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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

FEB 2 4 2011

MEMORANDUM

SUBJECT: *Aspergillus flavus* AF36 use on corn.

TO: Shanaz Bacchus, M.S., Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511P)

ACTION REQUESTED: Review of additional efficacy data from EUP field testing of AF36 in Texas to support a section 3 label amendment for use on field corn.

CONCLUSION: ACCEPTABLE – *Aspergillus flavus* AF36 applied to corn is demonstrated highly efficacious when used as directed and does not increase levels of cyclopiazonic acid on corn.

DATA REVIEW RECORD

Active Ingredient:	*Aspergillus flavus* AF36.
Product Name:	AF36.
Company Name:	Arizona Cotton Research and Protection Council.
EPA Reg. Nos.:	71693-EUP-2.
Chemical Number:	006456.
Decision Number:	424829.
DP Barcode:	385616.
MRID Nos.:	483301-01.

BACKGROUND:
Aspergillus flavus AF36 is currently registered for use on cotton.

***PREVIOUS* REVIEW SUMMARY:**
Study Type: Product Performance Test Guidelines (OPPTS 810.1000); General Considerations for Public Health Uses of Antimicrobial Agents (OPPTS 810.2000 - *Draft*).
MRID Nos.: 479351-01.
Test Material: *Aspergillus flavus* AF36.
Study Summary: The EUP label indicates AF36 applications should be made prior to or during silking from late May through June. An amendment to the December 17, 2007 EUP issued May 27, 2009 extends the EUP until January 4, 2011 allowing use on 3,000 acres per year in Arizona and 5,000 acres per year in Texas. Data from 2008 use in Texas is included in the current submission - an application to amend the section 3 registration to include use on corn in addition to cotton. Some testing in Grayson County was carried out independently by grain elevator operators and contains different end-points than data submitted by cooperators. Grayson County data is from V7-10 treated corn, the only data from corn treated per label instructions. Cooperator data from Grayson County contains only means without identification to other field data from a total of 300 acres, while data from grain elevators varies on what details are reported with most of that data submitted containing aflatoxin measurements from untreated fields without determination of AF36 presence on the few treated fields. Data reported from Ellis, San Patricio, Wharton, Jackson, Victoria, Hidalgo and Medina Counties in Texas all included %AF36 and ppb aflatoxin on individual samples. None of these cooperators reported total treated and untreated acreage, corn cultivars used, distances between fields, yields or harvest dates. In all Counties other than Grayson application of AF36 was made later than label instructions directed, in some cases too late to have any meaningful effect on aflatoxin levels. Cyclopiazonic acid levels and %AF36 were reported from 36 treated and 25 control samples. One fifth of AF36 treated samples had measurable cyclopiazonic acid levels compared to none detected in control samples. Units for measured cyclopiazonic acid, and the corn stage where AF36 was applied, were not provided.
Classification: SUPPLEMENTAL – data from 2009 and 2010 from both Texas and Arizona under the EUP should be submitted and include the following; corn planting dates, AF36 treatment dates, stage of corn at treatment, conditions at and shortly after treatment (i.e. irrigation or rain events), acres represented by each sample, number of samples per plot, type(s) of corn planted, distance(s) between plots, harvest dates, %AF36 with aflatoxin and cyclopiazonic acid levels for each sample. Additional discussion should include whether nearby areas to treated and untreated plots have been treated with atoxigenic *Aspergillus flavus* AF36 or *Aspergillus flavus* NRRL 21882 (AflaGuard) previously for any testing or commercial purposes.

***CURRENT* REVIEW SUMMARY:**
Study Type: Product Performance Test Guidelines (OPPTS 810.1000); General Considerations for Public Health Uses of Antimicrobial Agents (OPPTS 810.2000 - *Draft*).
MRID Nos.: 483301-01.
Test Material: *Aspergillus flavus* AF36.
Study Summary: The summary table 1 from MRID 479351-01 has the 2008 field testing data, while table 2 has the 2009 data with several additional endpoints addressed, including data on replication and distances between fields. Application levels are not listed but are assumed at the EUP label use

rate of 10 lbs./acre. Data reported from 2009 includes 7 sites with one field each in Grayson County Texas, 7 sites some with multiple fields in Ellis County Texas, 1 site with four fields in San Patricia County Texas, and 1 site each in Victoria and Hidalgo Counties in Texas. One site in Grayson County and the site in Hidalgo County had only measures of % AF36 and cyclopiazonic acid levels. Other sites all included %AF36 and ppb aflatoxin on individual samples and the majority of sites had measures of cyclopiazonic acid levels. Seven of 18 sites with control plots where aflatoxin was measured exceeded 100 ppb aflatoxin, and in several cases the level was >200 ppb up to a single site with 1,003 ppb in Ellis County. The highest aflatoxin concentration occurring from treated plots was 61 ppb and in 10 of the 18 fields tested aflatoxins were below the lowest FDA action level of 20 ppb for corn. All treated plots consistently showed a higher percentage of AF36 present on corn sampled, regardless of the proximity of treated to control plots, some of which were adjacent and others miles apart. Previous and/or nearby treated sites reported did not appear to play a significant role in efficacy of applied AF36 in 2009. Cyclopiazonic acid levels and %AF36 were reported from most sites. Regardless of treatment, cyclopiazonic acid levels ranged from 0.0-1.2 ppm with no distinguishable trend between treated and control plots (many treated and control plots both registered 0.0 ppm). Use of *Aspergillus flavus* AF36 on corn is demonstrated highly efficacious when used as directed and does not increase levels of cyclopiazonic acid on corn.

Classification: ACCEPTABLE.

*** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION ***

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.

EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Product Performance Test Guidelines (OPPTS 810.1000); General Considerations for Public Health Uses of Antimicrobial Agents (OPPTS 810.2000 - *Draft*).
MRID No.	483301-01.
Test Material	*Aspergillus flavus* AF36
Study Nos.	PR # 0378 B.
Sponsor	Arizona Cotton Research and Protection Council; 3721 E. Wier Avenue; Phoenix, Arizona 85040.
Testing Facility	Arizona Cotton Research and Protection Council; 3721 E. Wier Avenue; Phoenix, Arizona 85040.
Titles of Reports	Product Performance Data: Efficacy of AF36 in Corn 2009 Commercial Field Tests. Amendment #1 to MRID 47935101.
Authors	Peter J. Cotty, Phillip J. Wakelyn, Michael Braverman.
Studies Completed	December 13, 2010.
Study Summary	The summary table 1 from MRID 479351-01 has the 2008 field testing data, while table 2 has the 2009 data with several additional endpoints addressed, including data on replication and distances between fields. Application levels are not listed but are assumed at the EUP label use rate of 10 lbs./acre. Data reported from 2009 includes 7 sites with one field each in Grayson County Texas, 7 sites some with multiple fields in Ellis County Texas, 1 site with four fields in San Patricia County Texas, and 1 site each in Victoria and Hidalgo Counties in Texas. One site in Grayson County and the site in Hidalgo County had only measures of % AF36 and cyclopiazonic acid levels. Other sites all included %AF36 and ppb aflatoxin on individual samples and the majority of sites had measures of cyclopiazonic acid levels. Seven of 18 sites with control plots where aflatoxin was measured exceeded 100 ppb aflatoxin, and in several cases the level was >200 ppb up to a single site with 1,003 ppb in Ellis County. The highest aflatoxin concentration occurring from treated plots was 61 ppb and in 10 of the 18 fields tested aflatoxins were below the lowest FDA action level of 20 ppb for corn. All treated plots consistently showed a higher percentage of AF36 present on corn sampled, regardless of the proximity of treated to control plots, some of which were adjacent and others miles apart. Previous and/or nearby treated sites reported did not appear to play a significant role in efficacy of applied AF36 in 2009. Cyclopiazonic acid levels and %AF36 were reported from most sites. Regardless of treatment, cyclopiazonic acid levels ranged from 0.0-1.2 ppm with no distinguishable trend between treated and control plots (many treated and control plots both registered 0.0 ppm). Use of *Aspergillus flavus* AF36 on corn is demonstrated highly efficacious when used as directed and does not increase levels of cyclopiazonic acid on corn.
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Not GLP Compliant – conducted using accepted scientific and/or commercial practices hence the study information is considered valid.

A. Efficacy Data for AF36 applied to corn:

The EUP label approved March 27, 2009 states "Apply *Aspergillus flavus* AF36 to the surface of the soil under the plant canopy after last cultivation. Applications should be made prior to or during silking." Also "On corn, *Aspergillus flavus* AF36 has been shown to be effective when applied from late May through June." The specific claim to be evaluated is on the label, namely "*Aspergillus flavus* AF36 - For displacing aflatoxin producing fungi." An amendment to the December 17, 2007 EUP issued May 27, 2009 extends the EUP until January 4, 2011 allowing use on 3,000 acres per year in Arizona and 5,000 acres per year in Texas. Data from 2008 is included in the current submission. The registrant was specifically directed to include levels of aflatoxin and cyclopiazonic acid in treated and untreated corn.

Table 1: AF36® Efficacy studies with corn – Aflatoxin - 2008:

Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
0 lbs / acre – Control								
[redacted]	50	North	GRAYSON, TX	V7-10	49			110
	21				56			115
	73				122			140
	23				101			130
	65				116			80
	132				111			180
	83				98			100
	101				93			16
	112				94			140
	105				100			87
	72				92			160
	92				92			78
	37				92			170
	34				108			190
	78				92			61
	28				94			14
	162				80			25
	16				83			0
	225				89			40
	53				86			41
	22				98			55
	55				92			67
	55				109			140
	22				119			113
	182				101			37
		South >2mi				9/11/08		160
						9/11/08		3
						8/6/08		21
						9/8/08		560
						8/21/08		170
						9/5/08		1
						9/8/08		110

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Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
[redacted]						9/19/08		110
						9/19/08		22
						9/11/08		110
						9/4/08		160
						9/15/08		100
						8/5/08		308
						8/6/08		330
						9/19/08		110
						9/5/08		140
						9/8/08		35
						8/13/08		35
						8/13/08		170
						8/6/08		800
						8/7/08		780
						8/8/08		984
						8/21/08		73
						9/19/08		178
						8/13/08		160
						8/1/08		100
						8/5/08		52
						9/19/08		78
						8/13/08		78
						8/5/08		500
						9/5/08		110
						9/8/08		110
						9/19/08		52
						9/11/08		140
						9/5/08		52
						9/8/08		52
						9/11/08		160
						8/13/08		178
						9/11/08		24
						8/21/08		180
						9/11/08		3
						9/19/08		170
						8/5/08		74
						8/13/08		267
						9/5/08		140
						9/8/08		140
NTC-GC-SE						8/13/08		110
NTC-GC-SW						8/13/08		228
NTC-GC-EC						8/13/08		55
[redacted]		North	ELLIS, TX	V12-T			7	216
							18	66
							38	19
							7	131

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Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
[redacted]							8	91
							13	184
							20	84
							0	68
958		South					14	89
961							14	306
5083							0	142
[redacted]			SAN PATRICIO, TX	Post VT 1-2 weeks			93	15
							93	21
							0	58
							0	139
							0	25
							0	33
Bayer							13	55
Bayer							8	15
Bayer							0	24
Bayer							0	8
[redacted]							0	23
							0	20
							0	15
							13	187
							33	23
2757-11258			JACKSON / WHARTON TX	Post VT 2-4 weeks			93	0
2758-10091							43	0
2757-11299							42	2
695-11920							24	2
02B							0	440
2160-11890							0	48
778-10477							0	17
778-10766							0	50
1903-11720							0	26
2197-11893							0	3
Dupont			VICTORIA, TX	Post R1			0	13
Dupont							6.67	40
Dupont							13.33	20
Dupont							7.69	27
Bayer							20.00	0
Bayer							0.00	0
Bayer							0.00	17
Bayer							9.09	0
[redacted]							15.38	0
							7.69	21
							14.29	nd
							0.00	0
99-BCR			HIDALGO, TX	Post VT >4 weeks			0	6
99-BCR							7	36

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Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
M-14a							0	33
M-14b							60	32
M-14c							0	194
M-15a							91	7
M-15b							0	223
6+7 TJa							100	32
6+7 TJb							8	11
28a							0	8
28b							8	13
34a							0	64
34b							7	29
34c							0	31
[redacted]		≤2 mi.	MEDINA, TX	Not reported			57	0
							92	22
							92	0
							60	0
Airport							67	9
Airport							93	5
Airport							67	5
Airport							77	27
[redacted]							0	35
			10 lbs / acre – AF36®					
	71	North	GRAYSON, TX	V7-10	109			0
	79				119			0
NTC-Test		South				8/21/08		9
NTC-Test						8/21/08		22
NTC-Test						8/21/08		0
NTC GC-EC						8/13/08		24
[redacted]		North	ELLIS, TX	V12-T			80	60
							73	39
							87	0
							80	61
							87	25
							93	26
							92	0
							100	0
							87	69
							100	39
							43	0
860		South					100	8
864							93	27
865							100	58
933							100	14
953							82	64
958							100	2

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Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
961							83	27
[redacted]			SAN PATRICIO, TX	Post VT 1-2 weeks			100	25
							0	4
							27	64
							73	54
							0	14
							88	22
Bayer							87	5
Bayer							100	5
Bayer							87	6
Bayer							93	15
[redacted]							7	1
							100	0
							73	4
							0	6
							100	2
							100	0
							100	0
10407			JACKSON / WHARTON TX	Post VT 2-4 weeks			87	1
12076							53	47
11908							46	1
11284							45	1
11305							42	2
10099							38	0
01A							33	1
02A							21	1
695-17A							20	0
778-10645							13	3
778-10636							8	0
778-10417							7	0
778-10638							7	0
2160-23070							7	12
778-10422							7	3
1903-10443							0	1
2197-10626							7	1
778-10405							0	17
Dupont			VICTORIA, TX	Post R1			50.00	7
Dupont							84.62	16
Dupont							100.00	46
Dupont							100.00	2
Bayer							60.00	34
Bayer							71.43	34
Bayer							100.00	6
Bayer							53.85	3
[redacted]							21	0
							86	1

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Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
[redacted]							73	6
							100	20
M-11a			HIDALGO, TX	Post VT >4 weeks			54	93
M-11b							58	38
9MCa							100	31
9MCb							77	96
9MCc							100	9
2+3 TJa							79	35
2+3 TJb							50	1
2+3 TJc							0	3
25a							100	0
25b							82	22
25c							100	5
47WBCa							0	63
47WBCb							0	45
47WBAc							0	76
[redacted]			MEDINA, TX	Not reported			92	19
							80	20
							60	28
							86	7
							100	0
							100	0
							69	1
							100	0
							100	15
							100	15
							100	30
							100	0
BBN Hwy 90							100	1
BBN Hwy 90							93	1
BBN Hwy 90							100	2
BBN Hwy 90							80	26
[redacted]							100	18
							93	21
							100	18

[1] In some cases an approximate distance from control to treatment plot(s) was reported, though in most cases this data is absent; [2] Field corn cultivar and in some cases yield and harvest dates are supplied, though not consistently; [3] Aflatoxin (ppb) by commercial ELISA, samples with readings over 80ppb are diluted and re-run.

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Table 2: AF36® Efficacy studies with corn – Aflatoxin - 2009:

Farmer	Acres	Fields	Distance	Variety	Treatment	Trt. Date	Stage	Harvest Date	Yield[1]	N	% AF36	Aflatoxin[2]	CPA[3]	DST[4]
Grayson County, Texas														
[redacted]	165	1	0.1 mi.	P31G71	Treated	6/1	V1	8/21	76	2	93	[illegible]1	0.3	0.92
	105	1			Control				80	2	11	509	0.3-0.4	1.3
	150	2	0.26 mi.	NCT5433	Treated	6/1	V2	8/26	84	2	92	[illegible]4	0.2-0.3	3
	95	2			Control				86	2	28	179	0.0-0.3	3.4
	165	1	Adjacent	P33F85 & P33F87	Treated	6/1	V3	8/12-13	81	2	100	[illegible]	nd	>10
	20	1			Control					2	89	[illegible]7	nd	>10
	165	1	Adjacent	P33F85	Treated	6/1	V2	8/20	64	3	98	[illegible]	nd	>10
	56	1			Control					1	87	110	nd	>10
	165	1	0.08 mi.	PG71	Treated	6/1	V1	8/24-27	50-60	3	89	[illegible]5	0.0	2.6
	32	1			Control				45-50	1	7	172	0.0	2.6
	100	1	0.38 mi.	P31R65 & P31R70	Treated	6/3	V4	8/12-9/20	80	2	75	[illegible]5	0.1-0.6	0.15
	50	2			Control					5	25	[illegible]33	0.2-0.4	1.5
	nd	nd	nd	nd	Treated	nd	nd	nd	nd	1	87	[illegible]d	1.2	nd
	nd	nd	nd	nd	Control	nd	nd	nd	nd	1	33	[illegible]d	0.3	nd
Ellis County, Texas														
	122	2	Adjacent	BH9011/14	Treated	3/20	V5	8/4	66	4	89	[illegible]7	0.0	7.5
	175	2		UNK.	Control			8/18	UNK.	2	35	[illegible]6	0.0	8
	270	4	0.22 mi.	GARST	Treated	3/20	V2	8/6-13	71	9	97	[illegible]1	0.0-0.3	0.25
	214	2			Control					2	29	[illegible]8	0.1-0.3	1.6
	320	5	0.30 mi.	GARST	Treated				71	8	83	[illegible]	Ibid	5.25
	168	2			Control					2	50	[illegible]3	Ibid	5.5
	241	3	Adjacent	Dekalb67-23	Treated	3/20	V2	8/27-29	54	6	95	[illegible]3	0.3-0.4	9.5
	54	2			Control			8/25	52-56	4	56	169	0.6-1.1	9.8
	114	3	3 mi.	P31R87 & Dekalb67-86	Treated			9/3	58	4	98	[illegible]6	Ibid	7.2
	59	1			Control			8/6	83	1	54	[illegible]6	Ibid	9.6
	250	2	0.95 mi.	Dekalb67-23	Treated	3/20	V3	8/25	52	6	94	[illegible]6	Ibid	>10

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Farmer	Acres	Fields	Distance	Variety	Treatment	Trt. Date	Stage	Harvest Date	Yield[1]	N	% AF36	Aflatoxin[2]	CPA[3]	DST[4]
	46	1		& 67-86	Control			8/24	52-56	2	17	1003	Ibid	>10
[redacted]	282	5	Adjacent	P31R87 & NCT6962	Treated	3/20	V3	8/14-20		12	98	8	nd	>10
	68	2			Control			8/18-20		4	67	20	nd	>10
San Patricio County, Texas														
	80	1	Adjacent	GA2821	Treated	4/1	V6	7/8-22	104	1	100	10	0.4-0.7	0.6
	80	1			Control					2	36	15	0.1-0.2	0.75
	80	1	Adjacent	GA2821	Treated				104	2	87	12	Ibid	Adjacent
	80	1			Control					2	80	37	Ibid	Adjacent
	40	1	Adjacent	GA2821	Treated				68	2	84	8	Ibid	Adjacent
	40	1			Control					2	71	10	Ibid	Adjacent
	80	1	Adjacent	GA2841	Treated				95	1	100	0	Ibid	Adjacent
	80	1			Control					1	27	22	Ibid	Adjacent
Victoria County, Texas														
	300	1	Adjacent	BH88-95	Treated	5/1	V3	7/21	68	4	96	3	0.3	Adjacent
	135	1			Control					4	45	12	0.0	2008
Hidalgo County, Texas														
	nd	nd	nd	nd	Treated	nd	nd	nd	nd	2	53-57	nd	0.0	nd
	nd	nd	nd	nd	Control	nd	nd	nd	nd	2	33-85	nd	0.0-0.3	nd

1. Bushels per acre; 2. Parts per billion (ppb); 3. Parts per million (ppm); 4. Distance in miles from previously treated fields.

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Table 3: FDA Action levels for poisonous or deleterious substances - AFLATOXIN	
Commodity	**Action Level (ppb)**
Animal Feeds	
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)	300
Corn and peanut products intended for finishing swine of 100 pounds or greater	200
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20
Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20
Brazil nuts	20
Foods	20
Milk	0.5 (aflatoxin M1)
Peanuts and Peanut products	20
Pistachio nuts	20

RESULTS AND DISCUSSION:

The summary table 1 from MRID 479351-01 has the 2008 field testing data, while table 2 has the 2009 data with several additional endpoints addressed, including data on replication and distances between fields. Application levels are not listed but are assumed at the EUP label use rate of 10 lbs./acre. Data reported from 2009 includes 7 sites with one field each in Grayson County Texas, 7 sites some with multiple fields in Ellis County Texas, 1 site with four fields in San Patricia County Texas, and 1 site each in Victoria and Hidalgo Counties in Texas. One site in Grayson County and the site in Hidalgo County had only measures of % AF36 and cyclopiazonic acid levels. Other sites all included %AF36 and ppb aflatoxin on individual samples and the majority of sites had measures of cyclopiazonic acid levels. Seven of 18 sites with control plots where aflatoxin was measured exceeded 100 ppb aflatoxin, and in several cases the level was >200 ppb up to a single site with 1,003 ppb in Ellis County. The highest aflatoxin concentration occurring from treated plots was 61 ppb and in 10 of the 18 fields tested aflatoxins were below the lowest FDA action level of 20 ppb for corn. All treated plots consistently showed a higher percentage of AF36 present on corn sampled, regardless of the proximity of treated to control plots, some of which were adjacent and others miles apart. Previous and/or nearby treated sites reported did not appear to play a significant role in efficacy of applied AF36 in 2009. Cyclopiazonic acid levels and %AF36 were reported from most sites. Regardless of treatment, cyclopiazonic acid levels ranged from 0.0-1.2 ppm with no distinguishable trend between treated and control plots (many treated and control plots both registered 0.0 ppm). Use of *Aspergillus flavus* AF36 on corn is demonstrated highly efficacious when used as directed and does not increase levels of cyclopiazonic acid on corn.