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A REVIEW OF METHODS FOR THE RESIDUE ANALYSIS OF THE SYSTEMIC FUNGICIDES BENOMYL, CARBENDAZIM, THIOPHANATE METHYL AND THIABENDAZOLE

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A REVIEW OF METHODS FOR THE RESIDUE ANALYSIS OF THE SYSTEMIC FUNGICIDES BENOMYL, CARBENDAZIM, THIOPHANATE METHYL AND THIABENDAZOLE

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Abstract - A Review on the residue analysis of the benzimidazole fungicides is presented. The literature has been reviewed critically and in detail in respect of the single steps used in the analytical procedures in order to facilitate the comparison of the described extraction, clean up and determination/detection techniques. Recommendations for adequate analytical procedures are given. Relevant metabolites are included in the review.

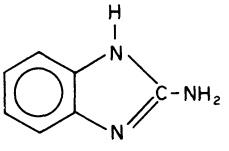
INTRODUCTION

Reviews on residue analysis of the benzimidazole fungicides have been published by Baker and Hoodless (7); Slade (73) and Watkins (89). The latter review also contains valuable information on the metabolism of these fungicides. A review on the chromatographic analysis of 66 fungicides was published by Sherma (69).

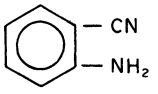
The present report reviews the literature on steps used in the various analytical procedures to facilitate comparison of extraction, clean up and determination/detection techniques.

Benzimidazole fungicides and metabolites and derivatives mentioned in this report

2-Aminobenzimidazole



o-Aminobenzonitrile



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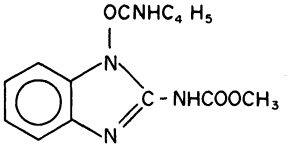

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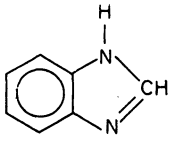
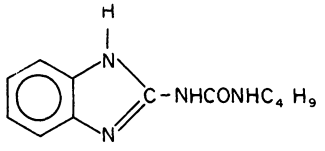
Benzimidazole



BBU



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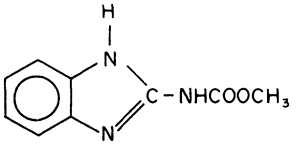
Carbendazim



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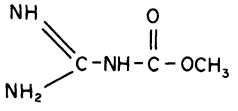
Methoxycarbonylguanidine



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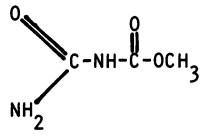
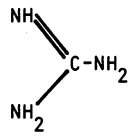


Guanidine



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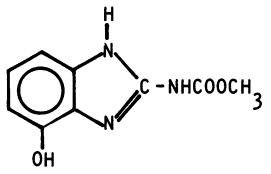

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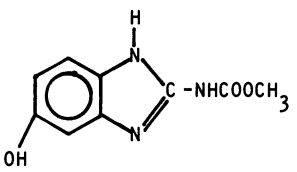

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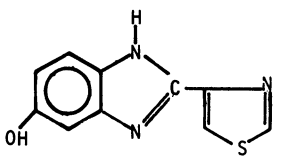
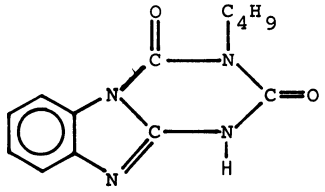
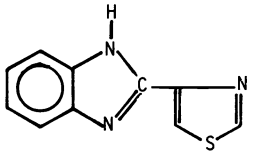


STB



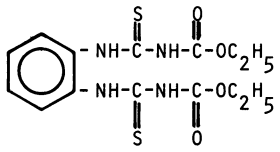
1,2,3,4-Tetrahydro-3-butyl-2,4-dioxo-s-triazino [a] benzimidazole

Thiabendazole



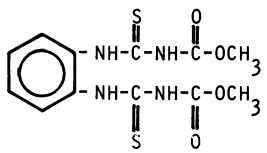
2-(4-Thiazolyl)benzimidazole

Thiophanate



1,2-Bis-(3-ethoxycarbonyl-2-thioureido)benzene

Thiophanate methyl



1,2-Bis-(3-methoxycarbonyl-2-thioureido)benzene

PROPERTIES OF BENZIMIDAZOLES RELEVANT TO ANALYSIS

Benomyl and thiophanate are characterized by their property of being easily transformed into the stable carbendazim under natural and artifical conditions.
Clemons and Sisler (19) observed the transformation of benomyl during the development of thin layer chromatograms and in aqueous solution. In the same year, carbendazim was reported to be the main metabolite of benomyl which still possesses fungitoxic action (Sims et al. (72). This observation has been subsequently confirmed by several authors.
Benomyl instability in water was demonstrated by Pease and Holt (59), Kirkland (40), White et al. (92) and Austin et al. (4). The latter reports the change to be complete within 24 hrs. at pH 3 and 20 °C.
The relative stability of benomyl in chloroform and acetone was referred to by Peterson and Edington (61), but Kilgore and White (39) showed that transformation in chloroform takes place. A systematic study on benomyl stability in various solvents was conducted by Chiba and Doornbos (16).

TABLE 1. Breakdown of benomyl to carbendazim in organic solvents (100 mg benomyl/50 ml solvent) on standing at room temperature (Chiba and Doornbos, (16))

Solvents	Time of first evidence of precipitate	Amount of precipitate 7 days after preparing solution (mg)
Benzene	Within 10 min	23.5
Ethyl ether	60 min	38.0
Ethanol	Approx. 20 hrs	49.3
Acetone	50 hrs	12.9
Ethyl acetate	50 hrs	6.8
Methylene chloride	80 hrs	6.5
Chloroform	100 hrs	2.6

Chiba (17) reports that benomyl is decomposed particularly fast in methanol and ethyl acetate at lower concentrations; for example, the half life at 10 µg/ml is less than 1 hr. The stability in water is largely dependent on the kind and amount of dispersing agent such as organic solvents and surface active agents.
Solvolysis and hydrolysis rate constants of benomyl dissolved in various organic solvents and methanol/water mixtures have been reported by Calmon and Sayag (15).
More recently Chiba and Cherniak (18) gave evidence that the solvolysis in organic solvents reaches an equilibrium, e.g. 88 % in chloroform at 25 °C.

In alkaline aqueous media, e.g. Bordeaux mixture, alkaline peeling solution, etc. benomyl is partly converted to STB (1,2,3,4-tetrahydro-3-butyl-2,4-dioxo-s-triazino[a]benzimidazole and BBU 1-(benzimidazol-2-yl)-3-butylurea (White et al. (92)). Conversion of benomyl to either carbendazim or STB and BBU is achieved artificially in most of the analytical procedures to obtain the stable carbendazim (or BBU) for determination.
For conversion of benomyl to carbendazim, Kirkland et al. (41) recommended refluxing for 4 hrs in 83 % methanol and 17 % 1 N HCl. Rouchaud and Decallonne (65) and Rouchaud et al. (66), however, report that benomyl is transformed during the clean-up when solutions are acidified.

The transformation during chromatography on alumina has been reported by Cox and Pinegar (22) and on MgO-Celite (1 + 1) by Aharonson and Ben Aziz (1). Thiophanate methyl is similarly metabolized but at a slower rate. This was demonstrated by Noguchi et al. (53), Fuchs et al. (32), Buchenauer et al. (14), Vonk and Sijpesteijn (85) and Soeda et al. (74).
Thiabendazole is a stable compound and no transformation to carbendazim was

observed under the conditions reported above with respect to benomyl.

EXTRACTION

A wide range of solvents has been used for extraction depending on the substrate, the metabolites and the conversion products.
Published methods of extraction use organic solvents such as: chloroform (Peterson and Edington (61); Corke et al. (20); Rajzman (64); Black and Neely (13)), methylene chloride (Wegman et al. (90)), ethyl acetate (Baker and Hoodless (6); Cox and Pinegar (22); Kirkland et al. (41); Pease and Gardiner (58); Vanden Heuvel et al. (84)), benzene (Rouchaud and Decallonne (65); Rouchaud et al. (66)) and acetone (Aharonson and Ben Aziz (1); Fleeker et al. (30)).
A mixed organic solvent, e.g. acetone, followed by a mixture of benzene/methanol/chloroform (1 + 1 + 1, v/v) is recommended by Siegel (71).
Quite common are mixed solvents using organic solvent and hydrochloric acid in various ratios. Sims et al. (72) used 95 % methanol containing 1 % by volume concentrated HCl to extract plants, and Kirkland et al. (41) used, 83 % methanol and 17 % 1 N HCl to extract soils. Polzhofer (63) used ethyl acetate, and NaOH at pH 8.3 for the extraction of apples and Dąbrowski and Czarnik (23) used ethyl acetate and NH_4OH for the extraction of mushrooms.
As reported in section 2, benomyl is converted totally or partially during extraction. For the same purpose the mixture acetone + M ammoniumchloride (1 + 1, v/v) is recommended by Austin and Briggs (3).
A comparison of the efficiencies of some organic solvents and methods in the extraction of tobacco is presented by Fernandes and Cole (29).

TABLE 2. Quantities of carbendazim (µg/g) extracted from tobacco by soxhlet and cold extraction using different solvents (Fernandes and Cole (29))

	Methods	
Solvents	Soxhlet	Cold (22 C)
Methanol	22.8	16.9
Ethanol	20.4	15.7
Aceton	15.2	9.9
Chloroform	16.2	9.4

Another comparison of extraction methods with respect to soil is presented by Austin and Briggs (3). In this study, 2-14-C-carbendazim and 2-14-C-benomyl were applied to soil in the field 1 week prior to the analysis.
The recoveries, expressed as percentages of radioactivity determined by combustion, are summarized in the following table.

TABLE 3. Recovery of 1 week old residues of carbendazim and benomyl "(see Note a)" (Austin and Briggs (3)

Extraction solvent	Radioactivity extracted in % of carbendazim
Acetone	15.4
M NH_4Cl	12.9
Acetone + M ammonium chloride (1 + 1)	71.3
Ethanol + M ammonium chloride (1 + 1)	68.0
Methanol + M ammonium chloride (1 + 1)	64.2
Methanol + M hydrochloric acid (4 + 1)	22.2

A comparison of the extraction efficiency of acetone/M ammonium chloride with that of chloroform shows that the efficiency of chloroform is very much dependent on the soil pH.

TABLE 4. Extraction of fresh carbendazim in soil by acetone/M ammonium chloride and by chloroform "(see Note a)" (Austin and Briggs (3))

pH	Acetone/M ammonium chloride [%]	Chloroform [%]
6.61	84.1	71.6
6.00	86.8	70.2
5.32	78.9	21.5
4.52	80.3	7.9

To remove aged residues from soils Baude et al. (10) used a mixture of methanol/hydrochloric acid (3 + 1, v/v) and boiled the sample for 24 hrs under reflux. 64.6 % of the total 14-C added as benomyl could be recovered from soil (Delaware) treated 12 months prior to the analysis with 5 lb a.i./ acre. The rest (35 %) could be recovered only after direct combustion of the sample and determination the $14\text{-}CO_2$.

A comparison with other extraction methods was not made by these authors. Information on the "total extractable residue" when extracting plants with various solvents is also present in the literature.
Siegel (71) extracted 14-C labeled residues from strawberry plants (grown in a 14-C-carbendazim spiked nutrient solution) with cold acetone followed by a further extraction with benzene/methanol/chloroform (1 + 1 + 1, v/v). The working up of the extracts of the foliage gave an organic and a water phase, both containing residue.

Note a. Extraction method: shaking for two hours.

As a result, after a period of 36 and 88 days after treatment he found that 65 % and 51 % respectively, of the radioactivity in the extracts, was contained in the organic phases; 19 % and 28 % respectively, in the water phases; 16 % and 21 % respectively, remained unextracted.
A similar distribution was observed by Siegel and Zabbia (70) after treatment of dwarf peas grown in nutrient solution. On acidic or enzymatic cleavage the composition of the water-soluble residue was found for both experimental periods to be 4 % carbendazim; 29 % and 25 % respectively, 2-amino-benzimidazole; and 67 % and 71 % respectively, remained unidentified (two non-fungitoxic substances). The liberation of a large percentage of radioactivity from the unextractable residue by NaOH, but not by acid treatment, suggests that a portion of these unidentified products were associated primarily with hemicelluloses of the plant cell walls.
In a similar experiment with melon plants grown in a nutrient solution spiked with H-3-carbendazim Rouchaud et al. (66) showed that carbendazim, 2-aminobenzimidazole and the metabolites benzimidazole and o-aminobenzonitrile can be extracted quantitatively with benzene as long as they are contained in the plant in the unconjugated form. Extraction with water following the benzene extraction removed some more radioactivity. In the nonhydrolyzed aqueous extracts from treated plants neither carbendazim nor 2-aminobenzimidazole were observed. On acidic hydrolysis carbendazim and 2-aminobenzimidazole, but none of the other previously mentioned metabolites, were detected. The distribution of detected compounds is as follows:

TABLE 5. Concentration and distribution of the identified metabolites of benomyl in melon leaves. Plants were exposed to 20 µg per ml benomyl during two months and the treatment was started at the five-leaf stage (Rouchaud et al. (66)

	Concentration in melon leaves (µg/g of fresh weight)	
	Free compounds (in benzene extract)	Conjugated compounds (in aqueous extract)
Metabolites		Acidic hydrolysis
Carbendazim	11.0	0.5
2-Aminobenzimidazole	3.0	1.1
Benzimidazole	1.2	0.0
o-Aminobenzonitrile	1.1	0.0
Aniline	0.2	0.0

An enzymatic digestion of animal tissue with a mixed glucuronidase/sulfatase preparation was used by Vanden Heuvel et al. (84) prior to extraction with ethyl acetate.
Special attention to the extracting procedure is necessary if the true residue of the unaltered benomyl is to be determined. The techniques referred to above are precluded, since carbendazim will continuously form from residual benomyl while in solution at these levels. Therefore, the extraction first involves macerating the crop tissue in 1 N aqueous NaOH and immediately refluxing the mixture (Baude et al. (9)). Under these conditions benomyl is converted to 1-(benzimidazole-2-yl)-3-butylurea (BBU) and carbendazim to 2-aminobenzimidazole (White et al. (92)). The reaction is almost quantitative (90 %) when the benomyl concentrations do not exceed 100 µg per ml.

After this first reaction step the BBU is extracted from the refluxed solutions using ethyl acetate (Baude et al. (9)). The same initial alkaline treatment of the sample has to be carried out when the intact benomyl residue in soil is to be determined (Baude et al. (10)).

CLEAN-UP PROCEDURES

The required efficiency of the clean-up procedure depends largely on the specificity and sensitivity of detection in the procedure selected for determination.
Obviously, the least demanding procedures with regard to the efficacy of clean-up are bioassay methods on thin layer plates. The extracts can be spotted onto the plates without further clean-up and can be developed (Fernandes and Cole (29); Wegman et al. (90); Baker and Hoodless (6); Cox and Pinegar (22); Ben Arie (11); Ellis and Sinclair (26); Homans and Fuchs (38)).
For all other methods of determination a more or less rigorous clean-up is necessary.

The weakly basic property of the =N-atom in the benzimidazoles can be utilized for an efficient partition step in the clean-up procedure. The residue dissolved in ethyl acetate (or benzene, Rouchaud and Decallonne (65) or chloroform, Rajzman (64)) can be extracted very easily with diluted hydrochloric acid and reextracted into ethyl acetate (Chloroform) upon adjusting the acid aqueous phase back to pH 8 - 9 (Sims et al. (72); Cox and Pinegar (22); Kirkland et al. (41); Aharonson and Ben Aziz (1); Austin et al. (2); Rouchaud and Decallone (65); Rouchaud et al. (66); Tanaka and Fujimoto (80)) or into chloroform after adjusting the aqueous phase to pH 6.5. After this clean-up step, the residue (carbendazim) can be reextracted with 0,1 M HCl and determined, e.g. by UV in the aqueous solution (see under section Colorimetric and Spectrophotometric Methods.

TLC was incorporated as a clean-up step in the UV-spectrophotometric determination by White and Kilgore (91) to eliminate interfering substances. For the separation of carbendazim from plant and other materials, column chromatography has been used in several cases. Florisil was used by Douch (25). A combination of two adsorbents; an upper layer of magnesium oxide/"Celite" (1 + 6) and a lower one of alumina (grade III) was used by Aharonson and Ben Aziz (1). Benomyl is converted to carbendazim in this column by the magnesium oxide/"Celite" layer. Thiabendazole is subsequently eluted with ethyl acetate and carbendazim with ethanol/ethyl acetate (1 + 1). It was found that interfering fluorescent compounds were eliminated.

Neutral alumina was used for the column chromatographic clean-up by Watkins (88) and alumina with 6 % water and chloroform or ethyl acetate as elution solvent by Cox and Pinegar (22). Siegel (71) used the cation exchange Dowex 50 x 8 - 200 resin in a column to separate carbendazim from interfering substances. The elution step was done in the batch mode using 4 N NH_4OH as eluant.
Thin layer chromatography and high speed liquid chromatography are separation techniques (or clean-up procedures), but they are usually regarded as detection/determination procedures and will, therfore, be discussed in the following section.

DETECTION AND DETERMINATION

Bioassay

For the detection of the benzimidazoles, fungal toxicity assays have been used by a number of authors.
The assay is carried out, in practically all cases, after thin-layer chromatography of the test solution. The plates are coated with an appropriate nutrient solution and sprayed with a suspension of the test organisms.
The inhibition zones developing after maintainance of appropriate cultivation conditions for a given time are in some instances correlated to the fungicide concentrations. A summary of the test species used are given in the table No. 6.

TABLE 6. Summary of test organisms used for the bioassay of benzimidazol fungicides

Test Organism	Author
Aspergillus fumigatus	Helling et al. (37)
Cladosporium	Homans and Fuchs (38)
Cladosporium cladosporoides	Wegman et al. (90)
Cladosporium cladosporoides	Baker and Hoodless (6)
Cladosporium cucumerinum	Wegman et al. (90)
Cladosporium ulmi	Black and Neely (13)
Diaporthe phaseolorum var. sojae	Ellis and Sinclair (26)
Diplodia zeae	Helling et al. (37)
Fusarium moniliforme	Helling et al. (37)
Monilinia fructicola	Phillips (62)
Neurospora crassa	Clemons and Sisler (19)
Penicillium	Homans and Fuchs (38)
Penicillium citrinum M 140	Cox et al. (21)
Penicillium citrinum M 140	Cox and Pinegar (22)
Penicillium chrysogenum	Helling et al. (37)
Penicillium cyclopium	Wegman et al. (90)
Penicillium expansum	Fernandes and Cole (29)
Penicillium expansum	Ellis and Sinclair (26)
Penisillium expansum	Black and Neely (13)
Penicillium rugulosum	Helling et al. (37)
Trichoderma viride	Helling et al. (37)
Verticillium albo atrum	Black and Neely (13)

Bioautographic detection of benzimidazole fungicides in residue analysis has been reported also by Ben-Arie (11) and Ben Aziz et al. (12); and was used by Buchenauer et al. (14) to detect photochemical transformation products of thiophanate and thiophanate methyl.

Colourimetric and Spectrophotometric Methods

To visualize carbendazim on thin-layer plates, N,2,6-trichlorobenzoquinone-imine has been recommended by von Stryk (77). However Baker and Hoodless (6) found that the colour development appeared to be due to traces of peroxide in the solvent. Further tests on other peroxide-containing solvents showed this to be the case.

Austin et al. (2) applied this reagent successfully to detect thiophanate and related compounds, while carbendazim gave a blue-green colour with bromphenol blue and silver nitrate. 2-Aminobenzimidazole was found to give blue coloured spots upon spraying with N-1-naphthylethylendiamine-dihydrochloride in aq. acetic acid and a followed by a spray with aq. HCl and heating 10 min to 110 °C.

Benomyl and carbendazim can be determined by colorimetry upon alkaline hydrolysis as 2-aminobenzimidazole, which upon bromination gives a red coloured compound (Pease and Gardiner (58)). Carbendazim forms a coloured substance with bromcresol-purple, sodium sulfate and acetic acid. It can be extracted with chloroform (Fleeker et al. (30)). The absorbance is read at 410 nm. This reaction is not specific (Sutherland (78); Stansbury (76)). A coloured copper-complex of benomyl has been described by Miller et al. (50). This colour reaction is applicable when higher concentrations of the fungicide are present (e.g. in spray mixtures). The benomyl is extracted from aqueous suspension into chloroform at pH 11 as a copper-benomyl-2-dimethyl-amino-2-methyl-1-propanol complex. A similar procedure is described for thiabendazole (Miller et al. (51)). In a more recent paper Miller et al. (52) applied this method to determine thiabendazole and benomyl in the presence of the fungicide folcidin in aqueous solutions.

Colorimetric methods for thiabendazole on citrus have been described by Hayward and Mc Cormack (36) and by Kröller (42). Both methods are based on that of Szalkowski and Kanova (79), thiabendazole is reduced with zinc dust in the presence of p-phenylene diamine to form a hydrogen sulfide complex. After subsequent oxidation a thiadiazine dye is formed.

Carbendazim and its precursors have strong characteristic absorptions in the range 260 - 300 nm which can be used for spectrophotometric determination (Gorbach and Künzler (33); BASF (8); Martens and Cus (44); White and Kilgore (91); Cox et al. (21); Mestres et al. (48); Austin et al. (2); van Wambeke and Assche (87); Dejonckheere et al. (24); Dabrowski and Czarnik (23). Cox and Pinegar (22)) recommended measuring the peak maxima at the wavelengths 282 and 275 nm from a base line at 310 nm. The peak ratio at 282/275 nm is constant at 1.20. Any variation greater than ± 0.02 will indicate interference from other UV-absorbing substances.
Spectrophotometric methods for the determination of thiabendazole were published by Mestres et al. (49); Mestres et al. (46); Mestres et al. (47); Tjan and Burgers (82); Norman et al. (55) and Rajzman (64).
Thiophanate and thiophanate methyl were detected and determined by spectrophotometry after transformation to carbendazim by Buchenauer et al. (14) and Fleeker et al. (30). The latter used this method for confirmation of the colour reaction with bromcresol purple.
The fluorescence spectrophotometric detection after conversion of benomyl and carbendazim to 2-amino-benzimidazole has been published by Pease and Gardiner (58), Pease and Holt (59). The direct fluorimetric measurement of carbendazim is described by Aharonson and Ben Aziz (1).
Fluorigenic labelling of carbendazim using dansyl chloride was proposed by Tedla (81).
Fluorescence spectrophotometric methods are also available for the determination of thiabendazole (Pest. Anal. Manual, Vol. II, 1969; Norman et al. (54); Norman et al. (55); Tocco et al. (83); Erwin et al. (27); Aharonso Ben Aziz (1); Otteneder and Hezel (57)). In situ fluorimetric determination of benomyl on thin layer chromatographic plates by scanning in a filter fluorimeter was proposed by Mc Neil and Kihichi (45). They suggested that a modification of the chromatographic system would permit carbendazim to be detected along with benomyl.
A similar method (determination of fluorescence quenching with an optical scanner) has been published by Sherma (69). Benomyl can be determined along with carbendazim and 2-amino-benzimidazole.
An in situ densitometric determination on TLC of benomyl and carbendazim after conversion (boiling with N HCl) to 2-aminobenzimidazole is reported by Polzhofer (63).

High Performance Liquid Chromatographic Methods

Measurement of absorbance in the UV or emission of light in fluorimetry are also the principles of determination of benomyl carbendazim and thiabendazole after separation from coextractives by high performance liquid chromatography.

For the determination benomyl and 2-aminobenzimidazole, Kirkland et al. (41) used the strong cation exchange packing Zipax SCX as the stationary phase and N-tetramethylammonium nitrate/nitric acid as the mobile phase. Benomyl is converted to carbendazim prior to the determination in the extraction process.
Two chromatographic systems were examined by Austin et al. (4): reversed phase chromatography using Permaphase ETH and Permaphase ODS as stationary phases, with methanol and water or buffer mixtures as solvents, and absorption chromatography using silica gel and hexane-propane-2-ol mixtures. The authors evaluated the retention times of "pure" solutions of common fungitoxic benzimidazoles and their metabolites.
A Li Chrosorb SI-60 silica column was used by Farrow et al. (28) to determine carbendazim and thiabendazole and a Li Chrosorb-COOH or Spherisorb ODS column was applied for confirmation in analysis of residues in citrus. For detection a UV spectrophotometer was used.
A fluorimetric detector was used by Maeda and Tsuji (43) for the determination of benomyl and thiabendazole by high performance liquid chromatography. Benomyl is transformed to 2-aminobenzimidazole prior to chromatography by successive acid and alkaline hydrolysis.

Gas Liquid Chromatographic Methods

Benomyl can be determined by gas liquid chromatography (glc)(Rouchaud and (Decaillonne (65)) when it is first converted to carbendazim and then trifluoracetylated with trifluoracetic anhydride in a sealed pyrex tube at 100° C for 35 min. After transfer of the solution and evaporation to dryness and heating up to 40 °C, the derivatisation is claimed to be 100 %. The glc is carried out on 1.50 m 5 % SE 30 on Chromosorb R using an electron capture detector.

Glc determination of the metabolites; 2-aminobenzimidazole, o-aminobenzonitrile and aniline after trifluoracetylation has been published by Rouchaud et al. (66). The glc method described by Rouchaud and Lhoest (67) and Rouchaud et al. (68) for the determination of benomyl and its metabolites carbendazim, 2-aminobenzimidazole, benzimidazole, o-aminobenzonitrile and o-phenylenediamine in melon plants, carrot, strawberry and apple. Thiabendazole can also be determined by glc using a Coulson conductivity detector (nitrogen mode) (de Vos and Bosma (86)) and a sulfur detector (Mestres et al. (46)). A gas chromatographic method, after derivatisation of thiabendazole, to N-methyl-thiabendazole, has been described by Tanaka and Fujimoto (84). Nose et al. (56) determined thiabendazole after reaction with pentafluorobenzoylchloride using an electron capture detector.
A combined gas-liquid chromatographic/mass spectrometric confirmatory assay, for thiabendazole and 5-hydroxythiabendazole at 0,1 mg/kg in animal tissue isolates, has been developed by Vanden Heuvel et al. (84). On-column methylation converts these compounds to their N-methyl and N,O-dimethyl derivatives, respectively. Identification and quantitation are achieved by selective ion monitoring of the M - 1, M and M + 1 ions from N-methyl-thiabendazole and the M and M - 15 ions from N,O-dimethyl-5-hydroxythiabendazole.

Other detection methods

A polarographic method to detect thiophanate was described by Martens and Cus (44).

The detection limits of benomyl/carbendazim for the various methods has been summarized by Watkins (89) (Table 7).

TABLE 7. Detection limits of benomyl/carbendazim for various methods (Watkins (89))

	mg/kg
Fluorimetry	0,1
Colorimetry	0,1
Ultraviolet spectrophotometry	0,1
High performance liquid chromatography	0,02
Gaschromatography	0,02
Bioassay	0,003
Dansyl	
a) In situ fluorimetry	0,0006
b) Spectrofluorimetry	0,003

MULTI-RESIDUE METHODS

The systemic fungicides can be separated by thin layer chromatography and detected either by UV light or by the use of a colorimetric spray reagent or by bioautography.
Baker et al. (5) separated 9 systemic fungicides on tlc plates but the method could not be applied to coloured crop extracts because of the interference with the visualization methods (UV and colorimetric).
Applying a two dimensional tlc technique, von Stryk (77) separated a solution of "pure" benomyl, thiophanate, thiophanate-methyl, carbendazim and the metabolites-benzimidazole and 2-aminobenzimidazole.
Austin et al. (2) described a tlc separation using a variety of solvents and recommended using this method as a clean-up procedure rather than for determination. The distinct zones can be scraped off and the compounds can be assayed by UV absorption. The R_f-values and the recommended developing solvents are given in Table 8.

TABLE 8. Thin layer chromatography of benzimidazoles on silica gel. R_f-values in different solvent systems (Austin et al. (2))

Compound	Solvent System* 1	2	3	4
Benomyl	0.81	0.82	0.99	0.77
Carbendazim	0.60	0.66	0.03	0.33
Thiophanate methyl	0.73	0.77	0.26	0.63
Thiophanate	0.79	0.81	0.54	0.76
2-Aminobenzimidazole	0.10	0.49	0	0

* 1 methanol/acetic acid/chloroform (10+1+89, v/v)
2 ethyl acetate/methanol/0.880 ammonia (10+25+1, v/v)
3 ethyl acetate/chloroform (1+9, v/v)
4 methanol/chloroform (1+9, v/v)

The separation and identification of thiabendazole, benomyl, carbendazim and thiophanate-methyl on citrus fruit using tlc and using the direct bioautographic method of Homans and Fuchs (38) has been published by Baker and Hoodless (6).
Further contributions to the separation and detection of the systemic fungicides and their degradation products were given by Fuchs et al. (32), and Soeda et al. (74,75). The tlc separation of carbendazim from its photodecomposition products has been published by Fleeker and Lacy (31). Carbendazim, 2-aminobenzimidazole, 4-hydroxy-2-(methoxycarbonylamino)-benzimidazole, 5-hydroxy-2-(methoxycarbonylamino)-benzimidazole, carbomethoxyguanidine, guanidine, carbomethoxyurea and dicarbomethoxyguanidine were separated on silica gel G using ethyl acetate/p-dioxane/methanol/conc. NH_3 (160 + 20 + 5 + 0,5).

Detection of the fungicides thiabendazole, carbendazim, thiophanatemethyl and benomyl by bioassay has been described by Wegman et al. (90). Interference from other fungicides such as binapacryl, dichloran, formaldehyde, mancozeb, PCNB, sulfur, tecnazene, trichlorotrinitrobenzene, zineb, captan, folpet and thioquinox was slight.
Austin et al. (4) investigated the direct assay of thiabendazole, benomyl thiophanate methyl, thiophanate, carbendazim and related compounds such as the metabolites 5-hydroxybenzimidazole and 2-aminobenzimidazole using high performance liquid chromatography. They used reversed phase systems (permaphase-ETA or ODS) as well as adsorption systems (Zorbax SIL or Merkosorb SI 60).
For the determination of thiophanate methyl and carbendazim, thiophanate is converted to carbendazim ($AgNO_3$ in ethanol) first and the total carbendazim is determined. In a second analysis carbendazim was determined after extraction of thiophanate methyl from acid (HNO_3) solution. The difference is calculated for thiophanate methyl (Fleeker et al. (30)). A glc separation and determination of metabolites of benomyl in carrots, as carbendazim, 2-aminobenzimidazole, benzimidazole, 2-aminobenzonitrile and o-phenylendiamine and the conjugates of carbendazim and 2-aminobenzimidazole is described by Rouchaud et al. (68).
The determination of benomyl, carbendazim and thiabendazole in citrus and in the presence of biphenyl and 2-phenylphenol by high performance liquid chromatography using an UV-spectrophotometric detector has been recommended by Farrow et al. (28). Benomyl is converted in this procedure to carbendazim during the clean up. As stationary phases Li Chrosorb SI-60 silica and Spherisorb ODS were used.

Special techniques are necessary to determine benomyl itself and in presence of carbendazim because of the tendency of benomyl to be transformed to the stable carbendazim (see under section Comments on the properties of benzimidazoles relevant to analysis). To overcome this problem, the base catalysed conversion of benomyl to BBU 1-(benzimidazol-2-yl)-3-butylurea and carbendazim to 2-aminobenzimidazole has been used (White et al (92); Baude et al. (9); Baude et al. (10)).
Recently preliminary work on the application of dansyl chloride for fluorigenic labelling of benomyl, BBU, and 2-aminobenzimidazole has become available (Tedla (81)). Benomyl and BBU are hydrolysed and the liberated butylamine will couple directly with dansyl chloride. 2-Aminobenzimidazole undergoes this reaction too. In situ fluorimetry on tlc plates or spectrofluorimetry metry using the extracts of the tlc spots can be used for estimation.

DISCUSSION AND RECOMMENDATIONS

Single residue methods

Benomyl. As far as the specific determination of benomyl is concerned, a reliable determination appear to be only possible after the base catalyzed transformation of benomyl to BBU.
All the relevant experiments described in the literature (Baude et al. (9); Baude et al. (10)), with the exception of those published by Tedla (81), made use of 14-C labelled benomyl and quantitation has been carried out using radiometric methods.
A routine analytical method for the specific determination of benomyl based on the conversion to BBU was not found in the literature.
However, the acidic degradation of benomyl (see under section Comments on the properties of benzimidazoles relevant to analysis) to carbendazim and determination of the latter is described and used as routine procedure by a number of authors.

Carbendazim. The reported data demonstrate that carbendazim can be extracted with a number of organic solvents preferably ethyl acetate and acetone from the neutral or weekly basic plant macerate.
The problem of not recovering all the carbendazim present as a residue does not seem to be great. Trials with radiolabelled carbendazim, added to nutrient solutions of plants, demonstrated that in the plants only a small percentage of the carbendazim is conjugated and thus is eliminated by the usual working-up procedures.
Moreover, it has been shown (Gorbach et al. (34)) that conjugates are less likly to occur when the fungicide is applied to the leaves, as it is done in practice, rather than via nutrient solutions, where the uptake of the chemical is forced.
One of the most efficient clean-up steps is partitioning of carbendazim from the organic extraction solvent into hydrochloric acid and back into, e.g. ethyl acetate after adjusting the aqueous phase to a pH of 4.5 - 9. Reextraction with hydrochloric acid ends up with an aqueous solution containing the carbendazim ready for spectrophotometry determination e.g. A number of authors found this clean-up (repeated twice) sufficient. Other authors introduced, in addition to the partition step, column chromatography or thin layer chromatography. Chromatography may be necessary when the detection limits have to be improved or in crops with highly interfering substances have to be analyzed.
For the extraction of carbendazim from soil the paper published by Austin and Briggs (3) gives validated information, indicating that a mixture of acetone and M ammonium chloride (1 + 1, v/v) is very efficient even for the extraction of aged residues.
As far as the determination is concerned, a fairly high percentage of the authors use UV-spectrophotometry.
For routine residue analysis of carbendazim, where detection limits around 0.05 to 0.1 mg/kg are sufficient, most of the important target crops can be analyzed using partition clean-up and UV-spectrophotometry.
For example, the author of this review was able to analyze the crops listed in the following table with an adequately low detection limit.

TABLE 9. List of crops analyzed for carbendazim by spectrophotometry, and observed detection limits (Gorbach (35))

Crop	Detection limits (µg/g)		
	range	average	s_*
apple	0.03 - 0.05	0.04	0.01
barley	0.06	-	-
beans (green)	0.06	-	-
cabbage red	0.03 - 0.05	0.04	-
coffee	0.06	-	-
cucumbers	0.03 - 0.05	0.034	0.014
gooseberry	0.05	-	-
lettuce	0.03 - 0.06	0.043	0.013
oats			
straw	0.05 - 0.06	0.058	0.005
seeds	0.03 - 0.05	0.035	0.01
oranges			
peel	0.05	-	-
pulp	0.05	-	-
raspberry	0.02	-	-
strawberry	0.1	-	-
wheat			
green	0.03 - 0.09	0.047	0.018
straw	0.03 - 0.09	0.059	0.019
seeds	0.01 - 0.06	0.041	0.017

s_* = standard deviation

Mean of the averages $x_A = 0.049$

Mean of the s $s_A = 0.017$

The reported detection limits were calculated following the guidlines given in the book "Methodensammlung zur Rückstandsanalytik von Pflanzenschutzmitteln" der Deutschen Forschungsgemeinschaft, Verlag Chemie GmbH, Weinheim/ Bergstr., 1972, Kapitel XI.
The consistency of the detection limits is quite good and shows an average of roughly 0.05 mg/kg with a standard deviation of 0.017 mg/kg.

Thiabendazole. The spectrofluorimetric and the spectrophotometric method is recommended using a clean-up very similar to that described for carbendazim (Norman et al. (55).

Thiophanate and Thiophanate methyl. As a result of the fate of thiophanate and thiophanate-methyl in plants and soil it seems that for routine analysis the determination of total carbendazim is advantageous.
To transform all residual parent thiophanate and thiophanate-methyl to carbendazim, the concentrated acetone extract of the substrate, for example, is heated with 1 % $AgNO_3$ in 95 % ethanol for 20 min (Fleeker et al. (30)).
The carbendazim can be determined in the usual way.

An excellent summary of the published detection limits observed for the systemic benzimidazol fungicides using the different clean-up and detection methods, has been given by Baker and Hoodless (7).

Multiresidue methods
A recommendation for a definite method cannot be given. For the time being, thin layer chromatography, for example, as described by Austin et al. (2)

may be the best of separating the different benzimidazoles.
They can be determined separately after scraping off the different zones in the chromatogram, eluting the substances and determining them using one of the already described procedures. The difficulties caused by the transformation especially of benomyl to carbendazim is (as with others) one of the greatest obstacles when using this separation technique.

A promising approach to establishing a multi method will become feasible by introducing high performance liquid chromatography, (Farrow et al. (28) and Maeda and Tsuji (43)). However, the published data (Austin et al. (2)) do not reflect that his method can be recommended to analyse a variety of normal crops in day-by-day routine at present.
The literature reveals that quite a number of authors use bio-assay techniques for the detection and quantitation of the benzimidazoles. The method is even used in routine analysis. The low demands in respect of clean-up and the specificity of detection is to some advantage in applying the technique for multi residue analysis.
Future endeavours should be concentrated predominately to the application of high performance liquid chromatography for the multiresidue analysis of normal crop samples.

REFERENCES

1. Aharonson, N., and Ben-Aziz, A.
Determination of Residues of Benomyl, Its Hydrolysis Product, and Thiabendazole in Various Crops
(1973) JAOAC, 56, 1330

2. Austin, D.J., Briggs, G.G., and Lord, K.A.
Problems in the Assay of Residues of Carbendazim and its Precursors
(1975) Proceedings 8th British Insecticide and Fungicide Conference

3. Austin, D.J., and Briggs, G.G.
A New Extraction Method for Benomyl Residues in Soil and its Application in Movement and Persistence Studies
(1976) Pestic. Sci. 7, 201

4. Austin, D.J., Lord, K.A., and Williams, J.H.
High Pressure Liquid Chromatography of Benzimidazoles
(1976) Pestic. Sci. 7, 211

5. Baker, P.B., Farrow, J.E., and Hoodless, R.A.
Agricultural fungicides
I. Identification of Systemic Fungicides using Thin-Layer Chromatography
(1973) J. Chromatogr. 81, 174

6. Baker, P.B., and Hoodless, R.A.
Agricultural fungicides
II. Detection and identification of Systemic Benzimidazole Fungicides on Citrus Fruit
(1973) J. Chromatogr. 87, 585

7. Baker, P.B., and Hoodless, R.A.
Analytical Methods for the Detection and Determination of Residues of Systemic Fungicides
(1974) Pestic. Sci. 5, 465

8. BASF, United Kingdom Limited,
(1973) Private Communication

9. Baude, F.J., Gardiner, J.A., and Han, J.C.Y.
Characterization of Residues on Plants Following Foliar Spray Application of Benomyl
(1973) J. Agr. Food. Chem. 21, 1084

10. Baude, F.J., Pease, R.L., and Holt, R.F.
Fate of Benomyl on Field Soil and Turf
(1974) J. Agr. Food. Chem. 22, 413

11. Ben-Arie, R.
Benzimidazole Penetration, Distribution and Persistence in Postharvest-Treated Pears
(1975) Phytopathology 65, 1187

12. Ben Aziz, A., Shabi, E., and Aharonson, N.
Persistence and Distribution of Three Benzimidazole Fungicides in Pear Trees Sprayed for the Control of Venturia pirina
(1974) Phytoparasitica 2, 117

13. Black, W.M., and Neely, D.
Root Uptake of Soil-Injected Benomyl and Its Detection within Ulmus americana
(1976) J. Agric. Food Chem. 24, 1016

14. Buchenauer, H., Edgington, L.V., and Grossmann, F.
Photochemical Transformation of Thiophanate-methyl and Thiophanate to Alkyl Benzimidazol-2-yl Carbamates
(1973) Pesti Sci. 4, 343

15. Calmon, J.P., Sayag, D.R.,
Instability of Methyl 1-(Butylcarbamoyl)-2-benzimidazole-carbamate (Benomyl) in Various Solvents
(1976) J. Agric. Food. Chem. 24, 426

16. Chiba, M., and Doornbos, F.
Instability of Benomyl in Various Conditions
(1974) Bull. Environ. Contam. Toxicol. 11, 273

17. Chiba, M.
Rate of Degradation of Benomyl in Common Organic Solvents and Water
(1975) Abstr. Papers, Am. Chem. Soc. 170 Meet., PEST 127

18. Chiba, M., and Cherniak, E.A.
Kinetic Study of Reversible Conversion of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl) to Methyl 2-Benzimidazolecarbamate (MBC) and n-Butyl Isocyanate (BIC) in Organic Solvents
(1978) J. Agric. Food Chem. 26, 573

19. Clemons, G.P., and Sisler, H.D.
Formation of a Fungitoxic Derivative from Benlate
(1969) Phytopathology 59, 705

20. Corke, A.T.K., Hunter, T., and Goldfinch, S.
(1971) Rep. agric. hort. Rs. Stu., Univ. Bristol, p. 147

21. Cox, j., Donegan, L. and Pinegar, J.A.
The Estimation of Surface Residues of Benomyl on Treated Bananas
(1974) Pestic. Sci. 5, 135

22. Cox, J., and Pinegar, J.A.
Benomyl Residues in Bananas
(1976) Pestic. Sci. 7, 193

23. Dabrowski, J., and Czarnik, W.
Untersuchungen über die Rückstände von Benomyl in Champignons
(1976) Nachrbl. Pflanzenschutz DDR 30, 254

24. Dejonchheere, W., Steurbaut, W., Van Damme, J., and Kips, R.H.
Évolution des Résidus de Bénomyl de Carbendazime et de Thiophanate-Méthyle Appliqués sur Laitues
(1976) Phytiatrie-Phytopharmacie 25, 141

25. Douch, P.G.C.
The Metabolism of Benomyl Fungicide in Mammals
(1973) Xenobioticy 3, 367

26. Ellis, M.A., and Sinclair, J.B.
Movement of Methyl 2-Benzimidazolecarbamete into Soybean Seeds after Foliar Application of Benomyl
(1975) Phytopathology 65, 1387

27. Erwin, D.C. Sims, J.J., Borum, D.E., and Childers, J.R.
Detection of the Systemic Fungicide Thiabendazole, in Cotton Plants and Soil by Chemical Analysis and Bioassay
(1971) Phytopathology 61, 964

28. Farrow, J.E., Hoodless, R.A., Sargent, M., and Sidwell, J.A.
Fungicide Residues, Part VI. Determination of Residues of Post-Harvest Fungicides on Citrus Fruit by High-Performance Liquid Chromatography.
(1977) Analyst 102, 752

29. Fernandes, D.L. and Cole, J.S.
Comparison of Solvent and Methods of Extracting Methylbenzimidazol-2-yl carbamate (MBC) from Tobacco
(1974) Pestic. Sci. 5, 239

30. Fleeker, J.R., Lacy, H.M., Schultz, I.R., and Houkom, E.C.
Persistence and Metabolism of Thiophanate-methyl in Soil
(1974) J. Agric. Food Chem. 22, 592

31. Fleeker, J.R. and Lacy, H.M.
Photolysis of Methyl 2-Benzimidazolecarbamate
(1977) J. Agric. Food Chem. 25, 51

32. Fuchs, A., Van den Berg, G.A., and Davidse, L.C.
(1972) Pestic. Biochem. Physiol. 2, 191

33. Gorbach, S., and Künzler, K.
Analysenmethode zur Bestimmung von Carbendazim in pflanzlichem Material
(1972) Farbwerke Hoechst AG, Frankfurt/Main, W-Germany, Analytical Method 3/72

34. Gorbach, S., Thier, W., and Schulze, E.F.
Untersuchung zum Rückstandsverhalten und Metabolismus von (14-C markiertem) Carbendazim an Gurkenpflanzen unter Gewächshausbedingungen
(1973) Farbwerke Hoechst AG, Report 01 Z53 0221-73

35. Gorbach, S.
(1976) private communication

36. Hayward, F.W., and Mc Cormack, A.A.
Packaging methods and diphenyl in relation to the keeping quality of tangerines and Valencia oranges
(1971) Fla. Agr. Exp. Sta. J. Ser. No. 4165

37. Helling, Ch. S., Dennison, D.G., and Kaufman, D.D.
Fungicide Movement in Soils
(1974) Phytopathology 64, 1091

38. Homans, A.L., and Fuchs, A.
Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances
(1970) J. Chromatogr. 51, 327

39. Kilgore, W.W., and White, E.R.
Decomposition of the Systemic Fungicide 1991 (Benlate)
(1970) Bull. Environ. Contam. Toxicol. 5, 67

40. Kirkland, J.J.
Method for High-Speed Liquid chromatographic Analysis of Benomyl and/or Metabolite Residues in Cow Milk, Urine, Feces, and Tissues
(1973) J. Agr. Food Chem. 21, 171

41. Kirkland, J.J., Holt, R.F., and Pease, H.L.
Determination of Benomyl Residues in Soils and Plant Tissues by High-Speed Cation Exchange Liquid Chromatography
(1973) J. Agr. Food Chem. 21, 368

42. Kröller, E.
Untersuchungen zur Restmengenbestimmung von Thiabendazol auf den Schalen von Apfelsinen
(1971) Deut. Lebensm. Rundschau 67, 229

43. Maeda, M., and Tsuji, A.
Determination of Benomyl and 2-(5-Thiazolyl)-Benzimidazole in Plant Tissues by High-Performance Liquid Chromatography using Fluorimetric Detection
(1976) J. of. Chrom. 120, 449

44. Martens, P.H., and Cus, M.
New oscillopolarographic method for the determination of thiophanate residues in vegetable
(1972) Meded. Fac. Landbouwwetenschappen, Gent, 37, 891

45. Mc Neil, J.D., and Kihichi, M.
A Rapid Method for the Quantitative Analysis of Benomyl on Cherries by Thin-Layer Chromatography and in Situ Fluorometry
(1974) J. Chromatogr. 101, 33

46. Mestres, R., Campo, M., and Tourte, J.
Pesticides residues, XXI Study and determination of thiabendazole in citrus fruits and bananas
(1970 a) Ann. Fals. Exp. Chim. 63, 160

47. Mestres, R., Campo, M., and Tourte, J.
Determination of thiabendazole in Citrus fruit, whole fruit, juice, and pulp
(1970 b) Trav. Soc. Pharm. Montpellier 30, 193

48. Mestres, R., Tourte, J., and Campo, M.
(1971) Trav. Soc. Pharm. Montpellier 31, 49

49. Mestres, R. Campo, M., and Tourte, J.
Study and determination of thiabendazole in cedrate fruits and bananas. Method adapted to new tolerance limits
(1972) Ann. Fals. Exp. Chem. 65, 315

50. Miller, V.L., Gould, C.J., and Csonka, E.
Estimation of Benomyl in the Milligram Range
(1974 a) J. Agr. Food Chem. 22, 93

51. Miller, V.L., Gould, C.J., and Csonka, E.
Estimation of Thiabendazole in the Milligram and Submilligram Ranges
(1974 b) J. Agr. Food Chem. 22, 90

52. Miller, V.L., Gould, C.L., and Csonka, E.
Measurement of Thiabendazole, Benomyl, and Folcidin Fungicides in Aqueous Suspensions
(1975) J. Assoc. Offic. Anal. Chem. 58, 971

53. Noguchi, T., Ohkuma, K., and Kosaka, S.
Chemistry and metabolism of thiophanate fungicides
(1971) IUPAC International Symposium on Pesticide Terminal Residues
(A.S. Tahori, ed.), Butterworths, London, p. 257

54. Norman, S.M., Fouse, D.C., and Craft, C.C.
Thiabendazole Residues on and in Citrus
(1972 a) J. Agric. Food Chem. 20, 1227

55. Norman, S.M., Fouse, D.C., and Craft, C.C.
Thin-Layer Chromatographic Separation and Spectrophotofluorometric Determination of Thiabendazole Residues on and in Citrus
(1972 b) J. AOAC 55, 1239

56. Nose, N., Kobayashi, S., Tanaka, A., Hirose, A., Watanabe, A.
Determination of Thiabendazole by Electron-Capture Gas-Liquid Chromatography after Reaction with Pentafluorbenzoyl Chloride
(1977) J. Chromatogr. 130, 410

57. Otteneder, H., and Hezel, U.
Quantitative routine Determination of Thiabendazole by Fluorimetric Evaluation of Thin-Layer Chromatograms
(1975) J. Chromatog. 109, 181

58. Pease, H.L., and Gardiner, J.A.
Fluorometric and Colorimetric Procedures for Determining Residues of Benomyl
(1969) J. Agr. Food Chem. 17, 267

59. Pease, H.L., and Holt, R.F.
Improved Method for Determining Benomyl Residues
(1971) J. AOAC 54, 1399

60. Pesticide Analytical Manual, Vol. II
(1969) Food and Drug Administration, Washington, D.C., Sec. 120.242

61. Peterson, C.A., and Edington, L.V.
Quantitative Estimation of the Fungicide Benomyl using a Bioautograph Technique
(1969) J. Agr. Food Chem. 17, 898

62. Phillips, D.J.
Detection and Translocation of Benomyl in Postharvesttreated Peaches and Nectarines
(1975) Phytopathology 65, 255

63. Polzhofer, K.
Determination of Benomyl, Carbendazim and 2-Aminobenzimidazole (2-AB) in Plant Materials
Part I: Apples, Red-currants, Grapes, Kale, and Sugar Beets
(1977) Z. Lebensm.-Untersuch.-Forsch. 163, 109

64. Rajzman, A.
Determination of Thiabendazole in Citrus Fruits by Ultraviolet Spectrophotometry
(1974) Analyst. 99, 120

65. Rouchaud, J.P., and Decallonne, J.R.
A Gas Chromatographic Method for the Analysis of MCB in Plants and Soil
(1974) J. Agr. Food Chem. 22, 259

66. Rouchaud, J.P., Decallonne, J.R., and Meyer, J.A.
Metabolic Fate of Methyl-2-Benzimidazole Carbamate in Melon Plants
(1974) Phytopathology 64, 1513

67. Rouchaud, J.P., Lhoest, G.J.
Metabolism of Benomyl in Carrot, Strawberry and Apple
(1977) Pestic. Sci. 8, 23

68. Rouchaud, J.P., Decallonne, J.R., and Meyer, J.A.
Metabolism of 2-Aminobenzimidazole and Benzimidazole in Melon Plants
(1977) Pestic. Sci. 8, 31

69. Sherma, J.
Chromatographic Analysis of Fungicides
(1975) J. Chromatog. 113, 97

70. Siegel, M.R., and Zabbia, A.J. jr.
Distribution and Metabolic Fate of the Fungicide Benomyl in Dwarf Pea
(1972) Phytopathology 62, 630

71. Siegel, M.R.
Distribution and Metabolism of Methyl 2-Benzimidazole-carbamate, the Fungitoxic Derivative of Benomyl, in Strawberry Plants
(1973) Phytopathology 63, 890

72. Sims, J.J., Mee, H., and Erwin, D.C.
Methyl 2-benzimidazole-carbamate, a Fungitoxic Compound Isolated from Cotton Plants Treated with Methyl 1-(butyl-carbamoyl)-2-benzimidazole-carbamate (benomyl)
(1969) Phytopathology 59, 1775

73. Slade, P.
IUPAC Commission on the Development, Improvement, and Standardization of Methods of Pesticide Residue Analysis
(1975) J. Assoc. Offic. Anal. Chem. 58, 1244

74. Soeda, Y., Kosaka, S., and Noguchi, T.
Identification of Alkyl 2-Benzimidazolecarbamates as a Major Metabolite of Thiophanates Fungicide in/on the Bean Plant
(1972 a) Agric. Biol. Chem. Tokyo, 36, 817

75. Soeda, Y., Kosaka, S., and Noguchi, T.
The Fate of Thiophanate-methyl Fungicide and Its Metabolites on Plant Leaves and Glass Plates
(1972 b) Agric. Biol. Chem. Tokyo, 36, 931

76. Stansbury, H.A. jr.
Glyodin"
(1964) G. Zweig, Anal. Methods Pestic. Plant Growth Regul. Food Additives 3, 99

77. Von Stryk, F.G.
Separation and Determination of some Systemic Fungicides and their Metabolites by Thin-Layer Chromatography
(1972) J. Chromatogr. 72, 410

78. Sutherland, G.L.
"Cyprex"
(1964) G. Zweig, Anal. Methods Pestic. Plant Growth Regul. Food Additives 3, 41

79. Szalkowski, C.R., and Kanora, J.
Spectrophotometric Determination of Thiabendazole in Feeds
(1965) J. AOAC 48, 288

80. Tanaka, A., and Fujimoto, Y.
Gas Chromatographic Determination of Thiabendazole in Fruits and Its Methyl Derivative.
(1976) J. Chromatogr. 117, 149

81. Tedla, F.M.
(1974) Sc. thesis,University of Bristol

82. Tjan, G.H., and Burgers. L.I.
Thin-Layer Chromatographic Detection of Thiabendazole and Benomyl in Fruits
(1973) J. AOAC 56, 223

83. Tocco, D.I., Egerton, I.R., Bowers, W., Christensen, V.W., and Rosenblum, C.
Adsorption, Metabolism and Elimination of Thiabendazole in Farm Animals and a Method for its Estimation in Biological Materials
(1965) J. Pharm. Exp. Ther. 149, 263

84. Vanden Heuvel, W.J.A., Wood, J.S., Di Giovanni, M., and Walker, R.W.
Gas-Liquid Chromatographic/Mass Spectrometric Confirmatory Assay for Thiabendazole and 5-Hydroxythiabendazole
(1977) J. Agric. Food Chem. 25, 386

85. Vonk, J.W., and Sijpesteijn, K.
Methyl benzimidazol-2-ylcarbamate, fungitoxic principle of methyl thiophanate
(1971) A. Pestic. Sci. 2, 160

86. De Vos, R.H., and Bosma, M.P.M.
(1970) M. Centraal Instituut voor Voedingsonderzoek TNO, Zeist, The Netherlands, Rpt. No. R 3199

87. Van Wambeke, E., and van Assche, C.
MBC Residues and Phytotoxicity in Gherkin Plants by Soil and Foliar Spray Applications with MBC Producing Compounds
(1975) Proceedings 8th British Insecticide and Fungicide Conference, Vol. 1 35

88. Watkins, D.A.M.
Photolysis of methyl benzimidazol-2-ylcarbamate
(1974) Chemosphere 3, 239

89. Watkins, D.A.M.
Benzimidazole Pesticides: Analysis and Transformation
(1976) Pestic. Sci. 7, 184

90. Wegman, R.C.C., Northolt, M.D., and Greve, P.A.
Optimization of the Determination of Fungicides by Bio-Assay
(1975) Meded. Fac. Landbouwwetensch. 40, No. 2, Pt.2, 1077

91. White, E.R., and Kilgore, W.W.
Determination of Systemic MBC Residues in Food Crops Treated with Benomyl Fungicide
(1972) J. Agr. Food Chem. 20, 1230

92. White, E.R., Bose, E.A., Ogawa, J.M., Manji, B.T., and Kilgore, W.W.
Thermal and Base-Catalyzed Hydrolysis Products of Systemic Fungicide, Benomyl
(1973) J. Agr. Food Chem. 21, 616