3/11/92 KF

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

MAR 11 1992

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: ID # 060101-618. Thiabendazole Phase V Review. Metabolism Studies: Wheat, Soybean and Sugar Beet. MRID # 418729-01,-02 &-03. CB # 8192. DP Barcode: D165718.

FROM: Leung Cheng, Chemist
Special Review Section II
Chemistry Branch II - Reregistration Support
Health Effects Division (H7509C)

THROUGH: Francis B. Suhre, Section Head
Chemistry Branch II - Reregistration Support
Health Effects Division (H7509C)

TO: Frank Rubis, PM # 51
Accelerated Reregistration Branch
Special Review/Reregistration Division (H7505C)

Merck Sharp & Dohme has submitted three plant metabolism reports to fulfill Requirement # 171-4 (a), Residue Chemistry - Plant Metabolism:

1) The Metabolic Fate of Thiabendazole (TBZ) in Wheat ABC Laboratories Project ID # 37724.

2) The Metabolic Fate of Thiabendazole (TBZ) in Soybean ABC Laboratories Project ID # 37725.

3) The Metabolic Fate of Thiabendazole (TBZ) in Sugar Beet ABC Laboratories Project ID # 37726.

TBZ is on list B for reregistration. The Phase IV review stated that the registrant had committed to conduct three plant metabolism studies (C. Olinger, 2/20/91). In other words, the submitted studies have not previously been reviewed.

Also attached is a letter (4/7/91) from ABC Labs stating that the precise rate of ^{14}C-TBZ (uniformly labeled in the phenyl ring) application to these plants could not be verified although residue values for the 2 hour samples confirm the application of TBZ.

All three metabolism studies were conducted on the property of ABC Labs, Inc in Columbia, MO. Analytical work was also done at ABC Labs. All three studies were conducted in a similar manner (equipment, sample collection and handling, instrumentation, extraction scheme, analysis technique, solvent system, etc). The plant metabolism studies will be discussed individually below.

THE METABOLIC FATE OF THIABENDAZOLE (TBZ) IN WHEAT (MRID # 418729-01)

The test plots were fertilized and spring wheat (Marshall variety) was planted on April 14, 1989. On May 31, 1989, a spray solution containing ^{14}C-TBZ (uniformly labeled in the phenyl ring) was applied to the wheat crop at ca 0.71 lb ai/A (maximum label rate). Samples of immature plants were taken before treatment, 2 hours post treatment, and 7 days after treatment (prior to booting representing forage). Subsequent samples were also collected at the early dough stage (37 days after application representing haylage), and at the mature stage (63 days; grain and straw).

Field samples were frozen (at about -20°C) before sample preparation. For sample preparation, dry ice was added and the mixture was ground in Cuisinart food processors. Wheat grain was further milled into a fine meal in the presence of dry ice. Processed samples were stored in a walk-in freezer after the dry ice was allowed to sublime.

The 2-hr wheat sample was reanalyzed after 10 months of frozen storage. TBZ was found to be stable in wheat by HPLC under these conditions.

Samples were combusted and counted. Radioactivity was analyzed by TLC (75:25:0.3% water:acetonitrile:phosphoric acid, v/v/v) and HPLC connected to an UV detector set at 279 nm (varying amounts of methanol buffered with 0.1M NaH_2PO_4 and 0.01 M $NaClO_4$ or 3:7 acetonitrile:0.04% phosphoric acid). For quantitation of released radioactive residues, a combination of HPLC and LSC was performed. The following standards were used: TBZ, 5-hydroxy TBZ, BNZ (benzimidazole), and BNZ carboxamide.

Review of the wheat metabolism study will concentrate on the results of mature straw and grain (i.e., at harvest). Identification of metabolites was conducted on mature wheat straw. For radioactivity distribution in wheat plants at earlier stages, see the summary table at the end of this section. The percentages of extractables and metabolites are averaged values.

Samples of mature straw and grain (63-day) underwent the following extraction steps. They were first extracted with methanol and the organic phase was analyzed by TLC and HPLC. The (unextracted) residues were treated with hot methanolic KOH, diluted with water, and extracted with ethyl acetate while basic

2

and then again when the aqueous phase was made acidic. The 2 ethyl acetate fractions were analyzed by HPLC whereas the aqueous phase was lyophilized, dissolved in methanol and analyzed. Residues remaining from the methanolic KOH treatment were further subjected to enzyme and chemical hydrolyses and quantitated by LSC.

Wheat straw contained 22.36 ppm TBZ equivalent which is some 200 times higher than the radioactivity found in wheat grain (0.12 ppm). The methanol fraction extracted 21-31% of straw activity of which 2.6-4.6 ppm (11-21% of TRR) was characterized by HPLC as unchanged TBZ. The identity of this fraction (retention time 18-24 min in HPLC solvent system A) was arrived at by mass spectroscopy with an authentic sample of TBZ in terms of GC retention time and fragmentation pattern (characteristic extrusion of HCN). The mass spectrum of the isolate (from prep HPLC) from wheat straw is superimposible with that of the standard; the GC retention times also match.

In addition to TBZ, the methanol extract also contained a fraction (ca 30% in the methanol extract; 9% straw activity) which eluted in a more polar region (4-10 min retention time) than TBZ. This fraction was suspected to be conjugates but was not further identified (see below however).

Hot methanolic KOH treatment yielded additional TBZ (5-28% straw activity) in the organic fraction after ethyl acetate extraction. From the ethyl acetate phase (retention time 4-8 min) was isolated 2 polar fractions (polars A and polars B) by preparative TLC; polars A is the major fraction. When polars A was hydrolyzed with glucosidase, a peak with a retention time same as BNZ was observed in HPLC, suggesting this fraction to be sugar conjugates of BNZ. When polars A was treated with acetic anhydride in pyridine, a reaction product which eluted on HPLC later (and hence less polar) than BNZ was obtained and was assumed to be an acetyl derivative. The identity of BNZ was confirmed by GC/MS on a sample of polars A that had been treated with acetic anhydride followed by glucosidase hydrolysis. For polars B, treatment with acetic anhydride yielded reaction products that by HPLC retention time consisted of BNZ and its acetate. There was not enough material of polars B for GC/MS identification work. Polars A and B amount to 14% of straw activity.

The registrant cited a journal article (J. Ag. Food Chem., 1975) in which TBZ was found to photodegrade (under sunlight) to BNZ and BNZ carboxamide (2-aminocarbonyl BNZ) as supporting evidence.

The aqueous phase from the hot methanolic KOH treatment was then acidified and again extracted with ethyl acetate. This latter fraction also contained the very polar materials (retention time 4-8 min) along with activity (ca 2% straw activity) spread over a wide range of retention times higher than 16 min. The report

suggested that this broad band of residues are "closely associated with endogenous matrix components."

The unextractable residues (3.76 ppm, 17% straw activity) left after the hot methanolic KOH treatment underwent additional hydrolyses: potassium phosphate buffer, ∝-amylase, a general protease, ethyleneglycol bis-(2-aminoethylether)-N,N'-tetraacetic acid, HCl, KOH, and H_2SO_4. The activity released from each of these fractions was less than 2% of the TRR in straw.

For wheat grain (TRR=0.12 ppm), 0.03 ppm or 23% of the activity was extracted into methanol which by HPLC consisted of essentially TBZ; a small amount (below minimum quantifiable limit) eluted in the same region as the polar fraction observed from the straw. The unextractable fraction, after methanolic KOH treatment, yielded 0.02 ppm (18% TRR) activity which had a retention time similar to that of the polar BNZ conjugate(s). The aqueous layer retained 14% of TRR (or 0.004 ppm) with 27% TRR (or 0.008 ppm) unaccounted for. The registrant stated that further work had not been pursued because of very low activity levels in wheat grain.

In summary, mature wheat straw bore practically all of the TBZ residues at harvest. Residues in straw consist of TBZ (26-40%), and BNZ and its conjugate(s) (22-31%). A similar residue profile was also found in grain (23% TBZ and 18% BNZ and conjugates).

Distribution of Radioactivity in Wheat

Crop Part	Days After Treatment	Total Recovered Radioactivity (ppm)	Percent of TRR				
			Organic Extractable	Aqueous Soluble	TBZ	BNZ and Its Conjugates	Unextractable
Foliage	2 h	67.46	97.2	1.1	97.2	ND	1.8
Forage	7	41.20	79.3	6.8	79.3	ND	14.0
Haylage	37	21.93	46.3	39.3	36.8	23.1	11.7
Mature Straw	63	22.36	60.1	23.1	33.1	33.5 (not a true average)	16.8
Mature Grain	63	0.12	23.2	32.0	23.2	18.3	17.5

THE METABOLIC FATE OF THIABENDAZOLE IN SOYBEANS (MRID # 418729-02)

Soybeans (Williams 82 variety) were planted 5/23/89 followed by 2 applications of carbon-14 labeled TBZ contained in a spray solution. The first application was made on 7/20/89 and the second on 8/3/89, both at ca 0.3 lb ai/A (maximum use rate and number of application). Samples were collected at 2 h (foliage), 27 days

(immature plant), 78 days (mature straw and seed) after the first treatment. Plant samples were handled, stored, and prepared in a manner similar to those from the wheat study (see above).

The 2 h foliage sample was reanalyzed following 8 months of freezer storage. HPLC analysis showed that TBZ is stable in this matrix under frozen conditions.

For HPLC analysis, the same solvent system for wheat was used (methanol and buffered water), the difference being minor variation in concentration and elution time.

The 2-hr soybean samples (93% extractable into 6N HCl/DMF) by HPLC analysis contained TBZ as the only radioactive component. Unextractable residues and aqueous soluble residues were not further pursued.

For the 27-day sample, 59% of TRR was extractable into methanolic KOH of which TBZ was the only significant component present. An additional 1-2% of TBZ was released from hot methanolic KOH treatment on the unextractable fraction. A small amount (1.4%) of BNZ and its conjugates were detected in the aqueous soluble phase.

Distribution of Radioactivity in Soybean

Crop Part	Days After First Treatment	Total Recovered Radioactivity (ppm)	Percent of TRR				
			Organic Extractable	Aqueous Soluble	TBZ	BNZ and Its Conjugates	Unextractable
Foliage	2 h	14.32	93.3	1.4	93.3	-	5.4
Immature	27 (13 after 2nd)	25.45	60.8	35.5	60.6	1.4	3.8
Mature Straw	78 (64 after 2nd)	10.15	47.3	41.4	43.6	7.3	11.2
Mature Seed	78	0.88	65.8	33.3	42.9	≈22	1.0

Since mature straw (10.15 ppm) contained more carbon-14 residues than the seed, isolation and identification work was done with straw. Activity was distributed among the organic phase (45%), aqueous phase (34%), and not extractable (21%). The organic phase contained TBZ (42%) essentially as the only component by HPLC and an isolate was confirmed by GC/MS to be TBZ. The aqueous phase was lyophilized and purified on C-18 solid phase extraction columns. HPLC analysis (count distribution not given) showed a peak at 4-8 min and a broad band of activity (10-40 min). The polar metabolites fraction (4-8 min) was acylated in pyridine, and the reaction products were analyzed by HPLC the retention time of

which compared well with those BNZ conjugates that were elucidated in the wheat study. After further purification these metabolites were treated with glucosidase and the resulting mixture had the same retention time as BNZ. The identity was confirmed to be BNZ by GC/MS.

For the mature soybeans (seeds) which were harvested at 64 days after the second application, cold methanolic KOH removed 66% of the TRR and by HPLC this fraction was found to contain only TBZ; the component also agreed with TBZ's R_f value on TLC. The report stated that the amount of TBZ was actually 43% of TRR or 0.38 ppm since HPLC analysis showed 11% "unaccounted for" radioactivity (Table VIII in the submission). The aqueous phase (22% TRR) after lyophilization and methanol dissolution was found to contain a fraction that eluted at the same retention time as the BNZ conjugates on HPLC. The unextractable residues (22%) were heated to reflux with methanolic KOH to yield 10% (0.08 ppm) organic soluble and 11% (0.1 ppm) aqueous soluble with 1% (0.009 ppm) remained. No chromatographic analysis on these later fractions was mentioned.

In sum, both soybean seeds and mature straw contain a mixture of TBZ and BNZ and its conjugates.

THE METABOLIC FATE OF THIABENDAZOLE (TBZ) IN SUGAR BEETS (MRID # 418729-03)

Sugar beets were planted on 5/17/89. Dates of the 5 applications made were 7/20, 8/3, 8/17, 8/31, and 9/14/89 and the rate was 0.36 lb ai/A. Samples of immature plants were taken before treatment, 2 hours after the first treatment, and immediately after the 5th treatment (day 56). Mature plants were collected 90 days after the first TBZ application.

Radioactivity in the 2-h sample (beet top) was distributed largely in the organic (HCl/DMF) soluble fraction (91%; TBZ), and much less in the aqueous phase (2%) or the unextractable fraction (7%). Activity in the latter two fractions was not investigated. Also, a freezer storage stability study was not conducted on sugar beet. However, TBZ has been found to be stable in young soybean and wheat plants when stored in a freezer for 8-10 months (see above).

For immature beet tops (24.66 ppm), cold methanolic KOH removed 54% activity. The supernatant fraction was concentrated, diluted with water, and extracted with ethyl acetate. HPLC analysis characterized 98% activity in the ethyl acetate phase as TBZ. The aqueous soluble fraction contained 31% beet tops activity of which a third eluted at the same retention time in HPLC as the BNZ and its conjugates (3.5-5.5 min), and the remaining two thirds consisted of a broad band of activity spread over a range of retention time (10 min and beyond). Hot methanolic KOH on the

unextractable residues released an additional 2% of TBZ (by HPLC), and the aqueous phase showed similar HPLC profile to that from the cold methanolic KOH treatment. Activity remained after all these extractions (unextractable) was 7%.

For immature beet roots (0.86 ppm), cold methanolic KOH extracted 56% of TBZ (by HPLC) and hot methanolic KOH released no additional TBZ (ca 1% activity extracted into the methanol phase). The aqueous phase (from cold methanolic KOH) contained 7% BNZ and its conjugates, 16% unknowns (many components over a range of retention time), and 7% "unaccounted for"; the aqueous phase (13%) from the hot methanolic KOH treatment was practically all BNZ and its conjugates, by HPLC retention time. There was practically no unextractable residue (0.2%) left after the hot methanolic KOH treatment.

Distribution of Radioactivity in Sugar Beets

Crop Part	Days After First Treatment	Total Recovered Radioactivity (ppm)	Percent of TRR				
			Organic Extractable	Aqueous Soluble	TBZ	BNZ and Its Conjugates	Unextractable
Tops	2 h	10.13	91.0	2.1	91.0	-	6.9
Immature tops	56	24.66	54.1	39.2	52.2	11.5	6.8
Immature roots	56	0.86	56.4	43.3	55.6	6.8	0.2
Mature tops	90 (34 days after 5th appl)	10.01	28.5	60.4	27.1	14.1	11.0
Mature roots	90	0.40	29.0	65.0	25.8	10.8	6.0

With mature beet tops (10.01 ppm), cold methanolic KOH extracted 24% of activity that was characterized by HPLC to be TBZ, and hot methanolic KOH released 3% more TBZ. Activity in the two aqueous phases was attributed to BNZ and its conjugates (14%) by HPLC retention time. There was also a broad band of residues (retention time 10 min and beyond) that accounted for ca 40% of the TRR. For mature beet roots (0.40 ppm), ca 26% TRR was characterized as TBZ, and 11% TRR was characterized as the polar metabolites (BNZ and its conjugates). A broad band of residues was also present starting at retention time 10 min (30% root activity).

In sum, residues found in young and mature sugar beets include TBZ and BNZ and its conjugates (27-52% TBZ and 11-14% BNZ and its conjugates in tops; 26-56% TBZ and 7-11% BNZ and its conjugates in roots).

CONCLUSIONS

Three plant metabolism studies were submitted: wheat, soybean, and sugar beet. Results indicate that translocation of TBZ-derived residues to wheat grain, soybean seed, or sugar beet root is minimal or very limited and the majority of the residues stay in wheat straw, soybean straw or sugar beet tops when the fungicide is foliarly applied to these young plants. TBZ and BNZ and its conjugates constitute the main fraction of the terminal residue in all three plant tissues. Identity of TBZ and BNZ were confirmed by gas chromatography/mass spectroscopy. Much of the remaining unidentified residue consists of numerous aqueous soluble metabolites, each accounting for only a very small portion of the TRR.

Codex Harmonization

Only the parent compound is being regulated in plant commodities under Codex (personal communication, F. Ives, 1/21/92).

RECOMMENDATION

Metabolism studies on 3 diverse crops have been reviewed and deemed satisfactory. Therefore, Residue Chemistry Requirement # 171-4(a) - Plant Metabolism, is fulfilled.

CBRS recommends that TBZ and BNZ and its conjugates be included for plant commodities in the definition of the total toxic residue. Consequently, the registrant should submit a residue method for plant commodities that is also capable of measuring conjugates of BNZ.

cc:Circ, RF, Thiabendazole (List B) File, Cheng, PIB/FOD
RDI:FSuhre:3/3/92:MMetzger:3/10/92:EZager:3/10/92
H7509C:CBII-RS:LCheng:CM#2:RM810:2/6/92:2/18/92:02: