UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

Date: 8/Jan./99

**SUBJECT:** **Thiabendazole Reregistration**. 860.1500 and 860.1340-Response to Agency Reviews for Magnitude of the Residue in Apples, Citrus, and Wheat; Analytical Method in Wheat and ILV, Magnitude of the Residue in Citrus and Banana Following Post-harvest Dip Application.

DP Barcode No.: D207850, D218096, D245782, D245785, and D245787.
Reregistration Case No.: 2670.
PC Code: 060101.
MRID No.: 43328301-43328303, 43328306, 43328307 and 43721901-43721904.

**FROM:** Sherrie L. Mason, Chemist
Reregistration Branch II
Health Effects Division (7509C)

**THROUGH:** Alan Nielsen, Branch Senior Scientist
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**TO:** William Sproat, Chemical Review Manager
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Special Review and Reregistration Division [7508W]

Attached is a review of studies submitted for the magnitude of the residue in apples, citrus and wheat; the residue analytical method in wheat and independent laboratory validation (ILV); and the magnitude of the residue in citrus and banana following post-harvest dip application of thiabendazole (TBZ). The submission was reviewed by Dynamac Corporation under the supervision of HED. This information has undergone secondary review in Reregistration Branch 2 and is consistent with current Agency policies.

1. **Wheat (MRID #: 43328301, 43328302 and MRID #: 43328303):**
The HPLC/fluorescence detection method (ABC Laboratories Report No. 39296E) for determining residues of total free and conjugated benzimidazole (BNZ) in/on wheat grain and straw is adequate. This method has undergone a successful ILV, and has been forwarded to the Analytical Chemistry Branch (ACB) for validation.

The available wheat processing study is adequate. Residues of TBZ and BNZ (free and conjugated)

do not concentrate in processed wheat commodities.

The available data on TBZ residues in/on wheat grain and straw crop field trials are adequate.

2. **Citrus (MRID #: 43328306 and MRID #: 43328307):**
Merck's HPLC/fluorescence detection method M-049 (revision 1) is adequate for determining residues of TBZ in/on whole citrus fruit, and Merck's HPLC/fluorescence detection method M-040 for determining residues of total BNZ (free and conjugated) in/on whole citrus fruits and dried pulp, are adequate.

The available citrus processing study is adequate and no additional data on BNZ residues in citrus processed fractions are required. Residues of TBZ do not concentrate in citrus juice or molasses, but concentrate by 1.5-1.6x in dried citrus pulp and by 1.7-2.7x in citrus oil.

Based on the available BNZ residue data for citrus and the information on metabolism of TBZ in citrus treated post-harvest, HED concurs that residues of BNZ (free and conjugated) are not likely to contribute significantly to the total TBZ residues in/on citrus fruits treated post-harvest. Therefore, no additional data on BNZ residues in/on citrus fruits treated post-harvest are required.

The available residue data reflecting post-harvest applications to citrus fruits support the established 10 ppm tolerance for TBZ residues in/on citrus fruit provided end-use product labels are amended to specify the following use directions. In all citrus producing regions except AZ and CA, a maximum of two post-harvest applications can be applied. The first as an aqueous dip of TBZ at up to 1,000 ppm for up to 3 minutes prior to de-greening, followed by second application (after de-greening and washing) of TBZ up to 3,500 ppm in wax (1 gal wax/3,500 lb of fruit). In AZ and CA, only a single post-harvest application is allowed as an application of TBZ in wax up to 5,000 ppm (1 gal wax/3,500 lb of fruit).

3. **Bananas (MRID #: 43721901 and MRID #: 43721902):**
Merck's HPLC/fluorescence detection method M-023 (revision 1) for determining residues of TBZ in/on whole green bananas and ripe banana pulp, and Merck's HPLC/fluorescence detection method M-056 for determining residues of total BNZ (free and conjugated) in/on whole green bananas, are adequate.

Provided that labels specify a maximum dip application time of 1 minute, the available data on TBZ residues in/on bananas are adequate and support the established 3 ppm tolerance for residues of TBZ in/on bananas.

4. **Apples and Pears (MRID #: 43721903 and 43721904):**
Merck's HPLC/fluorescence detection method M-041 is adequate for determining residues of total BNZ (free and conjugated) in/on apples and dry apple pomace.

The available apple processing study is adequate and no additional data on BNZ residues in apple

processed fractions are required. Residues of TBZ do not concentrate in apple juice, but concentrate by 3.5x in wet pomace and by 11.6x in dried pomace. As dried apple pomace is no longer a regulated apple commodity (OPPTS Guideline 860.1000, Table 1), the tolerance for TBZ residues in apple pomace, dried should be deleted. However, a tolerance for TBZ residues in wet apple pomace is required.

The Agency concurs that residues of BNZ (free and conjugated) are unlikely to comprise a significant portion of the total TBZ residue in/on pome fruits treated post-harvest with TBZ. Therefore, complete data for residues of BNZ (free and conjugated) in/on apples and pears are not required, and the deficiencies pertaining to the apple and pear residue studies are resolved. Provided labels are amended to specify a minimum of a 30-day interval between aqueous dip and wax applications of TBZ to pome fruit, the available pome fruit residue data are adequate and support lowering the tolerance for TBZ residues in/on apples to 5 ppm and lowering the tolerance for TBZ residues on pears to 5 ppm as well.

## EXECUTIVE SUMMARY

**Deficiencies in Wheat (MRID #: 43328301, 43328302 and MRID #: 43328303):** There are no deficiencies that would seriously compromise the interpretation of these data; however, **at the time of the Thiabendazole RED, the established 3 ppm tolerances for TBZ residues in wheat milled products (except flour) should be deleted from 40 CFR §185.5550 and §186.5550(a), and tolerances for TBZ residues in/on wheat grain and straw should be reduced to 0.2 and 0.5 ppm, respectively.**

**Deficiencies in Citrus (MRID #: 43328306 and MRID #: 43328307):** There are no deficiencies that would seriously compromise the interpretation of these data; however, **at the time of the Thiabendazole RED, the established tolerances for TBZ residues in citrus molasses and dried pulp should be deleted from 40 CFR §186.5550(a), a tolerance (concentration factor of 2.4x and tolerance of 15 ppm would be appropriate) is required for TBZ residues in citrus oil, and the end-use product labels must be amended to specify the following use directions.** In all citrus producing regions except AZ and CA, a maximum of two post-harvest applications can be applied. The first as an aqueous dip of TBZ at up to 1,000 ppm for up to 3 minutes prior to de-greening, followed by second application (after de-greening and washing) of TBZ up to 3,500 ppm in wax (1 gal wax/3,500 lb of fruit). In AZ and CA, only a single post-harvest application is allowed as an application of TBZ in wax up to 5,000 ppm (1 gal wax/3,500 lb of fruit).

**Deficiencies in Bananas (MRID #: 43721901 and MRID #: 43721902):** There are no deficiencies that would seriously compromise the interpretation of these data; however, **the labels should specify a maximum dip application time of 1 minute and at the time of the Thiabendazole RED, the established 0.4 ppm tolerance for residues of TBZ in banana pulp should be deleted.**

**Deficiencies in Apples and Pears (MRID #: 43721903 and 43721904):** There are no deficiencies that would seriously compromise the interpretation of these data; however, **the tolerance for TBZ residues in dried apple pomace should be deleted, a tolerance (3.5x concentration factor and 12 ppm**

**tolerance in wet apple pomace would be appropriate) for TBZ residues in wet apple pomace is required, and labels should be amended to specify a minimum of a 30-day interval between aqueous dip and wax applications of TBZ to pome fruit.**

**cc: Sherrie L. Mason (RRB2), Thiabendazole Reg. Std. File, Thiabendazole Subject File, RF, LAN. RDI: RRB2 Res. Chem. Team (12/30/1998).**

**7509C: RRB2: S. Mason: CM#2:Rm 718Q: 703-305-0563: 1/8/99.**

# THIABENDAZOLE
# Shaughnessy No. 060101; Case 2670
# (DP Barcodes D207850 and D218096)

Registrant's Response to Residue Chemistry Data Requirements

January 9, 1997

Contract No. 68-D4-0010

Submitted to:
U.S. Environmental Protection Agency
Arlington, VA

Submitted by:
Dynamac Corporation
1910 Sedwick Road
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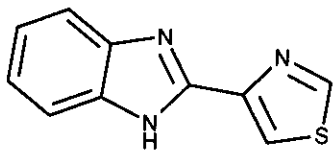
# Thiabendazole



(Shaughnessy No. 060101. Case No. 2670)

DP Barcode D207850 and D218096

## REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

### BACKGROUND

The Thiabendazole Phase IV Review (C. Olinger, 2/20/91) required data depicting thiabendazole (TBZ) residues in/on apples, citrus, banana, and wheat commodities treated with thiabendazole at the maximum label rates specified for the respective crops. In response, Merck & Company, Inc. submitted residue data reflecting post-harvest applications of TBZ to pome fruits, bananas, and citrus fruits along with apple and citrus processing studies. In its reviews of the post-harvest use data, the Agency ( DP Barcode D185173, R. Perfetti, 4/15/93; DP Barcode D186592, J. Abbotts, 7/30/93; and DP Barcode D196149, F. Suhre, 4/14/94) concluded that these studies were inadequate principally because residues of free and conjugated benzimidazole (BNZ) were not determined. In the banana study, the Agency also noted that the application method used did not reflect the most aggressive post-harvest application method for bananas. In the citrus study, the Agency likewise pointed out that the tests conducted in FL contained numerous deficiencies and that fruit from these tests had over-tolerance residues of TBZ.

In response to the Phase IV Review, Merck also submitted data on residues of TBZ and BNZ (free) in wheat and wheat processed commodities. After reviewing these submissions, the Agency (DP Barcode D190451, L. Cheng, 7/28/93) concluded that the data were inadequate primarily because the analytical method employed could not determine conjugated BNZ residues.

In its current submissions (1994; MRIDs 43328301-43328303, 43328306, and 43328307; and 1995, MRIDs 43721901-43721904), Merck has responded to the deficiencies cited in the earlier apple, banana, citrus, and wheat magnitude of the residue studies and the apple, citrus, and wheat processing studies. These submissions are reviewed in this report for adequacy in fulfilling outstanding residue chemistry data requirements. The Conclusions and

Recommendations stated in this document pertain only to analytical methodology, and magnitude of the residue in/on apples, bananas, citrus, and wheat, and in apple, citrus fruit, and wheat processed commodities.

The nature of the residue in plants and animals is adequately understood based on plant metabolism studies on soybean, sugar beet, and wheat, and animal metabolism studies on poultry, and goats. The HED Metabolism Committee has concluded that residues of concern in plants are TBZ and BNZ and its conjugates (L. Cheng, 3/11/92). The residues of concern in animals are TBZ, 5-OH-TBZ (free and conjugated), and BNZ (L. Cheng, 3/2/92).

Tolerances for residues of thiabendazole (2-(4-thiazolyl)benzimidazole) in or on raw agricultural commodities and processed foods and feeds are currently expressed in terms of TBZ *per se* [40 CFR §180.242(a), §185.5550, and §186.5550(a)]. Tolerance for residues of TBZ in animal commodities are currently expressed as the combined residues of TBZ and its metabolite 5-OH-TBZ [40 CFR §180.242(b)]. Methods are available for determining residues of TBZ *per se* in or on plant commodities and are listed in PAM, Vol. II, as Methods I, A, B, and C.

Codex Maximum Residue Limits (MRLs) for TBZ are presently expressed in terms of TBZ *per se* for plant commodities and in terms of the combined residues of TBZ and 5-OH-TBZ for animal commodities. Issues regarding the compatibility of the U.S. tolerances and Codex MRLs will be addressed when the reregistration eligibility decision for thiabendazole is made.

## CONCLUSIONS AND RECOMMENDATIONS

1a. Merck's HPLC/fluorescence detection method M-023 (revision 1) is adequate for determining residues of TBZ *per se* in/on whole green bananas and ripe banana pulp. The validated limit of quantitation (LOQ) for TBZ is 0.05 and 0.02 ppm in/on whole green bananas and ripe pulp, respectively.

1b. Merck's HPLC/fluorescence detection method M-049 (revision 1) is adequate for determining residues of TBZ *per se* in/on whole citrus fruit. The validated LOQ for TBZ is 0.05 ppm in/on whole citrus fruits.

1c. Merck's HPLC/fluorescence detection method M-040 is adequate for determining residues of total BNZ (free and conjugated) in/on whole citrus fruits and dried pulp. The validated LOQs for BNZ are 0.02 ppm in/on whole fruit and 0.08 ppm in dried pulp.

1d. Merck's HPLC/fluorescence detection method M-041 is adequate for determining residues of total BNZ (free and conjugated) in/on apples and dry apple pomace. The validated LOQs for BNZ are 0.025 ppm in/on apples and 0.05 ppm in dry pomace.



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1e. Merck's HPLC/fluorescence detection method M-056 is adequate for determining residues of total BNZ (free and conjugated) in/on whole green bananas. The validated LOQ for BNZ is 0.01 ppm in/on green bananas.

1f. The HPLC/fluorescence detection method (ABC Laboratories Report No. 39296E) for determining residues of total BNZ (free and conjugated) in/on wheat grain and straw is adequate. The validated LOQ for total BNZ is 0.1 ppm in/on wheat grain and straw, and the estimated LOD is 0.05 ppm. This method has undergone a successful independent laboratory validation (ILV), and should be forwarded to the Analytical Chemistry Branch (ACB) for validation.

2a. The Agency concurs that residues of BNZ (free and conjugated) are unlikely to comprise a significant portion of the total TBZ residue in/on pome fruits treated post-harvest with TBZ. Therefore, complete data for residues of BNZ (free and conjugated) in/on apples and pears are not required, and the deficiencies pertaining to the apple and pear residue studies are resolved.

2b. Provided labels are amended to specify a minimum of a 30-day interval between aqueous dip and wax applications of TBZ to pome fruit, the available pome fruit residue data are adequate and support lowering the tolerance for TBZ residues in/on apples to 5 ppm and lowering the tolerance for TBZ residues on pears to 5 ppm as well.

3a. Provided that labels specify a maximum dip application time of 1 minute, the available data on TBZ residues in/on bananas are adequate and support the established 3 ppm tolerance for residues of TBZ in/on bananas.

3b. Residues of TBZ *per se* were 0.96-2.25 ppm in/on whole green bananas sampled immediately following a post-harvested dip (1 minute) application of TBZ at 400 ppm (1x), and residues of BNZ (free and conjugated) were non-detectable ($<0.005$ ppm). In the earlier banana studies, residues of TBZ *per se* were 0.6-1.26 ppm in/on whole green bananas treated in Honduras with TBZ as a spray mist at 400 ppm (1x) and shipped to the U.S. in refrigerated commercial containers.

3c. At the time of the Thiabendazole RED, the established 0.4 ppm tolerance for residues of TBZ in banana pulp should be deleted as banana pulp is not a specifically regulated commodity of bananas.

4a. Based on the available BNZ residue data for citrus and the information on metabolism of TBZ in citrus treated post-harvest, HED concurs that residues of BNZ (free and conjugated) are not likely to contribute significantly to the total TBZ residues in/on citrus fruits treated post-harvest. Therefore, no additional data on BNZ residues in/on citrus fruits treated post-harvest are required.

4b. The available residue data reflecting post-harvest applications to citrus fruits support the established 10 ppm tolerance for TBZ residues in/on citrus fruit provided end-use product labels are amended to specify the following use directions. In all citrus producing regions except AZ and CA, a maximum of two post-harvest applications can be applied. The first as an aqueous dip of TBZ at up to 1,000 ppm for up to 3 minutes prior to de-greening, followed by second application (after de-greening and washing) of TBZ at up to 3,500 ppm in wax (1 gal wax/3,500 lb of fruit). In AZ and CA, only a single post-harvest application is allowed as an application of TBZ in wax at up to 5,000 ppm (1 gal wax/3,500 lb of fruit).

4c. In four tests conducted in FL, residues of TBZ were 2.7-4.7 ppm and residues of BNZ (free and conjugated) were $<0.01$ ppm in/on oranges, tangerines, and grapefruit collected immediately following a sequential post-harvest dip application of TBZ at 1000 ppm (3 minute) and a wax application containing TBZ at 3500 ppm (1 gal wax/3500 lb of fruit). In the four earlier citrus tests conducted in CA, residues of TBZ *per se* were 1.2-5.2 ppm in/on oranges, grapefruits, and lemon following a sequential post-harvest aqueous dip of TBZ at 5000 ppm for 3 minutes and a wax application at 5000 ppm (1 gal wax/3500 lb of fruit).

5. The available data on TBZ residues in/on wheat grain and straw are adequate. Based upon the available residue data, the combined residues of TBZ and BNZ (free and conjugated) are $<0.15$ ppm in/on wheat grain and $<0.23$ ppm in/on wheat straw harvested $\geq 60$ days following a single foliar application at tillering of TBZ at 0.67 lb ai/A (1x). At the time of the Thiabendazole RED, tolerances for TBZ residues in/on wheat grain and straw should be reduced to 0.2 and 0.5 ppm, respectively.

6. The available apple processing study is adequate and no additional data on BNZ residues in apple processed fractions are required. Residues of TBZ do not concentrate in apple juice, but concentrate by 3.5x in wet pomace and by 11.6x in dried pomace. As dried apple pomace is no longer a regulated apple commodity (OPPTS Guideline 860.1000, Table 1), the tolerance for TBZ residues in apple pomace, dried should be deleted. However, a tolerance for TBZ residues in wet apple pomace is required. Based upon the highest average field trial (HAFT) residues found in/on apples (3.4 ppm) and the 3.5x concentration factor, an appropriate tolerance for TBZ residues in wet apple pomace would be 12 ppm.

7. The available citrus processing study is adequate and no additional data on BNZ residues in citrus processed fractions are required. Residues of TBZ do not concentrate in citrus juice or molasses, but concentrate by 1.5-1.6x in dried citrus pulp and by 1.7-2.7x in citrus oil. Based upon HAFT residues of 5.2 ppm in/on whole fruit, a tolerance for dried citrus pulp is not necessary as the maximum expected residues in dried pulp (8.3 ppm) would be covered by the 10 ppm tolerance for residues in/on whole fruit. A the time of the Thiabendazole RED, the established tolerances for TBZ residues in

citrus molasses and dried pulp should be deleted from 40 CFR §186.5550(a). However, a tolerance is required for TBZ residues in citrus oil. Based upon the above HAFT residues and an average concentration factor of 2.4x, a tolerance of 15 ppm for residues of TBZ in citrus oil would be appropriate.

8. The available wheat processing study is adequate. Residues of TBZ and BNZ (free and conjugated) do not concentrate in processed wheat commodities. At the time of the Thiabendazole RED, the established 3 ppm tolerances for TBZ residues in wheat milled products (except flour) should be deleted from 40 CFR §185.5550 and §186.5550(a).

## DETAILED CONSIDERATIONS

### Residue Analytical Methods

In conjunction with the submissions pertaining to TBZ residues in/on apples, bananas, and citrus fruit, Merck submitted method descriptions (1995, MRID 43721902-43721904) for new HPLC/fluorescence detection methods for determining TBZ residues of concern in/on apple, banana, and citrus commodities along with method validation data. These methods are similar to the current enforcement methods in that residues are extracted with ethyl acetate (EtOAc) and are quantified fluorometrically. However, the new methods employ additional clean-up procedures and utilize HPLC/fluorescence detection for separating and quantifying residues.

*Merck Method M-023 (revision 1)* is for determining residues of TBZ *per se* in/on whole green bananas and ripe banana pulp. Green bananas are chopped and homogenized as is, and ripe pulp is diluted with water (1:1, w/w) and homogenized. Homogenized samples are diluted and mixed with pH 8 buffer, and residues are extracted into EtOAc. For clean-up, extracted residues are loaded onto a propyl sulfonic acid (PRS) solid phase extraction (SPE) column that has been preconditioned by washing successively with 1% $H_3PO_4$ in methanol (MeOH)/water (80/20, v/v), MeOH, and EtOAc. After sample loading, the SPE column is rinsed with EtOAc and allowed to run dry for approximately 15 minutes. Residues are eluted from the column using acetonitrile (ACN)/water (30/70, v/v) containing 0.1 M $KH_2PO_4$. Residues of TBZ are then determined by cation exchange (benzene sulfonic acid column) HPLC using an isocratic mobile phase of 0.05 M $KH_2PO_4$ in ACN/water (25/75, v/v) adjusted to pH 3.4. Residues are quantified using fluorescence detection (excitation - 305 nm, emission - 380 nm). The validated LOQs for TBZ are 0.05 and 0.02 ppm in/on whole green bananas and ripe pulp, respectively; the estimated limit of detection (LOD) is 0.01 ppm for TBZ in/on both green bananas and ripe pulp.

For method validation, triplicate control samples of whole green bananas were fortified with TBZ at 0.05, 1, 3, and 10 ppm, and triplicate control samples of ripe pulp were fortified with TBZ at 0.02, 0.1, 0.4, 2 ppm. Method recoveries of TBZ were 91.3-98.3% from fortified green bananas and 95.2-108.7% from ripe pulp. Apparent residues of TBZ were $<0.01$ ppm

in three control samples each of green fruit and ripe pulp. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-023 is adequate for determining residues of TBZ in/on bananas and banana pulp.

*Merck Method M-049 (revision 1)* is for determining residues of TBZ *per se* in/on whole citrus fruits. Homogenized samples are buffered to pH 8.0, and residues are extracted into EtOAc and dried over sodium sulfate. For clean-up, residues are loaded onto a pre-conditioned (see Method M-023 above) PRS SPE column. After sample loading, the SPE column is rinsed with EtOAc and allowed to run dry for approximately 15 minutes. Residues are then eluted from the column using ACN/water (30/70, v/v) containing 0.1 M $KH_2PO_4$. Residues of TBZ are determined by cation exchange HPLC using an isocratic mobile phase of 0.05 M $KH_2PO_4$ in ACN/water (25/75, v/v) adjusted to pH 3.4. Residues are quantified using fluorescence detection (excitation - 305 nm, emission - 380 nm). The validated LOQ for TBZ is 0.05 ppm in/on whole citrus fruits, and the estimated LOD for TBZ is 0.03 ppm in/on whole fruit.

For method validation, control samples of oranges were fortified with TBZ at 0.05 and 0.1 ppm in duplicate, and at 1-20 ppm in triplicate. Method recoveries of TBZ from orange fruit were 93.4 and 84.2% at 0.05 ppm (LOQ) and were 88.7-100.3% at 0.1-20 ppm. Apparent residues of TBZ were $<0.03$ ppm in five control samples. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-049 is adequate for determining residues of TBZ in/on citrus fruits.

*Merck Method M-040* is for determining total BNZ (free and conjugated) in/on citrus fruit and pulp. Prior to extraction of dried pulp, samples are re-hydrated with water (7 mL/g sample) overnight under refrigeration. Homogenized samples of each matrix are diluted with 0.025 M $KH_2PO_4$ and adjusted to pH 6.0-6.2. Residues of free BNZ are extracted with EtOAc. The remaining aqueous homogenate is filtered and any residual EtOAc is evaporated. The pH is adjusted to pH 5.3-5.5 fraction. Conjugated BNZ residues are then hydrolyzed with β-glucosidase at 38 C for 2 hours. The hydrolysate is cooled and adjusted to pH 6.0-6.2. Released BNZ residues are then extracted into EtOAc, combined with free BNZ residues, and dried over sodium sulfate.

For clean-up, extracted residues are loaded onto a pre-conditioned PRS SPE column. After sample loading, the SPE column is rinsed with EtOAc followed by MeOH. Residues are then eluted from the column using ACN/water (30/70, v/v) containing 0.1 M $KH_2PO_4$. Total BNZ residues are then determined by cation exchange HPLC using an isocratic mobile phase of 0.025 M $KH_2PO_4$/ACN (40/60, v/v) adjusted to pH 3.8-3.9. Residues are quantified using fluorescence detection (excitation - 265 nm, emission - 380 nm). The validated LOQs for total BNZ are 0.02 ppm in/on whole fruit and 0.08 ppm in dried pulp; and the estimated LODs are 0.01 ppm in/on whole fruit and 0.05 ppm in dried pulp.



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For method validation, the registrant fortified single control samples of homogenized whole fruit with BNZ at 0.02, 0.04, and 0.1 ppm and dried pulp with BNZ at 0.08 and 0.2 ppm. In addition, each sample was also fortified with TBZ at 15 ppm, although recoveries of TBZ were not reported. Enzyme activity was verified by incubating 4-methylumbelliferyl-α-D-glucoside with the β-glucosidase and monitoring the release of 7-OH-4-methylcoumarin by HPLC/fluorescence detection. Results of the method validation are presented in Table 1. Overall method recoveries of BNZ were 76-100% from whole fruit and ~88% from dried pulp. Apparent residues in single control samples were <0.01 ppm for whole fruit and <0.05 ppm for dried pulp. Adequate sample calculations and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-040 is adequate for determining residues of total BNZ (free and conjugated) in/on citrus fruit and pulp.

Table 1. Recovery of BNZ from fortified control samples of apple and orange commodities using Merck's HPLC/fluorescence detection Methods M-040 and M-041.

| Matrix | Fortification level (ppm) | % Recovery |
|---|---|---|
| Citrus fruit (*Method M-040*) | | |
| Whole fruit | 0.02, 0.04, 0.1 | 85, 100, 76 |
| Dry pulp | 0.08, 0.20 | 87, 88 |
| Apples (*Method M-041*) | | |
| Whole fruit | 0.025-0.10 | 88-90 |
| | 0.02-0.10 [a] | 80-88 |
| Dry pomace | 0.02-0.10 | 72-81 |
| | 0.05-0.20 [a] | 81-110 |

[a] Fortified aqueous filtrates from homogenized samples.

*Merck Method M-041* is for determining total BNZ (free and conjugated) in/on apple commodities. Prior to extraction of dry pomace, samples are re-hydrated with water (4 mL/g sample) overnight under refrigeration. The remainder of the method is essentially identical to Method M-040, which is described above. The validated LOQs for total BNZ are 0.025 ppm in/on apples and 0.05 ppm in dry pomace; and the estimated LODs are 0.01 ppm in/on apples and 0.02 ppm in dry pomace.

For method validation, the registrant fortified control samples of homogenized whole fruit and dry pomace with BNZ at 0.02-0.25 ppm. In another set of analyses, aqueous filtrates from control whole fruit and dry pomace were also fortified with BNZ at 0.02-0.2 ppm. Whole fruit and pomace samples from both analyses were also fortified with TBZ at 7.5 and 50 ppm, respectively, although recoveries of TBZ were not reported. Enzyme activity was verified by incubating 4-methylumbelliferyl-α-D-glucoside with the β-glucosidase and monitoring the release of 7-OH-4-methylcoumarin by HPLC/fluorescence detection. Results of the method



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validation are presented in Table 1. Overall method recoveries of BNZ were 80-90% from apples and 72-110% from dry pomace. Apparent residues in control samples were not reported. Adequate sample calculations were provided. These data indicate that the HPLC/fluorescence method M-041 is adequate for determining residues of total BNZ (free and conjugated) in/on apple commodities.

*Merck Method M-056* is for determining total BNZ (free and conjugated) in/on green bananas. Banana samples are initially homogenized with water (1:1, w/w). Residues are extracted from homogenized samples with 0.025 M HCl and centrifuged. The aqueous extract is adjusted to pH 5 and residues are incubated with β-glucosidase for 4 hours at 38 C. After cooling, the resulting hydrolysate is adjusted to pH 8, and residues are extracted into EtOAc. The EtOAc-soluble residues are then loaded onto a preconditioned PRS SPE column. After loading, the SPE column is allowed to run dry for 15 minutes, and residues are then eluted from the column using ACN/water (30/70, v/v) containing 0.1 M $KH_2PO_4$. Total BNZ residues are then analyzed by cation exchange HPLC using an isocratic mobile phase of 0.05 M $KH_2PO_4$ in ACN/water (25/75, v/v) adjusted to pH 3.2. Residues of BNZ are quantified using fluorescence detection (excitation - 265 nm, emission - 380 nm). The validated LOQ for BNZ residues is 0.01 ppm in/on green bananas, and the estimated LOD for BNZ is 0.005 ppm.

For method validation, the registrant fortified duplicate control samples of green bananas with BNZ at 0.01, 0.02, 0.1, and 3 ppm. Each sample was also fortified with TBZ at 3 ppm; however, recoveries of TBZ were not reported. Method recoveries of BNZ were 89.1-109.7% from green bananas. Apparent residues of each analyte were $<0.005$ ppm (LOD) in both control samples. Adequate sample calculations, raw data, and representative chromatograms were provided. These data indicate that the HPLC/fluorescence method M-056 is adequate for determining residues of BNZ in/on green bananas.

In addition to the above methods, Merck has also provided (1994, MRID 43328302) a description of a modified method for determining total BNZ (free and conjugated) in/on wheat commodities along with an ILV for the modified method. In a previous review (D190451, L. Cheng, 7/28/93), the Agency concluded that the available residue data for wheat and wheat processing commodities were inadequate because the analytical method employed could not determine conjugated BNZ residues as the method did not include an enzymatic hydrolysis step. The modified method is essentially identical to the previously described method except that the method now includes an enzymatic hydrolysis step.

In the modified method (ABC Laboratories Report No. 39296E), residues are extracted from homogenized wheat samples with MeOH and the residual solids are then reflexed in methanolic 3 N KOH for 16 hrs. The resulting extracts are combined, diluted with concentrated HCl to precipitate KCl, filtered, and concentrated. Residues are then diluted with 0.02 M $KH_2PO_4$, adjusted to pH 5 and hydrolyzed with β-glucosidase at 37 C for at least 2 hours. The resulting hydrolysate is adjusted to pH $<3$ with HCl and partitioned with EtOAc, discarding the organic fraction. The hydrolysate is then diluted with 2 N KOH and buffered by the addition of 2 N

$NA_2CO_3$. Residues are then partitioned into EtOAc, concentrated to dryness, and dissolved in 1% ammonium acetate. Total BNZ residues are determined by reverse-phase HPLC using an isocratic mobile phase of 0.01 M ammonium acetate in MeOH/water (25/75, v/v) with fluorescence detection (excitation - 261 nm, emission - 300 nm). The validated LOQ for total BNZ residues is 0.1 ppm in/on wheat grain, straw, and processed commodities, and the LOD for BNZ is 0.05 ppm.

For method validation, the developing laboratory fortified control samples of wheat grain and straw with BNZ at 0.1 and 0.5 ppm in duplicate and one sample of each matrix at 2 ppm. Recoveries of BNZ were 79-90% (ave. 84%) from grain and 70-76% (ave. 73%) from straw. Apparent residues of BNZ were <LOD in the control sample of each matrix. Enzyme activity was verified by incubating 4-methylumbelliferyl-α-D-glucoside with the β-glucosidase and monitoring the release of 7-OH-4-methylcoumarin by HPLC/fluorescence detection. Adequate sample calculations, raw data, and representative chromatograms were provided. This HPLC/fluorescence method is adequate for determining residues of BNZ (free and conjugated) in/on wheat grain and straw.

An independent method validation trial (1994, MRID 43328303) was also conducted on the above method by ABC Laboratories. The analytical laboratory fortified control samples of wheat grain and straw with BNZ each at 1 and 5 ppm in duplicate; the current tolerance for TBZ residues in/on wheat grain and straw is 1 ppm. Recoveries of BNZ were 83.1-93.6%from the four grain samples and 73.3-86.8% from the four straw samples. Apparent residues of BNZ were <0.05 ppm (<LOD) in/on control samples of each matrix. Adequate sample calculations, raw data, representative chromatograms, and standard curves were provided. A single sample set (6 samples) required 25.5 person-hours over a 3-day period for analysis. Although ABC Laboratories both developed this method and conducted the ILV, the personnel involved in the development phase were different from those conducting the ILV, and the report stated that there was no contact between the method validation team and the method developers until after the method was successfully validated in the first test run. The independent laboratory validation for the HPLC/fluorescence detection method for determining BNZ (free and conjugate) in/on wheat grain and straw is adequate.

Magnitude of the Residue in Plants

Pome fruit

Tolerances of 10 ppm have been established for residues of thiabendazole *per se* in/on apples and pears (post-harvest) [40 CFR §180.242(a)]. A REFS search dated 10/15/97 listed two thiabendazole end-use products, a 3.8 lb/gal FlC (EPA Reg. No. 100-889) and a 89% DF (EPA Reg. No. 100-891), registered to Novartis Crop Protection, Inc. for post-harvest treatment of apples and pears. These products are registered for post-harvest application to apples and pears as a dip, flood, or spray application of thiabendazole at 0.48 lb ai/100 gal.

The label specify a maximum treatment time of 3 minutes. Apples may be treated prior to and after storage, but pears may only be treated once. In addition, use directions for 98.5% FlC and WP labels also include one post-harvest wax treatment at 1000 ppm (EPA Reg. Nos. 2792-50, 5202-26, and 43410-7).

Residue data reflecting the above use pattern on apples and pears were submitted by Merck and reviewed by the Agency (R. Perfetti, 4/15/93). The Agency concluded that the available thiabendazole residue data on pome fruit were not adequate because residues of free and conjugated BNZ were not determined and methodology was not available for determining free and conjugated BNZ in/on pome fruits. The review also noted that supporting storage stability data were required along with the requested residue data on BNZ.

In its response (1995; MRID 43721903), Merck requested that data requirements for determination of BNZ (free and conjugated) in/on pome fruit be waived as residues of BNZ (free and conjugated) are unlikely to contribute significantly to the total TBZ residues in/on pome fruit treated post-harvest.

To support this argument, the registrant provided a copy of a published article (Jacob *et al.*, J. Agric. Food Chem. 1975, Vol. 23, No. 4 pp. 704-708) indicating that the formation of BNZ in plants results from photolysis of TBZ rather than by direct plant metabolism. Therefore, residues of BNZ are unlikely to form in/on apples treated post-harvest because treated apples are not exposed to sunlight during storage.

The registrant also submitted a description of a HPLC/fluorescence method (Merck Method M-041) for determining BNZ (free and conjugated) in/on pome fruits. This method is discussed in the above Residue Analytical Methods section and is adequate for determining BNZ residues in/on pome fruit.

Using Method M-041, the registrant reanalyzed selected samples from the original apple and pear studies for residues of BNZ (free and conjugated). Three samples each of apples and pears were analyzed along with control samples. Residues of TBZ in/on these samples were previously determined to be 2.8-3.3 ppm in/on apples and 3.1-5.0 ppm in/on pears. Total BNZ residues in/on these treated samples were $<0.01$ ppm (LOD) for both apples and pears. Apparent BNZ residues in/on control samples were $<0.01$ ppm, and concurrent method recoveries were 72-110% from control samples fortified with BNZ at 0.05-0.25 ppm. The analyses for BNZ residues were conducted after samples had been held in frozen storage for 34-48 months. Storage stability data are available indicating that BNZ is stable in frozen storage for up to 23 months in wheat grain and straw and for up to 24 months in mushrooms, potatoes, and wet potato peel. Although none of these studies had maximum storage intervals of 48 months, no decline in BNZ levels has been observed in any of these studies.

Based upon the above data, the Agency concurs that residues of BNZ (free and conjugated) are unlikely to comprise a significant portion of the total TBZ residue in/on pome fruits treated

post-harvest with TBZ. Therefore, complete data for residue of BNZ (free and conjugated) in/on apples and pears are not required, and the deficiencies pertaining to the apple and pear residue studies are resolved.

Provided labels are amended to specify a minimum of a 30-day interval between aqueous dip and wax applications of TBZ to pome fruit, the available pome fruit residue data are adequate and support the lowering the tolerance for TBZ residues in/on apples to 5 ppm and lowering the tolerance for TBZ residues on pears to ?? ppm. (note: Pear residue data in earlier review may be incorrect, need to review original pear MRID)

Bananas

Tolerances of 3 and 0.4 ppm have been established for residues of thiabendazole *per se* in/on bananas and banana pulp, respectively [40 CFR §180.242(a)]. A REFS search dated 10/15/97 identified two thiabendazole products, a 3.8 lb/gal FlC (EPA Reg. No. 100-889) and a 89% WDG (EPA Reg. No. 100-891), registered to Novartis Crop Protection, Inc. for use on bananas. Label directions for use of the 3.8 lb/gal FlC on bananas were included in the current submission; these directions specify a spray application of thiabendazole to bananas at concentrations of 187-373 ppm until runoff. Previous use directions indicate that bananas can be treated post-harvest with thiabendazole via spray to run-off, cascade, or dip application techniques at solution concentrations of 200-400 ppm.

In response to the Thiabendazole Phase 4 Review, Merck previously submitted data (1993, MRID 42868701) on residues of TBZ *per se* in/on green bananas and ripe banana pulp sampled following a spray mist application of thiabendazole at 400 ppm. In its review of these data, the Agency (F. Suhre, 4/14/94) concluded that the TBZ residue data on bananas were inadequate because the spray mist application used did not represent the most aggressive post-harvest application method (i.e. dip or cascade drench) allowed and because residues of BNZ (free and conjugated) were not determined.

In response to the Agency's review, Merck has submitted (1995, MRIDs 43721901 and 43721902) responses to the Agency's previous conclusions, descriptions of new HPLC/fluorescence methodology for determining TBZ and BNZ (free and conjugated) in bananas, and a new study on TBZ and BNZ (free and conjugated) residues in/on bananas treated post-harvest with TBZ as a dip application at 400 ppm. The registrant also requested that the Agency waive requirements for determining BNZ (free and conjugated) in/on bananas treated post-harvest.

In a single test conducted in HI during 1995, clusters of green bananas were harvested from a commercial plantation and were treated one day later with a post-harvest application of TBZ as a 15 or 60 second dip in a solution containing TBZ at 400 ppm (1x) and ammonium alum (10 g/L). Two commercial formulations of TBZ were applied in separate tests; Mertect 340-F®



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(3.8 lb/gal FlC) and Arbotect 20-S® (220 g/L of TBZ as a hypophosphite salt), which is identical to the formulation marketed in Central America (Mertect 20-S®). After application, the clusters were allowed to air dry and were then boxed for shipment at ambient temperature to the analytical laboratory (Merck), where clusters were stored at 14 C until processing for analysis. Fruit clusters were stored at ambient temperature to 14 C for a total 6 days prior to being sampled. A total of 10 samples were taken from each treatment, and four control samples were taken from the two control treatments (15 and 60 sec. dip without TBZ). Samples of whole green bananas were chopped, homogenized with dry ice, and placed in frozen storage until analysis. Samples were stored frozen for 17-24 days prior to analysis for TBZ and 57-59 days prior to analysis for total BNZ.

Data from the previous banana study indicate that TBZ is stable in frozen bananas and ripe banana pulp for up to 3 months. No storage stability data are available for BNZ in bananas; however, data are available indicating that BNZ is stable frozen mushrooms, wheat commodities, and potato commodities for >18 months. No additional storage stability data are required to support the current banana study.

Residues of TBZ were determined using an adequate HPLC/fluorescence detection method (Merck Method M-023, discussed in the Residue Analytical Methods section) that has a validated LOQ of 0.05 ppm for TBZ in/on whole green bananas. Apparent residues of TBZ were nondetectable (<0.01 ppm) in/on eight control samples, and concurrent method recoveries were 90.4-99.7% from ten control samples fortified with TBZ at 3 ppm. Residues of TBZ in/on treated bananas are presented in Table 2. Residues of TBZ resulting from the 60-second dip were higher than from the 15-second dip, and residues were higher in/on fruit treated with the 3.8 lb/gal FlC formulation than in fruit treated with the hypophosphite salt of TBZ.

After analysis for TBZ residues, the two samples bearing the highest TBZ residues from each treatment were also analyzed for residues of BNZ (free and conjugated) using Merck Method M-056, an adequate HPLC/fluorescence detection method with a validated LOQ of 0.01 ppm. Apparent residues of BNZ (free and conjugate)were <LOD (0.005 ppm) in/on four control samples, and concurrent method recoveries were 84.0-87.5% from four control samples fortified with BNZ at 0.1 ppm. Residues of BNZ in/on the four treated bananas were nondetectable (<0.005 ppm).



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Table 2. Residues of TBZ and total BNZ (free and conjugated) in/on whole green bananas following post-harvest dip application of TBZ at 400 ppm (1x).

| Formulation | Rate (ppm) | Dip time (sec) | Residues (ppm) [a] | |
|---|---|---|---|---|
| | | | TBZ [b] | BNZ [c] |
| Mertect 340-F® (3.8 lb/gal FlC) | 400 | 15 | 1.22-1.69 | <0.005 |
| | | 60 | 1.33-2.25 | <0.005 |
| Mertect 20-S® (hypophosphite salt of thiabendazole, 220 g/L) | 400 | 15 | 0.94-1.4 | <0.005 |
| | | 60 | 0.96-1.63 | <0.005 |

[a] Expressed in terms of each analyte.
[b] Range of residues from 10 samples/treatment.
[c] The two samples with the highest TBZ residues from each treatment were also analyzed for residues of BNZ (free and conjugated).

In the earlier banana studies, residues of TBZ *per se* were 0.6-1.26 ppm in/on whole green bananas treated in Honduras with TBZ (MERTECT 20-S® or MERTECT 340-F®) as a spray mist at 400 ppm (1x) and shipped to the U.S. in refrigerated commercial containers.

As in the case of post-harvest treatments for pome fruit and potatoes, the registrant has requested that data requirements for determination of BNZ (free and conjugated) be waived as residues of BNZ (free and conjugated) are unlikely to contribute significantly to the total TBZ residues in/on banana treated post-harvest. Again the registrant cited the published article (Jacob et al., 1975) indicating that formation of BNZ in plants results from photolysis of TBZ in sunlight, rather than by direct plant metabolism, indicating that BNZ is unlikely to form in fruit treated post-harvest.

Based on the above information and the available BNZ residue data, HED concurs that residues of BNZ (free and conjugated) are not likely to contribute significantly to the total TBZ residues in/on bananas treated post-harvest. Therefore, no additional data on BNZ residues in/on bananas are required.

Provided that labels specify a maximum dip application time of 1 minute, the available data on TBZ residues in/on bananas are adequate and support the established 3 ppm tolerance for residues of TBZ in/on bananas.

As banana pulp is not a regulated commodity of banana, the established 0.4 ppm tolerance for residues of TBZ in banana pulp should be deleted from 40 CFR §180.242(a) at the time of the Thiabendazole RED.

Citrus

A tolerance of 10 ppm has been established for residues of TBZ *per se* in/on citrus fruits (post-harvest) [40 CFR §180.242(a)]. A REFS search dated 10/15/97 listed no TBZ end-use products registered to Merck (or Novartis) for use on citrus. However, in a 6/26/94 meeting with the Agency and a subsequent letter (D. Miller, 8/10/94), Merck indicated that they were supporting the post-harvest use of TBZ on citrus as an aqueous dip of TBZ at 1,000 ppm for up to 3 minutes followed by second application (after de-greening and washing) of TBZ at up to 3,500 ppm in wax (1 gal wax/3,500 lb of fruit) except in CA. For use in CA, directions will specify a single post-harvest application of TBZ in wax at up to 5,000 ppm (1 gal wax/3,500 lb of fruit).

In response to the Thiabendazole Phase 4 Review, Merck previously submitted data (1992, MRID 42568001) on residues of TBZ *per se* in/on citrus fruits treated post-harvest at sites in CA and FL. Thiabendazole was applied to citrus fruits (oranges, grapefruit, and lemons) as sequential dip and wax applications at target rates of 5000 ppm in an aqueous dip solution and 5000 ppm in wax (1 gal wax/3500 lb of fruit). In its review of these data, the Agency (J. Abbotts, 7/30/93) concluded that the TBZ residue data on citrus were inadequate because residues of BNZ (free and conjugated) were not determined. In addition, the Agency noted numerous deficiencies in the residue data, particularly relating to the studies conducted in FL in which over-tolerance residues were found in/on fruit treated at 1x.

In a subsequent meeting with the Agency (D. Miller, 8/10/94), Merck indicated their belief that the over-tolerance residues found in/on citrus from the FL studies resulted from the misapplication of the product but that they were unable to verify the actual dosage applied. Accordingly, the registrant proposed conducting new residue studies to replace the flawed data from FL.

In their current submissions (1994, MRIDs 43328306 and 43328307; and 1995, MRID 43721904), Merck has provided responses to the Agency's previous conclusions, descriptions of new HPLC/fluorescence methodology for determining TBZ and BNZ (free and conjugated) in citrus commodities, and a new study on residues of TBZ and BNZ (free and conjugated) in/on citrus fruits treated post-harvest with TBZ in FL. The registrant also presented arguments for waiving requirements for determining BNZ (free and conjugated) in/on citrus treated post-harvest.

In four separate tests conducted in FL during the 1994-95 season (MRID 43721904), samples of mature oranges, grapefruits, and tangerines were harvested from commercial orchards and were treated 5-7 days later with a post-harvest application of TBZ (3.8 lb/gal SC/L) as an aqueous dip at a concentration of 1000 ppm for up to 3 minutes. After drying, fruits were placed in a de-greening room for ~24 hours and were then washed and dried prior to a second application of TBZ in wax at a concentration of 3500 ppm using 1 gal wax/3500 lb of fruit. After waxing and drying, two control and two treated samples of each variety were collected,

frozen, and shipped by freezer truck to Merck Research Laboratories, Three Bridges, NJ, where samples were held at <-15 C. Samples of whole fruit were homogenized with dry ice and kept in frozen storage until analysis. Samples were stored frozen for 90-98 days prior to analysis of TBZ and for 153-161 days prior to analysis for total BNZ.

Data from the previous citrus studies indicated that TBZ *per se* is stable in citrus fruit for up to 9 months. No storage stability data are available for BNZ in citrus; however, data are available indicating that BNZ is stable frozen mushrooms, wheat commodities, and potato commodities for >18 months. In addition, the registrant noted that BNZ is stable under acidic conditions (6N HCl at >90 C for 24 hours). No additional storage stability data are required to support the current citrus study.

Residues of TBZ were determined using Merck Method M-049 (discussed in the Residue Analytical Methods section), an adequate HPLC/fluorescence detection method with a validated LOQ of 0.05 ppm for TBZ in/on citrus fruits. Each control and treated sample was analyzed in triplicate, except for the grapefruit samples which were analyzed in quadruplicate. Apparent residues of TBZ were nondetectable (<0.03 ppm) in/on the four control samples of orange and two control samples of tangerines, but were 0.04 ppm in/on the two control samples of grapefruit. Concurrent method recoveries were 88.5-102% from nine control samples fortified with TBZ at 10 ppm. Residues of TBZ were 2.7-4.7 ppm (Table 3) in/on oranges, tangerines, and grapefruit collected immediately following a sequential post-harvest dip application of TBZ at 1000 ppm (3 minute) and a wax application containing TBZ at 3500 ppm (1 gal wax/3500 lb of fruit).

After analysis for TBZ residues, each sample was also analyzed for residues of BNZ (free and conjugated) using Merck Method M-040 (discussed in the Residue Analytical Methods section), an adequate HPLC/fluorescence detection method with a validated LOQ of 0.05 ppm. Apparent residues of BNZ (free and conjugate)were <LOD (0.01 ppm) in/on four control samples, and concurrent method recoveries were 80.2-93.6% from four control samples fortified with BNZ at 0.1 ppm. Residues of BNZ in/on the each sample of citrus fruit nondetectable (<0.01 ppm).



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Table 3. Residues of TBZ and total BNZ (free and conjugated) in/on citrus fruits following a post-harvest aqueous dip application and a wax application of TBZ at 1x.

| Commodity | Treatment rate (ppm) | | Residues (ppm) [a] | |
|---|---|---|---|---|
| | Aqueous Dip | Wax | TBZ [b] | BNZ [c] |
| Orange (Washington Navel) | 1000 | 3500 | 4.41, 4.84, 4.74 (4.7) | <0.01 |
| | | | 4.43, 4.44, 4.63 (4.5) | <0.01 |
| Orange (Hamlin) | 1000 | 3500 | 2.97, 3.07, 3.01 (3.0) | <0.01 |
| | | | 2.81, 2.72, 2.67 (2.7) | <0.01 |
| Tangerine (Sunburst) | 1000 | 3500 | 3.90, 3.46, 3.31 (3.6) | <0.01 |
| | | | 3.08, 3.52, 3.43 (3.3) | <0.01 |
| Grapefruit (Marsh) | 1000 | 3500 | 3.01, 2.81, 2.81, 2.54 (2.8) | <0.01 |
| | | | 3.65, 3.80, 3.55, 3.51 (3.6) | <0.01 |

[a] Expressed in terms of each analyte.
[b] Data are the average of triplicate analyses of each sample, except for grapefruit which were analyzed in quadruplicate; the average value for each sample is listed in parentheses.
[c] Each sample was analyzed once for residues of BNZ (free and conjugated).

To support their argument that BNZ residues are not a significant portion of the total TBZ residues in citrus, the registrant also submitted data (MRID 43328307) from the reanalysis of selected samples from the earlier citrus studies for residues of BNZ (free and conjugated) using Merck Method M-040. Five treated samples of whole fruit (grapefruit and orange) were analyzed, along with a sample of dry pulp from the processing study, for BNZ residues after 31-43 months of frozen storage. Apparent residues of BNZ were nondetectable in/on control samples of whole fruit and dry pulp. Concurrent method recoveries were 76-100% from three samples of whole fruit fortified with BNZ at 0.02-0.1 ppm and were 86 and 88% from two samples of dried pulp fortified with BNZ at 0.08 and 0.2 ppm. The original analysis of these samples found residues of TBZ *per se* at 7.8-15.0 (Table 4). Reanalysis found no detectable residues of BNZ (free and conjugated).



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Table 4. Residues of TBZ and total BNZ (free and conjugated) in/on citrus fruits following a post-harvest aqueous dip application and a wax application of TBZ[a].

| Commodity | Test No. | Samples No. | Residues (ppm) [b] | |
|---|---|---|---|---|
| | | | TBZ [c] | BNZ [d] |
| Grapefruit | 001-90-3056R | 2 | 8.6 | <0.02 |
| Orange | 001-90-3071R | 2 | 8.4 | <0.02 |
| Grapefruit | 001-90-3072R | 2 | 7.8 | <0.02 |
| Orange | 001-90-3073R | 2 | 15.0 | <0.02 |
| Orange | 001-90-3074R | 2 | 10.8 | <0.02 |
| Dried citrus pulp | 001-90-3058R | 12 | 12.5 | <0.05 |

[a] TBZ was applied at rates > 1x.
[b] Expressed in terms of each analyte.
[c] Average TBZ residues from original analysis conducted in 1991-1992 (MRID 42568001).
[d] Reanalyzed for residues of BNZ (free and conjugated) during 1994 after 31-43 months of frozen storage.

As in the case of post-harvest treatments for potatoes and other fruits, the registrant has also requested that data requirements for determination of BNZ (free and conjugated) be waived as residues of BNZ (free and conjugated) are unlikely to contribute significantly to the total TBZ residues in/on citrus fruits treated post-harvest. The registrant again cited the published article (Jacob et al., 1975) indicating that formation of BNZ in plants results from photolysis of TBZ in sunlight, rather than by direct plant metabolism, indicating that BNZ is unlikely to form in fruit treated post-harvest. The registrant also provided a copy of a published article by Rosenblum and Meriwhether (*J. Radioanal. Chem. Vol 6, 1970, pp 379-384*) in which oranges were dipped for 3 minutes in an aqueous solution containing of [$^{14}$C]thiabendazole at 1000 ppm and then stored at 10-21 C. After 4 weeks of storage, $^{14}$C-residues averaged 83.0 and 0.04 ppm in/on the peel and pulp, respectively. Extraction and analysis (TLC) of $^{14}$C-residues in peel after 4 weeks found that ≥93% of the radioactivity was associated with TBZ.

Based on the above information and the available BNZ residue data, HED concurs that residues of BNZ (free and conjugated) are not likely to contribute significantly to the total TBZ residues in/on citrus fruits treated post-harvest. Therefore, no additional data on BNZ residues in/on citrus fruits treated post-harvest are required. The available data support the established 10 ppm tolerance for TBZ residues in/on citrus fruit provided end-use product labels are amended to specify the following use directions. In all citrus producing regions except AZ and CA, a maximum of two post-harvest applications can be applied. The first as an aqueous dip of TBZ at up to 1,000 ppm for up to 3 minutes prior to de-greening, followed by second application (after de-greening and washing) of TBZ at up to 3,500 ppm in wax (1 gal wax/3,500 lb of fruit). In AZ and CA, only a single post-harvest application is allowed as an application of TBZ in wax at up to 5,000 ppm (1 gal wax/3,500 lb of fruit).

In the earlier post-harvest citrus studies conducted in CA, which are now deemed acceptable,

residues of TBZ *per se* were 1.2-5.2 ppm in/on citrus fruits treated sequentially with TBZ as a sequential dip at 5000 ppm for 3 minutes followed by a wax application at 5000 ppm (1 gal wax/3500 lb of fruit).

Wheat

Tolerances of 1 ppm have been established for residues of thiabendazole per se in/on wheat grain and straw [40 CFR §180.242(a)]. A REFS search dated 10/15/97 listed three thiabendazole products registered to Novartis Crop Protection, Inc. for use on wheat; these include a 2.7 lb/gal FlC (EPA Reg. No. 100-890), a 3.8 lb/gal FlC (EPA Reg. No. 100-889), and a 89% DF (EPA Reg. No. 100-891). Use directions for the 3.8 lb/gal FlC formulation allow a single application at tillering prior to elongation of the first node one at a maximum rate of 0.7 lb ai/A using ground or aerial equipment. A minimum application volume of 5 gal/A is specified for aerial applications. A 60-day PHI is specified and the label prohibits the grazing of livestock on treated green forage.

In Response to the Thiabendazole Phase IV Review, Merck previously submitted (1993, MRID 42718401) a description of method for determining TBZ and free BNZ in/on wheat commodities, an ILV for this method, storage stability data for TBZ and BNZ in wheat commodities, magnitude of the residue data on wheat grain and straw, and a wheat grain processing study. An Agency review (L. Cheng, 7/28/93) of these data concluded that the data were inadequate primarily because the analytical method employed could not determine conjugated BNZ residues as the method did not include an enzymatic hydrolysis step and because personnel involved in the ILV of the method were also involved in its development.

In response the Agency review, Merck submitted a description for a modified analytical method for determining total BNZ (free and conjugated) in wheat commodities along with a new ILV of this method (1994, MRIDs 43328302 and 43328303). The HPLC/fluorescence method is adequate and a successful ILV was conducted; these data are discussed above in the Residue Analytical Method section of this report. The registrant also reanalyzed selected samples of wheat grain and straw from the original study using the modified method. Residues of BNZ (free and conjugated) in/on these samples were $<0.1$ ppm.

In addition, Merck (1994, MRID 43328301) also responded to the Agency's questions pertaining to the personnel involved in the original ILV. The registrant explained that personnel who were involved in both the analysis of field samples and the ILV were involved only in sample receipt and initial processing of wheat grain and straw samples prior to analysis and were unfamiliar with the analytical method.

The available data on TBZ residues in/on wheat grain and straw are adequate. The combined residues of TBZ and BNZ (free and conjugate) are $<0.15$ ppm in/on wheat grain and $<0.23$ ppm in/on wheat straw harvested $\geq 60$ days following a single foliar application at tillering of TBZ at 0.67 lb ai/A (1x).



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At the time of the Thiabendazole RED, the tolerances for TBZ residues in/on wheat grain and straw should be reduced to 0.2 and 0.5 ppm, respectively.

Magnitude of the Residue in Processed Food/Feed

Apples

A tolerance of 33 ppm has been established for residues of TBZ *per se* in apple pomace, dried [40 CFR §186.5550(a)]. In response to the Phase IV Review, Merck previously submitted (1992, MRID 42515802) data from an apple processing study, in which apples bearing residues of TBZ at 3.9 ppm were processed into juice and wet and dry pomace. Residues of TBZ concentrated by 3.5x in wet pomace and by 11.6x in dry pomace.

The Agency review (R. Perfetti, 4/15/93) of these data concluded that the data were inadequate because residues of BNZ (free and conjugated) were not determined in apples and apple processed samples.

In its current submission (1995, MRID 43721903), Merck submitted a description of an analytical method (Merck Method M-041, discussed above) for determining total BNZ in apple processed commodities, along with data from the reanalysis of a sample of dried apple pomace from the original processing study. Using Method M-041, total BNZ residues (free and conjugated) were $<0.02$ ppm (LOD) in dried apple pomace that was previously shown to have residues of TBZ at 47 ppm.

As discussed under Magnitude of the Residue in pome fruits, the Agency has concluded that residues of BNZ (free and conjugated) are unlikely to comprise a significant portion of the total TBZ residue in/on pome fruits treated post-harvest with TBZ. Therefore, complete data for residues of BNZ (free and conjugated) in apple processed fractions are not required.

The available apple processing study is adequate and no additional data on BNZ residues in apple processed fractions are required. Residues of TBZ do not concentrate in apple juice, but concentrate by 3.5x in wet pomace and 11.6x in dried pomace. As dried apple pomace is no longer a regulated apple commodity (OPPTS Guideline 860.1000, Table 1), the tolerance for TBZ residues in apple pomace, dried should be deleted. However, a tolerance for TBZ residues in wet apple pomace is required. Based upon HAFT residues of 3.4 ppm for apples and the 3.5x concentration factor, an appropriate tolerance for TBZ residues in wet apple pomace would be 12 ppm.

Citrus

Tolerances of 20 and 35 ppm have been established for residues of TBZ in citrus molasses and citrus dried pulp, respectively [40 CFR §186.5550(a)].

A citrus processing study was submitted by Merck in conjunction with the original citrus residue studies and was reviewed by the Agency (J. Abbotts, 7/30/93). In two separate studies, residues of TBZ *per se* were determined in citrus juice, oil, molasses, and dried pulp processed from oranges bearing residues of TBZ *per se* at 5.37 ppm and grapefruit bearing residues of TBZ *per se* at 8.56 ppm. Residues of TBZ *per se* did not concentrate in juice and did not concentrate significantly in molasses (1-1.1x). However, residues of TBZ *per se* concentrated by 1.5-1.6x in dried citrus pulp and by 1.7-2.7x in citrus oil. The Agency concluded that the citrus processing study was inadequate because residues of BNZ (free and conjugated) were not determined in citrus processed commodities.

In response, Merck submitted data (MRID 43328307) on BNZ (free and conjugated) residues in a sample of dried citrus pulp from the original processing study bearing TBZ residues of 12.5 ppm. BNZ residues were determined using Merck Method M-040 and were nondetectable (<0.05 ppm) in this sample of dried pulp. As discussed under Magnitude of the Residue in citrus fruits, HED has concluded that residues of BNZ (free and conjugated) are unlikely to comprise a significant portion of the total TBZ residue in/on citrus fruits treated post-harvest with TBZ. Therefore, complete data for residues of BNZ (free and conjugated) in citrus processed fractions are not required. The available citrus processing study is adequate and no additional data on BNZ residues in citrus processed fractions are required.

Based upon HAFT residues of 5.2 ppm in/on whole fruit, a tolerance for dried citrus pulp is not necessary as the maximum expected residues in dried pulp (8.3 ppm) would be covered by the 10 ppm tolerance for residues in/on whole fruit. However, a tolerance is required for TBZ residues in citrus oil. Based upon the above HAFT residues and an average concentration factor of 2.4x, a tolerance of 15 ppm for residues of TBZ in citrus oil would be appropriate.

A the time of the Thiabendazole RED, the established tolerances for TBZ residues in citrus molasses and dried pulp should be deleted from 40 CFR §186.5550(a), and a tolerance for TBZ residues in citrus oil should be established under 40 CFR §185.5550.

Wheat

A tolerance of 3 ppm has been established for residues of TBZ *per se* in wheat milled products (except flour) [40 CFR §185.5550 and §186.5550(a)].

In Response to the Phase IV Review, Merck previously submitted (1993, MRID 42718401) data from a wheat processing study, in which wheat was treated at two test sites at tillering at 10x the maximum label rate. The combined residues of TBZ and free BNZ in/on wheat grain harvested ~ 100 days post-treatment from both tests were <0.1 ppm. Combined residues were also <0.1 ppm in dust, bran, middlings, shorts, red dog, low grade flour, and patent flour processed from these grain samples. The Agency review (D190451, L. Cheng, 7/28/93) of these data concluded that the data were inadequate because the analytical method employed determined only TBZ and free BNZ.

In its current submission (1994, MRID 43328302), Merck submitted a description of a modified analytical method for determining total BNZ (free and conjugated) in wheat commodities, along with data from the reanalysis of samples of red dog and bran from the original processing study. Using the modified method, residues of BNZ (free and conjugated) were $<0.1$ ppm in red dog and bran.

The available wheat processing study is adequate. Residues of TBZ and BNZ (free and conjugated) do not concentrate in processed wheat commodities. At the time of the Thiabendazole RED, the established 3 ppm tolerances for TBZ residues in wheat milled products (except flour) should be deleted from 40 CFR §185.550 and §186.5550(a).

## AGENCY MEMORANDA CITED IN THIS DOCUMENT

| | |
|---|---|
| CBRS No. | None |
| DP Barcode: | None |
| Subject: | Thiabendazole Livestock (Goat and Poultry) Metabolism. The Metabolism Committee Meeting Held on February 12, 1992. |
| From: | L. Cheng |
| To: | Metabolism Committee, HED |
| Dated: | 2/14/92 |
| MRID(s): | None |

| | |
|---|---|
| CBRS No. | 8192 |
| DP Barcode: | D165718 |
| Subject: | Thiabendazole Phase V Review. Metabolism Studies: Wheat, Soybean, and Sugar Beet. |
| From: | L. Cheng |
| To: | F. Rubis |
| Dated: | 3/11/92 |
| MRID(s): | 41872901 through 41872903 |

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|---|---|
| CBRS No. | 10954 |
| DP Barcode: | D185173 |
| Subject: | Response to the Thiabendazole Phase IV Review: Residue Chemistry Data. Magnitude of the Residue in Apples, Pears, and Apple Processed Commodities. |
| From: | R. Perfetti |
| To: | F. Rubis/ E Saito |
| Dated: | 4/15/93 |
| MRID(s): | 42515801 and 42515802 |

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|---|---|
| CBRS No. | 11216 |
| DP Barcode: | D186592 |

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|---|---|
| Subject: | Thiabendazole, Reregistration. Magnitude of the Residue in Citrus and Citrus Processed Commodities. |
| From: | J. Abbotts |
| To: | J. Ellenberger/C. Giles-Parker |
| Dated: | 7/30/93 |
| MRID(s): | 42568001 |

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|---|---|
| CBRS No. | 11792 |
| DP Barcode: | D190451 |
| Subject: | Thiabendazole. Response to Phase IV Review: Residue Chemistry Data. Magnitude of the Residue in Wheat and Wheat Processed Commodities. |
| From: | L. Cheng |
| To: | F. Rubis/A. Rathman |
| Dated: | 7/28/93 |
| MRID(s): | 42718401 |

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|---|---|
| CBRS No. | 12715 |
| DP Barcode: | D196149 |
| Subject: | Residue Chemistry Data Requirement 171-4(k) for Bananas. |
| From: | F. Suhre |
| To: | B. Briscoe |
| Dated: | 4/14/94 |
| MRID(s): | 42868701 |

| | |
|---|---|
| CBRS No. | 14013 |
| DP Barcode: | D205192 |
| Subject: | Response to Merck's June 30, 1994 letter re: 6/26/94 Thiabendazole Citrus Residue Study Meeting. |
| From: | D. Miller |
| To: | F. Rubis |
| Dated: | 8/10/94 |
| MRID(s): | None |



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## MASTER RECORD IDENTIFICATION NUMBERS

The citations for the MRID documents used in this review are presented below.

43328301 Johnson, N. (1994) Merck Responses to EPA Review Dated 8/4/93--Thiabendazole--Magnitude of the Residue in Wheat and Wheat Processed Commodities: Lab Project Number: 618-360-R/8/93. Unpublished study prepared by Merck Research Lab. 34 p.

43328302 Norton, J. (1994) Supplemental Enzyme Hydrolysis Method Final Report: Determination of the Magnitude of the Residues of the Fungicide Thiabendazole in Wheat Treated with Mertect DF: Lab Project Number: 618-360-93020: 93020: 39296E. Unpublished study prepared by ABC Labs, Inc. 34 p.

43328303 Cooper, J. (1994) Independent Laboratory Confirmation of the Enforcement Method for Benzimidazole and its Conjugates in Wheat Grain and Wheat Straw According to PR Notice 88-5 Guidelines: Lab Project Number: 618-360-41871: 41871. Unpublished study prepared by ABC Labs, Inc. 20 p.

43328306 Johnson, N. (1994) Merck Responses to EPA Review Dated 08/04/93--Thiabendazole--Magnitude of the Residue in Citrus and Citrus Processed Fractions: Lab Project Number: 618-360-R8/93C. Unpublished study prepared by Merck Research Labs. 50 p.

43328307 Norton, J. (1994) Determination of the Magnitude of the Residues of the Fungicide Thiabendazole in Citrus Treated with a Dip and Wax Treatment: Supplemental Data: Lab Project Number: SUPL.-93064: 93064: 001-90-3049R. Unpublished study prepared by Merck Research Labs. 72 p.

43371901 Arenas, R. (1995) Merck Responses to EPA Review Dated 5/16/94--Magnitude of residue--Thiabendazole on Bananas: Lab Project Number: 618-360-R-5/94. Unpublished study prepared by Merck & Co., Inc. 39 p.

43721902 Norton, J. (1995) Determination of the Magnitude of Residue of the Fungicide Thiabendazole (TBZ) in Green Banana Fruit By Post Harvest Dip Application: Lab Project Number: 618-360-94346: 94346. Unpublished study prepared by Merck & Co., Inc.403 p.

43721903 Arenas, R. (1995) Merck Response to EPA Review Dated 8/4/93--Magnitude of Residue--Thiabendazole in Apples, Pears, and Apple Processed Commodities: Lab Project Number: 618-360-R-8/93: 3109: 001-90-3060R. Unpublished study prepared by Merck & Co., Inc. 65 p.

43721904 Norton, J. (1995) Determination of the Magnitude of Residues of the Fungicide Thiabendazole in/on Citrus Treated with Aqueous Dip and Wax Treatment: Lab Project Number:



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618-360-34168: 4168: 94168. Unpublished study prepared by Merck & Co., Inc. 827 p.