Thiabendazole Residues on and in Citrus

Shirley M. Norman,* David C. Fouse, and Charles C. Craft

Citrus fruits coated with wax containing thiabendazole were assayed for thiabendazole by two procedures to determine what factors might affect methodology and quantitative reliability. Some factors studied were sampling, interference by citrus constituents, assay by surface stripping and by extracting ground whole fruit, spectrophotofluorometric and spectrophotometric measurements, commercial application, and experimental storage. Thin-layer chromatography eliminated citrus constituents that occasionally interfered with measurements of less than 1 ppm of thiabendazole. Surface stripping provided significantly higher residues than extracting ground whole fruit. Spectrophotometric and spectrophotofluorometric measurements were comparable; however, spectrophotofluorometric measurements were more sensitive. Thiabendazole residues varied two- to threefold from fruit-to-fruit with commercial application of thiabendazole in wax. Foam washing of lemons after storage removed 50–75% of the thiabendazole applied in wax before storage. Thiabendazole residues apparently decreased during storage.

Thiabendazole [2-(4-thiazolyl)benzimidazole] has been used commercially as a postharvest fungicide on citrus fruits since the official residue tolerance was established at 2 ppm in 1969 (Kirk, 1969). A temporary 6-ppm tolerance was established in 1971 (Johnson, 1971) for use on California and Arizona citrus. Numerous reports have been made concerning the efficacy of thiabendazole in controlling citrus fruit decay (Eckert *et al.*, 1969; Gutter, 1969; Harding, 1967; Harding and Schade, 1967; McCornack and Brown, 1968; Smoot and Melvin, 1970), but publications on residues have been limited.

Information on thiabendazole residues on and in citrus was obtained from storage tests and during the development of a thin-layer chromatography (tlc)–spectrophotofluorometric method (Norman *et al.*, 1972) to measure thiabendazole residues. Sampling techniques, sample preparation, fruit blanks, method of quantitation, fruit-to-fruit variation, and the effect of fruit type, size, and storage were studied to improve reliability of analytical techniques and residue values. This paper reports the results of these investigations.

METHODS

Thiabendazole residues determined by a tlc–spectrophotofluorometric procedure (tlc method) reported by Norman *et al.* (1972) and by a method developed by Merck and Co. (Pesticide Analytical Manual, 1969) do not differ statistically (Norman *et al.*, 1972). This report includes data obtained by both methods.

In the tlc method fruit (12 oranges, 18 lemons, or 8–10 grapefruit) are surface stripped or a ground fruit–Solka floc blend is extracted with ethyl acetate. Surface-stripping solutions, without prior cleanup, are separated by tlc. Extracts of ground fruit require preliminary cleanup before tlc. Thiabendazole is eluted from the chromatogram with methanol–0.1 *N* HCl (99 + 1) and measured spectrophotofluorometrically at 355 nm, with the excitation wavelength at 302 nm.

In the Merck method fruit (12 oranges, 18 lemons, or 8–10 grapefruit) are surface stripped or a buffered ground fruit blend is extracted with ethyl acetate. The ethyl acetate solutions are washed with 1 *N* NaOH and water, and then thiabendazole is partitioned into 0.1 *N* HCl. Thiabendazole in 0.1 *N* HCl is measured spectrophotofluorometrically at 360 nm, with the excitation wavelength at 302 nm.

All residue values reported are from experimental test fruit and high values are not typical of residues encountered commercially. Thiabendazole residues from ethyl acetate strippings of the surface of whole fruit are referred to as "surface residues," and values from ethyl acetate extracts of ground whole fruit are referred to as "whole-fruit residues."

Fluorescence and ultraviolet (uv) absorbance measurements were made with an Aminco–Bowman Model 4-8203 spectrophotofluorometer and a Bausch & Lomb 505 spectrophotometer, respectively.

RESULTS AND DISCUSSION

Background from Fruit Blanks. The tlc method provides a very low fluorescent background; untreated fruit samples read only slightly higher than the background from the thin layer and range from 0.01 to 0.05 ppm of equivalent thiabendazole. Figure 1 shows the fluorescence spectra of a typical orange blank from the tlc method and of typical orange and lemon blanks from the Merck method. Fruit blanks by the Merck method usually range from 0.01 to 0.39 ppm of equivalent thiabendazole; those in Figure 1 are equivalent to about 0.1 ppm of thiabendazole. Interference from citrus constituents, not removed in the cleanup by the Merck method, is encountered occasionally in lemons and grapefruit that have been stored for a few weeks. Citrus constituents sometimes interfere with measurements of less than 1 ppm of thiabendazole. Figure 2 illustrates the fluorescence spectra of a grapefruit blank with interference and with 1 ppm of thiabendazole added to the blank. Thiabendazole combined with this blank gives a distorted spectrum and the thiabendazole band appears as a shoulder on the curve. These interferences with the Merck method were separated into several fluorescent spots by tlc. No attempt was made to identify them. Tlc supplements the Merck method if interference is indicated by the fluorescence spectrum.

Quantitation. Both the tlc and the Merck methods use fluorometric measurements for quantitation. The tlc method gave 93% recovery, using uv absorption for quantitation, although fluorometric measurements were preferred (Norman *et al.*, 1972). Since spectrophotofluorometers are not always available, sample solutions from the Merck method were read both spectrophotofluorometrically and spectrophotometrically. The mean thiabendazole value, for 44 surface residues

Agricultural Research Service, U. S. Department of Agriculture, Pomona, California 91769.

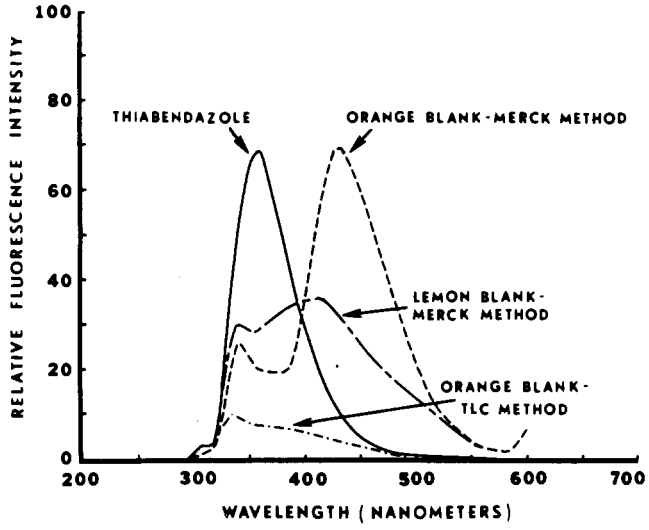

Figure 1. Fluorescence spectra of a typical orange blank from the tlc method and of thiabendazole and orange and lemon blanks from the Merck method

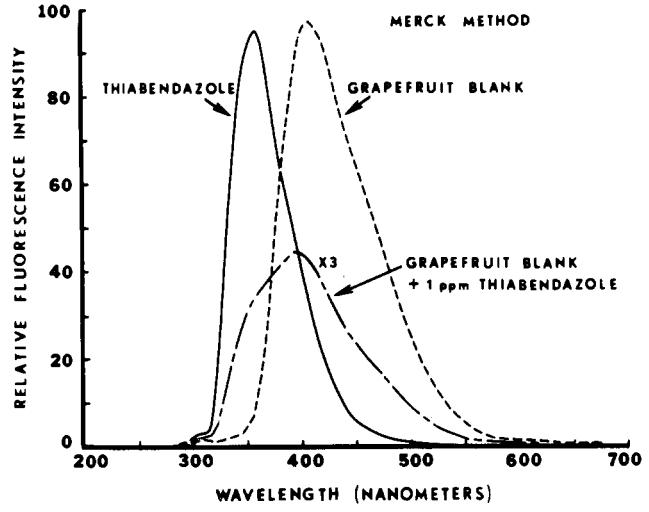

Figure 2. Fluorescence spectra of thiabendazole, a high grapefruit blank from the Merck method, and the grapefruit blank with 1 ppm of thiabendazole added

that ranged from 0.07 to 9.80 ppm of thiabendazole, was 2.26 ppm for fluorometric measurements and 2.32 ppm for absorbance measurements. The sensitivity of fluorometric measurements is not required for surface residues, except for very low values, and with appropriate adjustment of final sample volumes absorbance measurements provide comparable values. The sensitivity of the spectrophotofluorometer is required for analyses of trace residues remaining in ground-stripped fruit and pulp. Large samples are required for absorbance measurements by the Merck method for unstripped-ground fruit.

Thiabendazole Residues Following Commercial Application. To illustrate fruit-to-fruit variations, one composite sample (12–18 fruits) and 12 individual fruits were analyzed for thiabendazole surface residues for several lots of lemons and oranges treated with different levels of thiabendazole. Fruit-to-fruit variations within one lot were often two- to threefold (Table I), indicating that the amount of thiabendazole applied commercially is not uniform. Realistic compliance requirements should reflect variability of commercial wax application. Additional data would be required to establish a statistical

Table I. Thiabendazole Residues on the Surface of Individual Fruits and on Multiple Fruit Samples of Commercially Treated Arizona Lemons (Size 140) and California Oranges (Size 113)

Fruit no.	Thiabendazole, ppm						
	Lemons, lot no.					Oranges, lot no.	
	1	2	3	4	5	1	2
1	6.9	2.5	6.9	6.8	0.6	8.4	6.4
2	6.0	3.9	6.9	6.0	0.8	5.5	8.2
3	4.3	3.6	6.2	4.1	2.3	3.4	7.2
4	8.0	1.7	5.1	5.3	1.1	4.5	8.1
5	11.8	3.1	6.7	7.2	1.9	5.6	8.3
6	11.1	3.6	5.4	6.3	1.6	6.0	6.3
7	9.5	4.0	4.8	5.8	1.3	4.2	7.5
8	8.6	1.3	6.9	6.6	1.6	6.5	5.1
9	8.8	2.8	5.2	4.2	1.7	3.9	9.0
10	6.9	2.2	4.4	6.4	2.6	7.6	9.3
11	9.1	2.7	4.9	1.3	1.6	5.9	9.1
12	6.6	2.7	5.2	6.1	2.3	5.2	7.7
Mean	8.1	2.8	5.7	5.5	1.6	5.6	7.7
Range	7.5	2.8	2.5	5.9	2.0	4.9	4.2
Std dev	2.2	0.8	0.9	1.7	0.6	1.5	1.3
% C. V.	26.4	29.8	16.5	31.3	36.7	26.3	16.7
Composite	8.5[a]	3.1[a]	6.3[a]	5.7[a]	1.5[a]	5.8[b]	7.0[b]

[a] One 18-fruit composite sample. [b] One 12-fruit composite sample.

Table II. Thiabendazole Residues on the Surface of California Oranges and Lemons at Different Intervals During a Day's Application in the Packinghouse

Hours after start of application	Thiabendazole, ppm				
	Lemons,[a] lot no.		Oranges,[b] lot no.		
	1	2	1	2	3
1	7.8	7.8	4.6	9.5	8.1
2	7.2	8.3	6.8		6.5
3	8.2	7.3	5.3	9.6	6.3
4	6.6	6.5	5.2		6.3
5			5.4		5.8
6			4.5	9.3	5.6
7	6.0		4.4	8.2	5.2
8				8.9	5.0
Mean	7.2	7.5	5.2	9.1	6.1

[a] 18 fruit per sample. [b] 12 fruit per sample.

guide for sampling, but large numbers of fruit per sample with several replications are indicated if a sample is to be representative of the mean thiabendazole residue. Samples should be replicated since size of samples for surface stripping is limited by the capacity of laboratory equipment. Large numbers of fruit per sample can be ground, mixed thoroughly, and subsampled for ground fruit blends.

Thiabendazole residues on oranges of different commercial sizes were measured. Residues for sizes 72, 113, and 163 for one test were 3.5, 3.1, and 4.4, respectively. Residues for sizes 72 and 163 in other tests were, respectively, 1.9 and 2.0, 2.1 and 2.3, and 1.7 and 2.7. Variation among sizes was less than among fruits within one size (Table I), therefore, no conclusions regarding size can be drawn from the present data.

Citrus fruits were sampled at intervals during packinghouse application of wax containing different levels of thiabendazole to determine the variation of thiabendazole deposited on citrus fruit during a day. The amount of thiabendazole applied to oranges and lemons during a day's run varied (Table II), but not as much as the fruit-to-fruit variations within one sampling (compare Tables I and II). The individual oranges and composite sample for orange lot 1 in Table I (mean 5.6 ± 1.5 ppm) are from the same lot as the hourly samples for orange lot 1 in Table II (mean 5.2 ppm).

Table III. Thiabendazole Residues on the Surface of California Lemons During Packinghouse Operations

Packinghouse operation	Thiabendazole, ppm[a] Test 1	Test 2	Test 3
Before storage wax	0	0	0
After storage wax	0.7	2.7	0.3
After 11 weeks' storage at 58°F	0.5	2.2	0.2
After foam washing	0.2	0.5	0.2
After shipping wax	0.2	0.6	0.1
After simulated marketing (5 weeks at 58°F plus 1 week at 70°F)	0.1	0.5	0.1

[a] 18 fruit per sample.

Table IV. Thiabendazole Residues on the Surface of Whole Fruit Compared with That in Extracts of Whole Ground Fruit

Lot no.	Thiabendazole, ppm[a] Surface	Whole fruit	Lot no.	Surface	Whole fruit
	Oranges[b]			Oranges[b]	
1	2.8	2.4	16	2.0	1.4
2	2.0	1.4	17	2.5	2.1
3	2.8	2.1	18	4.9	4.6
4	5.0	4.3	19	3.0	2.5
5	2.8	2.1	20	3.6	2.9
6	3.4	3.0	21	10.9	6.7
7	9.6	6.5	22	2.1	1.3
8	1.7	1.4	23	7.5	4.5
9	2.5	2.0	24	3.7	3.2
10	4.4	3.8		Lemons[c]	
11	2.1	1.8	25	3.8	2.8
12	2.9	2.9	26	2.2	1.3
13	6.3	5.4	27	3.9	2.9
14	3.1	2.4	28	1.4	1.0
15	3.5	2.9			
Mean (orange and lemon residues combined)				3.8	2.9

[a] t = 4.493 (27 d.f.); P (0.01) = 2.771. [b] 12 fruit per sample. [c] 18 fruit per sample.

Table V. Thiabendazole Residues on the Surface of Whole Oranges Compared with That in Extracts of Whole Ground Oranges Before and After 3 Weeks' Storage at 46°F

Lot no.	Thiabendazole in ppm before storage[a] Surface	Whole fruit	Thiabendazole in ppm after storage[a] Surface	Whole fruit
1	2.0	1.4	1.7	1.4
2	2.8	2.1	2.5	2.0
3	5.0	4.3	4.4	3.8
4	2.8	2.1	2.1	1.8
5	3.4	3.0	2.9	2.9
6	9.6	6.5	6.3	5.4
Mean	4.3	3.2	3.3	2.9

[a] 12 fruit per sample.

Table VI. Thiabendazole Residues of California Navel Oranges during 5 Weeks' Storage at 42°F

Replication no.	Length of storage in weeks Initial	1	2	3	4	5
	Thiabendazole surface residues in ppm[a]					
1	2.3	2.4	1.6	1.5	1.2	1.2
2	2.3	2.2	1.9	1.8	1.2	1.3
3	2.3	2.6	2.1	1.9	1.5	1.4
Mean	2.3	2.4	1.9	1.7	1.3	1.3
	Thiabendazole whole fruit residue after surface stripping in ppm[a]					
1	0.1	0.3	0.1	0.2	0.3	0.2
2	0.1	0.2	0.1	0.2	0.3	0.2
3	0.1	0.2	0.1	0.2	0.3	0.2
Mean	0.1	0.2	0.1	0.2	0.3	0.2
	Total thiabendazole residue					
	2.4	2.6	2.0	1.9	1.6	1.5

[a] 12 fruit per sample.

Lemons often are waxed with a water-emulsion storage wax and stored 2–4 months at 58° for degreening. After storage, lemons are foam washed and then waxed with a solvent shipping wax. To determine whether thiabendazole incorporated in the storage wax could be washed off the fruit before shipping, three lots of lemons were analyzed for thiabendazole surface residues before and after each packinghouse step. About 20–40% of the thiabendazole disappeared from the surface of the lemons during storage and, except for the low-residue fruit, 50–75% of the remaining surface thiabendazole was removed during the foam washing (Table III). Little change was noted in the residue between foam washing and the shipping wax applications. Thiabendazole surface residues continued to decrease during the simulated marketing period. These experiments indicate that considerable but not all of the thiabendazole can be washed off lemons under normal packinghouse procedures, which is in agreement with the findings of other workers (Hayward and McCornack, 1971) on oranges.

Comparison of Surface Residues and Whole Fruit Residues. Surface stripping provided significantly higher residues than ground whole fruit analyses in 28 lots of fruit (Table IV). Apparently thiabendazole is not completely extracted from fruit blends. One sample with a surface residue of 10.9 ppm and 0.3 ppm remaining in the ground stripped fruit yielded only 6.7 ppm of thiabendazole when a ground fruit blend without prior stripping was assayed. Thiabendazole residues (in ppm) for additional subsamples of this same ground fruit blend using exhaustive extraction techniques with different solvents were 7.2 with acidified methanol, 6.4 with methanol, 6.1 with acidified methyl ethyl ketone, 7.5 with boiling 6 N HCl, and 7.2 with ethyl acetate without buffer. Other solvents (0.1 N HCl, dimethyl sulfoxide, and acetonitrile) were investigated but gave low values. An average of 6.8 ppm of thiabendazole was measured in ground fruit blends when acidified with HCl, dried in an oven at 100°, and then extracted exhaustively with ethyl acetate. Values from the exhaustive extractions did not approach the 10.9 ppm obtained by surface stripping. With the exception of ethyl acetate, the solvents used for the exhaustive extractions are impractical for routine analyses because additional citrus constituents extracted with thiabendazole make the extracts extremely difficult to clean up for quantitation.

Rosenblum and Meriwether (1970) reported that 8–21% of the thiabendazole applied to the surface of oranges with a 0.1% aqueous suspension diffused into the inner peel during 4 weeks' storage. For this reason, quantitative measurement of thiabendazole residues in citrus should require either the surface residue plus the content of the ground stripped fruit or analyses of ground fruit blends without prior surface stripping. Even after storage, however, residues from surface stripping were higher than from ground fruit analyses for six lots of fruit (Table V). After 3 weeks' storage, the average surface residue decreased about 23% and whole fruit residues decreased about 11% from the initial values. The greater loss in surface residue, as compared to the whole fruit residue, could indicate that some thiabendazole diffused into the fruit. However, the surface residue value was still 13% higher than the whole fruit residue.

About 98% of thiabendazole added to surface strippings and 85% of thiabendazole added to ground fruit can be recovered when fortified between 0.2 and 6.0 ppm (Norman *et al.*, 1972). It was not determined whether the thiabendazole is degraded or is bound in some way by the citrus constituents to prevent extraction from the ground fruit. Until more efficient procedures are developed to extract thiabendazole from ground fruit, residues from the surface and the ground stripped fruit should be combined.

To determine the change in thiabendazole residues on navel oranges during 5 weeks' storage at 42°, three replications of oranges were sampled weekly (Table VI). Surface residues and ground stripped fruit residues were determined. Little difference was noted the first week, but total residue decreased 18, 21, 31, and 38% of the initial value after 2, 3, 4, and 5 weeks, respectively. The surface residue decreased with storage and the ground stripped fruit residue did not increase substantially. Therefore, for highest residue values after storage, residues from the surface and ground stripped fruit should be combined. The stability of thiabendazole on citrus and the effect of constituents normally found in citrus on thiabendazole are not known. Until more information is available, the apparent loss of thiabendazole during storage cannot be explained.

Stability of Citrus-Stripping Solutions. Stripping solutions must be analyzed as soon as possible for highest residue values. Nineteen stripping solutions with an average of 1.86 ppm of thiabendazole decreased during 4 weeks' storage at 50° to an average of 1.32 ppm, a loss of 29%. Cause of this loss has not been determined. Our experience shows that stripping solutions can be kept in a freezer for a few days, but long storage studies have not been carried out.

Interlaboratory Variation. Thiabendazole results from separate samples from the same treatments by three or four different laboratories were compared. The results varied as follows: (test 1) 2.8, 2.5, 2.6, and 3.1; (test 2) 2.1, 1.5, 1.8, and 2.0; (test 3) 5.0, 4.6, 4.3, and 4.7; (test 4) 2.8, 2.3, and 2.3; (test 5) 3.4, 4.0, and 3.4; and (test 6) 9.4, 9.9, and 9.4. The differences among laboratory results are small, since several days lapsed between some analyses, methods varied among laboratories, and samples and sample sizes were not identical. With standardization of methods, adequate sampling, and the combination of surface residues and the residue in the ground stripped fruit, interlaboratory variation should be minimized.

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Determination of Systemic MBC Residues in Food Crops Treated with Benomyl Fungicide

Earl R. White* and Wendell W. Kilgore

A procedure for the determination of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] and its decomposition product, MBC (methyl 2-benzimidazole carbamate), on various food crops is described. The compounds are first extracted with benzene and partitioned into 0.1 *N* hydrochloric acid. The acidic layer is washed several times with chloroform and then neutralized to pH 7.8–8.2 with concentrated sodium hydroxide. The single residual product, MBC, is partitioned into ethyl acetate, concentrated by evaporation, and subsequently developed on a commercially prepared thin-layer chromatogram containing a fluorescent indicator. The spot on the developed chromatogram having the same retention time as the standard methyl 2-benzimidazole carbamate is extracted with methanol and measured in an ultraviolet spectrophotometer at 287 mμ. The lower limit of sensitivity for this method is 0.05 ppm. Overall average recovery of benomyl residues obtained from fortified control samples was 87%. Recovery from grown-in labeled MBC residues averaged 83%.

Benlate benomyl fungicide (du Pont) [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] has been registered for use in the control of certain diseases of stone fruits (EPA, 1971). The decomposition product, MBC (methyl 2-benzimidazole carbamate), is also a broad spectrum fungicide and is potentially useful at the same low dosage rates as benomyl (Kilgore and White, 1970).

Current analytical procedures for the determination of benomyl residues are based upon the fluorometric and colorimetric methods developed by Pease and Gardiner (1969). The present paper describes a simple but sensitive thin-layer chromatographic and ultraviolet spectrophotometric procedure designed for the positive identification and quantita-

Department of Environmental Toxicology, University of California, Davis, California 95616.