6-24-93

MEMORANDUM

Subject: Imazalil (List B; Case 2325; Chemical 111901): Analytical Method for the Determination of Imazalil and R14821 in/on Bananas, Citrus, and Citrus Processed Commodities. Research Method and Enforcement Method Comparison. DP Barcode D182707. MRID Nos. 42454803, 42454804, and 42454805. CBRS No. 10602.

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Imazalil, or 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole, is a fungicide used as a postharvest treatment for citrus and bananas. A tolerance of 10 ppm has been established for the combined residues of imazalil (R23979) and its metabolite 1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)-1-ethanol (R14821) in/on citrus fruit (40 CFR 180.413(a)). A food additive tolerance of 25 ppm exists for citrus oil (40 CFR 185.3650), and a feed additive tolerance of 25 ppm has been created for dried citrus pulp (40 CFR 186.3650). Tolerances of 3.0 ppm and 0.20 ppm have been established for the combined residues of imazalil (R23979) and its metabolite 1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)-1-ethanol (R14821) in/on whole bananas and banana pulp (40 CFR 180.413(a)), respectively.

Enforcement methods (PAM II, Method 1 and Method 2) exist for the determination of imazalil and 1-(2,4-dichlorophenyl)-2-(1H-imidazole-1-yl)-1-ethanol in citrus, processed citrus commodities, bananas, cottonseed, small grains, bovine tissue, and milk. The methods require the use of blank control material for calibration.

The Phase 4 Reregistration DCI required field trials for bananas, a processing study for citrus fruit (grapefruit, orange, lemon) treated postharvest with imazalil, and storage stability studies for citrus and the processed commodities and bananas. A citrus processing study and a citrus commodity storage stability study have been reviewed and found acceptable (S. Funk, CBRS No. 10563, 02/17/93; CBRS No. 11335,

03/02/93). Banana field trials (postharvest treatment) were submitted and reviewed (A. Aikens, CBRS No. 8846, 08/13/92), and additional data were requested. For all these studies, modifications of the existing enforcement method were utilized. The present submission is a validation of the data collection methods, a comparison of the enforcement and data collection methods, and validation of the enforcement method for bananas.

The structures of imazalil (R23979), R14821, and an analytical method internal standard R30617 are as follows:

Conclusions

1. The analytical method presented for bananas may be acceptable for enforcement purposes and is fully acceptable for data collection. The method requires the preparation of standards in the matrix (banana), and this is not usually acceptable for enforcement methods. However, the existing enforcement method (PAM II, Method 1) requires a control matrix. The new method replaces the packed column with a megabore column and eliminates a sodium hydroxide extraction step. Otherwise, the

new method and the PAM methods are similar. Adequate accuracy, precision, and validation data have been presented for use of the new method with bananas for data collection purposes, such as the previously reported postharvest banana trials (A. Aikens, CBRS No. 8846, 08/13/92). Because an adequate enforcement method exists, and because the new method continues to require the use of the control matrix, it will not be validated by EPA. The existing PAM method, EPA-validated for oranges and bananas, is deemed adequate for bananas and banana pulp.

2a. The internal standard method for citrus has been demonstrated to be fully acceptable for data collection purposes, such as the citrus processing study previously reviewed (S. Funk, CBRS No. 10563, 02/17/93). The method provides for acceptable extraction of imazalil and R14821 from citrus and citrus processed commodities. The gc determination provides chromatographic resolution of imazalil and derivatized R14821. Method accuracy and precision are acceptable. Fortified commodity recoveries ranged from 87% to 110%, and the relative standard deviation of the mean recovery ranged from 0.2% to 10.4%. Demonstrated limits of quantitation were substantially below the established tolerances.

2b. The external standard method for citrus is a modification of the existing PAM II procedure. The significant changes are in the extraction procedure. In the modified method, an initial mixing of the matrix with acid is not performed, hexane is added with the sulfuric acid in the acid extraction step, and BSTFA (not BSA) is used to derivatize the R14821. The registrant claims that the initial acid treatment to release conjugated residues is not needed because the citrus is naturally acidic, that hexane facilitates extraction, and that the BSTFA provides enhanced reactivity for derivatization.

The proposed enforcement method does not differ substantially from the existing enforcement method, which has been deemed acceptable for citrus and citrus processed commodities. Therefore, the Agency will not pursue validation of the new method and will not require an independent laboratory validation.
Extensive sample and spiked matrix data were provided to compare the modified method to the internal standard method. There is little difference in the results obtained. The internal standard method gives slightly improved accuracy and precision.

Recommendation

CBRS finds the existing enforcement method (PAM II, Method 1) acceptable for bananas, banana pulp, citrus, citrus pulp, and citrus processed commodities and does NOT recommend for a validation of the new enforcement methods submitted.

CBRS further finds the data collection methods used for bananas (MRID 42058701, CBRS No. 8846) and citrus/citrus processed commodities (MRID 42454806, CBRS No. 10563) fully acceptable. No further data are required for analytical methods used with the referenced studies.

Detailed Consideration

Banana Enforcement and Data Collection Methods (MRID 42454803; MRID 42454805)

The existing enforcement method (PAM II, Method 1) for citrus has been adapted and validated for bananas. The modifications include the substitution of a 50 m fused silica megabore (0.53 mm) column with a 95% dimethyl siloxane / 5% phenyl siloxane phase for a packed column and elimination of a sodium hydroxide extraction in the sample workup.

Bananas, at least 10 with or without peel, are cut into pieces and homogenized in a blender. A 1 g subsample is mixed for one hour with 2.5 ml of 0.1 N HCl. Fortifications are made by the addition of a spike after the HCl addition. Concentrated ammonia is added to a pH $\geq$ 9. This requires about 0.5 ml of the concentrated ammonia. The solution is extracted with 95/5 (v/v) heptane/isoamyl alcohol (2 X 5 ml). The combined organic layers are brought to 10 ml by the addition of heptane/isoamyl alcohol. A 3 ml fraction is carefully evaporated at 50°C under nitrogen to dryness. The residue is treated with 40 µl of N,O-bis(trimethylsilyl)acetamide (BSA) and diluted after 1 minute with 1.5 ml of toluene. After sealing in a gc autosampler vial, the solution is heated for 30 minutes at 80°C.

This extraction differs from the enforcement method extraction. The enforcement method uses 0.01N HCl (25 ml per 10 g sample) in the initial 1 minute homogenization, as opposed to the one hour homogenization of the modified method. A 1 g subsample is mixed for 10 min with 1 ml of 1N NaOH and 4 ml of heptane-isoamyl alcohol (95/5). The modified method uses NH_4OH, not NaOH, in the initial extraction. The registrant claims that ammonium hydroxide affords better separation of the aqueous and organic phases. In the PAM method, the mixture is centrifuged, the organic layer is removed, and the aqueous layer is again extracted with 4 ml of heptane-isoamyl alcohol. The aqueous layer is discarded, the combined organic extract is extracted with 3.0 ml of 0.1N H_2SO_4, and the acid extract is made alkaline with NH_4OH and extracted with heptane-isoamyl alcohol. The final extract is evaporated to dryness and derivatized with BSA in toluene. The H_2SO_4 extraction of the heptane-isoamyl extract is eliminated in the modified procedure.

Analyses are conducted on a gc equipped with an electron capture detector. In the modified method of this submission, the 2 m X 3 mm i.d. glass column packed with 3% SP-2250 DB is replaced with a 50 m megabore (0.53 mm) with 95% dimethyl siloxane / 5% phenyl siloxane. Originally (MRID 42454805), a 15 meter column was utilized. The column temperature is 250°C, versus 245°C for the packed column; and the helium carrier flow rate is 20 ml/min. Nitrogen make-up gas (50 ml/min) is required for the ec detector. Injection volume is 1.5 µl, versus 0.5 µl for the packed column. The retention time (50 m column) for imazalil is 5.7 minutes. The retention time for R 148821 (silanized) is 4.9 minutes. The comparable retention times on the packed column are 2.8 minutes and 2.0 minutes. The column degradation noted in the EPA validation

would be reduced by use of the megabore.

For both methods, calibration is by external standards. Standard solutions are spiked into a control banana matrix and extracted and analyzed by the same procedure as samples. Log/log plots of concentration versus response may be needed to achieve linearity. The use of a rac matrix for the preparation of calibration curves is not normally permitted for enforcement methods, but the existing enforcement method requires the use of the same control matrix. The PAM method does not specify log/log plots.

Results for the metabolite R14821 may be confirmed qualitatively by the analysis of an unsilanized fraction. There is no confirmatory procedure for the parent. About 3.3 hours is needed to prepare and analyze one sample. The existing enforcement method does not have a confirmatory procedure.

A variation of the modified PAM method was used for the banana field trial study. An internal standard, 1-[2-(2,4-dichlorophenyl)heptyl]-1H-imidazole mononitrate (R 30617), was added at a concentration of 1 ppm immediately after the addition of HCl to the homogenized matrix. The internal standard procedure was used for data collection only and is not proposed as an enforcement method.

The limit of quantitation (loq) for both the external standard and internal standard methods is 0.05 ppm for imazalil and 0.08 ppm for R14821. These values are calculated assuming that a signal 10X background is the limit of quantitation. Chromatograms presented for a 0.05 ppm imazalil standard and a 0.09 ppm R14821 support the defined loq's. These loq's are below the tolerances for banana pulp (0.2 ppm) and whole bananas (3.0 ppm) and are acceptable.

Accuracy data (recovery) were reviewed previously (A. Aikens, CBRS No. 8846, 08/13/92) for the internal standard variation. In the present submission, extraction recovery data were presented for both the external and internal standard methods. Final control analyte extracts (before silylation) were fortified with imazalil and R14821 at concentrations identical to those used for calibration curves. Curves were constructed from the control fortifications and compared to the calibration curves. The t-test (2-tail with 95% confidence level) showed no difference in the slopes of the calibration curve and the spiked control extracts for green bananas, yellow bananas, and banana pulp, for both external and internal standard methods. This is interpreted as constant recovery over the fortification range. Likewise, calculated t-values were less than critical t-values for the y-intercepts except for imazalil in pulp by external standard and imazalil in yellow banana by internal standard, indicating no statistical difference in the y-intercepts or 100% recoveries. Conventional recoveries were also calculated and are summarized in Table 1. No raw data (peak heights) were provided, and the results given in Table 1 cannot be confirmed.

Table 1: Recovery of Imazalil and R14821 from Bananas		
Commodity	Imazalil (R23979)	R14821

	Fortification Range[1] (ppm)	Average Recovery (%)	Recovery Range (%)	Fortification Range (ppm)	Average Recovery (%)	Recovery Range (%)
External Standard						
green banana	0.05 - 5	100	93 - 108	0.05 - 0.5	101	93 - 122
yellow banana	0.05 - 5	99	93 - 103	0.05 - 0.5	102	77 - 139
pulp	0.05 - 1.0	90	84 - 96	0.05 - 0.2	86	81 - 92
Internal Standard						
green banana	0.05 - 5	96	88 - 99	0.05 - 0.5	95	83 - 100
yellow banana	0.05 - 5	107	100 - 113	0.05 - 0.5	-[2]	91 - 151
pulp	0.05 - 1.0	109	107 - 116	0.05 - 0.2	99	92 - 102

[1] n = 6 for yellow bananas and green bananas. n = 5 for pulp.
[2] Not reported; statistically significant difference in the intercepts of the calibration and the spike analyses.

Precision was determined by spiking 9 to 11 subsamples of banana with imazalil and the metabolite R14821 and determining the concentrations in each subsample. The test was conducted by both the external standard method and the internal standard method, but the spiking concentrations were not reported. Raw data (peak heights) were provided and the results have been verified. The relative standard deviations (in peak heights) are summarized in Table 2.

Table 2: Precision (% RSD) for External and Internal Standard Methods

Commodity	Method	Imazalil (% RSD)	R14821 (% RSD)
Green Banana	External Standard	4.00	1.73
	Internal Standard	3.26	4.39
Yellow Banana	External Standard	4.85	13.3
	Internal Standard	1.65	11.6
Pulp	External Standard	4.21	15.6
	Internal Standard	3.27	11.3

The internal standard method did not provide substantially improved precision.

Regression analysis and the Wilcoxon T-test were used to show that the internal standard method and the external standard method provide comparable results for green banana, yellow banana, and banana pulp.

Accuracy and precision data were also presented for the 15 meter column (MRID 42454805), and a comparison of the external and internal standard methods was made for imazalil only. The results are similar to those reported above. For banana pulp spiked at 1.50 µg/g with imazalil and at 0.15 µg/g of R14821, the relative standard deviations were 3.57% and 12.4%, respectively. For the same spike levels, recoveries (n = 4 for imazalil and n = 5 for R14821) ranged from 86.5% to 100.4% for imazalil and from 86.5% to 133% for R14821. Imazalil was determined by internal standard procedure, and R14821 was determined by external standard procedure.

Citrus and Citrus Processed Commodities Enforcement and Data Collection Methods (MRID 42454804)

External and internal standard methods are described for the determination of imazalil and the metabolite R14821 in/on citrus and citrus processed commodities. The

external standard method is proposed by the registrant as an enforcement method. The gc method employs a 0.5 m X 3 mm i.d. glass column silanized and packed with 3% OV-17. The existing PAM II method uses the same packing (SP2250), but a longer 2 m column. The shorter column is operated at 210° C; the PAM column is operated at 245° C. Average retention times in the proposed method are reported as 2.6 minutes for imazalil (R23979), 2.2 minutes for the metabolite R14821 (derivatized), and 4.6 minutes for the internal standard 1-[2-(2,4-dichlorophenyl)heptyl]-1H-imidazole mononitrate (R 30617). Average retention times in the PAM method are 2.8 minutes for imazalil and 2.0 minutes for R14821 (derivatized).

The sample extraction and workup differs from both the existing PAM method and the method described above for bananas. Six fruits are homogenized, or solid processing fractions, such as frits, are pulverized. A 1 g subsample of the homogenate, pulverized material, or liquid processed fraction is spiked with internal standard contained in 100 µl of methanol. The internal standard is added for data collection only and is not used for enforcement purposes. The mixture is made alkaline with 3 ml of 0.1 M NaOH. The initial mixing with acid of the banana method and PAM method is not utilized. Acidification is needed to release bound or conjugated residues, but the citrus commodities are inherently acidic. The mixture is extracted with heptane-isoamyl alcohol (95/5, v/v; 3 X 3.5 ml). In the PAM and banana methods, only two extractions (2 X 4 ml, 2 X 5 ml, respectively) are conducted. The organic extracts are combined, and a 4 ml aliquot is added to 4 ml of heptane and 3 ml of 0.05 M H_2SO_4. The mixture is rotated at 10 rpm for 10 minutes, followed by centrifugation. The organic layer is discarded, and the aqueous layer is washed with heptane (5 ml) and alkalinized with 200 µl of concentrated ammonia. The alkaline aqueous fraction is extracted with heptane-isoamyl alcohol (95/5, v/v, 1X 2.5 ml), and the organic extract is evaporated to dryness (60° C). The sulfuric acid extraction is not used in the banana method (above), but a very similar extraction is used in the PAM method. The PAM method does not add heptane with the sulfuric acid. According to the registrant, the heptane reduces the solvent polarity and facilitates extraction into the acid. The residue is treated with 50 µl of BSTFA, or N,O-bis(trimethylsilyl)-trifluoroacetamide, 500 µl of acetonitrile, and 1 ml of toluene. The acetonitrile is not used in the PAM procedure, and both the PAM and banana methods use BSA, or N,O-bis(trimethylsilyl)acetamide, as the derivatizing agent. The registrant claims that BSTFA provided better reactivity. The mixture is mixed for 20 seconds and then heated at 80°C for 30 minutes. The mixture is diluted with 1.5 ml of toluene. Injection size is 1 µl.

Calibrations require the spiking of control commodity samples with imazalil, R14821, and the internal standard (data collection samples only). The commodities are extracted and analyzed in the identical fashion as samples. Log-log calibration curves of analyte peak height (or analyte peak height to internal standard peak height ratio) versus concentration are constructed. No mention of the need to perform log-log transformations is mentioned in the PAM method. Slope and intercept are determined, as in the PAM and banana methods. The lowest calibration point was 0.050 µg/ml(g) for imazalil in all commodities except finisher pulp, peel frits, chopped peel, and dried

peel. Of these commodities, the lowest imazalil calibration point was 0.50 µg/ml(g). Taken as the defined limit of quantitation, these concentrations are below the current combined tolerance for imazalil and its metabolite in citrus (10 ppm). The lowest calibration point, and defined limit of quantitation, for R14821 was 0.20 µg/ml(g) for all commodities except finisher pulp, peel frits, chopped peel, and dried peel. For these commodities, the lowest calibration point ranged from 0.10 µg/g to 0.25 µg/g. Again, these values are substantially below the existing tolerance (10 ppm).

Example chromatograms were provided for whole fruit extracts, dried peel extracts, chopped peel extracts, cold pressed oil extracts, molasses extracts, and fresh juice extracts. Chromatograms of various fortified commodities were also provided. Adequate resolution of the analytes is shown in all cases, and the claimed limits of quantitation are demonstrated.

One analyst can prepare, extract, and analyze 48 samples in 3 days, or an average of 16 samples per 8 hour day.

Validation of the internal standard method for accuracy and precision is reported. Samples of the various commodities were spiked with the target analytes, and concentrations were measured. The recoveries are summarized in Table 3. Precision (RSD) was also reported, but the nature of the three replicate determinations per commodity is not disclosed. The replicates may be independent subsamples or may be multiple analysis of one sample. The former is required to measure precision of the total method. The fortification levels do encompass the current tolerance values for whole fruit, citrus pulp, and citrus oil. All recoveries are acceptable. However, the reported recoveries cannot be verified because no raw data (peak heights) were provided.

The internal standard method and the external standard (modified enforcement) method were compared by the analysis of randomly selected processing study (MRID 42454806) samples by both methods. Results only are reported for 53 samples for imazalil and 46 samples for R14821. The relative percent difference of the concentrations found by the two methods varied from 0.0% (fresh juice) to 23.5% (pressed oil) for R14821 and from 0.4% (pressed oil) to 22.0% (whole fruit) for imazalil. For R14821, the RPD was less than 15% for 89% of the samples. For imazalil, the RPD was less than 15% for 90% of the samples. The methods provide comparable results.

The registrant also presented results of a paired t-test with relative differences and a weighted linear regression analysis to demonstrate comparability of the methods. Also, accuracy and precision data from the analysis of spiked commodity samples by the two methods were compared. For 36 spiked commodity samples, the mean relative error for the external standard method was 1.5% $\pm$ 6.2%; for the internal standard method, the relative error was 1.3% $\pm$ 6.0%. Based on 12 spiked samples with replicate analyses, the mean relative standard deviation was 4.4% for the external standard

method and 3.3% for the internal standard method.

Table 3: Accuracy and Precision of Internal Standard Method for Imazalil and R14821[1]

Analyte	Commodity[2]	Fortification (µg/ml or µg/g)	Mean Recovery[3] (%)	RSD (%)
Imazalil	Whole Fruit	0.250	97.2	4.5
		10.0	110	1.8
	Fresh Juice	0.250	101	4.2
		1.00	103	3.1
	Molasses	1.00	102	0.8
		10.0	109	0.6
	Dried Peel	2.50	86.8	4.6
		25.0	103	1.8
	Pressed Oil	0.250	102	10.4
		25.0	110	2.1
R14821	Whole Fruit	0.250	98.8	1.4
		1.00	105	4.8
	Fresh Juice	0.025	104	9.1
		0.250	103	1.8
	Molasses	0.250	98.8	6.3
		1.00	103	2.6
	Dried Peel	0.250	96.0	4.0
		2.50	93.6	0.2
	Pressed Oil	0.250	100	2.0
		2.50	106	3.9

[1] Raw data not provided. Results cannot be verified.
[2] Type of citrus (lemons, oranges, grapefruits) not specified.
[3] n = 3 for whole fruit molasses, dried peel, and oil; n = 6 for juice.

cc: Imazalil List B File, Imazalil Subject File, RF, circ., S. Funk.

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