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

NOV 22 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Thiabendazole.** Magnitude of the Residue Field Trials with Mushrooms.
DP Barcodes: D186572; CBRS No. 11161; MRID No.: 425989-01; Case No. 2670.

FROM: David J. Miller, SA HSO, US Public Health Service
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THRU: Francis B. Suhre, Section Head
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TO: Franklin Rubis, PM Team 51
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Attached is a review of the registrants response to the magnitude of the residue data requirements for mushrooms. This information was reviewed by Dynamac Corporation under the supervision of CBRS/HED. The data assessment has undergone secondary review in the Branch and has been revised to reflect Branch policies.

CBRS makes the following conclusions with respect to the submitted studies:

- Despite the fact that only the parent thiabendazole and free BNZ were determined, the residue study on mushrooms is considered adequate. CBRS has sufficient information to conclude that it is only the parent thiabendazole and (possibly) free BNZ residues which are the residues of potential concern in mushrooms, and will not require either the development of a method to detect

BNZ conjugates in mushrooms or the submission of additional data regarding BNZ conjugates in mushrooms.

- The registrant must amend the label limiting the maximum single direct spray application to 0.12 lb ai/1000 ft^2 or propose an increase in the tolerance for thiabendazole residues in on mushrooms (TOX considerations permitting).

- The submitted storage stability data indicate that thiabendazole and benzimidazole are stable in mushrooms stored at -23 °C for a least 189 days (6 months). Samples from the submitted residue study were stored frozen for up to 18 months prior to analysis. The registrant has indicated that the storage stability study will be continued up to 18 months. Conclusions regarding the adequacy of storage stability data for mushrooms are reserved pending review of the 18-month storage interval data.

If you need additional information, please advise.

cc: RF, SF, List B File, Circ., Dynamac, DJM.
RDI: FSuhre:11/12/93;MMetger:11/15/93;EZager:11/17/93.
EF:11/18/93

THIABENDAZOLE
(Chemical Codes 060101 & 060102)
(CBRS No. 11161; DP Barcode D186572)

TASK 2B

Phase 5 - Reregistration Review
Residue Chemistry

March 30, 1993

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

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THIABENDAZOLE

(Chemical Codes 060101 & 060102)

(CBRS No. 11161; DP Barcode D186572)

PHASE 5 - REREGISTRATION REVIEW RESIDUE CHEMISTRY

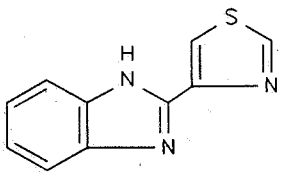
Task 2B

BACKGROUND

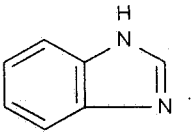
The Thiabendazole Phase 4 review dated 2/91 required data depicting residues of thiabendazole and its regulated metabolites in or on mushrooms following sequential applications with thiabendazole at a seasonal rate of 0.6 lb ai/1000 ft^2. The first application was to be at 0.24 lb ai/1000 ft^2, with three subsequent applications at 0.12 lb ai/1000 ft^2 each. A PHI of 12 hours was to be observed. In response, Merck Research Laboratories submitted data (1992; MRID 42598901) depicting residues of thiabendazole in or on mushrooms. The registrant also analyzed for free benzimidazole, a metabolite previously found in plants exposed to sunlight. This submission is reviewed here to determine its adequacy in fulfilling residue chemistry data requirements. The Conclusions and Recommendations stated in this review pertain only to thiabendazole residues in or on mushrooms.

The nature of the residue in plants is adequately understood. The residues of concern are the parent compound thiabendazole and benzimidazole (BNZ) and its conjugates. Methods are available for determining residues of thiabendazole per se in or on plant commodities and are listed in PAM, Vol. II, as Methods I, A, B, and C. Method I has undergone a successful EPA validation trial; recoveries were 81-105% from fruit samples fortified with thiabendazole at 0.04-4 ppm. The Agency has determined that a method must be developed for plants that is capable of measuring BNZ and its conjugates (L. Cheng; CBRS No. 8192, 3/11/92).

Tolerances for residues of thiabendazole (2-(4-thiazolyl)benzimidazole) in or on raw and processed plant commodities are currently expressed in terms of thiabendazole per se [40 CFR §180.242(a), §185.5550, and §186.5550(a)]. There is no Codex MRL for thiabendazole residues in/on mushrooms. Therefore, there are no questions regarding the compatibility of the U.S. tolerance with Codex.



thiabendazole



benzimidazole (BNZ)

CONCLUSIONS/RECOMMENDATIONS

1. Despite the fact that only the parent thiabendazole and free BNZ were determined, the residue study on mushrooms is considered adequate. While CBRS has earlier concluded that the tolerance expression should be revised to include the combined residues of thiabendazole and BNZ and conjugates, CBRS will not require that data regarding BNZ conjugates be generated for mushroom (see Conclusion #5).

2. Residues of thiabendazole per se in or on mushrooms harvested 12 hours posttreatment were $\leq$13 ppm following each of four sequential irrigation applications at 0.12-0.24 lb ai/1000 ft^2 and were 20-39 ppm following repeated direct spray applications at 0.12 lb ai/1000 ft^2. However, following an initial direct spray application at 0.24 lb ai/1000 ft^2, thiabendazole residues in or on mushrooms were 50 ppm, exceeding the established 40 ppm tolerance. Residues of free benzimidazole were $<$0.01 ppm in all samples, but BNZ conjugates were not determined and are not expected to have been liberated by the analytical methodology. The registrant must amend the label limiting the maximum single direct spray application to 0.12 lb ai/1000 ft^2 or propose an increase in the tolerance for thiabendazole residues in on mushrooms (TOX considerations permitting).

3. Merck method S.A.P. 500-M-021 is adequate for collecting data on thiabendazole and free benzimidazole residues in or on mushrooms. This method does not determine conjugated residues of BNZ. Nevertheless, CBRS concludes that conjugated BNZ residues are not expected to be of concern in or on mushrooms and will not require further information regarding these conjugates (see Conclusion #5).

4. The submitted storage stability data indicate that thiabendazole and benzimidazole are stable in mushrooms stored at -23 °C for a least 189 days (6 months). Samples from the submitted residue study were stored frozen for up to 18 months prior to analysis. The registrant has indicated that the storage stability study will be continued up to 18 months. Conclusions regarding the adequacy of storage stability data for mushrooms are reserved pending review of the 18-month storage interval data.

5. CBRS does not believe that BNZ conjugates are of significant concern in mushrooms and will not require that this data be developed for mushrooms for the following reasons: 1) BNZ and BNZ carboxamide are products of thiabendazole in light (J. Ag. Food Chem. 1975. Vol. 23 no. 4, pp. 704-708) and thus would not be expected to occur in mushrooms; 2) to the extent that BNZ and BNZ carboxamide do occur in plants exposed to light, these compounds are of lesser significance than the parent TBZ; 3) the short (12 hour) PHI and absence of detectable free BNZ in the residue study for mushrooms mean that the conjugates of this metabolite would not be expected to be a significant component of the residue; and 4) mushrooms are a minor crop of minimal dietary significance and are not expected to significantly contribute to dietary exposure. CBRS has sufficient information to conclude that it is only the parent thiabendazole and (possibly) free BNZ residues which are the residues of potential concern, and will not require either the development of a method to detect BNZ conjugates in mushrooms or the submission of additional data regarding BNZ conjugates in mushrooms.

DETAILED CONSIDERATIONS

Residue Analytical Methods

In conjunction with the residue study, Merck Research Laboratories submitted an analytical method description (1992; MRID 42598901). Thiabendazole residues were determined using Merck Standard Assay Procedure, S.A.P. 500-M-021, a modification of Method B in PAM Vol. II. For Method S.A.P. 500-M-021, residues in homogenized mushroom samples are extracted into EtOAc (3x) and pooled. The pooled EtOAc fraction is then split for distinct thiabendazole and benzimidazole assays.

For thiabendazole determinations, the EtOAc fraction is cleaned up by sequential partitioning with 2N NaOH and NaCl saturated water, and residues are partitioned into 0.1N HCl. The acidic aqueous fraction is then neutralized with 2N NaOH and NaCl, and residues are partitioned into EtOAc. The residues are then partitioned back into 0.1N HCl and analyzed spectro-fluorometrically. For benzimidazole determinations, residues in the EtOAc fraction are partitioned into 0.1N HCl. The resulting aqueous fraction is neutralized with 2N NaOH, and residues are partitioned back into EtOAc and concentrated. Benzimidazole is determined by HPLC using a fluorometric detector.

For method validation, samples of mushrooms were fortified with thiabendazole (0.1-50 ppm) and benzimidazole (0.02 and 0.05 ppm). Method recoveries of thiabendazole and benzimidazole were 94-103% and 84-95%, respectively. The validated detection limits for the method are 0.1 ppm for thiabendazole and 0.02 ppm for benzimidazole in mushrooms. Sample calculations, standard fluorescence spectra, and HPLC chromatograms were provided. These data indicate that Merck method S.A.P. 500-M-021 is adequate for

collecting data on residues of thiabendazole and benzimidazole in/on mushrooms. This method does not determine conjugated residues of BNZ; as cited by L. Cheng (memorandums of 3/11/92 and 7/28/93, CBRS No. 11792), previous plant metabolism data indicated that BNZ conjugates are stable towards hot methanolic KOH and are released as BNZ (only) after glucosidase hydrolysis.

Storage Stability Data

In conjunction with the submitted residue study, Merck submitted data (1992; MRID 42598901) depicting the stability of thiabendazole in mushrooms. Control samples of mushrooms were fortified with thiabendazole at 0.1 and 50 ppm and benzimidazole at 0.02 and 0.1 ppm. Fortified samples were analyzed after 0, 96, and 189 days of storage at -23 °C. Recoveries of thiabendazole and benzimidazole were 89-103% and 88-120%, respectively.

The submitted storage stability data indicate that thiabendazole and free BNZ are stable at -23 °C for at least 189 days (6 months) in mushrooms. Actual residue samples from the mushroom studies were stored from 456-555 days. Storage stability data in the current submissions do not support the entire storage interval for residue samples from these studies, but the registrant stated that the storage stability study is ongoing and will terminate after 18 months of storage.

Magnitude of the Residue in Plants

Miscellaneous Commodities

The Merck Research Laboratories thiabendazole 3.8 lb/gal FlC (EPA Reg. No. 618-75) is registered for use at a maximum seasonal rate of 0.6 lb ai/1000 ft^2 on mushrooms. Use directions for 3.8 lb/gal FlC label include applications in irrigation water or by direct sprays at casing, fuzzing, pinning, and between breaks. The first application can be made at up to 0.24 lb ai/1000 ft^2, with three subsequent applications at a maximum of 0.12 lb ai/1000 ft^2 each. A PHI of 12 hours is in effect.

Mushrooms. A tolerance of 40 ppm has been established for residues of thiabendazole in or on mushrooms [40 CFR §180.242(a)]. Merck Research Laboratories submitted data (1992; MRID 42598901) from six tests conducted in CA(3) and PA(3) depicting residues of thiabendazole in or on mushrooms following direct spray or irrigation applications of thiabendazole at 0.12-0.24 lb ai/1000 ft^2 for a seasonal rate of 0.6 lb ai/1000 ft^2, which corresponds to 1x the maximum label rate. Irrigation applications were applied at 150 gal/1000 ft^2, and broadcast sprays were made at 1 gal/1000 ft^2. In the CA tests, thiabendazole was applied at 0.24 lb ai/1000 ft^2 at pinning and was reapplied at 0.12 lb ai/1000 ft^2 after the first, second and third breaks (fruitings). In the PA tests, thiabendazole

was applied at 0.24 lb ai/1000 ft^2 after the first break and was reapplied at 0.12 lb ai/1000 ft^2 after the second, third, and fourth breaks.

Mushrooms were harvested 12 hours after each break application and placed in frozen storage at -17 to -23 °C within 1-3 hours. The CA and PA tests yielded 3 and 4 harvests of mushrooms, respectively. Samples were stored at approximately -23 °C for 456-555 days prior to analysis. Residues of thiabendazole and benzimidazole in or on mushroom samples were determined fluorometrically using the Merck S.A.P. 500-M-021 method.

Thiabendazole residues in or on mushrooms following irrigation and direct spray applications of thiabendazole are summarized in Table 1. Apparent residues of thiabendazole were ≤0.01 ppm in/on nine control samples from the CA tests and were <0.01-0.15 ppm in/on 12 control samples from the PA tests. Thiabendazole residues in control samples from PA resulted from a nutritional supplement that contained 0.08% (w/w) thiabendazole and was added to one bed. Residues of free benzimidazole were ≤0.01 ppm in or on all samples.

Table 1. Residues of thiabendazole in/on mushrooms harvested 12 hours following repeated applications of thiabendazole in the irrigation water or as a direct spray.

		Application			
Method - #		Interval (days)[a]	Rate (lb ai/1000 ft^2)	# of Samples	Thiabendazole Residues (ppm)[b]
Irrigation	1	-	0.24	2	5.9, 9.4
	2	1-8[c]	0.12	4	1.9-7.2
	3	7-10	0.12	4	2.1-13
	4	4-10	0.12	4	2.4-12
Direct spray	1	-	0.24	1	50
	2	1-7[c]	0.12	2	26, 38
	3	9-10	0.12	2	20, 35
	4	5-8	0.12	2	31, 39

[a]Days between successive applications. [b]Each residue value represents duplicate or triplicate analysis of single samples. [c]In the CA tests, the second application was applied within 1-2 days of the initial application.

Geographic representation is adequate. The test states of CA(13%) and PA (49.5%) accounted for 63% of the U.S. mushroom production in 1987 (Census of Agriculture, 1987, p. 401). These data indicate that thiabendazole residues in or on mushrooms are not likely to exceed the established tolerance of 40 ppm for residues of thiabendazole in/on mushrooms

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following repeated irrigation applications of thiabendazole at the maximum label rate. Residues of free benzimidazole were <0.01 ppm in all samples.

In addition, thiabendazole residues in/on mushrooms did not exceed the tolerance following direct spray applications at 0.12 lb ai/1000 ft^2. However, thiabendazole residues in/on mushrooms did exceeded the 40 ppm tolerance following the initial direct spray application at 0.24 lb ai/1000 ft^2. The registrant must either amend its label decreasing the rate of the initial direct spray from 0.24 to 0.12 lb ai/1000 ft^2 or propose an increase in the tolerance for mushrooms (TOX considerations permitting).

The registrant provided arguments for waiving the requirement for data on BNZ conjugates in mushrooms. First, BNZ and BNZ carboxamide are products of thiabendazole in light (J. Ag. Food Chem. 1975. Vol. 23 no. 4, pp. 704-708) and, thus, would not be expected to occur in mushrooms. Second, the short PHI and absence of detectable free BNZ in the residue study for mushrooms mean that the conjugates of this metabolite would not be expected to be a significant component of the residue. CBRS agrees with the registrant's argument for the following additional reasons:

- to the extent that BNZ and BNZ carboxamide do occur in plants exposed to light, these compounds are of lesser significance than the parent TBZ (see J. Ag Food Chem., *op. cit.*): in this published article, parent TBZ accounted for 78% of the total radioactivity present in sugar beet plants which were exposed to sunlight for the equivalent of 14-8 hour days with the remaining 22% representing chemically altered products which appear to be induced by exposure to direct sunlight. CBRS has also reviewed previous metabolism studies submitted by the registrant regarding the prevalence of TBZ with respect to BNZ: an early study by the registrant reflected post-harvest treatment of whole fruit with ^{3}H-labelled thiabendazole, in which most of the residue (84%) remaining after several weeks was the parent TBZ (C. Olinger, 7/16/91, CB No. 2670). More recent submissions regarding wheat, sugarbeet, and soybean show that in foliage of wheat and soybean and tops of sugar beats, parent TBZ represents $>50\%$ of total residue in all cases, with BNZ and its conjugates representing in all cases no more that 15% of total residues.

- mushrooms are a minor crop of minimal dietary significance and are not expected to significantly contribute to dietary exposure.

CBRS has sufficient information to conclude that it is only the parent thiabendazole and free BNZ residues which are the residues of potential concern in mushrooms, and will not require either the development of a method to detect BNZ conjugates in mushrooms or the submission of additional data regarding BNZ conjugates in mushrooms.

References

Citations for the MRID documents referenced in this review are presented below.

42598901 Norton, J.; Nelson, R. (1992) Determination of the Magnitude of the Residues of the Fungicide Thiabendazole in Mushrooms Treated With Mertect 340-F in Irrigation Water and by Direct Application: Laboratory Project Study #93041. Unpublished Study prepared by Merck Research Laboratories. 683 p.

Agency Memoranda

CBRS No. 8192
Subject: Thiabendazole Phase V Review. Metabolism Studies: Wheat, Soybean, and Sugar Beet.
From: L. Cheng
Dated: 3/11/92
MRID(s): 41872901, -02, and -03