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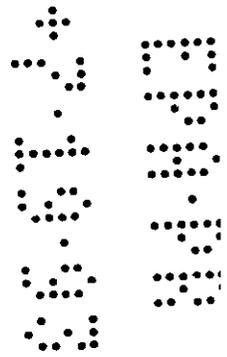
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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA subsection 10(d)(1)(A), (B) or (C).

Company: Merck & Co., Inc.

Company Agent: *Louis S. Grosso* Date: 07/27/93
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CERTIFICATION OF GOOD LABORATORY PRACTICE

Study No. 93670, "Method Validation HPLC-Fluorescence Method to Determine the Total Toxic Residue of MK-0244 and Its Metabolites, on Vegetables, Including Leafy Vegetables and Cole Crops," has been performed in compliance with the current EPA FIFRA Good Laboratory Practice Standards (40 CFR Part 160) by Analytical Research Department, Merck Research Laboratories with the following exceptions:

1. Chemical characterization of the test substances (analytical standard or authentic substances) was conducted following FDA Good Manufacturing Practice regulations.
2. Chemical characterization of the test substances was conducted following EPA FIFRA GLP subsequent to their use in this study.

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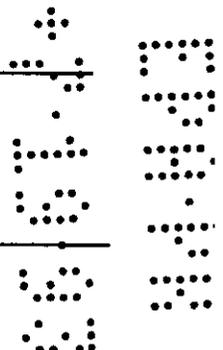
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**MERCK & COMPANY, INC.
CRITICAL PHASE OPERATIONS
Q.A.U. INSPECTIONS AND REPORT DATES**

Title of Study: HPLC-Fluorescent Method to Determine the Total Toxic Residue of
D-0244 and Its Metabolites in Vegetables, including Leafy
Vegetables and Cereals - Analytical Research Method 044-93-0

Study Number: 370

Protocol Number: 8

Location: Rahway, NJ

Q.A.U. Dates of Inspection	Critical Phase	Report Date
May 20, 1992	Protocol review*	Sept...
February 25, 1993	Analysis of celery samples and Fortification of lettuce samples **Raw data audit and Review of draft report	February 25, 1993

*The protocol was reviewed by the QAU on April 24, 1992 and comments were handled directly with the study director.

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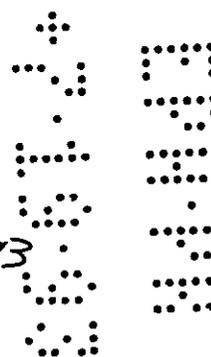
Date: 10 May 93

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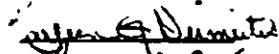
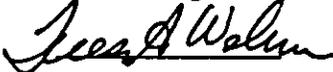
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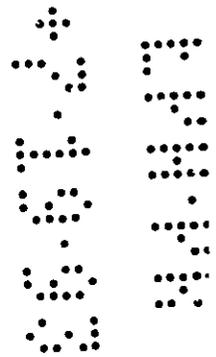
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STUDY NO. 93670

VALIDATION OF THE

HPLC-FLUORESCENCE METHOD TO DETERMINE THE TOTAL TOXIC
RESIDUE OF MK-0244 AND ITS METABOLITES, ON VEGETABLES,
INCLUDING LEAFY VEGETABLES AND COLE CROPS

ANALYTICAL RESEARCH METHOD 244-92-3
Data Requirement: 171-4

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618-244-93670

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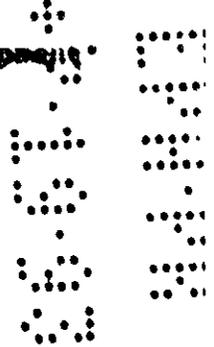
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-	In volumes 2 through 5 of this study	
•	The method, certificates of analysis, etc. have been included in volume 7 of Study No. 93336, entitled: *Determination of the Magnitude of Residues of MX-244 and Its Metabolites In/On the Raw Agricultural Commodity Group, Cole Crops, From MX-244 0.16 EC Applied with Non-Ionic Surfactant by Ground Equipment*	



I. SUMMARY

MX-0244 (4'-deoxy-4'-epi-methylamino avermectin B1) is proposed as an insecticide to be applied at low levels to various vegetables such as lettuce, celery, broccoli and cabbage. It is a mixture of two homologs, which differ by a methylene group, defined as not less than 90% B1a and not more than 10% B1b.

A method was developed and published (Prabhu, et al, 1991) to determine the residue of the parent MX-0244 (B1a and B1b) as well as an expected photodegradation product, the delta 8,9 Z isomer, in celery and lettuce. Plant metabolism studies (Crouch et al, 1992) have more recently determined that a method may be needed to monitor residues of not only the parent MX-0244 (B1a and B1b) and its delta 8,9 isomer (Z) photodegrade but also other possible degradation products: 4'-epi-amino-4'-deoxy avermectin B1 (L-653,649 = L'649), 4'-epi-(N-formyl)amino-4'-deoxy avermectin B1 (L-(17,831 = L'831) and 4'-epi-(N-methyl-N-formyl)amino-4'-deoxy avermectin B1 (L-660,599 = L'599). The degradates (also termed metabolites or photometabolites) in addition to the parent MX-0244 may be considered part of the total toxic residue. The published method was unable to adequately recover the L'599 and L'831 residues so the method was revised.

The revised method (244-92-3) was developed and validated at the Merck Research Laboratories in Rahway, New Jersey to determine all of these compounds. The method consists of extracting the residue from the matrix with organic solvent, removing the co-extractives with solid phase or liquid-liquid extractions and derivatizing to detect fluorescent derivatives on a reverse phase high pressure liquid chromatograph with fluorescence detection. The method separates the ionizable compounds from the neutral compounds and there are also separate solutions injected onto the HPLC to quantitate the different compounds. The method validation was performed by fortifying the matrix with solutions of various standards or authentic substances and then assaying the samples as described in the method.

This report describes the results of the validation. Table I summarizes the average method recoveries found for leafy vegetables (lettuce and celery) and cole crops (cabbage and broccoli) for each of the compounds investigated. The validation has demonstrated that the method will achieve acceptable recoveries ranging from 70-120% for both the B1a and B1b components of parent MX-0244. Acceptable recoveries for the degradates, which are expected to be minor components of the total toxic residue, are defined as being in the range of 45-110%. Fortifications to demonstrate the quantitation

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range were made at concentrations from approximately 5 ng/g to 240 ng/g. The lower limit of quantitation established for all matrices was 5 ng/g. Following the completion of the experimental work in the validation, criteria were established for the performance of the method and the method has been used for the determination of incurred residues from field trials.

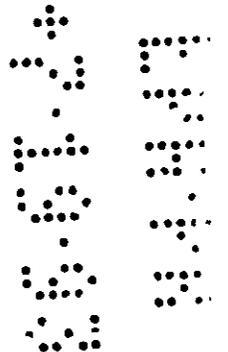


Table I

**Summary of Average % Recoveries Over All Concentrations
for MK-0244 and its Metabolites (Degradates)
(Study No. 93670)**

Compound Fortification (Conc. Range)	Celery	Matrix		Cabbage	All Matrices
		Lettuce	Broccoli (%)		
MK-0244 B1a (5 - 120 ng/g)	94	102	80	100	94
MK-0244 B1b (5 ng/g)	98	110	83	107	100
8,9 Z (5 - 52 ng/g)	87	77	58	84	76
MK-0244 B1a + 8,9 Z (10 - 240ng/g)	100	90	68	85	86
L'649 (5 - 120 ng/g)	85	83	55	80	75
L'599 (5 ng/g)	43	61	64	90	65
L'831 (5 ng/g)	NA	54	NA	64	59
L'599 + L'831 (10 - 240 ng/g)	57	55	54	72	60

NA = Not individually fortified in this matrix.
Numbers rounded to nearest unit.
Calculated as described in method.

II. PROCEDURES

A. Sample Handling

Samples of the various matrices were initially obtained from commercial sources (grocery stores) for methods development. When samples from residue field trials became available, with a known pesticide treatment history, extra material from the control samples collected in various trials was used in the validation. Table II tracks the sample history of the untreated control samples from residue field trials used in the validation. More details about specific trials can be found in the appropriate trial reports. Samples from the grocery stores (used only during methods development) were obtained fresh and then were frozen before or after processing. Samples from field trials were received frozen and were kept frozen until processing. All samples were stored frozen at or below -10 degC except during limited handling such as processing and analysis. The samples were processed at Three Bridges using a Hobart food processor and shipped frozen with dry ice to the laboratories in Rahway, New Jersey. The validation consisted of assaying untreated control matrix samples as well as fortified control matrix samples. The assays were performed during 1992.

Table II

**History of Trial Samples Used for the Method 244-92-3 Validation
(Study No. 93670)**

Matrix	Trial Sample ID	Date Processed	Date Shipped to Rahway	Date Rec'd at Rahway
CELERY	001-91-1019R-10	04/03/92	04/03/92	04/03/92
	001-91-0004R-1	03/14/92	04/14/92	04/14/92
	001-91-0006R-1	04/21/92	04/21/92	04/21/92
LETTUCE	001-90-6017R-1	03/09/92	03/09/92	03/09/92
	001-90-6017R-4	03/09/92	03/09/92	03/09/92
BROCCOLI	001-91-1033R-9	05/19/92	05/19/92	05/19/92
	001-91-1033R-10	05/19/92	05/19/92	05/19/92
CABBAGE	001-91-0027R-10	03/19/92	03/24/92	03/25/92
	001-91-0027R-14	03/20/92	03/24/92	03/25/92

B. Method

(1) Method Summary

A representative 10 gram subsample from each processed sample was homogenized with a mixture of ethyl acetate, water and acetonitrile. In those samples from which method recovery was determined, the matrix was fortified first with an acetonitrile solution of standards and/or authentic substances and that volume of acetonitrile replaced the same volume of acetonitrile added for extraction. The ethyl acetate extract and macerate were shaken and the mixture centrifuged. The supernatant ethyl acetate extract was removed and additional ethyl acetate transferred to the macerate for shaking, extraction and centrifugation. The combined ethyl acetate extracts were loaded onto a prepared propylsulfonyl (PRS) cation exchange cartridge column. The ionizable compounds, MK-0244, its 8,9 isomer (Z) and the amino degradate (L'649), were retained on the column and the neutral degradates, the N-formyl (L'831) and the N-formyl-N-methyl (L'599) compounds, were eluted through the column and collected in the ethyl acetate load. The ethyl acetate was retained until the neutral fraction isolation was completed as described below.

The PRS column containing the ionizable compounds was eluted with a solution of ammonium acetate in methanol to isolate MK-0244, its 8,9 isomer and L'649. The methanolic eluant was usually split in half. One half was retained for possible reassay and the other half was evaporated to 1 mL. Water and ethyl acetate were added and the avermectin residue was extracted into the ethyl acetate phase, leaving the ammonium acetate in the aqueous phase. The ethyl acetate was evaporated to 1 mL and acetonitrile was added. The samples and corresponding external standard solutions were derivatized with trifluoroacetic anhydride in the presence of N-methylimidazole. The derivatized samples and standards were injected on a reverse phase HPLC with fluorescence detection. Quantitation was based on comparison with the external standards.

The neutral compound fraction of the samples was isolated by evaporating the ethyl acetate eluent (collected from the PRS column) to dryness and reconstituting in methylene chloride and hexane. This mixture was loaded onto a prepared aminopropyl normal phase solid phase extraction cartridge column. The column was washed with toluene and isopropanol in hexane and eluted with a solution of ethanol in ethyl acetate. The ethanolic ethyl acetate was usually split in half. One half was retained for possible reassay and the other half was

evaporated to dryness. Methanol was added to reconstitute the residue; water and hexane were added and the degradates (or metabolites) were extracted into the hexane phase. The hexane extracts were evaporated to dryness and reconstituted in acetonitrile. The samples and corresponding external standards were derivatized with trifluoroacetic anhydride in the presence of N-methylimidazole. The derivatized standards and samples were injected on a reverse phase HPLC with fluorescence detection. Quantitation was based on comparison with the external standards.

The neutral compounds have sufficiently stable rotational isomers (rotamers) that the isomers resulted in incompletely resolved but distinct HPLC bands or peaks. Thus, total peak area responses within the retention time region of interest have been used to measure the total amount of L'831 B1 and/or L'599 B1 present. Under the cleanup and chromatographic conditions used in this method, it was not possible to separately quantitate L'831 from L'599 except in those instances where only one or the other was added for method recovery.

The method was able to distinguish as separate residues, the amino-avermectin degradate (L'649 B1), the B1a component as well as the minor B1b component of the parent MK-0244 (including its delta 8,9 isomer) and a combination of the neutral N-formyl-N-methyl (L'599 B1) and N-formyl (L'831 B1) degradates.

The method had a limit of detection of 1 ng/g (0.001 mg/kg) for each separate resultant value (i.e. L'649 B1; MK-0244 B1a + 8,9-Z isomer B1a; MK-0244 B1b [+ 8,9-Z isomer B1b by analogy]; L'599 B1 + L'831 B1). The method was validated so that the lower limit of quantitation is 5 ng/g (0.005 mg/kg) for each residue value group. The samples were assayed in batches or sets; each set had at least one untreated control sample to demonstrate the lack of contamination in the method. Multiple recovery samples were run in each set to validate the method's performance. There were different combinations of standards (or authentic substances) fortified onto the matrix as well as some individual compounds fortified onto the matrix, as described in the protocol, to verify the effectiveness of the method in the presence of the other compounds.

More details about the method used for the determination of the residues reported herein may be found in the method description, Analytical Research Method 244-92-3. For the sake of convenience, the two parts of the assay, ionizable and neutral, were frequently described as the A assay (or A-side) and the B assay (or B-side), respectively.

(2) Fortification Procedures and Standards

Table III-A lists the stock standard solutions used in the validation while Table III-B lists the working standard solutions. Table III-C summarizes the combination of compounds examined in the fortification experiments. This table summarizes the number of samples for which the specified fortifications were determined by completing both the A (ionizable) and B (neutral) sides of the assay. The combinations examined the various permutations possible for incurred residue, to show that the method was valid regardless of the combination of analytes present in the sample. Sample combinations a through m were specified in the protocol and were assayed as part of the full validation. Lettuce and cabbage were the matrices studied in the full validation described in the protocol. Samples beyond those specified in the protocol were assayed either as part of the limited validation or additional combinations. The limited validation was performed on celery and broccoli and was intended to demonstrate the utility of the method without change for these matrices.

In general, standards for quantitation or fortification were prepared from the reference standard or authentic substances cited below. The material was weighed using an analytical balance and dissolved in acetonitrile to make a concentrated stock standard solution in a volumetric flask. Working standards were prepared by diluting aliquots of the stock standard solution with additional acetonitrile in a volumetric flask. The test substances (either reference standard or authentic substances) used in the validation are listed in Table III-A. The materials were originally characterized under the USFDA Good Manufacturing Practices (21CFR211) regulations but have been recently characterized following the USEPA FIFRA Good Laboratory Practices regulations (40CFR160). Copies of the certificates of analyses are included in the appendix.

MK-0244 B1a (from reference standard L-656,748-052S003 containing both B1a and B1b) was used as the standard for comparison for the B1a component as well as for the B1b component. It is also used for their corresponding delta 8,9-Z isomers since the delta 8,9-Z isomer yields the same derivative as the parent MK-0244. The delta 8,9-Z isomer of the B1a component (authentic substance L-695,638-001C001, also designated as 8,9 Z or just Z) was prepared and this relationship was established by chromatographic comparison of the derivatized authentic samples. The delta 8,9-Z isomer of the B1b component is considered to behave in an analogous manner. Consequently, the residue quantitated at the

retention time of the parent derivative represents the sum of the parent plus its delta 8,9-Z isomer. The Blb and Bla components were resolved.

The authentic substance of the ionizable degradate 4"-epi-amino-4"-deoxy avermectin B1 (L'649 B1 from L-653,649-005S001) was used for the quantitation of the L'649 residues. The Bla and Blb components were usually incompletely resolved and so the total peak area was used for the quantitation of their sum.

Solutions of each neutral compound B1 (prepared from authentic substances L-657,831-000S008 and L-660,599-000N004) as well as an approximately 1:1 mixture of L'831 and L'599 were used to quantitate the neutral compounds.

Fortifications were prepared by aliquoting solutions of the stocks or working standards onto the matrix. Multiple aliquots of different solutions were needed to fortify a small known volume onto the matrix and to have various combinations of both neutral and ionizable compounds in the fortification. As discussed above, the different combinations were designed to document the performance of the method for both parts of the assay procedure in the presence of the other compounds.

HPLC standards were prepared by aliquoting 0.10, 0.20, 0.50, 0.80 or 1.00 mL of individual standard solutions of either MK-0244 Bla, the delta 8,9-Z isomer of the Bla component, and L'649 for the ionizable assays or L'831 and L'599 (both individually and in combination) for the neutral compounds. MK-0244 Bla standards were also injected with the neutral compounds, for future reference. The resultant concentrations of the HPLC standards, following derivatization, ranged from approximately 1 ng/mL to 10 ng/mL for MK-0244 Bla, 8,9 Z, L'649 B1, L'831 B1 and L'599 B1 or approximately 2 to 20 ng/mL for the combined standard of L'831 B1 plus L'599 B1.

Solutions of the MK-0244 standard were prepared before this study began, during methods development, and fresh solutions were prepared for the validation. These solutions of different ages were compared as a measure of standard solution stability under the conditions of storage (MEMO: Hicks to Wehner, August 7, 1992, revised May 3, 1993). Solutions of the metabolites have also been compared (MEMO: Morneweck to Wehner, December 14, 1992). The solutions were stable under the conditions of use and for the time periods used.

Table III-A
Summary of Stock Standard Solutions
(Study No. 93670)

Std No.	Compound	L-Number	As is Purity %	Final Concentration mcg/mL
#402	MK-0244 B1	L-656,748-052S003	93.6	97.53
	MK-0244 B1a		3.8	3.96
	MK-0244 B1b			
#399	8,9 isomer B1a	L-695,638-001C001	92.9	108.1
#396	L'649 B1	L-653,649-005S001	95.6	100.6
#393	L'599 B1	L-660,599-000N004	94.4	106.1
#292	L'831 B1	L-657,831-000S008	89.4	101.1

Table III-B
Summary of Working Standard Solutions
(Study No. 93670)

Std. No.	Compound	Std. Used for Prep.	Final Concentration ng/mL
#408	MK-0244 B1	#402	6050 (B1a), 245 (B1b)
409	MK-0244 B1	402	2540 (B1a), 103 (B1b)
410	MK-0244 B1	409	254 (B1a), 10 (B1b)
411	MK-0244 B1	409	50.7 (B1a), 2.1 (B1b)
#412	8,9 isomer B1a	#399	6060
413	8,9 isomer B1a	399	2600
414	8,9 isomer B1a	413	260
415	8,9 isomer B1a	413	51.9
#416	L'649 B1	#396	6030
417	L'649 B1	396	2410
418	L'649 B1	417	241
419	L'649 B1	417	48.3
#420	L'599 B1	#393	5940
421	L'599 B1	393	2550
422	L'599 B1	421	255
423	L'599 B1	421	50.9
#424	L'831 B1	#292	6070
425	L'831 B1	292	2430
426	L'831 B1	425	243
427	L'831 B1	425	48.5
#428	MK-0244	#409	50.7
	L'649	417	48.3
#429	L'599	#421	50.9 99.4 (Total
	L'831	425	48.5 L'599 + L'831)

Table III-C

**Combinations of Compounds Fortified in
Leafy Vegetables or Cole Crops
(Study No. 93670)**

n	Protocol ID	Compounds (in ng/g approx. conc.)					
		<u>MK-0244</u>	<u>B1a</u>	<u>B1b</u>	<u>8.9</u>	<u>L'649</u>	<u>L'599</u>
15	a	0	0	0	0	0	0
1	b	120	5	0	0	0	0
4	c	5	NI	0	5	0	0
2	d	0	0	0	5	0	5
5	e	0	0	5	0	0	0
8	f	0	0	5	5	5	5
4	g	120	NI	120	120	120	120
7	h	5	NI	5	5	5	5
4	i	50	NI	50	50	50	50
2	j	0	0	5	5	0	5
4	k	5	NI	5	0	5	0
3	l	5	NI	0	0	0	5
5	m	0	0	0	0	5	5
3		120	-5	0	50	50	50
3		120	NI	0	50	50	50
2		50	NI	0	120	5	5
3		120	-5	0	0	120	120
3		0	0	50	5	5	0
4		5	NI	0	5	5	5
8		120	NI	0	120	120	120
2		5	NI	0	120	5	5
2		0	0	0	5	5	0
2		NI	5	0	0	0	0
1		0	0	5	0	5	5
1		0	0	5	10	0	5

n = number of samples successfully performed in the validation.

NI = not determined because below or above standard curve.

(3) Conditions

Four different combinations of HPLC instruments were used in the method validation.

HPLC Instrument C:

Pump: Spectra-Physics 8700XR.
Autoinjector: Spectra-Physics 8780XR;
 injection volume = 50 microliters
Column Heater: FIATron CH-30, set at 35 degC.
Detector: Waters 470 fluorescence; excitation set
 at 365 nm and emission set at 470
 nm; Xenon lamp.
Integrator: Spectra-Physics Chromjet model 4400;
 chart speed 0.25 cm/min.
Column: ES Industries Chromegabond MC-18
 (15 cm x 4.6 mm id).
Guard Column: ABI/Rainin OD-GU RP-18; (30 x 4.6 mm.)
Mobile Phase: 94:6 methanol:water at 1 mL/min.

HPLC Instrument F:

Pump: Spectra-Physics 8800.
Autoinjector: Spectra-Physics 8780;
 injection volume = 50 microliters
Column Heater: FIATron CH-30, set at 35 degC.
Detector: Waters 470 fluorescence; excitation set
 at 365 nm and emission set at 470
 nm; Xenon lamp.
Integrator: Varian 4270; chart speed 0.25 cm/min.
Column: ES Industries Chromegabond MC-18
 (15 cm x 4.6 mm id).
Guard Column: ABI/Rainin OD-GU RP-18; (30 x 4.6 mm.)
Mobile Phase: 94:6 methanol:water at 1 mL/min.

HPLC Instrument R80L-123:

Pump: Spectra-Physics 8800.
Autoinjector: Waters WISP 710B;
 injection volume = 50 microliters
Column Heater: FIAtron CH-30, set at 35 degC.
Detector: Hitachi F-1050 fluorescence; excitation
 set at 365 nm and emission set at
 470 nm; Xenon lamp.
Integrator: Spectra-Physics model 4270;
 chart speed 0.25 cm/min.
Column: ES Industries Chromegabond MC-18
 (15 cm x 4.6 mm id).
Guard Column: Alltech Absorbosphere C18 Cartridge;
 (10 X 4.6 mm)
Mobile Phase: 93:7 methanol:water at 1 mL/min.

HPLC Instrument M:

Pump: Spectra-Physics 8700XR.
Autoinjector: Waters WISP 710B;
 injection volume = 50 microliters
Column Heater: None - run at ambient temperature.
Detector: Waters 470 fluorescence excitation set at
 365 nm and emission set at 470 nm;
 Xenon lamp.
Integrator: Spectra-Physics Chromjet Model 4400;
 chart speed 0.25 cm/min.
Column: ES Industries Chromegabond MC-18
 (15 cm x 4.6 mm id).
Mobile Phase: 93:7 methanol:water at 1 mL/min.

(4) Data Handling and Calculations

The samples were calculated versus the external standard curves, as mentioned in the method summary above, using the peak areas at the appropriate retention times. Standards for the ionizable compounds consisted of MK-0244 B1a, delta 8,9 isomer B1a and L'649 B1. Standards for the neutral compounds consisted of L'831 or L'599 alone and an approximately equal mixture of L'831 and L'599 to obtain a combined peak area for a total value. A linear regression fit to the standard curve data (plotting peak area versus concentration) generated an equation which was then used to calculate the concentration of the samples. The procedure used for calculating the results was described in a memo Wehner to Egan, dated July 27, 1992 and entitled "Validation of RS/1 Procedure to Calculate Residue Results." Table IV summarizes the standard curve information for each set.

The recovery of the delta 8,9 isomer was calculated versus a standard curve of its authentic substance as well as versus a standard curve of the MK-0244 B1a standard. The recovery of MK-0244 B1b was determined from a MK-0244 B1a standard curve. The recovery of L'599 fortified without the presence of L'831 was calculated versus a standard curve of the L'599 authentic substance as well as versus a standard curve of a mixture of L'599 and L'831. The L'831 individual recoveries were similarly calculated versus its own authentic substance as well as versus the mixture. This was to demonstrate the use of a more limited number of standard curves for the determination of incurred residue which could contain an unknown combination of MK-0244 + delta 8,9 isomer or L'599 + L'831.

(5) Deviations to the Method and Protocol

There were no deviations to Analytical Research Method 244-92-3, as described in the June 22, 1992 version, since this validation was intended to demonstrate what procedures worked and the appropriate stopping places. There was a protocol deviation in that 2 samples were not spiked and successfully recovered for compound combination b. This did not have a material impact on the results of this study because there were many other combinations investigated, including some not specified in the protocol.

Table IV

**Summary of HPLC Injection Set Parameters for Validation
of MK-0244 Total Toxic Residue Method 244-92-3 (Study No. 93670)**

Date Assay Started	HPLC Date	A or B Fraction Assayed	HPLC Table ID	Slope	Interc.	R-sq
CELERY						
05/18/92	05/19/92	B	CEL B1	24010	-15790	0.9851
•	05/20/92	A6	CELA1	18160	-786.3	0.9905
•	05/20/92	AP	CELA1P	21260	-3457	0.9955
•	05/26/92	AP	CELA1RP	17330	1623	0.9723
•	05/26/92	A6	CELA1R	16520	-147.5	0.9869
05/26/92	05/26/92	A6	CELA2	35360	-2252	0.9808
•	05/26/92	AP	CELA2P	38310	6312	0.9944
•	06/01/92	B	CELB2R	59600	-63830	0.9798
•	06/01/92	BP	CELB2PR	•	•	•
06/01/92	06/03/92	AP	CELA3P	54050	-13030	0.9885
•	06/03/92	A6	CELA3	50910	-16020	0.9907
•	06/03/92	AZ	CELA3Z	48130	-23470	0.9935
•	06/05/92	B	CELB3	60200	-32900	0.9953
•	06/05/92	B5	CELB35	•	•	•
•	06/05/92	BP	CELB3P	•	•	•
06/09/92	06/09/92	AP	CELA4P	56440	1817	0.9998
•	06/09/92	AZ	CELA4Z	50740	-16530	0.9836
•	06/09/92	A6	CELA4	52290	1425	0.9976
•	06/11/92	B	CELB4	49950	-17020	0.9952
•	06/11/92	B5	CELB45	51370	-27710	0.9806
•	06/11/92	BP	CELB4P	•	•	•
•	06/12/92	B	CELB4R	56270	-45150	0.9978
LETTUCE						
05/26/92	05/27/92	B	HLV02B58	22420	-13030	0.9988
•	05/27/92	B8	HLV02B8	20030	-6379	0.9972
•	05/27/92	B5	HLV02B5	18810	-2910	0.9880
•	05/27/92	BP	HLV02B	•	•	•
06/02/92	06/03/92	B8	HLV03B8	20990	-2833	0.9810
•	06/03/92	B	HLV03B58	22760	2626	0.9951
•	06/03/92	B5	HLV03B5	23660	-2317	0.9940
•	06/03/92	BP	HLV03B	•	•	•
06/10/92	06/10/92	AP	HLV04A2	17860	1924	0.9936
•	06/10/92	AZ	HLV04AZ	16920	2406	0.9906
•	06/10/92	A6	HLV04A6	17130	-2538	0.9992
06/15/92	06/15/92	AP	HLV05A2	19750	3086	0.9936
•	06/15/92	AZ	HLV05AZ	17050	402.6	0.9931
•	06/15/92	A6	HLV5A6	15790	3205	0.9940
•	06/18/92	B	HLV05BRI58	21150	-10400	0.9924
•	06/18/92	B5	HLV05BRI5	20620	-3822	0.9983
•	06/18/92	BP	HLV05BRI	•	•	•

A = Ionizable Fraction - AP = Parent MK-0244 standard curve (calculations include the 8.9 isomer when present)

A6 = L'649 B1 standard curve

AZ = B1a 8.9 isomer standard curve

B = Neutral Fraction - B = Metabolites L'599 + L'831 standard curve

B5 = L'599 B1 standard curve

B8 = L'831 B1 standard curve

BP = MK-0244 B1a standard curve derivatized with B assay

Interc. = Intercept of Linear Regression Equation

R-sq = Coefficient of Determination * = Not calculated

Table IV (Continued)

**Summary of HPLC Injection Set Parameters for Validation
of MK-0244 Total Toxic Residue Method 244-92-3 (Study No. 9367^o)**

Date Assay Started	HPLC Date	A or B Fraction Assayed	HPLC Table ID	Slope	Interc.	R-sq
BROCCOLI						
06/01/92	06/01/92	A6	VPB01A6	22390	-1589	0.9789
*	06/01/92	AP	VPB01AP	23720	255.7	0.9935
*	06/02/92	B	VPB01B	30060	-11100	0.9920
*	06/04/92	B	VPB01BS	25750	-7067	0.9952
*	06/04/92	BP	VPB1BSP	18740	955	0.9994
06/04/92	06/04/92	A6	VPB026	23030	-9332	0.9736
*	06/04/92	AZ	VPB02Z	14990	3927	0.9891
*	06/04/92	AP	VPB02P	22650	-5462	0.9872
*	06/05/92	B	VPB02B	27880	-20450	0.9909
*	06/05/92	B	VPB2558	27880	-20450	0.9909
*	06/05/92	B5	VPB025	25750	-2737	0.9987
*	06/05/92	BP	VPB02BP	21060	-8796	0.9772
*	06/08/92	AZ	VPB02ZD	14200	8114	0.9869
*	06/08/92	A6	VPB026D	26020	-11900	0.9843
*	06/08/92	AP	VPB02PD	24230	-5555	0.9848
*	06/08/92	AP	VPB2AZDP	24230	-5555	0.9848
CABBAGE						
05/13/92	05/15/92	AP	MV01RAP	5497	-1700	0.9909
*	05/15/92	AZ	MV01RAZ	5075	-389.9	0.9896
*	05/15/92	A6	MV01RA6	4582	-855.2	0.9885
*	05/18/92	B5	MV01B5	5856	-445.4	0.9960
*	05/18/92	B8	MV01B8	6251	-2013	0.9868
*	05/18/92	B	MV01B	6425	-2970	0.9982
05/21/92	05/22/92	AP	MV02AP	4888	-1719	0.9867
*	05/22/92	AZ	MV02AZ2	5481	-3469	0.9893
*	05/22/92	A6	MV02A6	4106	-1504	0.9708
*	05/22/92	B5	MV02B5	6140	-1070	0.9987
*	05/22/92	B8	MV02B8	5728	-2874	0.9796
*	05/22/92	B	MV02B	5756	-1571	0.9952
05/27/92	05/28/92	AP	MV03AP	5665	-3015	0.9777
*	05/28/92	AZ	MV03AZ	5498	-2882	0.9911
*	05/28/92	A6	MV03A6	4849	-1646	0.9852
*	06/01/92	B5	MV03B5	6323	-997.5	0.9998
*	06/01/92	B8	MV03B8	4334	-723.3	0.9362
*	06/01/92	B	MV03B	5646	-2690	0.9923
*	06/01/92	BP	MV03PB	4439	-1543	0.9816

A = Ionizable Fraction - AP = Parent MK-0244 standard curve (calculations include the 8,9 isomer when present)

A6 = L'649 B1 standard curve

AZ = B1a 8,9 isomer standard curve

B = Neutral Fraction - B = Metabolites L'599 + L'831 standard curve

B5 = L'599 B1 standard curve

B8 = L'831 B1 standard curve

BP = MK-0244 B1a standard curve derivatized with B assay

Interc. = Intercept of Linear Regression Equation

R-sq = Coefficient of Determination

* = Not calculated

Table IV (Continued)

**Summary of HPLC Injection Set Parameters for Validation
of MK-0244 Total Toxic Residue Method 244-92-3 (Study No. 93670)**

Date Assay Started	HPLC Date	A or B Fraction Assayed	HPLC Table ID	Slope	Interc	R-sq
CABBAGE (cont'd)						
06/03/92	06/03/92	AP	MV04AP	6550	-405.5	0.9969
*	06/03/92	AZ	MV04AZ	5619	-495.9	0.9801
*	06/03/92	A6	MV04A6	5317	311.4	0.9942
*	06/04/92	B5	MV04B5	6624	-446.8	0.9997
*	06/04/92	B8	MV04B8	5304	-1184	0.9949
*	06/04/92	B	MV04B	5990	2959	0.9972
*	06/04/92	BP	MV04PB	5486	-644.2	0.9998
05/27/92	06/10/92	AP	MV03AP	6571	213.8	0.9994
*	06/10/92	AZ	MV03AZ	6290	1217	0.9981
*	06/10/92	A6	MV03A6	5096	1948	0.9942
*	06/11/92	B5	MV03B5	5226	584.7	0.9973
*	06/11/92	B8	MV03B8	4740	198.2	0.9966
*	06/11/92	B	MV03B	5150	-441.2	0.9970
*	06/11/92	BP	MV03PB	4403	1647	0.9940

A = Ionizable Fraction - AP = Parent MK-0244 standard curve
(calculations include the 8.9 isomer when present)
A6 = L.649 B1 standard curve
AZ = B1a 8.9 isomer standard curve
B = Neutral Fraction - B = Metabolites L.599 + L.831 standard curve
B5 = L.599 B1 standard curve
B8 = L.831 B1 standard curve
BP = MK-0244 B1a standard curve derivatized with B assay
Interc = Intercept of Linear Regression Equation
R-sq = Coefficient of Determination
* = Not calculated

III. RESULTS AND DISCUSSION

A. Recovery and Quantitation

The validation demonstrated that the method would achieve acceptable recoveries ranging from 70-120% for both the B1a and B1b components of parent MK-0244. Acceptable recoveries for the degradates, which are expected to be minor components of the total toxic residue, range from 45-110%. During the validation, some samples deviated from these ranges of performance, but generally, the method performed within the ranges specified. Fortifications were made at concentrations from approximately 5 ng/g to 240 ng/g. Criteria were established for the performance of the method following the completion of the experimental work in the validation. These criteria were summarized separately from the method in a report entitled: "Criteria for Performance of the MK-0244 Total Toxic Residue Method, Analytical Research Method 244-92-3."

A full validation, as described in the protocol, was conducted on both lettuce and cabbage. The full validation was designed to demonstrate (1) lack of interaction between the compounds fortified, (2) reproducibility and (3) method performance. The more limited validation was used on broccoli and celery. Their validation was designed to demonstrate (1) the equivalence between matrices, (2) reproducibility and (3) method performance. Each matrix was handled by one analyst during the course of the validation. Each analyst handled only one matrix during the validation, although some of the analysts were involved in the methods development and were more experienced with each of the matrices. The statistical evaluation of the data, including the examination of the relationships between standard curves, is the subject of a separate report to be prepared by the Merck Research Laboratory Biometrics Research Department.

As mentioned in the Data Handling and Calculations section above, the delta 8,9 isomer and the L'831/L'599 were calculated from their own standard curves as well as from the parent MK-0244 or mixed L'831-L'599 standard curve. Table V summarizes the recoveries observed from both types of curves. The results demonstrate that the recoveries of the delta 8,9 isomer were essentially the same whether calculated versus its own curve or the MK-0244 B1a curve. This is reasonable since they form the same derivative. The recoveries of L'831 or L'599 also were essentially the same whether they were calculated from their individual standard curves or from the combined standard curves. The method recoveries of the

individually fortified compounds were also approximately the same as the mixed (L'599 + L'831 or MK-244 Bla + delta 8,9) recoveries, indicating that the method performed as well on the combination as on the individual compounds. Tables VI-A through -E list the recoveries observed for the compounds in each of the four matrices.

B. Assay Timing

The method validation also demonstrated those points in the procedure during which the samples could be stored frozen during some period of time (generally overnight or over a weekend) and maintain acceptable recovery. The data reported here reflect samples which were stored at the recommended places or not stored at all. The method description (version dated November 11, 1992) recommends the appropriate stopping points.

In general, the validation has demonstrated that the method can be conducted on any number of samples up to 12-15 samples simultaneously. The number of samples to be assayed in a set depends on the purpose of the assay. One successful scheme for conducting the assays of 12 samples, including untreated control and method recovery samples is for the analyst to complete the ionizable portion of the assay on one day and to complete the isolation and determination of the neutral compounds on the second day. The first and second days conclude with automatic injection of the samples and standards overnight on the HPLC, so that one has results for the ionizable compounds by the beginning of the second day and results for the neutral compounds by the beginning of the third day. An analyst can complete both portions of the assay procedure in one day, with both fractions (ionizable and neutral) being assayed overnight consecutively on the HPLC. Fewer samples can be more easily assayed in one day.

C. Storage

The stability of the MK-0244 formulation under the conditions of use is the subject of a separate study. The stability of the MK-0244 and degradate standards in solution was investigated during the course of this method validation and there was no problem with the standard stability. The data are summarized in attached memos. The storage stability of the residues on leafy vegetables (lettuce) and cole crops (cabbage) is the subject of a separate, ongoing freezer storage study (Study number 93698).

Raw data, including chromatograms and standard curves, are presented in Appendix A by analysis set. The original analytical raw data, including chromatograms and study specific notebooks, will be archived. The statistician's report on the use of the standards based on the data generated in the validation will be prepared and archived separately from the validation report. The validation data as well as the method validation final report will be archived in the Animal Science Communication Center, presently located at Merck & Co., Inc, Metropolitan Corporate Plaza, Building C, Iselin, New Jersey 08830, WBC-125. This study was initiated on May 13, 1992 when the study director signed the protocol. The study completion date will be recorded when the final report is signed by the study director.

D. Conclusions

Method 244-92-3 has been demonstrated to be valid for use on leafy vegetables (such as lettuce and celery) and cole crops (such as cabbage and broccoli) to determine the total toxic residue of MK-0244. The lower limit of quantitation has been defined as 5 ng/g for each of the individual groups determined: MK-0244 B1a (or B1b) plus its delta 8,9 isomer, L'649 B1, and L'599 B1 plus L'831 B1. The limit of detection for each group has been defined as 1 ng/g. The validation has further demonstrated that the method will achieve acceptable recoveries ranging from 70-120% for both the B1a and B1b components of the parent MK-0244. The method achieved recoveries for the degradates (or photometabolites), which are expected to be minor components of the total toxic residue, ranging from approximately 40% to 110%. Criteria were established for the use of the method whereby the degrade recoveries must be in the range of 45% to 110% for the assay of residue trial samples to be acceptable. The maximum time required to assay 12 samples for all components is 3 days, from start to finish. During the validation, convenient stopping points were identified and have been added to an updated version of the method description.

Table V
Comparison of Average % Recoveries Calculated
from Different Standard Curves
(Study No. 93670)

<u>Compound</u> <u>Forts Level</u>	<u>Matrix</u>							
	<u>Celery</u>		<u>Lettuce</u>		<u>Broccoli</u>		<u>Cabbage</u>	
	<u>Own</u>	<u>Comb</u>	<u>Own</u>	<u>Comb</u>	<u>Own</u>	<u>Comb</u>	<u>Own</u>	<u>Comb</u>
	<u>(%)</u>							
delta 8,9								
- 5.2 ng/g	105	92	89	80	NA	60	88	84
52	93	81	NA	NA	77	56	NA	NA
delta 8,9 + B1a								
10.3 ng/g	115	100	110	98	NA	68	NA	93
103	NA	NA	90	80	NA	NA	NA	77
242	NA	NA	102	92	NA	NA	NA	86
L'599								
5.1 ng/g	46	43	62	61	56	64	81	90
L'831								
4.9 ng/g	NA	NA	57	54	NA	NA	68	64
L'599 + L'831								
- 10 ng/g	NA	56	NA	52	NA	52	NA	72
- 100	NA	63	NA	51	NA	55	NA	72
240	NA	51	NA	62	NA	53	NA	74

Own = Calculated from standard curve of individual compound fortified

Comb = Calculated from MK-0244 B1a (for 8,9) or from combination of L'599 + L'831 (for L'831 or L'599).

NA = Not determined.

Results rounded to nearest unit.

Table VI-A
Individual Recoveries for MK-0244 B1
in Leafy Vegetables and Cole Crops (Study No. 93670)

COMPOUND	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY	
MK-0244 B1a	-5.1	89	98	77	96	
		93	114	83	96	
		99	87	89	118	
		90	102		114	
		85			110,\$93	
		89			114,\$85	
		89			77	
		89			96	
		AVERAGE	90.4	100	83.0	99.9
		% RSD	4.5	11	7.2	14
		50.8			73	
					81	
		AVERAGE			77	
		% RSD			7.3	
		MK-0244 B1a	121	99	107	83
102	99			85		
88				60		
88				84		
101				86		
94						
101						
98						
110						
99						
118						
91						
85						
91						
AVERAGE	97.5			103	79.6	
% RSD	9.3	5.5	14			
MK-0244 B1a	<hr/>					
AVERAGE AT ALL LEVELS	94	102	79.9	99.9		

\$ = Value of split, analyzed and calculated as separate sample

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618-244-93670

Table VI-A (Continued)

**Individual Recoveries for MK-0244 B1
in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	ROCCOLI % RECOVERY	CABBAGE % RECOVERY
MK-0244B1b	4.9	102	110	86	108
		94	110	80	106
				61	
				92	
				98	
	AVERAGE	98	110	83.4	107
	% RSD	5.7	0	17	13

S = Value of split, analyzed and calculated as separate sample

Table VI-B

**Individual Recoveries for MK-0244 B1a Delta 8,9 Isomer, with or without
MK-0244 B1a in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND@	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY		
8,9 Z	5.19		94[91] 67[56]				
		AVERAGE % RSD		80.5[73.5] 24[34]			
	5.2		108[94] 102[90]	90[85] 112[106] 62[50] 92[77]	[62] [58]	79[77] 62[62] 67[67] 92[96] 67[69] 83[85] 77[75], \$62[56] 119[115], \$106[98] 104[102], \$92[87] 100[87] 100[87] 112[96]	
		AVERAGE % RSD	105[92] 40[3.1]	89[79.5] 23[29]	[60] [4.7]	88.1[83.9] 21[19]	
		52		94[82] 92[80]		73[54] 81[58]	
			AVERAGE % RSD	93[81] 1.5[1.8]		77[56] 7.3[5.1]	
		8,9 Z AVERAGE AT ALL LEVELS		99[86.5]	84.8[76.5]	[58]	88.1[83.9]

@ = 8,9 Z = Delta 8,9 Z isomer of MK-0244 B1a; B1a+8,9 Z = Sum of MK-0244 B1a and B1a delta 8,9 Z isomer

* = Different dilution of same sample preparation

() = Average of different dilutions of same sample preparation used to calculate Average Recovery

\$ = Value of split, analyzed and calculated as separate sample

[] = Calculated from an alternate standard curve, i.e., 8,9 Z calculated from MK-0244 B1a Std Curve or B1a+8,9 Z calculated from 8,9 Z Std Curve

Table VI-B (Continued)

**Individual Recoveries for MK-0244 B1a Delta 8,9 Isomer, with or without
MK-0244 B1a in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND [®]	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY	
B1a+8,9 Z	10.3	100[1.3]	107[113]	65	85	
		100[117]	105[111]	71	99	
			87[105]		101	
			93[111]		103	
					115,\$89	
					109,\$82	
					78	
					72	
		AVERAGE	100[115]	98[110]	68	93.3
		% RSD	0[2.5]	9.8[3.1]	6.2	15
103			91[97]		64,*66(65)	
			68[82]		91	
					83,\$68	
					78	
		AVERAGE		79.5[89.5]		77
% RSD		20[12]		14		
242			107[112]		83	
			76[91]		98	
					97,\$82	
					70	
		AVERAGE		91.5[102]		86
% RSD		24[15]		14		
B1a+8,9 Z						
AVERAGE AT ALL LEVELS		100[115]	89.7[101]	68	85.4	

[®] = 8,9 Z = Delta 8,9 Z isomer of MK-0244 B1a; B1a+8,9 Z = Sum of MK-0244 B1a and B1a delta 8,9 Z isomer

* = Different dilution of same sample preparation

() = Average of different dilutions of same sample preparation used to calculate Average Recovery

\$ = Value of split, analyzed and calculated as separate sample

[] = Calculated from an alternate standard curve, i.e., 8,9 Z calculated from MK-0244 B1a Std Curve or B1a+8,9 Z calculated from 8,9 Z Std Curve

**Table VI-C
Individual Recoveries for L'649 B1
in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND [⊙]	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY
L'649	4.82	73	66	52	71
		77	87	44	48
		79	100	48	75
		87.*93(90)	95	56	62.*58(60)
		64	89	52	77
		73	62	54	93
			81	56	64
			79	75	108.*104(106)
			93	68	54.552
					89.585
					93.587
					100.595
					83.585
					81
					79
			85		
			62		
			54		
	AVERAGE	76	83.6	56.1	77.3
	% RSD	11	15	17	21
	9.64				84
48.2	48.2	88	83	68	68.*71(70)
		81	79	59.*55(57)	93.*102(97)
		95		41.*39(40)	73.571
		99			79
		83			
		114			
	AVERAGE	93.3	81	55	78.4
	% RSD	13	3.5	26	14
121	121	76	99	54	78.*79(79)
		88	69	52	96.*102(99)
		78			83.583
		86			51
		89			
		93			
		89			
		76			
		87			
		85			
84					
	AVERAGE	84.6	84	3	79
	% RSD	6.7	25	2.7	22
L'649					
AVERAGE AT ALL LEVELS		84.6	82.9	54.7	79.1

⊙ = L'649 = L-453,649 B1
 * = Different dilution of same sample preparation
 () = Average of different dilutions of same sample preparation used to calculate Average Recovery
 S = Value of split, analyzed and calculated as separate sample

Table VI-D

**Individual Recoveries for L'599 B1 or L'831 B1
in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND [ⓐ]	PORT LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY
L'599	5.1	49(47)	65(63)	55(63)	59(63)
		43(39)	59(57)	57(65)	73(80)
			57(55)		84(100), 94(96)
		65(69)			78(94), 110(112)
					71(86)
	AVERAGE	46(43)	61.5(61)	56(64)	81.3(90.1)
	% RSD	9.2(13)	6.7(10)	2.5(2.2)	21(17)
L'831	4.86		58(58)		53(53)
			64(64)		58(60)
			58(58)		53(56)
			58(49)		60(53)
			49(41)		60(56)
					68(64)
					79(76), 88(84)
			76(66), 570(68)		
	AVERAGE		57.4(54)		67.7(63.6)
	% RSD		9.3(17)		20(16)

ⓐ = L'599 = L-660,599 B1
L'831 = L-657,831 B1

[] = Calculated from an alternate standard curve, i.e., L'599 or L'831 (also) calculated from L'599+L'831 Std Curve

S = Value of split, analyzed and calculated as separate sample

ⓐ = Results from Std Curve with $r^2 > 0.9362$ (due to L'831 reagent change)

**Table VI-E
Individual Recoveries for L'599 B1 Plus L'831 B1 Combined
in Leafy Vegetables and Cole Crops (Study No. 93670)**

COMPOUND®	FORT. LEVEL (ng/g)	CELERY % RECOVERY	LETTUCE % RECOVERY	BROCCOLI % RECOVERY	CABBAGE % RECOVERY
L'599+L'831	1.97	61			
		76			
		76			
		51			
		71			
	AVERAGE	67			
	% RSD	16			
	9.96	58	57	50.552	67
		56	62	48.547	64
		62	63	51.548	59
		60	57	56.552	47
		65	54	47.555	50
		60	34	59.554	70
		60	32	48.556	86.581
		43	55	51	75.591
53		59	55	580	
45			62	83	
AVERAGE	56.2	52.6	52.4	71.5	
% RSD	13	22	8.3	19	
99.6	65	62	55.559	62	
	57	40	55.556	70	
	66		50.557	83.572	
AVERAGE	62.7	51	55.3	71.8	
% RSD	7.9	31	5.4	12	
240	46	68	56	56	
	55	50	50	73.592	
	52	69		73	
	52				
	59				
	60				
	61				
	65				
	50				
	43				
	41				
	38				
	54				
AVERAGE	52	62.3	53	73.5	
% RSD	16	17	8	20	
L'599+L'831					
AVERAGE AT ALL LEVELS	59.5	55.3	53.6	72.3	

® = L'599+L'831 = Sum of 1:1 mixture of L'599 B1 and L'831 B1
 S = Value of spin, analyzed and calculated as separate sample

References

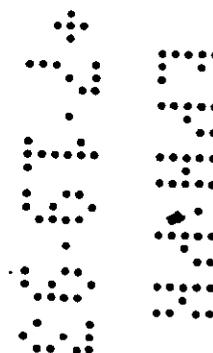
Crouch, Louis, and others in Animal and Exploratory Drug Metabolism, Pesticide Metabolism and Environmental Safety Group Report for Study PLM6

Prabhu, S.V., T. A. Wehner, R. S. Egan and P. C. Tway, (1991) "Determination of 4"-Deoxy-4"-(epimethylamino)avermectin B1 Benzoate (MK-0244) and Its Delta 8,9-Isomer in Celery and Lettuce by HPLC with Fluorescence Detection." J. Agric. Food Chem, 39, 2226-2230.

Appendix A

Raw Data

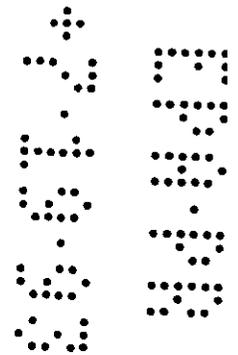
Chromatograms, RS/1 Tables and Standard Curves



040 A

618-244-93670

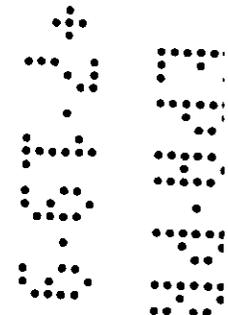
Broccoli Raw Data
Study No. 93670
Method Validation

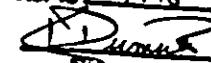


MK244 TOTAL TOXIC RESIDUE METHOD VALIDATION, DATA FOR L'649 B1 FROM L'649 B1
 STD. CURVE, BROCCOLI 001-91-1033R DAY 7 CONTROL PORTIFICATIONS
 TABLE VPB01A6, LDP, 90206-7, 2 JUNE 1992

STD/ ID	TYPE	TOTAL CONC ng/mL	TOTAL PEAK AREA	VLXD PV ML	V2 FRAC	NG/G	DIL	PORT LEVEL NG/G	Rec
*SP6.1	1 JUN	1.0mL/428	9.7	235981					
SP6.8		.8mL/428	7.7	156418					
		"	7.7	155433					
SP6.5		.5mL/428	4.8	113094					
		"	4.8	103664					
SP6.2		.2mL/428	1.9	42150					
		"	1.9	43741					
SP6.1		.1mL/428	1.0	20374					
		"	1.0	21714					
VPB01A	CON DAY7	0.0	0	5.0	0.5	0.0	10		
VPB02A	CON DAY7	0.0	0	5.0	0.5	0.0	10		
VPB03A	.2mL/418	2.5	54013	5.0	0.5	2.5	10	4.82	52
VPB04A	.2mL/418	2.1	45545	5.0	0.5	2.1	10	4.82	44
VPB05A	.2mL/418	2.3	49571	5.0	0.5	2.3	10	4.82	48
VPB06A	.2mL/418	2.7	58459	5.0	0.5	2.7	10	4.82	56
VPB10A	.2mL/418	2.5	53632	5.0	0.5	2.5	10	4.82	52
VPB11A	.2mL/418	2.6	55934	5.0	0.5	2.6	10	4.82	54
VPB12A	.2mL/418	2.7	59886	5.0	0.5	2.7	10	4.82	56
VPB06ADX10	.2mL/417	3.3	72705	50.0	0.5	33.0	100	48.20	68
VPB07ADX10	" DILX10	2.7	58998	52.5	0.5	28.4	110	48.20	59
VPB08ADX10	" "	2.0	43104	50.0	0.5	20.0	100	48.20	41
VPB07ADX3	.2mL/417	8.8	195758	15.0	0.5	26.4	30	48.20	55
VPB08ADX3	"DILX3	6.2	137979	15.0	0.5	18.6	30	48.20	39

COEFFICIENT OF DETERMINATION = 0.9789
 Amount injected = 50uL Total sample size = 10g
 * One SP6 injection not used due to interference peak.



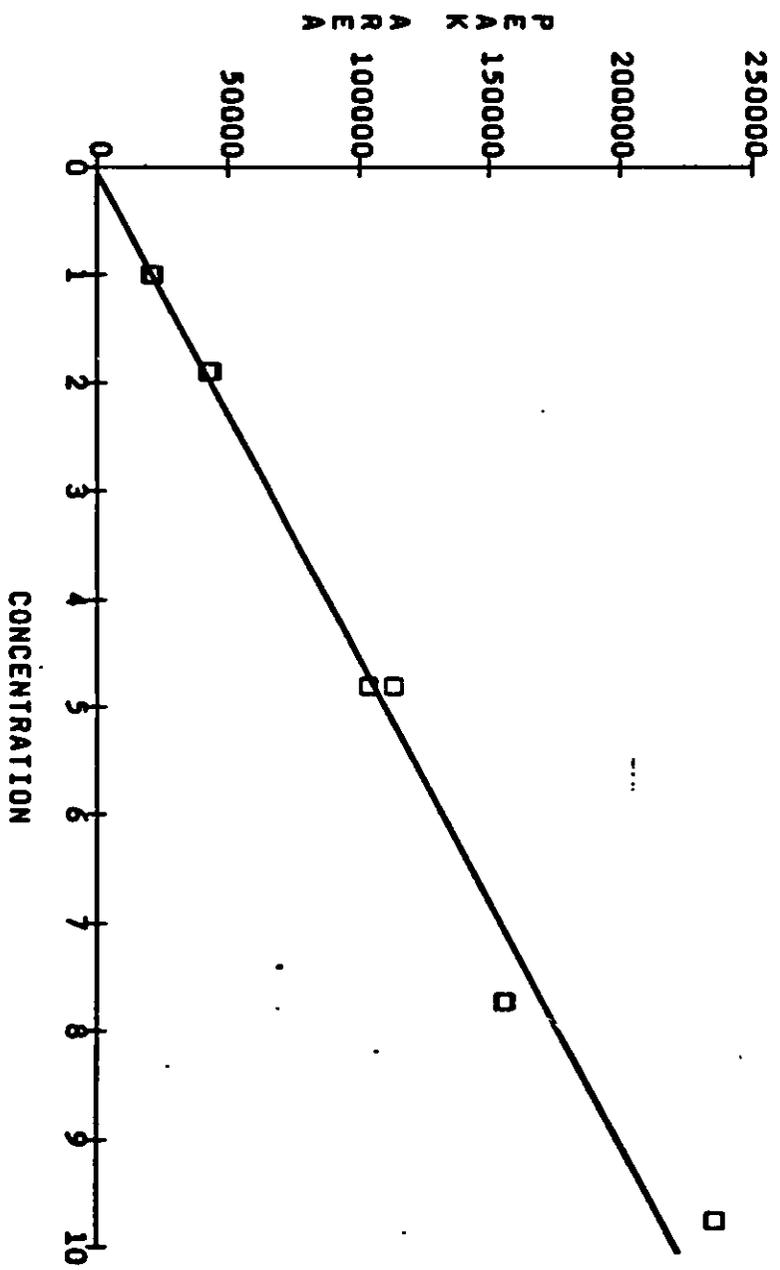
This is to certify that this document
 is a reduced copy of the original.
 The Reduction Factor is 77%

 Signature
March 21, 1993
 Date

042

618-244-93670

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STD CURVE L'649 B1, BRCCOLI 001-91-1033H
GRAPH VP801A6G, 90206-7, 2 JUNE 1992

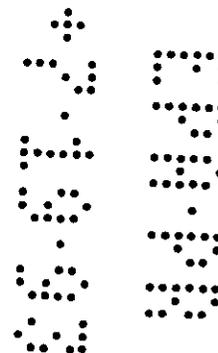


□ TOTAL
PEAK
AREA
22390 * X + -1589

NK244 TOTAL TOXIC RESIDUE METHOD VALIDATION, DATA FOR NK244 B1a, B1b +
 8,9-Z ISOMER FROM NK-0244 B1a STD CURVE, BROCCOLI 001-S1-1033R DAY 7
 CONTROL FORTIFICATIONS, TABLE VPB01AP, LDP, 90206-S, 2 JUNE 1992

STD/ ID	TYPE	TOTAL CONC ng/mL	TOTAL PEAK AREA	V1XD FV mL	V2 FRAC	WG/G	DIL	PORT LEVEL NG/G	%Rec
SP61 1 JUN	1.0mL#428	10.1	232978						
		10.1	253776						
SP6.8	.8mL#428	8.1	190075						
	"	8.1	180627						
SP6.5	.5mL#428	5.1	126839						
	"	5.1	125098						
SP6.2	.2mL#428	2.0	44353						
	"	2.0	48293						
SP6.1	.1mL#428	1.0	24367						
	"	1.0	23946						
VPB01A	CON DAY7	0.0	0	5.0	0.5	0.0	10		
VPB02A	CON DAY7	0.0	0	5.0	0.5	0.0	10		
VPB03A	.2mL#410	3.9	93937	5.0	0.5	3.9	10	5.08	77
VPB04A	"	4.2	98787	5.0	0.5	4.2	10	5.08	83
VPB05A	"	4.5	105833	5.0	0.5	4.5	10	5.08	89
VPB09A	.2mL#414Z	3.2	76391	5.0	0.5	3.2	10	5.20	62
VPB10A	" 8,9-Z	3.0	72327	5.0	0.5	3.0	10	5.20	58
VPB11A	.2mL#414Z	6.7	158743	5.0	0.5	6.7	10	10.30	65
VPB12A	.2mL#410P	7.3	173834	5.0	0.5	7.3	10	10.30	71
VPB06ADX10	.2mL#408	10.0	237468	50.0	0.5	100.0	100	121.00	83
VPB07ADX10	" DILX10	9.8	233619	52.5	0.5	103.0	110	121.00	85
VPB08ADX10	" B1a	7.2	171180	50.0	0.5	72.0	100	121.00	60
VPB06ADX3	.2mL#408	1.4	32619	15.0	0.5	4.2	30	4.90	86
VPB07ADX3	" DILX3	1.3	30301	15.0	0.5	3.9	30	4.90	80
VPB08ADX3	" B1b	1.0	24540	15.0	0.5	3.0	30	4.90	61

COEFFICIENT OF DETERMINATION = 0.9935
 Amount injected = 50uL Total sample size = 10g
 NK-0244 B1b calculation



PP#6F4628. Emamectin Benzoate on Broccoli, Brussels Sprouts, Cabbage, Cauliflower, Lettuce, and Celery.

"Method Validation: HPLC-Fluorescence Method to Determine the Total Toxic Residue of MK-0244 and Its Metabolites on Vegetables, Including Leafy Vegetables and Cole Crops," by T.A. Wehner, dated 6/3/93.

EPA ADDENDUM

- 1) ACB substituted equivalent equipment and materials.
 - A) ACB used an IKA Works T25 for the high speed homogenizer to extract samples with good results.
 - B) The commodities were homogenized in a RobtCoupe R 301 Ultra without dry ice.
 - C) In place of the Kryorack ice water bath for chilling the sample extracts before derivatizing, ACB placed the samples extracts in a freezer for 5 minutes.
- 2) ACB used a Hewlett Packard (HP) 1050 LC with a HP 1046A Fluorescence detector.
 - Column: Supelcosil LC-18, 15 cm x 4.6 mm, 3um.
 - Mobile Phase: 6% water in methanol
 - Elution: isocratic, run time 15 min.
 - Flow Rate: 1 ml/min
 - Injection volume: 50 ul
 - Xenon lamp: Excitation 365 nm, Emission 470 nm, Cut-off filter 450 nm
 - PMT gain: 18, Lamp 220 hz, Response time: 1000 msec
- 3) Users of the method as written need to incorporate these suggestions.
 - A) ACB and the petitioner's laboratory used 50 ul injection volume, and the ILV laboratory used 100 ul. The method needs to state in the section D. 'HPLC Apparatus and Chromatographic Conditions', the acceptable range of injection volume.
 - B) The 1-Methylimidazole (NMIM) and trifluoroacetic anhydride (TFAA) were kept in a desiccator in the refrigerator for storage by ACB. They were removed from the refrigerator and allowed to warm to room temperature in the desiccator to prevent moisture from condensing on the interior walls when the bottles were opened.

- C) ACB found that in the ionizable fraction of ethyl acetate (step 24) often minute amounts of water became apparent when concentrated to 1 ml. Care needs to be taken to remove this water, ACB did this by reextracting with 1 or 2 ml ethyl acetate, centrifuging and transferring the ethyl acetate to a fresh tube for concentration to 1 ml. again.
- D) ACB found that even the smallest amount of water or methanol will prevent the derivatization reaction and extreme care should be used to eliminate the presence of these solvents. ACB found that the reaction could fail even when white vapor was formed with the addition of TFAA solution.