dithioate, 756-80-9; monomethyl phosphorothioate, 106191-34-8; S-methylglutathione, 2922-56-7.

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Determination and Persistence of Several Fungicides in Postharvest-Treated Apples during Their Cold Storage

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This work describes the storage stability of fungicide residues usually used in postharvest treatment of pome fruits: Benomyl, Carbendazim, Methylthiophanate, Thiabendazole. Fungicide treatments were carried out by immersion of apples, cvs. Starking and Golden Delicious, in aqueous solutions of each fungicide. Fruits were stored at 0 °C (cv. Starking) or +2 °C (cv. Golden Delicious) both in 85–90% relative humidity. Samples were taken monthly, and each fungicide compound was determined in peel, two zones of pulp, and whole fruit by high-performance liquid chromatography. Benzimidazole residues decreased during storage; Thiabendazole, Benomyl, and Methylthiophanate residues found at 160 days of storage were 30–25% (cv. Starking) or 45–55% (cv. Golden Delicious) of the initial amount recovered after treatment. Carbendazim showed the greater storage stability (40–65%) in both cultivars. Most residues were found on the peel, and amounts in the pulp decreased toward the core.

Benzimidazole fungicides, Benomyl [methyl [1-(butylcarbamoyl)-1*H*-benzimidazol-2-yl]carbamate], Carbendazim [methyl 1*H*-benzimidazol-2-ylcarbamate], Methylthiophanate [dimethyl 4,4′-*o*-phenylenebis(3-thioallophanate)], and Thiabendazole [2-thiazol-4-ylbenzimidazole] (Figure 1), have extensively used to control the most important postharvest diseases in pome fruits as *Penicillium expansum* (Spalding and Hardenburg, 1971; Cargo and Dewey, 1970), *Gloesporium spp.* (Bompeix and Morgat, 1970; Burchill and Edney, 1972), and *Botrytis cinerea* (Hardenburg, 1974).

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These compounds are systemic fungicides so they can prevent the development of disease on regions of the plant away from the side of application (Marsh, 1977; Ben-Aziz and Aharonson, 1974; Solel and Edgington, 1973), and their penetration into the fruit was reported on pears (Ben-Arie, 1975).

Systemic fungicides are subject to a number of natural processes that will alter their chemical structure, and the following degradative pathways may be distinguished: first, purely chemical breakdown processes; second, the metabolic systems present in the fruit. Benomyl rapidly loses the butylcarbamate (Carbendazim, MBC; Clemons and Sisler, 1969), and this compound is also formed when benomyl is applied to plants (Baude et al., 1973). Ring cyclization of Methylthiophanate to (benzimidazol-2-yl)-

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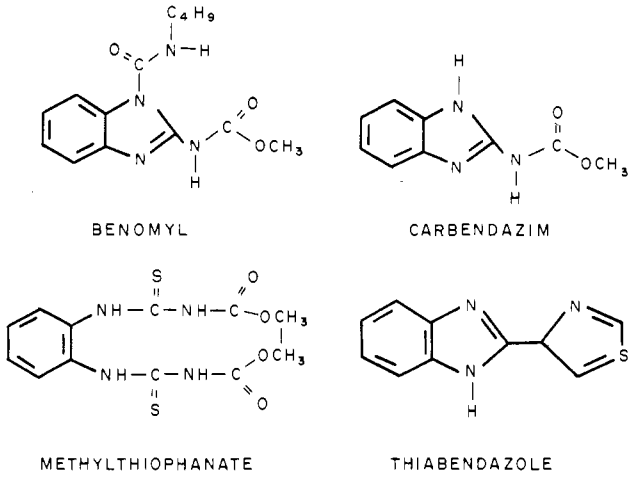

Figure 1. Structure of the benzimidazole fungicides.

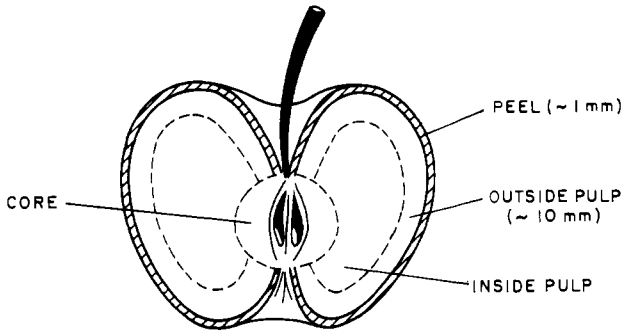

Figure 2. Zones of fruits: residue analysis.

carbamate (MBC), which is responsible for its activity, has been described (Soeda et al., 1972). Carbendazim and thiabendazole are rather resistant to attack by plants, showing a greater rate of disappearance (Ben-Aziz and Aharonson, 1974).

The present work was carried out to study the penetration, persistence, and distribution of benzimidazole fungicides on apples when then were applied after harvest by the immersion method. This paper also describes some modifications of previously reported high-performance liquid chromatographic methods of analysis of these compounds in order to improve their determination on apples.

EXPERIMENTAL SECTION

Apparatus. Residues were determined by high-performance liquid chromatography on a constant-pressure apparatus using a Waters liquid chromatograph equipped with a Model 441 wavelength detector, a Model 6000A pump, and a Model UGK injector, and a Hewlett-Packard Model 3390A integrator recorded and measured the peaks for comparison of areas by external method.

Chromatography. A μ-Bondapack C_{18} reversed-phase (250 × 4.0 i.d., 10 μm; Waters) column was employed. Operating conditions: solvent flow rate 1 mL/min (Benomyl, Carbendazim, Thiabendazole) or 0.7 mL/min (Methylthiophanate); room temperature; injection volume 25 μL; detection at 280 nm; attenuation setting corresponding to 0.1 AUFS; chart speed 0.5 cm/min. The mobile phases were methanol–water–ammonium hydroxide (60:40:0.6) for Benomyl, Carbendazim, and Thiabendazole or acetonitrile–water (50:50) for Methylthiophanate, degassed in a sonic bath and filtered through a Millipore filter (0.45 μm) prior to use.

Chemicals. Water was destilled twice and filtered through a Millipore apparatus; ethyl acetate, chloroform, and dichloromethane were suitable for pesticide analysis (Merck); methanol and acetonitrile were HPLC-grade solvents (Merck). Analytical samples of each fungicide compound were donated by their respective manufacturer.

Table I. Program of Fungicide Treatments Applied to Apple Fruits

fungicide	formulation	concn active ingred, ppm	immersion time
Benomyl	BENLATE	500	1 min
Carbendazim	BAVISTIN	500	1 min
Methylthiophanate	PELT	1000	1 min
Thiabendazole	TECTO 60	1000	35 s

Sampling and Extraction Procedure. Apples (*Pyrus malus* L.) cvs. Starking and Golden Delicious were grown in Málaga, Spain. Fruits were transported to the pilot plant of the Instituto del Frío, Madrid, where they were selected for uniformity of size and maturity. Mean weights of apples were 170 g (72-mm diameter) and for Starking and 222 g (80-mm diameter) for Golden Delicious; injured fruits were discarded.

Over 24 h from harvest, apples were treated by immersion in aqueous solutions of each fungicide with concentrations of active compound and immersion times showed in Table I. Fruits were then placed in open plastic boxes and stored in 85–90% relative humidity at 0 °C (cv. Starking) or +2 °C (cv. Golden Delicious). Residues were determined 24 h after fungicide treatment and at monthly intervals during storage. This experiment was repeated in two consecutive years.

To determine each fungicide residue on different zones of fruit, 10 individual apples were peeled with a potato peeler to an uniform depth (1 mm). Peeled fruits were then cut perpendicularly to the core, and two zones of pulp were removed; outer pulp was taken to 10-mm depth and inner pulp below 10-mm depth.

Carbendazim and Thiabendazole. Samples (50 g of peel or 100 g of pulp) were homogenized with an Osterizer Pulsematic 16 for 10 min, and the homogenates were placed in a Sorvall R Omnimixer and extracted with 220 mL of ethyl acetate, blending for 10 min. The samples were centrifuged at 4500 rpm for 15 min, and the organic layer was placed in a separatory funnel. The solids were extracted again with 100 mL of ethyl acetate, centrifuged, and added to the first extract. The ethyl acetate extracts were evaporated until dry in a rotary evaporator at 50 °C bath temperature. The residues were taken up three times with 35-mL portions of 0.5 N H_2SO_4 and filtered through a fritted-glass filter. The filtrate was collected in a separatory funnel.

Benomyl. Samples (50 g of peel or 100 g of pulp) were homogenized with an Osterizer Pulsematic 16 for 10 min, and the homogenates were blended with 50 mL of 2 M HCl for 10 min. The mixture was transferred in a round-bottoned flask with a water-cooled condenser and refluxed for 30 min. The cold solution was centrifuged at 4500 rpm for 15 min, and the liquid layer was transfered to a separatory funnel to do the cleanup.

In this extraction procedure Benomyl was completely hydrolyzed to Carbendazim, and its variable degradation during extraction and cleanup are avoided.

Methylthiophanate. Samples (50 g of peel or 100 of pulp) were homogenized with an Osterizer Pulsematic 16 for 10 min, and the homogenates were placed in a Sorvall R Omnimixer and extracted with 100 mL of benzene. After centrifugation (5 min at 3000 rpm), the organic layer was separated, 10 mL was evaporated to dryness under reduced pressure (T = 40 °C), and the residue was dissolved with 1.0 mL of eluting mixture (acetonitrile–water, 50:50). Cleanup was not necessary in this procedure.

Table II. Residues of Benomyl (ppm) Determined as Methyl Benzimidazol-2-ylcarbamate (MBC) in Apples during Cold Storage[a]

	storage time, days						
	0	31	56	88	119	141	169
			Golden Delicious at +2 °C				
peel	2.218	1.869	1.457	1.070	0.909	0.853	0.722
whole fruit	2.071	1.827	1.683	1.424	1.283	0.901	0.578
outer	0.235	0.123	0.152	0.108	0.103	0.087	0.121
inner	0.014	0.047	0.021	0.033	0.045	0.022	0.051
			Starking at 0 °C				
peel	2.399	1.921	1.659	1.387	0.952	0.862	0.705
whole fruit	1.973	1.605	1.412	1.128	0.992	0.813	0.673
outer	0.199	0.157	0.125	0.095	0.122	0.107	0.092
inner	0.010	0.025	0.072	0.018	0.059	0.042	0.037

[a] Results are means of duplicate samples and corrected with standard deviation.

Table III. Residues of Carbendazim (ppm) in Apples during Cold Storage[a]

	storage time, days						
	0	31	56	88	119	141	169
			Golden Delicious at +2 °C				
peel	2.451	2.031	1.862	1.622	1.483	1.402	1.471
whole fruit	2.013	1.630	1.412	1.203	1.257	1.249	1.293
outer	0.547	0.349	0.209	0.094	0.083	0.079	0.051
inner	0.093	0.145	0.115	0.084	0.044	0.053	0.020
			Starking at 0 °C				
peel	2.150	1.429	1.321	1.064	0.974	0.722	0.438
whole fruit	1.893	1.792	1.529	1.293	1.210	0.925	0.700
outer	0.325	0.297	0.193	0.158	0.242	0.095	0.048
inner	0.052	0.095	0.081	0.032		0.058	0.027

[a] Results are means of duplicate samples and corrected with standard deviation.

Table IV. Residues of Methylthiophanate (MT) and Carbendazim (MBC) (ppm) in Apples Treated with Methylthiophanate (PELT) during Cold Storage[a]

		storage time, days						
		0	22	46	74	109	129	156
				Golden Delicious at +2 °C				
peel	MT	1.540	1.022	1.071	0.985	0.877	0.782	0.736
	MBC	0.027	0.323	0.281	0.427	0.281	0.431	0.315
whole fruit	MT	1.204	1.115	0.973	0.908	0.825	0.746	0.483
	MBC	0.113	0.308	0.479	0.215	0.182	0.164	0.110
outer	MT	0.029	0.502	0.621	0.331	0.288	0.182	0.141
	MBC	0.007	0.065	0.120	0.092	0.105	0.203	0.237
inner	MT	0.008	0.042	0.081	0.051	0.064	0.042	0.053
	MBC		0.013	0.037	0.016	0.051	0.062	0.013
				Starking at 0 °C				
peel	MT	1.386	1.123	1.037	0.968	0.897	0.782	0.694
	MBC		0.437	0.321	0.216	0.205	0.152	0.199
whole fruit	MT	1.127	1.093	0.991	0.807	0.722	0.557	0.367
	MBC		0.278	0.559	0.592	0.214	0.136	0.143
outer	MT	0.063	0.633	0.571	0.419	0.365	0.159	0.281
	MBC	0.010	0.105	0.137	0.064	0.165	0.097	0.111
inner	MT		0.121	0.097	0.071	0.065	0.091	0.063
	MBC	0.039	0.082	0.032	0.011	0.032	0.082	0.025

[a] Results are means of duplicate samples and corrected with standard deviation.

Cleanup Procedure. The aqueous acid filtrates collected in a separatory funnel were extracted three times with 75-mL portions of chloroform. After phase separation the organic layers were discarded, and the aqueous extract was adjusted to pH 8.5-9.0 with 60% NaOH solution and extracted twice with 50 mL of dichloromethane. The combined extracts were dried with anhydrous Na_2SO_4 and evaporated to total dryness on a rotary evaporator. The residues were immediately dissolved with 1.0 mL of mobile phase (methanol–water–ammonium hydroxide, 60:40:0.6), and filtered through an organic Millipore filter (0.45 μm).

Recoveries. The efficiency of the procedures was determined against a control sample fortified with 1.0, 0.5, and 0.2 ppm of each fungicide compound prior to start the extraction procedure.

RESULTS AND DISCUSSION

In repeated tests, extracting apple samples with these procedures produced acceptable recoveries (85–90%) in all fungicide products studied. Methanol and acetonitrile were compared as extractants of Methylthiophanate from whole fruit, but too many components were extracted and recoveries were low. Thiabendazole recovery was 95–98% and carbendazim 87–90%, extracting the tissue homogenate twice with ethyl acetate. Benomyl recovery also oscillated between 87 and 90%, measured as Carbendazim.

Table V. Residues of Thiabendazole (ppm) in Apples during Cold Storage[a]

	storage time, days						
	0	37	62	90	125	146	174
			Golden Delicious at +2 °C				
peel	4.560	4.232	3.734	3.026	2.114	1.937	1.812
whole fruit	3.307	2.859	2.321	2.251	1.902	1.995	1.821
outer	0.544	0.112	0.194	0.273	0.117	0.098	0.165
inner	0.060	0.022	0.071	0.168	0.037	0.054	0.079
			Starking at 0 °C				
peel	4.634	3.945	3.821	2.562	1.598	1.322	0.833
whole fruit	3.457	2.980	2.611	2.057	1.862	1.403	0.824
outer	0.723	0.171	0.184	0.266	0.104	0.127	0.146
inner	0.155	0.042	0.092	0.287	0.138	0.105	0.040

[a] Results are means of duplicate samples and corrected with standard deviation.

The extraction of Methylthiophanate with benzene provided no interfering materials, and cleanup was not necessary; recoveries of 89–96% (Methylthiophanate) and 93–95% (Carbendazim) were obtained with this extraction procedure.

Separation and retention of Benomyl, Carbendazim, and Thiabendazole were best with ammonium hydroxide in the mobile phase because this product eliminated completely the peak tailings. Methylthiophanate and its metabolite (Carbendazim) showed retention times of 4.02 and 4.90 min, respectively, which allowed their simultaneoius quantitative determination.

Benzimidazole fungicides were highest in the peel and declined toward the core (Tables II–V). Generally, the amounts of active products on whole fruit decreased during storage, and at the end (160 days) there were very low residues of each fungicide (less than 0.3 ppm) in the flesh of both cultivars. Residues of Thiabendazole, Benomyl, and Methylthiophanate on whole fruit and in the peel were 30–25% (cv. Starking) or 45–55% (cv. Golden Delicious) of the amount recovered immediately after treatment. Carbendazim showed greater storage stability, 40–65% on both cultivars.

Benomyl and Carbendazim showed a similar persistence on both apple cultivars, maybe because the fungicide treatments were applied at the same concentration of active product (Tables II and III). However, lower concentrations of residues were found on fruits treated with Methylthiophanate than those treated with Thiabendazole at the same treatment concentration (Tables IV and V).

Most residues were retained in the peel, showing a declining residue gradient from peel to core and with depth of penetration proportional to concentration of fungicide treatment; similar results were reported by Ben-Arie (1975) for postharvest-treated pears.

In the two experiments, 1982 and 1984, with apples stored for 170 days similar residue trends were found for Benomyl and Carbendazim lots (Tables II and III), and the final residue concentrations detected in the pulp, outer and inner zones, were lower than 0.1 ppm of active product. At this date, similar amounts of Methylthiophanate and Carbendazim were found on the fruits treated with Methylthiophanate.

The residue analysis of fruits treated with Methylthiophanate indicated the simultaneous systemicity of this product and its metabolism to Carbendazim into the fruit. There was a balance between a physical process of penetration (Methylthiophanate residues) and a biochemical process (Carbendazim residues). Methylthiophanate residues in the peel were diminishing while the residue concentration into the fruit was increasing.

The Thiabendazole stability in this experiments was greater than the Benomyl one; Thiabendazole appeared to be less mobile than Benomyl in the apple tissue, although the total degradation of both fungicides was very similar. Norman et al. (1973) reported similar losses of Thiabendazole during the storage of treated citrus fruits.

In conclusion, high-performance liquid chromatography provide a sensitive and rapid means for quantitative routine determination of benzimidazole fungicides on apples. The concentrations of residues found on apples after storage were remarkably low, and their toxicological importance could be subordinate to the legal limits admitted in each country.

Registry No. Benomyl, 17804-35-2; Carbendazim, 10605-21-7; Thiabendazole, 148-79-8; Methylthiophanate, 23564-05-8.

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