Primary Evaluator W. Cutchin, Chemist Date: 10/13/04
Technical Review Branch
Registration Division (7505C)

Approved by J. R. Tomerlin, Plant Pathologist
Fungicide Branch
Registration Division (7505C)

STUDY REPORTS:

MRID No. 46345401 A. Phillips (1/30/02) Residues of Spinosad in Wheat Processed Fractions: Lab Study ID: 000138. Unpublished study prepared by Regulatory Laboratories - Indianapolis Lab, Dow AgroSciences, LLC. 86 pages.

EXECUTIVE SUMMARY:

Dow AgroSciences LLC has submitted processing study data for spinosad on wheat grain. Spinosad was used in 4 trials on wheat in TX (2), IN, and MS. At each test location, spinosad was applied three times by foliar broadcast, at tilling, bootstage, and 16-21 days PHI. The spinosad formulation NAF-85 was applied by foliar broadcast to the grain at a treatment level ranging from 1.353-2.872 lg a.i./A/season. An adjuvant was not added to the spray mixture for all applications. Wheat grain was processed using simulated commercial practices.

Dow AgroSciences draft method from Dow databook E0724 and method GRM 00.04 were used for data collection. Samples were extracted by acetonitrile:water, purified using a solid phase extraction column, eluted, evaporated to dryness and reconstituted. Analysis was conducted using HPLC/MS. The limit of detection (LOD) and limit of quantitation (LOQ) are reported as 0.003 and 0.01 ppm, respectively. Concurrent recoveries of Spinosad A and D from grain samples fortified at 0.01-5 ug/g ranged from 79-108% (avg. 94.4% ± 6.12%). The methods are adequate for data collection purposes. Spinosad had been showed to be stable in frozen matrices up to 930 days which is adequate for this study.

The results from these trials show that for wheat processed commodities only wheat bran (2.25x) and wheat AGF (171.96x) showed concentration factors of regulatory interest.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processed commodity residue data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D304201.

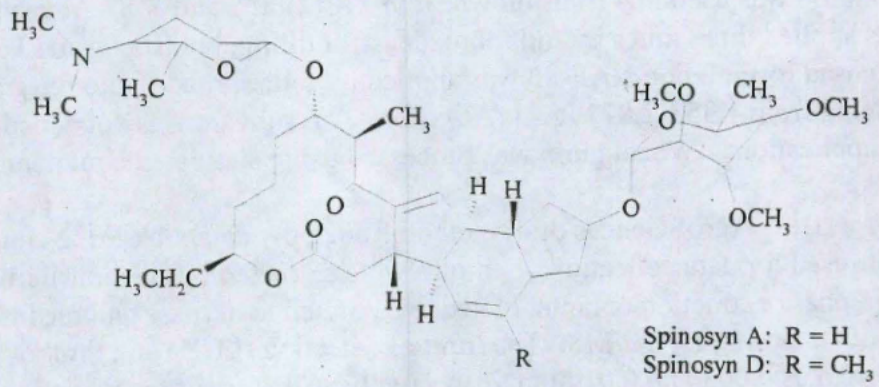
COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. Some climatological, maintenance, sample weight, and crop data were not conducted in accordance to GPL standards and some entries in a laboratory databook were not signed and dated at the time of entry. These deviations from regulatory requirements did not impact the validity of the study.

A. BACKGROUND INFORMATION

Spinosad is a fermentation product of *Saccharopolyspora spinosa* typically at an 85:15 ratio of Spinosyn A and Spinosyn D. Spinosad affects nicotinic acetylcholine receptors characterized by excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and paralysis. Spinosad is intended for use to control lepidopterous and other insect pests.

TABLE A.1. Test Compound Nomenclature

Compound	Chemical Structure
	
Common name	Spinosad
Company experimental name	XDE-105
IUPAC name	**Spinosyn A**:(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-αa-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-ββ-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione **Spinosyn D:** (2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-αa-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-ββ-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione

IUPAC name	**Spinosyn A**:(2*R*,3a*S*,5a*R*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-αa-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-ββ-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione **Spinosyn D:** (2*S*,3a*R*,5a*S*,5b*S*,9*S*,13*S*,14*R*,16a*S*,16b*R*)-2-(6-deoxy-2,3,4-tri-*O*-methyl-αa-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-ββ-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1*H*-8-oxacyclododeca[*b*]*as*-indacene-7,15-dione
CAS name	**Spinosyn 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 **Spinosyn 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
CAS #	**Spinosyn A**: 131929-60-7 **Spinosyn D:** 131929-63-0
End-use product/(EP)	NAF-313

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value			Reference
Melting point/range	Spinosad A: 84-99.5°C Spinosad D: 161.5-170°C			EPA Pesticide Fact Sheet
pH	7.74			
Density	0.512 @ 20°C			
Water solubility (20°C)	Spinosad A: 89.4 ppm Spinosad D: 0.469 ppm			
Solvent solubility (mg/L)		Spinosad A g/mL	Spinosad D g/mL	
	acetone	16.8	1.01	
	ACN	13.4	0.255	
	DCM	52.5	44.8	
	hexane	0.448	743 ppm	
	methanol	19.0	0.252	
	1-octanol	0.926	0.127	
	toluene	45.7	15.2	
Vapour pressure at 25°C	Spinosad A: 3.0 x 10E-11 Spinosad D: 2.0 x 10E-11			
Dissociation constant (pK_a)	N/A			
Octanol/water partition coefficient Log(K_{ow})		Spinosad A	Spinosad D	
	pH 5	2.8	3.2	
	pH 7	4.6	4.5	
	pH 9	5.2	5.2	
UV/visible absorption spectrum	N/A			

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.1. Study Use Pattern.

Location (City, State/Year)	EP [1]	Application			
		Grain	Method/ Timing	Vol. mL	Rate ug/g
KSU, Manhattan, KS 2002	NAF-315 SC	wheat	metered spray to grain stream entering auger	not indicated	1.16
Purdue, West Layfayette, IN 2002	NAF-315 SC	corn	metered spray to grain stream entering auger	not indicated	1.54

[1]EP = End-use Product

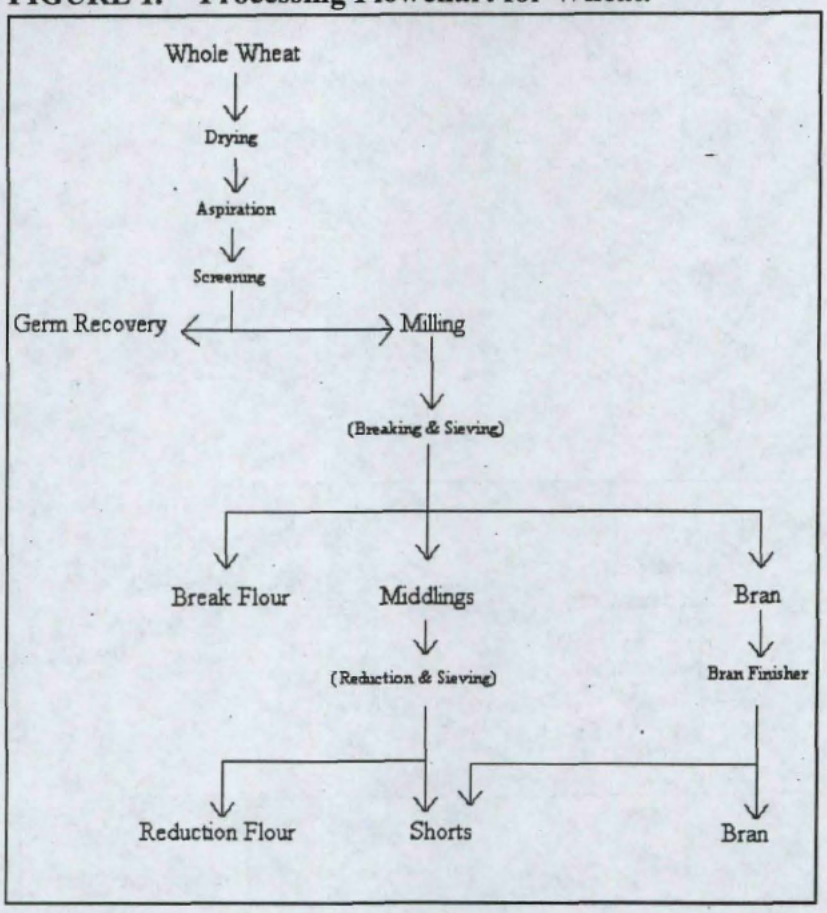
B.2. Sample Handling and Processing Procedures

After the treatment and ambient storage interval samples were shipped frozen to the analytical laboratory. Samples were prepared for analysis by freezing with liquid nitrogen and then ground, mixed, and placed into plastic storage containers until extraction.

Wheat Processing

After determination of grain moisture content, sample is placed in a dust generation room which generates movement in the sample while aspiration is applied. The cleaned wheat is moisture adjusted and the germ is separated out using an impact rotor and sifter. The wheat is run through break rolls, producing break flour, middlings, and coarse bran. The middlings are fed into a reduction roller. The products are sieved to produce reduction flour and shorts. The break flour and reduction flour are mixed with agitation to produce standard mill run flour. The coarse bran is finished by being fed into a beating machine followed by screening. Material passing through the screen is added to shorts while the retained material is bran. The mill flour is treated with water and the resulting slurry separated into gluten and starch. The system simulates commercial practices.

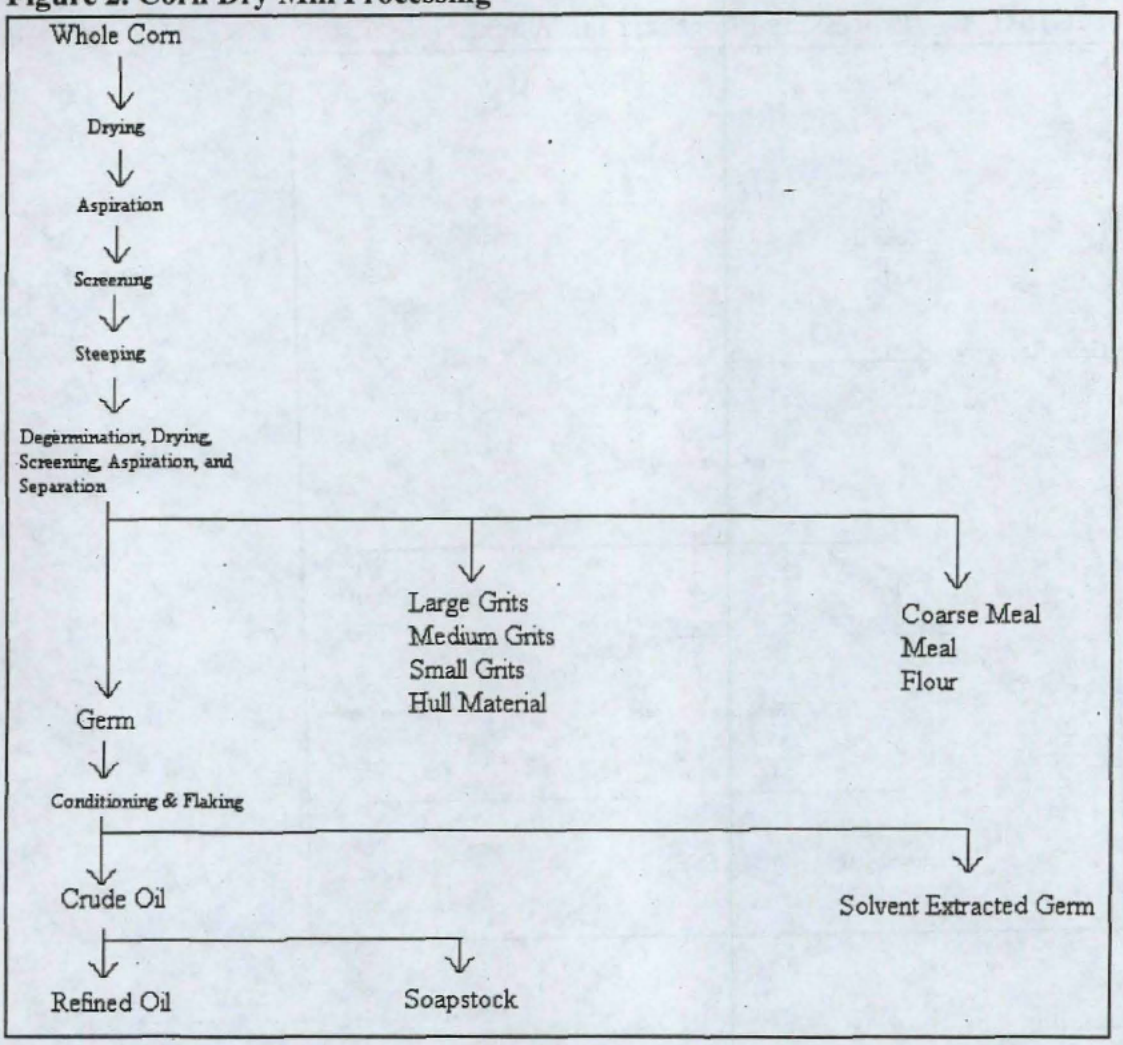
FIGURE 1. Processing Flowchart for Wheat.



Corn Processing

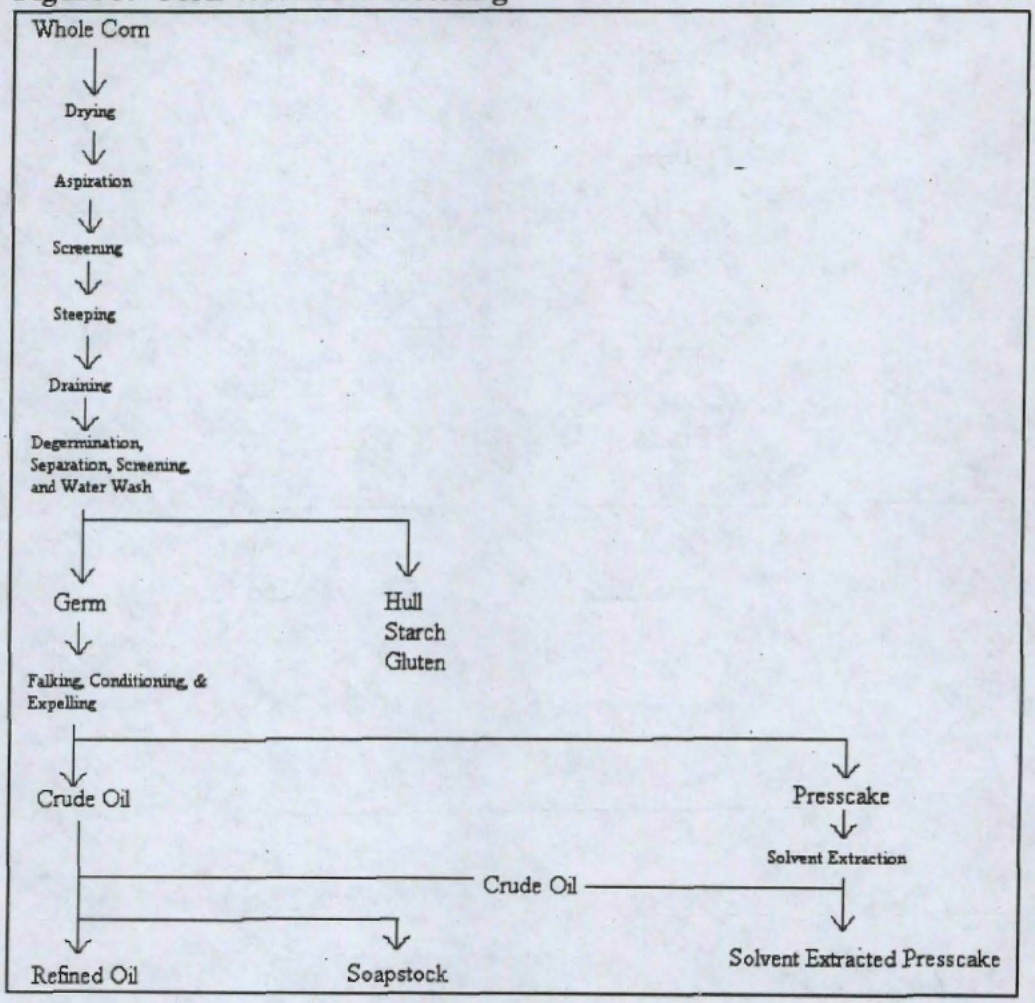
Dry Mill: After determination of grain moisture content, sample is placed in a dust generation room which generates movement in the sample while aspiration is applied. The corn is moisture tempered and treated in a mill impacter. The treated corn is passed over a shaker screen. The material above the screen is further processed into large grits, germ, and hulls. The material through the screen is separated into medium and small grits, course meal, meal, and flour. The germ is flaked and the crude oil and presscake is produced through solvent extraction. The crude oil is further processed into refined oil and soapstock. The system simulates commercial practices.

Figure 2. Corn Dry Mill Processing



Wet Mill: After determination of grain moisture content, sample is placed in a dust generation room which generates movement in the sample while aspiration is applied. The corn grain is steeped in weak sulfurous acid then milled. The milling process produces germ, hulls, and cornstock. The germ is flaked and pressed producing crude oil and presscake with residual crude oil. The oily presscake is extracted with solvent to produce presscake and crude oil. The two crude oil fractions are combined and is further processed into refined oil and soapstock. The cornstock is processed into bran, starch, and gluten. The system simulates commercial practices.

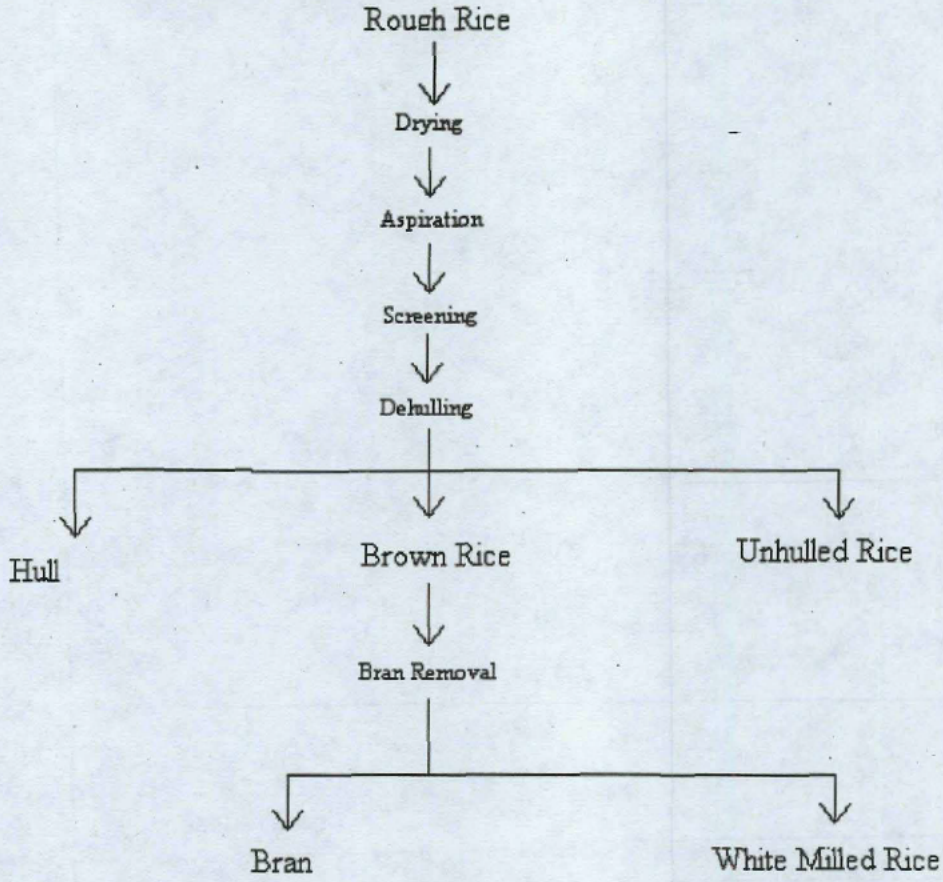
Figure 3. Corn Wet Mill Processing



Rice Processing

After drying, the rough rice is aspirated. The rice is screened and dehulled to remove the hulls from the brown rice. The brown rice is processed in an abrasion mill and screened. The material on top of the screen is white milled rice while that passing through is bran. The system simulates commercial practices.

Figure 4. Rice Processing



B.3. Analytical Methodology

Analysis of the grain samples was conducted using the method designated GRM 00.04. Samples were extracted with acetonitrile:water (80:20). The resulting extract was diluted with acetonitrile and purified using a strong cationic exchange solid phase extraction column. The analytes were eluted with acetonitrile:methanol (1:1) with 0.1 M ammonium acetate. The eluate was evaporated to dryness and reconstituted in acetonitrile:methanol:water (4:4:2). Analysis was conducted by HPLC/MS using a YMC ODS AM column with a mass selective detector operating in the positive ion atmospheric pressure chemical ionization mode (APCI). The LOD and LOQ are reported as 0.002 and 0.01 ppm, respectively.

C. RESULTS AND DISCUSSION

No storage stability data were submitted with this study. Samples in this study were stored frozen for up to 422 days prior to analysis after the ambient exposure interval. Storage stability data have been previously submitted for the following crops showing adequate frozen storage stability for the durations noted: almonds and apples, 6 months; celery and spinach, 7 months; cabbage, 12 months; cottonseed, 9.5 months; and tomatoes, 11 months (DP Barcodes: D237013, D242939, D242941, D243796, G. Herndon, 4/21/99). In addition, radiolabeled spinosad residues were found to be stable in cabbage leaves for up to 930 days (PP# 6F4761/6H5754, DP Barcode: D228434 & D228510, CB Nos. 17404 & 17670, S. Willett, 1/23/97). Existing storage stability data is adequate to support the frozen storage interval of the samples in this study.

Dow AgroSciences method GRM 00.04 (HPLC/MS) was used for data collection. The LOD and LOQ are reported as 0.002 and 0.01 ppm, respectively. Concurrent recoveries of Spinosad A and D from grain matrices fortified at 0.01-200ug/g ranged from 65-124% (avg. 99.0% ± 9.96%). The registrant suppled adequate sample chromatograms that indicate the control samples of various crop matrices are free from interferences. The method is adequate for data collection purposes. The registrant indicated that the processing procedures for wheat, corn, and rice simulated commercial practices.

The results from these trials show that for wheat, corn, and rice processed commodities generated from grains treated at 1-1.54 ug ai/g grain (mg/kg 1 ug ai/g) and stored at ambient temperatures for up to 6 months, only wheat gluten (1.29x), corn wet mill refined oil (1.20x), rice hulls (4.45x) and bran (1.31x), and wheat (317.03x) and corn (125.52x) aspirated grain fraction (AGF) showed concentration factors of regulatory interest. These concentration factors were generally at or below the theoretical concentration factors.

TABLE C.1. Summary of Concurrent Recoveries of Spinosad A and D from Grain Matrices.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)		Mean ± std dev	
			A	D	A	D
Wheat						
wheat grain	0.01	11	90,101,102,105, 99,95,77,79, 102,104,103	93,93,100,99, 101,96,79,82, 112,113,93	96.1±9.5	96.5±10.0
	0.1	3	89,83,98	92,83,89	90.0±6.2	88.0±3.7
	1.5	2	103,105	105,106	104.0±1.0	105.5±0.5
wheat gluten	0.01	1	108	103	108±0	103±0
wheat bran	0.01	1	98	93	98±0	93±0
	0.1	1	99	93	99±0	93±0
	200	1	90	91	90±0	91±0
wheat flour	0.01	1	100	90	100±0	90±0
	0.1	1	98	92	98±0	92±0

TABLE C.1. Summary of Concurrent Recoveries of Spinosad A and D from Grain Matrices.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)		Mean ± std dev	
			A	D	A	D
wheat germ	0.01	1	106	101	106±0	101±0
	0.1	1	102	99	102±0	99±0
wheat middlings	0.01	1	98	91	98±0	91±0
	0.1	1	96	89	96±0	89±0
wheat shorts	0.01	1	98	93	98±0	93±0
	0.1	1	98	91	98±0	91±0
wheat AGP	200	2	83,96	86,98	89.5±6.5	92.0±6.0
Barley						
barley grain	0.01	8	106,108,101,99, 94,97,98,94,	109,109,89,92, 93,93,91,90	99.6±4.8	95.8±7.8
	1.5	2	90,91	90,90	90.5±0.5	90±0
Corn						
corn grain	0.01	10	92,95,97,102, 107,122,124, 105,110,97	99,97,99,104, 100,119,117, 107,108,93	105.1±10.4	104.3±8.1
	0.1	2	99,101	100,102	100.0±1.0	101.0±1.0
	1.5	4	97,94,114,115	97,94,110,112	105.0±9.6	103.3±7.9
	2.0	2	90,88	89,85	89.0±1.0	87.0±2.0
corn flour	0.01	2	111,110	111,108	110.5±0.5	109.5±1.5
	0.1	2	110,107	108,106	108.5±1.5	107.0±1.0
corn grits	0.01	1	109	106	109±0	106±0
	0.1	1	110	107	110±0	107±0
corn meal	0.01	1	113	114	113±0	114±0
	0.1	1	111	109	111±0	109±0
	200	1	94	97	94±0	97±0
corn starch	0.01	2	92,70	88,65	81.0±11.0	76.5±11.5
	0.1	1	70	65	70±0	65±0
Oats						
oat grain	0.01	2	101,94	80,74	97.5±3.5	77.0±3.0
	1.5	2	89,90	82,86	89.5±0.5	84.0±2.0
Rice						
rice grain	0.01	8	113,111,93,94, 109,108,106, 103	113,113,97,98, 109,108,104,102	104.6±7.0	105.5±5.9
	0.1	4	97,94,106,102	97,94,100,98	99.8±4.6	97.3±2.2
	1.5	1	102	102	102±0	102±0
rice hulls	0.01	2	106,106	106,106	106.0±0	106.0±0
	0.1	2	110,112	103,108	111.0±1.0	105.5±2.5

TABLE C.1. Summary of Concurrent Recoveries of Spinosad A and D from Grain Matrices.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)		Mean ± std dev	
			A	D	A	D
	10	1	98	97	98±0	97±0
rice polished grain	0.01	2	115,105	115,104	110.0±5.0	109.5±5.5
	0.1	2	106,102	102,102	104.0±2.0	102.0±0
rice bran	10	1	93	96	93±0	96±0
				Avg	100.0±9.6	98.0±10.4

TABLE C.2. Summary of Storage Conditions.

Matrix (RAC)	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
wheat	-20	442	930
corn			
rice			

TABLE C.3. Residue Data from Grain Storage after Treatment with Spinosad.

Trial ID (City, State/Year)	Total Rate, (ug/g)	Crop	Commodity or Matrix	Storage Interval (mo)	Spinosad A (ppm)	Spinosad D (ppm)	Total (ppm)	Proc. Factor	Avg PF
KSU, Manhattan, KS 2002	1.16	wheat	grain	6	0.785 0.830 0.845	0.124 0.133 0.138	0.909 0.963 0.983	Avg: 0.952 ppm	NA
			bran	6	0.813,0.783	0.127,0.122	0.923	0.97	0.97
			middlings	6	0.245	0.040	0.285	0.30	0.30
			shorts	6	0.908	0.146	1.054	1.11	1.11
			flour	6	0.280	0.048	0.328	0.34	0.34
			gluten	6	1.045	0.187	1.232	1.29	1.29
			germ	6	0.586	0.098	0.684	0.72	0.72
			starch	6	0.006	<LOD	0.007	0.01	0.01
			AGF	6	258.046	43.768	301.814	317.03	317.03
Purdue, West Layfayette, IN 2002	1.54	corn	grain	0	ND(3),1.128 0.961 1.279,1.612	ND(3),0.170 0.142 0.214,0.235	0.434 1.103 1.670	Avg: 1.069ppm	NA
				6	0.707 0.580 0.708	0.115 0.093 0.113	0.822 0.673 0.821	Avg: 0.722ppm	NA
			grits	0	0.079	0.013	0.092	0.09	0.09
				6	0.064	0.010	0.074	0.09	
			meal	0	0.210,0.209	0.034,0.034	0.397	0.37	0.26
				6	0.097	0.017	0.114	0.15	
			flour	0	0.264	0.050	0.314	0.29	0.22

TABLE C.3. Residue Data from Grain Storage after Treatment with Spinosad.

Trial ID (City, State/Year)	Total Rate, (ug/g)	Crop	Commodity or Matrix	Storage Interval (mo)	Spinosad A (ppm)	Spinosad D (ppm)	Total (ppm)	Proc. Factor	Avg PF
				6	0.092	0.017	0.109	0.14	
			dry mill refined oil	0	0.318	0.055	0.373	0.35	0.32
				6	0.187	0.037	0.224	0.29	
			wet mill refined oil	0	1.092	0.140	1.232	1.15	1.20
				6	0.858,0.847	0.107,0.106	0.959	1.24	
			starch	0	0.002	ND	0.003	<0.01	<0.01
				6	0.001	ND	0.002	<0.01	
			AGF	0	134.987	22.673	157.660	147.48	125.52
				6	68.432	11.520	79.952	103.56	
DAS, Fresno, CA 2003	1	rice	grain	0	ND 0.509 0.352 0.388,0.385	ND 0.082 0.059 0.065,0.064	0.001 0.591 0.411 0.451	Avg: 0.364ppm	NA
				3	0.562 0.650 0.531	0.091 0.102 0.082	0.653 0.752 0.613	Avg: 0.673ppm	NA
			hulls	0	1.797	0.305	2.102	5.78	4.45
				3	1.790	0.313	2.103	3.13	
			brown	0	0.095	0.015	0.110	0.30	0.19
				3	0.047	0.007	0.054	0.08	
			milled	0	0.022	0.004	0.026	0.07	0.04
				3	0.006	0.001	0.007	0.01	
			bran	0	0.606	0.099	0.705	1.94	1.31
				3	0.397	0.065	0.462	0.46	

D. CONCLUSION

This study is adequate to determine the processing factors for spinosad on grain processed commodities.

E. REFERENCES

F. DOCUMENT TRACKING

RDI: J. R. Tomerlin (9/30/04).
Petition Number(s): 3F6754
DP Barcode(s): D304201
PC Code: 110003

Template Version September 2003