Quantitative Determination of Thiabendazole in Fruit Juices and Bulk Juice Concentrates Using a Thiabendazole Monoclonal Antibody

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A thiabendazole [2-(4-thiazolyl)-1*H*-benzimidazole, TBZ] monoclonal antibody has been employed in an enzyme immunoassay (EIA) procedure to analyze TBZ in fruit juices and bulk juice concentrates. Samples were prepared by partitioning into methylene chloride and analyzed by EIA and HPLC. The average recovery for 50 juices and concentrates spiked at 5–1000 ppb was 93%. Forty-one market samples containing TBZ in the range from 0.6 to 280 ppb were analyzed, and the analyses by the two methods showed excellent agreement ($r = 0.988$). The detection limits were 0.5 ppb for juice and 2 ppb for bulk concentrates. Because a quicker and simpler sample preparation scheme would be desirable, seven positive juice samples were analyzed following direct dilution. For these samples, the correlation between EIA and HPLC was still very good ($r = 0.92$). Bulk juice concentrates generally required partitioning to completely eliminate matrix effects.

Keywords: *ELISA; thiabendazole; fruit juices; concentrates*

INTRODUCTION

The need for monitoring food for pesticide residues has gained increased interest with the recent publication of *Pesticides in Diets of Infants and Children* (National Research Council, 1993). One approach to obtaining residue data quickly and inexpensively is to use enzyme immunoassay (EIA) (National Research Council, 1987; Office of Technology Assessment, 1988). Immunoassay methods for analyzing pesticide residues in food are becoming widely accepted (Bushway and Fan, 1995) and increasingly available in kit form.

Thiabendazole (TBZ), a member of the benzimidazole fungicide group (Figure 1), is commonly applied postharvest to fruits and vegetables. The tolerance ranges from 10 to 25 ppm depending upon the fruit or vegetable, which is much higher than is normally found on produce. Previous enzyme immunoassay procedures used for analysis of TBZ in juices and concentrates demonstrated significant cross-reactivity with carbendazim, the breakdown product of other widely used fungicides, namely benomyl and thiophanate methyl (Newsome and Collins, 1987; Mountford et al., 1994; Bushway et al., 1994). In addition, carbendazim itself is used as a fungicide outside the United States. One immunoassay reported for TBZ in produce (Brandon et al., 1993) employs a monoclonal antibody that does not cross-react with carbendazim and can thus detect TBZ in samples which also contain benomyl and thiophanate methyl residues. Since benomyl, thiophanate methyl, and TBZ can each be used on several kinds of fruits, a method that can distinguish among them would be most useful, especially for mixed juices and concentrates.

This paper describes an enzyme immunoassay procedure developed for the analysis of thiabendazole in fruit juices and bulk juice concentrates using the monoclonal antibody described by Brandon et al. (1992).

MATERIALS AND METHODS

Materials. Thiabendazole pesticide standard (97.7%) was obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC. All solvents were of HPLC grade and were purchased from EM Science (Gibbstown, NJ). Phosphate salts, ammonium chloride, sodium sulfate, and ammonium hydroxide were bought from VWR (Boston, MA).

Fruit juices were purchased from local supermarkets in the Bangor, ME, area; bulk concentrates were obtained from Coca Cola Foods (Auburndale, FL) and Ocean Spray, Inc. (Lakeville-Middleboro, MA).

Liquid Chromatography System. The HPLC consisted of a Waters 510 pump (Waters Associates, Milford, MA), a Valco pneumatic injector (VICI Instruments, Houston, TX) containing a 10-μL loop, a Waters 470 fluorescence detector, and a Hewlett-Packard 3396 integrator (Avondale, PA).

EIA Materials. Monoclonal antibody 448, the horseradish peroxidase (HRP) conjugate of 5-succinamido-TBZ, and the general assay procedure were previously described (Brandon et al., 1992). However, several modifications were made to the assay procedure. Antibody 448 was bound to polystyrene microtiter wells by ImmunoSystems, Inc. (Scarborough, ME), using their proprietary procedure. Arrays of 1 × 12 strips were used, permitting up to 96 samples and standards to be processed simultaneously. The HRP conjugate was used as a 1:1800 dilution in a proprietary stabilizing diluent (ImmunoSystems). The substrate used was a one-component formulation of tetramethylbenzidine (ELISA Technologies, Lexington, KY), and 1 N HCl was employed as "stop solution". These plates are commercially available from Millipore Corp.

Methods. *The extraction procedure* used was a modification of a soil method for TBZ developed by Cayley and Lord (1980).

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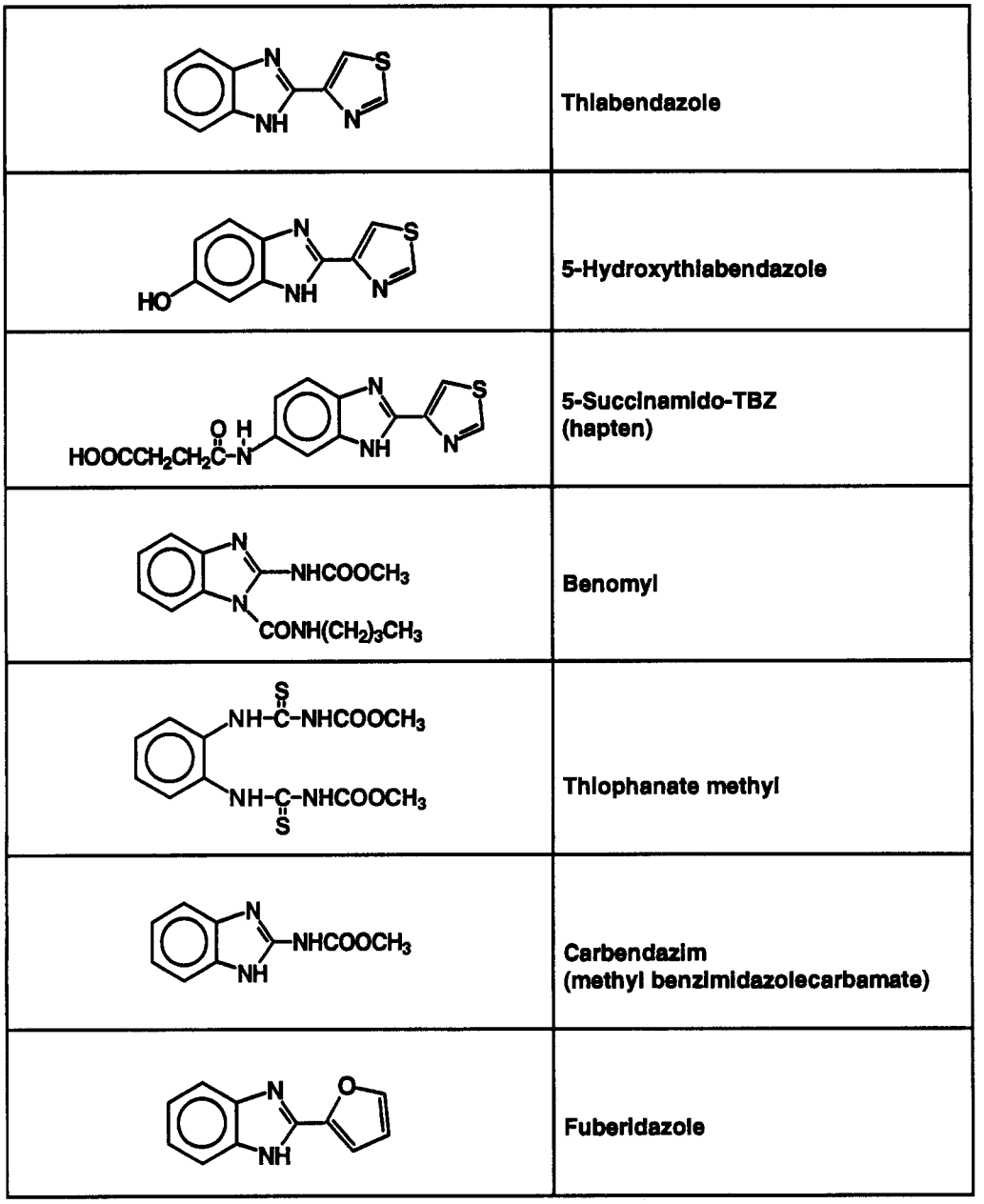

Figure 1. Benzimidazole and thiophanate pesticides and related compounds including the metabolite of TBZ (5-OH-TBZ) and the hapten.

Either 5 mL of juice or 2 g of bulk juice concentrate was extracted by placing it into a 50-mL conical polypropylene centrifuge tube followed by 5 mL of 95% ethanol and 15 mL of 2 M ammonium chloride adjusted to pH 9.5 with 14.5 N ammonium hydroxide. Finally, 20 mL of methylene chloride was added before the mixture was homogenized for 2 min at medium speed with a Polytron Model 1S (Brinkman Instruments, Westbury, NY).

Once homogenized, the samples were vigorously hand shaken for 2 min and then centrifuged for 3 min at 5000*g*. The methylene chloride (bottom layer) was placed into a 20-mL glass scintillation vial containing 0.5 g of anhydrous sodium sulfate. A 10-mL aliquot was removed and evaporated to dryness under nitrogen, and the residue was redissolved in 1 mL of HPLC mobile phase (see Liquid Chromatography Conditions). This milliliter of solution was transferred to a 1.5-mL polypropylene centrifuge tube and centrifuged for 5 min at 10000*g*. A 0.1-mL aliquot was added to 0.9 mL of water before analysis by immunoassay.

Direct Juice Method. One-tenth milliliter of juice was added to 0.9 mL of HPLC mobile phase/0.2 M phosphate buffer, pH 7.0/water (10:40:50 v/v/v). This sample was then applied to the antibody-coated microwells as descibed below.

Preparation of Standard. A stock solution of thiabendazole was prepared at a concentration of 0.84 mg/mL in acetonitrile. From the stock standard an intermediate solution of 0.163 ug/mL in 10% mobile phase was made. Working standards were prepared by removing 6.1, 12.5, 25, 50, and100 μL of intermediate standard and diluting each aliquot to 5 mL with 10% mobile phase. This yielded standards containing 0.2, 0.4, 0.8, 1.6, and 3.2 ng/mL thiabendazole.

HPLC standard was prepared by taking 10 μL of stock solution and adding it to a total volume of 10 mL using mobile phase. This gave a working standard having 0.84 ng/μL thiabendazole.

Liquid Chromatography Conditions. Operating conditions were as follows: injection volume, 10 μL; flow rate, 1.0 mL/min; column, Ultracarb 30 ODS, stainless steel, 15 cm × 4.6 mm i.d. (Phenomenex, Torrance, CA); mobile phase, 500 mL of water/260 mL of acetonitrile/70 mL of methanol/0.1 mL of monoethanolamine; excitation wavelength, 305 nm; emission wavelength, 345 nm; attenuation, 8; gain, 100; filter, 1.5 s. For more details on the HPLC method see Bushway et al. (1995)

EIA Method. Standards and samples in single were analyzed by adding 100 μL to each assay well, followed by 100 μL

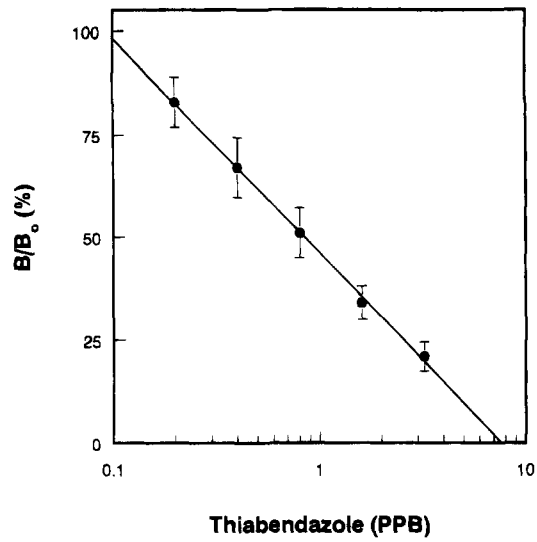

Figure 2. Standard curve for thiabendazole ELISA. Conditions are given in the text.

of enzyme conjugate. The wells were incubated for 60 min at room temperature and then rinsed four times with water to remove unbound sample and conjugate. One hundred microliters of substrate solution was added to each rinsed well and incubated for 30 min before 100 μL of 1 N HCl was added to stop the reaction and change the color from blue to yellow.

Each well was read at 450 nm by employing a strip plate reader (Millipore Corp., Bedford, MA). The percent B_0 (upper asymptote) values were calculated and a standard curve was plotted as percent B_0 vs log TBZ concentration. "Percent B_0" is the percent of color developed by the standards or samples compared to the color of the negative control and is calculated as: A_{450}(standard) or A_{450}(sample)/A_{450} (negative control) × 100.

Recovery Study. Juices and bulk juice concentrates were fortified with TBZ at the following concentrations: 5, 25, 50, 200, and 1000 ng/g. This study was used to ascertain the accuracy of the immunoassay and the efficiency of the thiabendazole extraction technique.

Reproducibility Study. Several juices and juice concentrates were analyzed several times on the same day and different days to determine the intraassay and interassay variation of the EIA method.

RESULTS

Dissolving standards and samples in 10% mobile phase with or without phosphate buffer yielded the same standard curve, which was linear from 0.2 to 3.2 ppb (Figure 2). If one compares the linear range of this assay to previous work by Brandon et al. (1993) (linear range 1−10 ppb), the organic solvent and/or the assay format seems to shift the thiabendazole linear range (0.2−3.2) to make the assay reported here more sensitive. Samples containing thiabendazole concentrations greater than 3.2 ppb were diluted using 10% mobile phase so that they could be quantified from the standard curve.

Detection limits were 0.5 ppb for juices and 2 ppb for bulk concentrates, while the limit of quantitation was ascertained as 1.0 ppb for juices and 3 ppb for bulk concentrates for the immunoassay as determined according to the procedure recommended by the American Chemical Society (ACS, 1980).

The HPLC method employed had a detection limit of 1 ppb for juice and 2 ppb for bulk concentrates and quantitation limits of 2.5 ppb for juice and 4.5 ppb for juice concentrates (ACS, 1980).

Table 1. Reproducibility of the Thiabendazole (TBZ) Immunoassay for Standards Prepared in 10% Mobile Phase with or without Buffer

	CV (%)	
TBZ std (ppb)	intra-assay[a]	interassay[b]
0.2	3.9	7.3
0.4	5.6	11
0.8	4.6	12
1.6	6.4	12
3.2	7.3	17

[a] Percent coefficients of variation based on the $\%B_0$ values for 6 determinations in 1 day. [b] Percent coefficients of variation based on the $\%B_0$ values for 46 determinations performed over a period of 4 months.

Table 2. Reproducibility of the Thiabendazole (TBZ) Immunoassay for Juices by the Partition Method

		CV (%)	
sample	TBZ (ppb)	intra-assay[a]	interassay[b]
apple cider 1	159	4.6	19
apple mix	5.1	11	10
grapefruit	126	11	15
pear	221	9.1	14
apple cider 2	257	7.5	17

[a] Percent coefficients of variation based on 6 determinations in 1 day. [b] Percent coefficients of variation based on 6 determinations on 6 different days except for apple cider 2 interassay, which was based on 5 determinations in 5 days.

Table 3. Reproducibility of the Thiabendazole (TBZ) Immunoassay for Juices by the Direct Method

		CV (%)	
sample	TBZ (ppb)	intra-assay[a]	interassay[b]
apple cider 1	84	13	8
apple cherry	9.2	12	21
grapefruit 1	21	7.5	19
lemon	12	1.7	30
pear	214	13	15
grapefruit 2	166	6.0	5
apple mix	8.2	24	16

[a] Percent coefficients of variation based on 4 determinations in 1 day except for apple mix, which was based on 3 determinations in 1 day. [b] Percent coefficients of variation based on 4 determinations performed on 4 different days.

Juices could be analyzed either by directly diluting the sample in 10% mobile phase containing 40% 0.2 M phosphate buffer, pH 7.0, or by partitioning into methylene chloride. However, the bulk concentrates required partitioning into methylene chloride before analysis. Matrix effects were encountered using the direct dilution method for concentrates other than apple. For example, eight TBZ-positive concentrates analyzed by EIA yielded TBZ levels 20−50% higher than the HPLC results.

With any analytical technique, the reproducibility of the results between runs and over time is very important. Table 1 shows the data on the reproducibility of the TBZ standards, conducted over a 4-month period. The intra- and interassay reproducibilities were excellent, with coefficients of variation (CVs) ranging from 3.9 to 17%. Only the interassay CV for the highest standard exceeded 12%.

Table 2 shows the data on reproducibility of the EIA of juices by the partition method. The CVs averaged 8.6% for intra-assay and 15% for interassay comparisons. Table 3 shows the corresponding data for juice analyzed by direct dilution. The CVs averaged 11% for intra-assay and 16.2% for interassay compari-

Table 4. Reproducibility of the Thiabendazole (TBZ) Immunoassay for Bulk Concentrates by the Partition Method

sample	TBZ (ppb)	CV (%) intra-assay[a]	CV (%) interassay[b]
apple 1	133	19	3.8
apple 2	4.1	10	12
apple 3	89	9.7	9.7
apple 4	24	8.1	3.2
apple 5	23	13	11
apple 6	14	10	16

[a] Percent coefficients of variation based on 4 determinations in 1 day. [b] Percent coefficients of variation based on 4 determinations performed on 3 different days.

Table 5. Accuracy of Thiabendazole (TBZ) Immunoassay for Juices and Concentrates[a]

sample[b]	TBZ added (ppb)	mean, TBZ found (ppb)	mean rec (%)	CV (%)
mix juice	5.0	5.5	109	19
mix juice	25	22	87	5.8
mix juice	50	42	83	9.0
mix juice	200	162	81	8.9
mix juice	1000	850	85	13
mix concentrate	5.0	5.5	110	23
mix concentrate	25	23	92	8.1
mix concentrate	50	44	88	13
mix concentrate	200	204	102	17
mix concentrate	1000	920	92	6.2

[a] Means and percent coefficients of variations based on 5 determinations except mix conc. 200, which is based on 4 determinations. [b] Mix juice means that the following juices were used in the recovery study: apple juice, apple grape juice, cranberry raspberry juice, apple sweet potato, apple cherry, and fruit punch. Mix conc. means that the following kinds were used in the recovery study: apple, orange, and raspberry. The word "mix" means that for each spiking level there were four or five different juices used and these juices were taken from the different ones listed under the juice and concentrate section.

son. Neither intra- nor interassay precision was significantly different for the partition vs direct dilution.

Table 4 illustrates the reproducibility of the EIA analysis of six apple concentrates by the partition method. The intra-assay reproducibility was similar to that for juices, but the interassay precision was significantly greater (average CV 9.3% compared to 15%, $p < 0.1$).

To determine the efficiency of the extraction method and the accuracy of the assay, juice and concentrate samples were fortified at levels from 5 to 1000 ppb and then analyzed. Recoveries ranged from 81 to 110% (Table 5) and indicated that the EIA partition procedure produced accurate results. The procedure was precise as well, with CVs ranging from 5.8 to 23. Excluding the lowest (5 ppb) standards, the average CV was 10%.

To ascertain the effectiveness of the immunoassay method for TBZ, correlation studies were performed. Table 6 shows the results of EIA and HPLC analyses of 41 samples of juice and bulk concentrates containing detectable levels of TBZ. For these samples, ranging in concentration from 0.6 to 284 ng/mL, excellent linear correlation was achieved ($r = 0.988$) with a slope of 0.950.

Table 7 shows the results of direct dilution EIA and HPLC analyses for seven TBZ-positive juice samples. The concentrations ranged from 4.5 to 233 ppb, and the linear regression of HPLC vs EIA had a slope of 1.03 ($r = 0.92$).

Table 6. Comparison of immunoassay and HPLC for the Determination of Thiabendazole (TBZ) in Juices and Industrial Strength Concentrates

sample	immunoassay TBZ[a] (ppb)	HPLC TBZ[a] (ppb)
apple cider juice	280	284
apple cider juice	92	124
apple raspberry juice	1.0	1.1
apple cranberry juice	8.4	11
grapefruit juice	18	18
grapefruit juice	16	15
lemon juice	16	17
lime juice	1.1	1.1
grapefruit juice	19	23
apple juice	3.0	3.1
apple cherry juice	0.7	1.3
apple cherry juice	3.3	3.6
apple grape juice	7.6	5.5
lime juice	0.6	1.5
fruit punch juice	76	72
pear juice	216	220
lime juice	2.2	4.9
grapefruit juice	135	187
apple mix juice	4.2	5.1
apple concentrate	135	133
apple concentrate	126	109
apple concentratec	3.7	5.4
orange concentrate	3.7	2.7
orange concentrate	15	9.3
apple concentrate	8.6	5.6
apple concentrate	3.0	2.7
apple concentrate	36	35
apple concentrate	2.6	4.4
peach concentrate	3.5	3.0
apple concentrate	9.6	5.7
apple concentrate	23	16
apple concentrate	15	14
peach concentrate	5.3	4.7
apple concentrate	77	118
apple concentrate	168	172
apple concentrate	8.5	6.8
apple concentrate	32	32
apple concentrate	2.0	3.4
apple concentrate	3.2	4.0
apple concentrate	220	242
apple concentrate	70	84

[a] These single values were generated from the partition method.

Table 7. Comparison of the Direct Immunoassay and HPLC for the Quantitation of Thiabendazole (TBZ) in Juices

sample	direct immunoassay TBZ[a] (ppb)	HPLC TBZ[a] (ppb)
apple cider	84	172
apple cherry	9.2	3.6
grapefruit 1	21	19
lemon	12	17
pear	214	233
grapefruit 2	166	136
apple mix	8.2	4.5

[a] Values were based on a single analysis.

DISCUSSION

The excellent agreement between EIA and HPLC analyses reflects the specificity of the TBZ antibody. Data on specificity were presented earlier (Brandon et al., 1992). However, since we are describing a modified format and different diluents in our present method, cross-reactivity was revisited. Table 8 shows the results of a cross-reactivity study using 25 pesticides and 1 TBZ metabolite, 5-OH-TBZ. Only one pesticide, fuberidazole, a benzimidazole fungicide, demonstrated any reactivity at 2 ppm or less. The IC_{50} was 2.5 ppb and the LLD was 0.5 ppb, while TBZ had an IC_{50} of 0.83 ppb and an

Table 8. Specificity of Monoclonal Antibody 448 in a Competitive EIA

compound[a]	IC_{50}[b] (ppb)	LLD[c] (ppb)
TBZ	0.83	0.2
fuberidazole	2.5	0.5
5-OH-TBZ	0.8	0.2

[a] The following list of pesticides did not show cross-reactivity at 2 ppm: carbendazim, CIPC (chloropropham), benomyl, captan, captofol, fluchloralin, vinclozolin, chlorothalonil, 2-4-D, imazalil, iprodione, metribuzin, thiophanate, thiophanate methyl, bayleton, fenarimol, imazaquin, imazapyr, TCNB (tetrachloronitrobenzene), procymidone, tetradifon, diazinon, balan, and buthidazole. [b] Concentration of compound that inhibits binding of the HRP-hapten conjugate by solid-phase antibody by 50%. [c] Concentration of compound that yields 85% of $\%B_0$.

LLD of 0.2 ppb. 5-OH-TBZ showed binding identical to that of TBZ. From this study and that of Brandon et al. (1992), it appears that the antibody can recognize either an unsubstituted thiazole or a furan ring attached to the benzimidazole portion of the molecule with stronger binding than the unsubstituted thiazole ring. Such specificity was developed particularly for the analysis of TBZ residues in food animal tissues, in which the U.S. federal tolerances are expressed as the sum of TBZ and 5-OH-TBZ, which occurs in animal tissues and fluids but not in plant tissues (Zbozinek, 1984). Fuberidazole does not have any U.S. tolerances for use on food or feed but instead is used as a fungicide on seed grain.

It is apparent from the data in Tables 6 and 7 that many "market basket" samples of juices and concentrates contain residues of TBZ. The positive samples represent 37% of all the samples studied (a total of 112 samples analyzed). It should be noted that most of these levels are in the range of 0.2–37 ppb [the acceptable daily intake (ADI) has been set at 0.1 ppm, making some of these juices higher than the ADI, whereas typical concentrations in positive samples of fresh produce are considerably higher (Brandon et al., 1993; Bushway et al., 1995)]. The most important reason for this difference stems from the predominant use of TBZ as a postharvest fungicide, applied as a dip or spray (Davidse, 1986). Consequently, TBZ reaches the internal areas of the fruit by penetration of the peel, and the levels in the flesh are dependent on conditions of treatment as well as storage (Ben-Arie, 1975). In the previously cited studies from our laboratories and others (Friar and Reynolds, 1991), particularly high levels of TBZ were found in the peel fraction. In addition, residues decline during storage of commodities (Norman et al., 1972; Ben-Arie, 1975; Cano et al., 1987). Although extensive washing can remove most of TBZ residues in potatoes (Ministry of Agriculture, Food, and Fisheries, 1982), it appears that further heat processing does not affect residues, at least in potatoes (Friar and Reynolds, 1991; Bushway, unpublished results).

The data in Table 6 show that juices and concentrates from apples and pears have the highest amounts of TBZ. Although peels are not usually processed into juice, certain fruits with thin skin, like pears and apples, are processed by techniques that could extract some of the TBZ from the peel. Once the juice is expressed, it is evaporated and clarified. Although the heat used for evaporation is not sufficient to destroy TBZ, some of the clarification techniques, such as the use of absorbents, could remove TBZ.

The data presented indicate that the accuracy of the EIA method was improved when juices and concentrates were first partitioned into methylene chloride rather than diluted directly. These results are very similar to what was found during the EIA analysis of carbendazim in blueberries (Bushway et al., 1992). In that case, methanol extracts analyzed directly yielded less precision and accuracy than methanol extracts that were partitioned in methylene chloride.

However, as shown by an admittedly limited number of juice samples analyzed by direct dilution, this simple and rapid sample preparation afforded very good agreement with the HPLC determination. As a practical matter, the EIA should have more widespread utility as a rapid method performed on directly diluted samples.

This simple direct dilution technique described for juices can also be applied to apple concentrates. Other concentrates, however, required further cleanup, such as the methylene chloride partition step, for accurate quantitation.

In summary, this EIA method is sufficiently rapid and specific to be used by the juice industry and by government and private laboratories for monitoring TBZ residues in juice and bulk concentrates. In addition, it correlated very favorably with a standard—more conventional—HPLC method. Convenient and affordable immunodiagnostic methods, such as the one described in this paper, should facilitate the collection of data needed for a modern food safety program which ensures the availability of a safe and wholesome food supply.

LITERATURE CITED

ACS. Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry. *Anal. Chem.* **1980**, *52*, 2242–2249.

Ben-Arie, R. Benzimidazole penetration, distribution, and persistence in postharvest-treated pears. *Phytopathology* **1975**, *65*, 1187–1189.

Brandon, D. L.; Binder, R. G.; Bates, A. H.; Montague, W. C., Jr. A monoclonal antibody-based ELISA for thiabendazole in liver. *J. Agric. Food Chem.* **1992**, *40*, 1722–1726.

Brandon, D. L.; Binder, R. G.; Wilson, R. E.; Montague, W. C., Jr. Analysis of thiabendazole in potatoes and apples by ELISA using monoclonal antibodies. *J. Agric. Food Chem.* **1993**, *41*, 996–999.

Bushway, R. J.; Fan, T. S. Detection of pesticide and drug residues in foods by immunoassay. *Food Technol.* **1995**, *49* (2), 108–115.

Bushway, R. J.; Kugabalasooriar, J.; Perkins, L. B.; Harrison, R. O.; Young, B. E. S.; Ferguson, B. S. Determination of methyl 2-benzimidazolecarbamate in blueberries by competitive inhibition enzyme immunoassay. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 323–327.

Bushway, R. J.; Young, B. E. S., Paradis, L. R.; Perkins, L. B. Determination of thiabendazole in fruits and vegetables by competitive inhibition enzyme immunoassay. *J. AOAC Int.* **1994**, *77*, 1243–1248.

Bushway, R. J.; Li, L.; Paradis, L. R.; Perkins, L. B. Determination of thiabendazole in potatoes, fruits and their processed products by high-performance liquid chromatography. *J. AOAC Int.* **1995**, in press.

Cano, P.; de la Plaza, J. L.; Munoz-Delgado, L. Determination and persistence of several fungicides in postharvest-treated apples during their cold storage. *J. Agric. Food Chem.* **1987**, *35*, 144–148.

Cayley, G. R.; Lord, K. A. The extraction and assay of thiabendazole in strongly adsorbing soils. *Pestic. Sci.* **1980**, *11*, 9–14.

Davidse, L. C. Benzimidazole fungicides: mechanisms of action and biological impact. *Annu. Rev. Pathopathol.* **1986**, *24*, 43–65.

Friar, P. M. K.; Reynolds, S. L. The effects of microwave-baking and oven-baking on thiabendazole residues in potatoes. *Food Addit. Contam.* **1991**, *8*, 617–626.

Ministry of Agriculture, Fisheries, and Food. Report of the Working Party on Pesticide Residues (1977–1981); Food Surveillance Paper 9; HMSO: London, 1982.

Mountfort, K. A.; Reynolds, S. L.; Thorpe, S. A.; White, S. N. Comparison of ELISA and HPLC techniques for the analysis of carbendazim an thiabendazole residues in fruit and vegetables. *Food Agric. Immunol.* **1994**, *6*, 17–22.

National Research Council. *Regulating Pesticides in Food. The Delaney Paradox;* National Academy Press: Washington, DC, 1987.

National Research Council. *Pesticides in the Diets of Infants and Children;* National Academy Press: Washington, DC, 1993.

Newsome, W. H.; Collins, P. G. Enzyme-linked immunosorbent assay of benomyl and thiabendazole in some foods. *J. Assoc. Off. Anal. Chem.* **1987**, *70*, 1025–1027.

Norman, S. M.; Fouse, D. C.; Craft, C. C. Thiabendazole residues on and in citrus. *J. Agric. Food Chem.* **1972**, *20*, 1227–1230.

Office of Technology Assessment. *Pesticide Residues in Food;* U.S. Government Printing Office: Washington, DC, 1988.

Zbozinek, J. V. Environmental transformations of DPA, SOPP, benomyl, and TBZ. *Residue Rev.* **1984**, *92*, 114–155.

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