003), (0.004)	0.128, 0.209
10	Fresno, CA	1	0.071, 0.087	0.013, (0.011)	(0.004), (0.004)	(0.007), (0.005)	0.013, (0.007)	0.084, 0.098
		3	0.027, 0.035	(0.005), (0.008)	(0.004), (0.007)	<0.003, <0.003	<0.003, <0.003	0.032, 0.043
Cabbage without wrapper leaves								
1	Berks, PA	1	(0.005), 0.014	<0.004, <0.004	<0.004, <0.004	(0.003), <0.003	<0.003, <0.003	<0.009, <0.018
			(0.005), (0.006)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.009, <0.010
		3	(0.007), (0.010)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	(0.005), <0.003	<0.011, <0.014
			<0.004, <0.004	<0.004, (0.004)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.008, <0.008
2	Greensville, VA	1	0.024, 0.034	<0.004, (0.004)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.028, 0.038
		3	(0.006), 0.027	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.010, <0.031
3	Seminole, FL	1	0.125, 0.182	0.017, 0.025	0.004, 0.005	<0.003, <0.003	<0.003, <0.003	0.142, 0.207
		3	<0.004, <0.004	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.008, <0.008
		5	<0.004, <0.004	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.008, <0.008

(continued; footnotes follow)

Table 21 *(continued)*.

Test Location		PTI	Spinosyn Residues (ppm) [a]					
Region	County, State	(days)	A	D	B	K	N-Demethyl D	Total A + D
Cabbage without wrapper leaves (continued)								
5	Hancock, IN	1	(0.004), (0.005)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, (0.004)	<0.008, <0.009
			0.017, 0.026	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.021, <0.030
		3	<0.004, (0.004)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.008, <0.008
			(0.009), (0.011)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.013, <0.015
6	Wharton, TX	1	0.300, 0.335	0.039, 0.043	0.019, 0.040	<0.003, <0.003	(0.003), (0.005)	0.339, 0.378
		3	0.082, 0.112	(0.012), 0.017	0.015, 0.012	<0.003, <0.003	<0.003, <0.003	0.094, 0.129
		5	0.044, 0.181	(0.006), 0.025	(0.007), 0.022	<0.003, <0.003	<0.003, (0.003)	0.050, 0.206
10	Fresno, CA	1	0.018, 0.027	<0.004, (0.006)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.022, 0.033
		3	0.016, 0.023	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.020, <0.027
Mustard greens								
2	Greensville, VA	1	4.718, 5.184	0.567, 0.670	0.174, 0.180	0.013, 0.013	0.022, 0.022	5.285, 5.854
		3	2.873, 3.135	0.406, 0.443	0.115, 0.124	0.014, 0.010	0.013, 0.014	3.279, 3.578
	Greensville, VA	1	0.856, 0.867	0.128, 0.148	0.098, 0.087	<0.003, <0.003	0.018, 0.015	0.984, 1.015
		3	0.403, 0.415	0.058, 0.061	0.032, 0.033	(0.008), (0.006)	(0.006), (0.005)	0.461, 0.476
		5	0.157, 0.184	0.022, 0.024	0.016, 0.015	(0.007), (0.006)	(0.003), (0.005)	0.179, 0.208
4	Washington, MS	1	3.805, 3.903	0.436, 0.451	0.242, 0.273	(0.008), (0.008)	0.037, 0.043	4.241, 4.354
		3	0.073, 0.197	(0.011), 0.029	(0.006), 0.019	<0.003, <0.003	<0.003, <0.003	0.084, 0.226
		5	0.014, 0.018	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.018, <0.022
5	Hancock, IN	0	3.425, 5.381	0.424, 0.677	0.168, 0.189	0.014, 0.014	0.016, 0.022	3.849, 6.058
		1	1.734, 2.380	0.329, 0.278	0.129, 0.188	0.010, 0.014	(0.005), 0.014	2.063, 2.658
		3	0.065, 0.102	(0.010), 0.017	(0.008), (0.011)	(0.003), (0.003)	<0.003, <0.003	0.075, 0.119
		5	0.017, 0.023	<0.004, (0.005)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.021, <0.027
		7	(0.012), 0.028	<0.004, (0.004)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.016, 0.032
		10	(0.006), (0.007)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.010, <0.011

(continued; footnotes follow)

Table 21 *(continued)*.

Test Location		PTI	Spinosyn Residues (ppm) [a]					
Region	County, State	(days)	A	D	B	K	N-Demethyl D	Total A + D
Mustard greens (continued)								
6	Wharton, TX	1	0.039, 0.041	(0.004), (0.005)	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.043, 0.046
			2.808, 3.354	0.424, 0.462	0.103, 0.124	<0.004, (0.004)	0.012, 0.014	3.232, 3.816
		3	(0.011), (0.011)	<0.004, <0.004	<0.004, <0.004	<0.003, <0.003	<0.003, <0.003	<0.015, <0.015
			3.297, 3.351	0.440, 0.506	0.136, 0.121	(0.005), (0.005)	0.015, 0.017	3.737, 3.857
10	Yuma, AZ	1	4.365, 5.422	0.525, 0.657	0.230, 0.330	(0.006), (0.008)	0.029, 0.042	4.890, 6.079
	Fresno, CA		4.262, 5.999	0.509, 0.765	0.224, 0.277	0.010, 0.010	0.031, 0.039	4.771, 6.764
	Yuma, AZ	3	3.488, 5.114	0.432, 0.603	0.250, 0.321	(0.009), 0.013	0.031, 0.041	3.920, 5.717
	Fresno, CA		2.873, 3.056	0.382, 0.388	0.181, 0.199	(0.008), (0.009)	0.024, 0.027	3.255, 3.444

[a] Residues in treated samples were not corrected for concurrent method recovery. Residue values in parentheses are greater than the LOD but less than the LOQ.

A total of eight field trials were conducted with broccoli in Regions 6 (1 trial), 10 (6 trials), and 12 (1 trial), a total of eight field trials were conducted with cabbage in Regions 1 (2 trials), 2 (1 trial), 3 (1 trial), 5 (2 trials), 6 (1 trial), and 10 (trial), and a total of eight field trials were conducted with mustard greens in Regions 2 (2 trials), 4 (1 trial), 5 (1 trial), 6 (2 trials), and 10 (2 trials). The number and location of field trials is adequate to support the proposed uses on the head and stem subgroup and greens subgroup of Brassica (cole) leafy vegetables.

Conclusions: The submitted field trial data on Brassica (cole) leafy vegetables are adequate. They indicate that residues of spinosyns A and D will not exceed the proposed subgroup tolerances for the head and stem subgroup (2.0 ppm) and greens subgroup (15.0 ppm) of the Brassica (cole) leafy vegetables in/on samples harvested one day following the last of four sequential foliar broadcast applications, with 3- to 5-day retreatment intervals, of the 80% WDG formulation at ~0.089 + 0.089 + 0.134 + 0.134 lb ai/A (1x the proposed maximum seasonal rate). The combined residues in/on representative commodities, treated as described above, were 0.105-0.599 ppm in/on broccoli (16 samples), <0.014-1.198 ppm in/on cabbage with wrapper leaves (16 samples), and <0.043-6.764 ppm in/on mustard greens (16 samples).

Data from the residue decline studies indicate that spinosad residues decline rapidly in broccoli and mustard greens harvested at 3, 5, 7, and 10 days following treatments. The petitioner determined that residues of spinosad had a half-life of approximately 4-5 days in broccoli and 2 days in mustard greens.

Based on the highest residue value obtained from samples harvested at the proposed 1-day PHI and treated at the proposed maximum use rates, the proposed tolerance level of 2.0 ppm for the head and stem subgroup is appropriate. However, the petitioner should submit a revised section F to reduce the proposed tolerance for the greens subgroup of Brassica (cole) leafy vegetables from 15 to 10 ppm.

OPPTS GLN 860.1520: Processed Food/Feed

Apples

DowElanco submitted the following data depicting the potential for concentration of spinosad residues in the processed commodities of apples:

> MRID 44058829 Bolles, H.; Robb, C. (1996) Magnitude of Residue of Spinosad in Processed Products of Apples: Lab Project Number: RES95041: 95.05. Unpublished Study Prepared by DowElanco. 145 p.

In one test conducted in WA, mature apple fruits were harvested 7 days following the last of five foliar sequential broadcast applications, with 28- to 49-day retreatment intervals, of the

80% WDG formulation at ~0.223, 0.313, 0.449, 0.450, and 0.801 lb ai/A in ~200 gal/A using ground equipment. The total seasonal application rate was 2.236 lb ai/A (5x the proposed maximum total seasonal rate).

Control and bulk treated samples were collected (hand picked) from the designated test plots. The harvested fruit samples were shipped frozen on blue ice by Federal Express to DowElanco Global Environmental Chemistry Laboratory (Indianapolis, IN). Samples for processing were shipped under ambient conditions to Wm. J. Englar and Associates, Inc. (Moses Lake, WA) within 30 minutes of harvest. Samples were stored chilled (~3 C) until processing; the fruit was stored for a total of 5 days prior to processing. Samples were processed according to simulated commercial procedures into washed apples, fresh juice, and wet pomace. A brief description of the processing procedure follows. Apples were first washed for five minutes and leaves, stems, and other debris were removed. Washed apples were crushed to a pulp and heated with steam to 40-50 C. Pectic enzyme was added to the heated apple pulp and after approximately two hours, the pulp was pressed and wet pomace collected. The raw juice collected from the apple press was screened through a filter and fresh juice was collected. Duplicate subsamples of the processed fractions were collected and stored frozen (-22 C) at the processing facility. Processed samples were shipped overnight on dry ice by Federal Express to DowElanco for analysis.

Residues in/on treated and untreated apples and apple processed commodities were determined using DowElanco method GRM 95.05. Apparent residues of spinosyns A, D, B and K and N-demethyl spinosyn D were each less than the LOQ (<0.01 ppm) in/on one sample each of untreated fruit (RAC), unwashed and washed fruit (from processor), juice, and wet pomace, except that residues of spinosyn D in untreated wet pomace, could not be distinguished from a low level matrix interference. Because treated wet pomace samples contained residues of spinosyn D well above the LOQ (0.01 ppm), control samples were not reanalyzed. Residues in treated samples are presented in Table 22.

Table 22. Residues of spinosyns A, D, B, and K and N-demethyl spinosyn D in the processed commodities of apples harvested 7 days following the last of five foliar applications at 5x the proposed maximum seasonal rate.

Substrate	Spinosyns					
	A	D	B	K	N-demethyl D	Total (A+D)
Uncorrected Residues (ppm)						
Apples	0.2676	0.0367	0.0089	<0.003	0.0087	0.3043
Apples, unwashed (processor)	0.2175	0.0286	0.0094	<0.003	0.0051	0.2461
Apples, washed	0.1545	0.0208	0.0066	<0.003	<0.004	0.1753
Juice	0.0193	0.0036	0.0018	0.0035	0.0050	0.0229
Wet pomace	1.1568	0.1581	0.0460	0.0151	0.0172	1.3149
Concentration/Reduction Factors [a]						
Apples, washed	0.7x	0.7x	0.7x	--	<0.8x	0.7x
Juice	0.1x	0.1x	0.2x	>1.2x	1x	0.1x
Wet pomace	5.3x	5.5x	4.9x	>5x	3.4x	5.3x

[a] Concentration factors were calculated using the residues in/on the unwashed apple sample from the processor.

Conclusions: The submitted apple processing data are adequate. The data indicate that total residues of spinosyns A and D concentrated 5.3x in wet pomace processed from apples bearing detectable residues. No concentration of residues was observed in juice processed from treated apples.

Based on the available field trial data, the HAFT (Total A+D) for apples harvested 7 days following treatment at the maximum proposed seasonal application rate (0.45 lb ai/A) is 0.089 ppm. The maximum total spinosyns A and D residues expected in apple wet pomace, based on the HAFT and a concentration factor of 5.3x, would be 0.48 ppm. The available data support the proposed tolerance of 0.5 ppm for residues of spinosyns A and D in apple wet pomace.

OPPTS GLN 860.1480: Meat, Milk, Poultry, Eggs

Milk, and the Fat, Meat, and Meat Byproducts of Cattle, Goats, Hogs, Horses, and Sheep

DowElanco submitted the following data pertaining to the magnitude of the residue of spinosad and metabolites spinosyn B and N-demethyl spinosyn D in milk, meat, and meat byproducts of dairy cattle following oral dosing of spinosad:

MRID 44058826 Rutherford, B.; Robb, C. (1996) Magnitude of the Residue of Spinosad in Meat and Milk from a 28-Day Dairy Feeding Study: Lab Project Number: RES95126: 128-006-10. Unpublished Study Prepared by DowElanco and Bio-Life Associates, Ltd. 447 p.

The in-life portion of the study was conducted by Bio-Life Associates, Ltd. (Neillsville, WI) and the analytical portion of the study was conducted by DowElanco (Indianapolis, IN). Sixteen dairy cows were orally dosed once daily with spinosad at levels equivalent to 1 ppm (three cows), 3 ppm (three cows), and 10 ppm (seven cows). Animals were dosed via a gelatin capsule administered orally using a balling gun. An additional three cows were not treated and served as control animals.

The petitioner calculated theoretical dietary burdens for beef and dairy cattle of 0.69 and 0.37 ppm, respectively, based on diets consisting of wet apple pomace, wet tomato pomace, and dry tomato pomace (the petitioner intends to seek a registration for spinosad on fruiting vegetables). We note that tomato pomace is no longer considered a significant livestock feed item (Table 1, OPPTS 860.1000).

CBTS has calculated the maximum theoretical dietary burden of spinosad to beef and dairy cattle to be 0.50 ppm and 0.25 ppm, respectively, based on a diet consisting of wet apple pomace at 40% and 20%, respectively (0.50-ppm proposed tolerance; 40% dry matter). Based on these dietary burdens, the dosing levels of 1, 3, and 10 ppm represent 2x, 6x, and 20x the maximum theoretical dietary burden to beef cattle and 4x, 12x, and 40x the maximum dietary burden to dairy cattle.

The cattle were milked twice daily (a.m. and p.m.) and were fed dairy ration, alfalfa hay cubes, and baled hay at each milking; water was provided *ad libitum*. Information pertaining to daily average milk production and average body weights by group as well as general health of the test animals was included in the submission.

The morning and evening milk collections were weighed and a composite sample was prepared proportional to the amount of milk produced at the morning and evening milkings. In addition, on days 14 and 28 of the study, the composited milk collections were separated into cream and skim milk. Milk samples were then stored frozen (-20 C) until shipment on dry ice to DowElanco.

All but four animals, from the 10-ppm dose group, were sacrificed after 28 days of dosing; sacrifice occurred within 24 hours of the final dose. The remaining cows received no more doses of spinosad and were sacrificed 8, 15, 29, and 57 days after the final dose. Samples of fat (peritoneal, omental, and somatic), muscle (composite of flank, loin, and leg), liver, and kidney were collected and stored frozen (-20 C) until shipment on dry ice to DowElanco.

The milk and tissue samples were analyzed for residues of spinosyns A, D, and B and N-demethyl spinosyn D using HPLC method GRM 95.03. In addition, samples were analyzed by immunoassay method GRM 95.14 to determine total spinosad-related residues. The results of the feeding study are presented in Tables 23 (HPLC analyses) and 24 (immunoassay analyses). Apparent residues of spinosad and its metabolites, as determined by HPLC, were nondetectable (<0.003 ppm each) in 24 samples of milk, six samples each of cream and skim milk, and three samples each of fat, kidney, liver, and muscle, except that detectable residues of spinosyn B were observed in one sample of milk (0.005 ppm, day 10). Apparent residues of spinosad and its related metabolites, as determined by immunoassay, were nondetectable (<0.003 ppm) in 39 samples of milk and three samples each of kidney, liver, and muscle.

Spinosyn residues appeared to reach a plateau in milk between the 7th and 10th days of dosing for all dose groups. In the 10-ppm dose group, residues increased on the 14th day of dosing and then returned to the plateau level in subsequent sampling intervals. The petitioner noted that in tissues, residues determined by HPLC were lower than those determined by immunoassay, indicating that immunoassay detected additional spinosad-related metabolites other than those determined by HPLC. The HPLC chromatograms revealed additional minor peaks, which the petitioner stated could be due to spinosyn-related compounds known to be present in the test substance or spinosyn-related metabolites.

Table 23. Residues of spinosyns A, D, and B and N-demethyl spinosyn D, as determined by HPLC method GRM 95.03, in dairy cattle matrices following oral administration of the test substance at 1 ppm, 3 ppm, and 10 ppm for 28 consecutive days.

Dose Level (ppm)	Dosing or Sampling Day [a]	Number of Samples	Residues (ppm) [b]				
			Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D	Total
Milk							
1	1	3	<0.003	<0.003	<0.003	<0.003	<0.012
	3	3	0.036-0.038	<0.003	<0.003	<0.003	<0.045-<0.047
	5	3	0.033-0.049	<0.003	<0.003	<0.003	<0.042-<0.058
	7	3	0.032-0.052	<0.003-(0.004)	<0.003	<0.003	<0.041-<0.062
	10	3	0.041-0.053	(0.004)	<0.003-(0.004)	<0.003	<0.051-<0.063
	14	3	0.041-0.052	(0.003)-(0.004)	<0.003	<0.003	<0.050-<0.062
	21	3	0.041-0.048	<0.003-(0.004)	<0.003	<0.003	<0.050-<0.057
	28	3	0.038-0.053	<0.003	<0.003	<0.003	<0.047-<0.062
3	1	3	0.006-0.010	<0.003	<0.003	<0.003	<0.015-<0.019
	3	3	0.072-0.112	(0.004)-(0.007)	<0.003-(0.003)	<0.003	<0.082-<0.125
	5	3	0.114-0.128	<0.003-(0.009)	(0.004)	<0.003	<0.124-<0.144
	7	3	0.129-0.147	(0.007)-0.010	(0.004)-(0.005)	<0.003	<0.144-<0.164
	10	3	0.097-0.146	(0.006)-0.010	(0.004)-(0.006)	<0.003	<0.110-<0.165
	14	3	0.103-0.149	(0.005)-(0.009)	(0.003)-(0.005)	<0.003	<0.114-<0.166
	21	3	0.131-0.159	(0.007)-0.011	(0.005)-(0.007)	<0.003	<0.148-<0.179
	28	3	0.119-0.177	(0.006)-0.012	<0.003-(0.005)	<0.003-(0.003)	<0.131-0.197
10	1	7	0.011-0.037	<0.003	<0.003	<0.003	<0.020-<0.046
	3	7	0.248-0.455	0.014-0.028	(0.009)-0.014	<0.003	<0.274-<0.497
	5	7	0.325-0.549	0.019-0.031	0.011-0.014	<0.003	<0.359-<0.594
	7	7	0.357-0.629	0.019-0.034	(0.009)-0.014	<0.003	<0.392-<0.678
	10	7	0.296-0.644	0.016-0.040	0.011-0.017	<0.003	<0.326-<0.704
	14	7	0.359-1.349	0.016-0.080	0.012-0.035	<0.003	<0.391-1.464
	21	7	0.327-0.715	0.014-0.039	0.013-0.026	<0.003-(0.005)	<0.357-0.785
	28	7	0.372-0.693	0.016-0.038	(0.009)-0.017	(0.005)-(0.008)	0.402-0.756

(continued; footnotes follow)

Table 23 *(continued)*.

Dose Level (ppm)	Dosing or Sampling Day [a]	Number of Samples	Residues (ppm) [b]				
			Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D	Total
Cream							
1	14	3	0.110-0.237	(0.007)-0.012	<0.003-(0.004)	<0.003	<0.123-<0.255
	28	3	0.153-0.222	(0.007)-0.011	(0.003)	<0.003	<0.166-<0.239
3	14	3	0.485-0.557	0.023-0.033	(0.009)-0.011	<0.003	<0.520-<0.604
	28	3	0.484-0.708	0.026-0.047	(0.009)-0.019	<0.003	<0.522-<0.777
10	14	7	1.583-3.131	0.074-0.165	0.035-0.133	<0.003	1.700-3.429
	28	7	1.095-3.012	0.047-0.159	0.016-0.049	<0.003	1.158-3.220
Skim Milk							
1	14	3	(0.005)-(0.009)	<0.003	<0.003	<0.003	<0.014-<0.018
	28	3	(0.004)-(0.006)	<0.003	<0.003	<0.003	<0.013-<0.015
3	14	3	0.011-0.013	<0.003	<0.003	<0.003	<0.020-<0.022
	28	3	0.010-0.023	<0.003	<0.003-(0.003)	<0.003	<0.019-<0.032
10	14	7	0.024-0.077	<0.003-(0.004)	(0.003)-(0.007)	<0.003-(0.003)	<0.033-0.091
	28	7	0.059-0.123	<0.003-(0.005)	(0.004)-0.010	<0.003	<0.069-<0.140
Fat							
1	28	3	0.506-0.530	0.022-0.030	0.011-0.014	(0.003)-(0.005)	0.542-0.578
3	28	3	0.616-1.380	0.032-0.067	0.014-0.036	(0.006)-0.014	0.678-1.497
10	28	3	2.954-6.172	0.126-0.255	0.142-0.312	(0.003)-(0.005)	3.225-6.744
	36	1	3.020	0.142	0.153	(0.005)	3.320
	43	1	0.255	(0.008)	0.012	<0.003	<0.278
	57	1	0.023	<0.003	<0.003	<0.003	<0.032
	85	1	0.159	<0.003	(0.003)	<0.003	<0.168

(continued; footnotes follow)

Table 23 *(continued)*.

Dose Level (ppm)	Dosing or Sampling Day [a]	Number of Samples	Residues (ppm) [b]				
			Spinosyn A	Spinosyn D	Spinosyn B	N-Demethyl Spinosyn D	Total
Kidney							
1	28	3	0.032-0.057	<0.003	(0.009)-0.012	<0.003	<0.047-<0.075
3	28	3	0.143-0.170	(0.006)-(0.008)	0.037-0.065	(0.003)-(0.005)	0.194-0.222
10	28	3	0.355-0.502	0.013-0.019	0.142-0.187	(0.009)-0.011	0.526-0.715
	36	1	0.175	(0.008)	0.037	(0.003)	0.223
	43	1	0.027	<0.003	(0.007)	<0.003	<0.040
	57	1	(0.004)	<0.003	<0.003	<0.003	<0.013
	85	1	0.023	<0.003	(0.004)	<0.003	<0.033
Liver							
1	28	3	0.068-0.108	(0.004)-(0.007)	0.014-0.026	(0.004)-(0.006)	0.090-0.144
3	28	3	0.216-0.300	0.010-0.020	0.047-0.090	(0.007)-0.016	0.280-0.426
10	28	3	0.708-1.198	0.024-0.033	0.165-0.364	0.022-0.033	0.950-1.628
	36	1	0.252	0.012	0.066	(0.008)	0.338
	43	1	0.034	<0.003	(0.009)	<0.003	<0.049
	57	1	(0.003)	<0.003	<0.003	<0.003	<0.012
	85	1	(0.009)	<0.003	(0.004)	<0.003	<0.019
Muscle							
1	28	3	0.010-0.020	<0.003	(0.003)-(0.006)	<0.003	<0.019-<0.032
3	28	3	0.020-0.055	<0.003	(0.008)-0.013	<0.003	<0.034-<0.074
10	28	3	0.108-0.233	(0.006)-(0.009)	0.017-0.049	<0.003	<0.134-<0.293
	36	1	0.178	0.010	0.023	(0.003)	0.214
	43	1	0.014	<0.003	(0.005)	<0.003	<0.025
	57	1	<0.003	<0.003	<0.003	<0.003	<0.012
	85	1	0.016	<0.003	<0.003	<0.003	<0.025

[a] Dosing ceased on Day 28.

[b] Residues of spinosad were not corrected for procedural recoveries. Residue values in parentheses are greater than the LOD (0.003 ppm) but less than the LOQ (0.01 ppm). Individual residues levels < LOD were not included in the total if residues of spinosyn A were greater than 1 ppm.

Table 24. Residues of spinosad, as determined using immunoassay method GRM 95.14, in dairy cattle matrices following oral administration of the test substance at 1 ppm, 3 ppm, and 10 ppm for 28 consecutive days.

Commodity	Dosing or Sampling Day	Residues (ppm) [a]		
		1 ppm	3 ppm	10 ppm
Milk	1	<0.003 [3]	(0.005)-0.012 [3]	0.011-0.038 [7]
	2	0.018-0.025 [3]	0.051-0.074 [3]	0.168-0.298 [7]
	3	0.032-0.037 [3]	0.085-0.127 [3]	0.303-0.444 [7]
	4	0.030-0.052 [3]	0.094-0.160 [3]	0.323-0.555 [7]
	5	0.026-0.040 [3]	0.098-0.117 [3]	0.338-0.489 [7]
	6	0.029-0.045 [3]	0.087-0.164 [3]	0.379-0.557 [7]
	7	0.035-0.042 [3]	0.086-0.138 [3]	0.364-0.617 [7]
	10	0.034-0.053 [3]	0.085-0.153 [3]	0.305-0.795 [7]
	12	0.046-0.070 [3]	0.125-0.204 [3]	0.414-0.664 [7]
	14	0.057-0.067 [3]	0.124-0.202 [3]	0.481-1.085 [7]
	16	0.056-0.090 [3]	0.130-0.205 [3]	0.326-0.904 [7]
	21	0.040-0.043 [3]	0.257-0.305 [3]	0.250-0.734 [7]
	28	0.047-0.053 [3]	0.231-0.377 [3]	0.298-0.679 [7]
	29	--	--	0.324-0.828 [4]
	30	--	--	0.145-0.569 [4]
	31	--	--	0.118-0.359 [4]
	32	--	--	0.062-0.294 [4]
	33	--	--	0.054-0.279 [4]
	34	--	--	0.033-0.230 [4]
	35	--	--	0.040-0.229 [4]
	36	--	--	0.030-0.165 [3]
	37	--	--	0.026-0.162 [3]
	38	--	--	0.014-0.130 [3]
	39	--	--	0.017-0.107 [3]
	40	--	--	0.016-0.102 [3]
	41	--	--	0.011-0.077 [3]
	42	--	--	0.011-0.081 [3]
	49	--	--	(0.004), 0.047
	56	--	--	<0.003, 0.033
	70	--	--	0.017
	84	--	--	(0.005)
Kidney	29	0.052-0.067 [3]	0.236-0.299 [3]	0.596-0.828 [3]
	36	--	--	0.297
	43	--	--	0.059
	57	--	--	(0.005)
	84	--	--	0.035
Liver	29	0.116-0.181 [3]	0.421-0.644 [3]	1.547-2.542 [3]

(continued; footnotes follow)

Table 24 *(continued)*.

Commodity	Dosing or Sampling Day	Residues (ppm) *		
		1 ppm	3 ppm	10 ppm
	36	--	--	0.662
	43	--	--	0.078
	57	--	--	0.011
	84	--	--	0.018
Muscle	29	0.016-0.030 [3]	0.036-0.078 [3]	0.137-0.329 [3]
	36	--	--	0.246
	43	--	--	0.027
	57	--	--	<0.003
	84	--	--	0.021

* Residues are uncorrected for concurrent method recovery. Residue values in parentheses are greater than the LOD (0.003 ppm) but less than the LOQ (0.01 ppm). Each residue value represents one sample unless otherwise indicated in brackets.

Conclusions: The submitted dairy cattle feeding data are adequate. They indicate that tolerances for residues of spinosad are required for milk and the fat, meat, and meat byproducts of cattle, goat, hogs, horses, and sheep. Detectable residues of spinosad were observed in the milk, fat, kidney, and liver of cattle fed spinosad at ~2x the maximum theoretical dietary burden (the lowest dosing level) for 28 days.

The available data do not support all the proposed tolerances for animal tissues (0.05 ppm for meat, 0.2 ppm for meat byproducts, and 1.0 ppm for fat), milk (0.02 ppm) and milk fat (0.5 ppm). While the meat byproduct and milk fat tolerances are appropriate, the meat and milk tolerances should be revised to 0.04 ppm (i.e., the sum of the LOQs for the four moieties. Milk residues from the maximum expected dietary burden are estimated to be as high as 0.03 ppm by extrapolation of the feeding study results. The fat tissue tolerance should be lowered to 0.4 ppm. The petitioner must submit a revised Section F proposing these tolerances. In addition, the petitioner should modify the proposed tolerance levels for horse fat, meat, and meat byproducts to coincide with the tolerance levels for cattle, goat, hogs, and sheep.

Eggs, and the Fat, Meat, and Meat Byproducts of Poultry

There are no poultry feed items associated with this petition. Therefore, data pertaining to the magnitude of spinosyn residues in poultry commodities are not required.

OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops

No confined or field rotational crop studies were submitted with this petition. A rotational crop study with wheat, radish, and lettuce was submitted and reviewed in conjunction with PP#6G04692. The results of the confined rotational crop study indicate that the spinosad molecule was metabolized to the point where it entered the general carbon pool. It did not appear that the parent compound was taken up and/or translocated within the rotational crops tested. Extensive/limited rotational crop field studies need not be conducted and tolerances for rotational crops need not be established to support this permanent tolerance request.

Other Considerations

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

List of Attachments

I. International Residue Limit Status Sheet

AGENCY MEMORANDA CITED IN THIS DOCUMENT

CBTS No.:	None
DP Barcode:	D219016, D224608, D223898, D223899
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.
From:	G. J. Herndon
To:	G. LaRocca/A. Heyward
Dated:	4/24/96
MRID(s):	43450301 through 43450306, and 43727401 through 43727411

CBTS No.:	None
DP Barcode:	D226824 and D227014
Subject:	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.
From:	G. J. Herndon
To:	G. LaRocca/A. Heyward
Dated:	6/17/96
MRID(s):	None

CBTS No.:	None
DP Barcode:	D228791
Subject:	PP#6G04692 and PP#F04735. Spinosad (XDE-105) on Cotton Commodities. Results of the Petition Method Validation Request.
From:	G. J. Herndon
To:	A. Heyward/G. LaRocca
Dated:	8/13/96
MRID(s):	None

Attachment I

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL: Spinosad

CODEX NO. _____

CODEX STATUS:

✓ No Codex Proposal Step 6 or above

Residue (if Step 8): _____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. PP#6F04761/FAP#6H05754

CB Reviewer _____

Residue: Combined residues of spinosyns A and D for plant commodities; combined residues of spinosyns A, D, B, and N-demethyl spinosyn D for animal commodities

Crop(s)	Limit (mg/kg)
Apples	0.20 ppm
Brassica (cole) leafy vegetables, head and stem subgroup	2.0 ppm
Brassica (cole) leafy vegetables, greens subgroup	15.0 ppm
Apple pomace, wet	0.5 ppm
Fat of cattle, goats, hogs, and sheep	1.0 ppm
Meat of cattle, goats, hogs, and sheep	0.05 ppm
Meat byproducts of cattle, goats, hogs, and sheep	0.2 ppm
Fat, meat, and meat byproducts of horses	1.0 ppm
Milk, fat	0.5 ppm
Milk, whole	0.02 ppm

CANADIAN LIMITS:

✓ No Canadian limit

Residue: _____

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

✓ No Mexican limit

Residue: _____

Crop(s)	Limit (mg/kg)

NOTES: This form was filled in based on a 11/26/96 TELCON between D. Martinez and F. Ives.