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

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Memorandum

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Subject: PP#2E4051. CGA-169374 (Difenoconazole, **Dividend**) in Imported Wheat, Barley, and Rye Grain. **First Food Use**. CBTS# 9029, 9895. MRID# 420900-01 to -04, -32 to -59, 423039-01. DP Barcode# D172067, D178394.

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10/22/92

To: James Stone/Cynthia Giles-Parker (PM22)
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CIBA-GEIGY Corporation, Greensboro, NC, proposes import tolerances for residues of the fungicide **CGA-169374** (1-{2-[4-(4-chlorphenyl)-2-chlorphenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1*H*-1,2,4-triazole; proposed common name: **difenoconazole**) of 0.1 ppm on wheat, barley, and rye grains as the result of seed treatment. This represents the first food use for this chemical.

Conclusions

1. **Product Chemistry.** **a)** The petitioner should specifically state whether their technical is a racemic mixture or is enriched with one or more enantiomers. For future domestic registrations, the fungicidally active enantiomers should be identified. **b)** The petitioner should revise the Confidential Statement of Formula for the technical product to include the CAS number for the active ingredient(s), and if necessary provide a new EPA Form 8570-4 which lists the fungicidally active enantiomers separate from the fungicidally inactive enantiomers. The latter should be listed as impurities. In addition, the petitioner should indicate if the CSF for the end-use product is appropriate for the **Dividend 150SF** or **3SF** formulation. If different CSFs are appropriate for the two formulations, the petitioner should submit both. **c)** The petitioner should indicate the reaction time of Steps 1 and 2 of the manufacturing process. **d)** CBTS notes that while the petitioner has come up with reasonable scenarios for the formation of observed

impurities, the possibility of other impurities that may result from additional side reactions will need discussion for any future tolerance petition. Also, the petitioner should indicate if nitrosamine formation is possible by any route other than decomposition of starting material. **e)** The petitioner has included a method for determination of the cis/trans ratio of the parent compound, CGA-169374 (see below). However, nowhere is the actual ratio discussed. The petitioner must submit information for the five batches concerning the cis/trans ratio of the active ingredient, as well as any information available concerning the relative activities of the two isomers. Also, the petitioner should provide the chemical name of the technical product using the (RS) notation. **f)** The petitioner has not discussed the justification for the certified limits. The petitioner should submit an explanation of how they arrived at their certified limits. **g)** The method for determination of the cis/trans ratio of the active ingredient involves use of a "Spectra-Physics SP 8773" detector. The petitioner should indicate what type of detector it is. **h)** For both Methods AW-128/2 and AK-128/2, the petitioner suggests that the methods were validated at an outside laboratory. The petitioner should submit the reports from the outside laboratory. Those reports should include at least the name and location of the laboratory, a description of the procedure, a record of any communication between that laboratory and the petitioner concerning the execution of the method, sample chromatograms, and results. **i)** For Method AG-20/1, the petitioner should submit the results of an analysis of the technical product for nitrosamines for our review. **j)** The information submitted for Section 63-13, stability of the TGAI, is incomplete. The petitioner must also submit data describing the stability of difenoconazole in the presence of metal and metal ions, and also in sunlight, for any future tolerance request requiring registration of this product. **k)** The petitioner should also refer to p. 10 of the Confidential Appendix for further comments about the validation of the analytical method for several impurities.

2. **Nature of the Residue - Plants. a) Wheat.** The petitioner has NOT adequately determined the nature of the residue in wheat. (However, for our conclusions for this seed treatment use, see Conclusion 2d.) For future domestic tolerance requests, these following major deficiencies associated with the submitted studies will need to be resolved: **1)** The petitioner has been able to characterize and/or quantitate only a small fraction of the activity in any sample, but particularly for those samples originating from seed-treated plants. In many cases, such as grain and stalk samples in MRID# 420900-33, no characterization was achieved. In other examples, such as the grain and stalk samples in MRID# 420900-34, where the activity was (partially) characterized, only rarely was the petitioner able to quantitate the activity. In addition, large portions of the activity (15% - >25%) could not be accounted for after the extraction procedures. The petitioner must be able to characterize and quantitate the residues in wheat grain and stalks before CBTS and TOX can assess the need to regulate metabolites of CGA-169374. **2)** The petitioner did not carry out all the extraction or analysis procedures that would be expected. For example, several times no attempt was made to analyze the organic layer of grain or stalk sample separations. Also, the petitioner did not use enzymes besides β-glucosidase to release bound residues. Since significant fractions (up

to 65%) of the activity were "bound", the petitioner must demonstrate that all reasonable efforts have been made to release and identify those residues.

b) Tomatoes. The petitioner has adequately determined the nature of the residue in tomatoes following foliar application. The major terminal residues are the parent compound, CGA-169374, and its metabolite triazole alanine, CGA-131013.

c) Potatoes. The petitioner has established that the primary metabolic fate of CGA-169374 in potatoes following foliar application is cleavage of the phenyl-triazole bridge. Triazole-labeling studies indicate that side of the molecule becomes triazole alanine, CGA-131013, while phenyl-labeling studies indicate the side group becomes conjugated with a number of naturally occuring substrates. The petitioner has made reasonable attempts to identify the unknown metabolites, and no further characterization is necessary. The major terminal residues are the parent and triazole alanine (CGA-131013).

d) In MRID# 420900-50, "Analytical Method for the Determination of Total Residues of CGA-64250 (Propiconazole) in Crops as 1,2,4-Triazole", the petitioner states (p. 12) that interfering signals arising from natural products range from <0.10 ppm to 3.5 ppm in various plant matrices. This indicates that it is possible that naturally occuring levels of 1,2,4-triazole occur in significant quantities in plants. If this is the case, since the proposed use is a seed treatment use, which implies low residues, any triazole alanine formed in the plant from application of the pesticide would be negligible compared to naturally occuring amounts of triazole-containing crops (see PP#4F3074, A. Smith memo of 12/31/86). CBTS concludes that the major terminal residue of regulatory concern **for this use only** is the parent compound (based on the low residues expected from seed treatment use). For future tolerance request petitions, and depending on the results of the requested metabolism studies in grains, triazole alanine may have to be regulated as triazole alanine per se, or the petitioner may demonstrate that triazole alanine is naturally occuring. CBTS will consider this issue on a case-by-case basis.

3. **Nature of the Residue - Animals.** CBTS concludes that the nature of the residue in animals is adequate for this imported crop from treated seed use. For any future tolerance request, the petitioner must address the following concerns: **a)** The petitioner has failed to take all reasonable steps to identify or release aqueous phase and conjugated metabolites which comprise up to half of the observed activity in several tissues. The petitioner has not noted the effects of acidic or basic hydrolysis on these metabolites, nor has mass spectrometry been used to identify the metabolites. **b)** While the metabolite CGA-205375 appears to be the major organic-soluble metabolite, inconsistencies between the triazole- and phenyl-labeled experiments bring the measured residue levels of this compound into question. Specifically, bridge-intact metabolites should appear in approximately the same proportion in the two studies, and they do not. CBTS asks the petitioner to resolve these inconsistencies. **c)** The petitioner should also explain the large radioactivity recoveries ($>>120\%$) reported for several samples. If the petitioner has

stored extra samples from these studies under frozen conditions, reanalysis of the tissues may be attempted, taking care to address the concerns listed above. In that event, the petitioner should also supply information detailing the stability of the chemical and metabolites under the storage conditions. Otherwise, the petitioner will have to reconduct these studies.

4. **Residue Data.** The data indicate that residues of difenoconazole (parent only) will not exceed 0.1 ppm in wheat, barley, or rye grains or in their processed fractions after seed treatment at the proposed rate. For any future domestic registrations on cereal grains for which the petitioner cites these studies in support, we will withhold our conclusions until deficiencies associated with the determination of the nature of the residue in plants have been satisfied. If additional metabolites are determined to be of concern, the petitioner will either have to conduct new field trials to detect those compounds, or reanalyze any stored samples available from the above trials. If the latter option is chosen, appropriate storage stability data will have to be provided.

5. **Analytical Methods.** a) CBTS will withhold its conclusions concerning the suitability of Method AG-575 for enforcement purposes pending completion of the method validation trial as discussed in Conclusion 5c. For future domestic tolerance requests, pending satisfaction of the deficiencies associated with the plant metabolism studies, residues in addition to the parent may need to be regulated. If that is the case, additional enforcement methodology will need to be developed, or the petitioner may demonstrate the sensitivity of this method to any metabolites of concern. b) The petitioner must demonstrate that interferences from other pesticides registered for use on cereal grains will not occur with this method. Also, the petitioner must clarify whether "a 24 hour period" refers to 24 working hours or one calendar day. c) The method has been forwarded to EPA's Beltsville laboratories for a petition method validation. If additional deficiencies are noted as a result of that trial, they will be forwarded to the petitioner as they become available. d) The petitioner has submitted a report describing multiresidue testing of difenoconazole and its metabolites in plant and animal tissues. The study has been sent to FDA.

6. **Proposed Use.** CBTS concludes that the proposed use is adequately described.

7. **Storage Stability.** The petitioner has established that difenoconazole (parent only) is stable in potatoes and tomatoes under frozen storage conditions for at least 2 years. For the purposes of this import tolerance request, CBTS will to translate this data to wheat, barley, and rye grain. Should the petitioner wish to pursue a domestic registration for use of CGA-169374 on any wheat, barley, or rye commodity in the future, storage stability data on a cereal grain crop (including grain, forage, and fodder) will have to be provided. Also in that case, any conclusions concerning the adequacy of the storage stability data would be withheld until the nature of the residue in cereal grains has been determined.

8. **Meat, Milk, Poultry, and Eggs.** The petitioner has not submitted any animal feeding studies. However, based on the low residue levels resulting from the seed treatment use, CBTS concludes that tolerances on animal commodities are not necessary at this time. This evaluation will have to be reconsidered if additional uses and tolerances are proposed for CGA-169374 on animal feed commodities. The petitioner must assure us that this product will not be registered overseas for foliar use. If such a use is or will be registered, CBTS will require proposed meat (except poultry) and milk tolerances, appropriate analytical methodology, and animal feeding studies.

9. **Other Considerations. a)** There are no Codex, Canadian, or Mexican tolerances for difenoconazole on wheat, barley, or rye grains. Therefore, no compatibility problems are anticipated with this tolerance request. **b)** No Craven data is associated with this petition.

Recommendations

CBTS recommends against the proposed imported tolerances of 0.1 ppm for difenoconazole on wheat, barley, and rye grains for the reasons outlined in Conclusions 1(a-e, i, and k), 5(a-b), and 8. For any future domestic tolerance or registration requests, the petitioner will also have to address the deficiencies in Conclusions 1(f-h and j), 2(a), 3(a-c), 4, 5(a), 7, and 8.

Note to PM: This memo contains the review of the tolerance petition, as indicated under Barcode No. D172067. However, we note here receipt of the amendment, containing a GLP statement, to MRID# 420900-55, which was forwarded to CBTS under Barcode No. D178394. That information has been included in our petition file.

Detailed Considerations

Product Chemistry (MRID# 420900-01, -02, -03)

The product chemistry data requirements are listed in subpart C of 40 CFR §158. Detailed information on the data requirements is given in the Pesticide Assessment Guidelines, Subdivision D.

Note to PM: Product chemistry data (MRID# 420900-04) for the end-use product, **Dividend**, have been sent to CBTS. It is our understanding that this is a tolerance request for treated import commodities. It is not under our purview to review this information, which should be sent to the Product Chemistry Review Section of RSB/RD when and if the petitioner wishes to register this product in the USA.

CBTS has the following conclusions concerning the product chemistry of technical difenoconazole. Conclusions (a-e), (i), and (k) pertain to the proposed tolerance; the remainder pertain to any future domestic registration.

a) The petitioner should specifically state whether their technical is a racemic mixture or is enriched with one or more enantiomers. The fungicidally active enantiomers should be identified.

b) The petitioner should revise the Confidential Statement of Formula for the technical product to include the CAS number for the active ingredient(s), and if necessary provide a new EPA form 8570-4 which lists the fungicidally active enantiomers separate from the fungicidally inactive enantiomers. The latter should be listed as impurities. In addition, the petitioner should indicate if the CSF for the end-use product is appropriate for the **Dividend 150SF** or **3SF** formulation. If different CSFs are appropriate for the two formulations, the petitioner should submit both.

c) The petitioner should indicate the reaction time of Steps 1 and 2 of the manufacturing process.

d) CBTS notes that while the petitioner has come up with reasonable scenarios for the formation of observed impurities, the possibility of other impurities that may result from additional side reactions will need discussion for any future tolerance petition. The petitioner is referred to §61-3(b) and (c) for discussion of the Agency's requirements for this part of the product chemistry submission. In general, the petitioner should discuss the formation of impurities from a theoretical standpoint first, then refer to the actual impurities found, rather than the reverse (as was done in this case). Also, the petitioner should indicate if nitrosamine formation is possible by any route other than decomposition of starting material.

e) The petitioner has included a method for determination of the cis/trans ratio of the parent compound, CGA-169374 (see below). However, nowhere is the actual ratio discussed. The petitioner must submit information for the five batches concerning the cis/trans ratio of the active ingredient, as well as any information available concerning the relative activities of the two isomers. Also, the petitioner should provide the chemical name of their technical product using the (RS) notation.

f) The petitioner has not discussed the justification for the certified limits. The petitioner should submit an explanation of how they arrived at their certified limits.

g) The method for determination of the cis/trans ratio of the active ingredient involves use of a "Spectra-Physics SP 8773" detector. The petitioner should indicate what type of detector it is.

h) For both Methods AW-128/2 and AK-128/2, the petitioner suggests that the methods were validated at an outside laboratory. The petitioner should submit the reports from the outside

laboratory. Those reports should include at least the name and location of the laboratory, a description of the procedure, a record of any communication between that laboratory and the petitioner concerning the execution of the method, sample chromatograms, and results.

i) For Method AG-20/1, the petitioner should submit the results of an analysis of the technical product for nitrosamines for our review.

j) The information submitted for Section 63-13, stability of the TGAI, is incomplete. The petitioner should also submit data describing the stability of difenoconazole in the presence of metal and metal ions, and also in sunlight for any future tolerance request requiring registration of this product.

k) The petitioner should also refer to p. 10 of the Confidential Appendix for further comments about the validation of the analytical method for several impurities.

Details of the reviewed product chemistry data are presented below.

Series 61 - Product Identity and Composition

Proposed ANSI name:	difenoconazole
Company code name:	CGA-169374
Trade name:	Dividend
Chemical name:	1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole
Chem. Abstracts No.:	119446-68-3

Information concerning the formulation (CSF) of this product (§61-1), the beginning materials and manufacturing process (§61-2), and all theoretical discussion about the formation of impurities in the manufacturing process (§61-3) is **Confidential Business Information (CBI)** and is included in a Confidential Appendix.

Series 62 - Analysis and Certification of Product Ingredients

Preliminary (5-batch) analysis of product samples (§62-1), certification of ingredient limits (§62-2), and the description of the analytical methods used to verify those limits (§62-3), are discussed in the Confidential Appendix.

Series 63 - Physical and Chemical Characteristics

Information on the physical and chemical characteristics of the technical grade active ingredient has been submitted. A summary of this information, accompanied by our comments, follows.

Series	Property	
63-2	Color	beige-greyish
63-3	Physical state	crystalline
63-4	Odor	sweetish
63-5	Melting Point	78.6 °C
63-7	Density	1.37 g/cm^3 typical at 20°C
63-8	Solubility	Solubilities (g/100 ml at 25 °C, except as noted): water: 3.3 ppm @ 20°C 1-octanol: 35 acetone: 88 ethanol: 89 toluene: 77 n-hexane: 0.5
63-9	Vapor Pressure	2.5 x 10^{-10} mmHg @ 25 °C
63-10	Dissociation Constant	$pK_a < 0$
63-11	Octanol/Water Partitition Coef.	log K_{ow} = 4.2 @ 25 °C
63-12	pH	6-8 typical at 20 °C (saturated solution)
63-13	Stability	Original comp.: 94.5% At 20-25 °C: 6 months: 94.4% 12 months: 94.3% 24 months: 95.5%

At 35 °C:
3 months: 95.1%
6 months: 94.7%
12 months: 94.9%
24 months: 95.1%

At 54 °C:
0.5 months: 93.1
3 months: 94.9%

CBTS notes that the information submitted for 63-13 is incomplete. The petitioner must submit stability data for the product in the presence of metals and metal ions, and also in sunlight.

Proposed Use

Dividend 150FS, a flowable concentrate containing 150 g a.i./litre (15% ai by weight), is proposed as a seed treatment for control of seed- and soil-borne diseases on wheat, barley, and rye. It is to be applied undiluted to seed at 0.102-0.272 g a.i./lb seed, according to the following schedule:

Crop	g a.i./100 lb seed	Target Pathogen
Wheat	10.2	*Tilletia caries, Tilletia controversia, Septoria nodorum, Urocystis agropyri, Ustilago tritici, Cochliobolus sativus*
	27.2	*Gerlachia nivalis, Fusarium spp., Gaeumannomyces graminis*
Barley	10.2	*Pyrenophora graminea, Pyrenophora teres*
Rye	27.2	*Urocystis occulta*

[10.2 g ai/100 lb = 22.5 g ai/100 kg seed.] Recommended treatment equipment includes on-farm equipment such as a concrete mizer, a continuous-flow seed treater, or a rotating drum or fluidized bed system.

CBTS concludes that the proposed use is adequately described.

Nature of the Residue - Plants

The petitioner has submitted studies describing the metabolism of CGA-169374 in wheat, tomatoes, and potatoes. GLP statements have been signed for all metabolism studies.

Wheat. The petitioner has investigated the fate of both ^{14}C-phenyl and -triazole labeled CGA-169374 in wheat upon either seed treatment or foliar spray. Wheat plants were grown in both greenhouse and field conditions. The analytical methodology used was common to all three

studies. Samples were frozen in dry ice and homogenized in a Waring blender. Total radioactivity was measured by combustion; approximately 0.200 g of plant sample was oxidized. The combustion gas was trapped in Oxosol scintillation cocktail and radioassayed. The limit of quantitation for plant samples was about 20 ppb. Calculation of residue levels was made for all samples having cpm activity approximately twice that of the control samples, which registered about 50 cpm.

Distribution of plant activity >0.05 ppm into polar and non-polar phases was determined by sequential extraction by the Bligh-Dyer (organic) and Ting-Dugger (aqueous) methods (BD/TD) (see CG SOP 4.65, MRID# 401140-10). Briefly, the plant sample is extracted with a chloroform/methanol/water solution and filtered. The filter cake is rinsed with the organic and aqueous phases of the Bligh-Dyer solvent (a 100/100/90 chloroform/methanol/water mix), and the filtrate separated into organic and aqueous phases. The remaining filter cake is re-extracted with water and chloroform/methanol, and then filtered and rinsed with the organic and aqueous phases of the Ting-Dugger solvent (a 122/123/150 chloroform/methanol/water mix). The filtrate is separated and each phase combined with the appropriate phase from the Bligh-Dyer method. Aliquots from each phase are then analyzed for radioactivity by liquid scintillation counting.

Further identification of the residues was carried out by extraction of plant subsamples in MeOH:water (9:1). Anion exchange chromatography and/or gel filtration chromatography were used in profiling the aqueous layer. Both aqueous and organic layers were also profiled with two-dimensional thin layer chromatography (using different solvent systems). The chromatographic identification of certain metabolites was confirmed by mass spectroscopy. Attempts to release bound residues from aqueous fractions were made by enzyme hydrolysis (β-glucosidase) followed by partitioning with ethyl acetate. Released residues remained in the aqueous phase.

Individual studies are summarized below.

MRID# 420900-32, "Uptake and Metabolism of ^{14}C-CGA-169374 by Wheat Resulting From Foliar Spray Application in a Greenhouse Environment. Ciba-Geigy Study No. ABR-90011." The test substance was applied as a spray to spring wheat on four dates as an EC formulation at a rate of 100 g ai/A (~6.32 mg/pail total) for each application. Each bucket contained 25 plants. There were 10 treated buckets per label and 5 controls; phenyl and triazole labeled plants were kept separate. The phenyl-labeled compound had a specific activity of 19.0 μCi/mg, and a radiochemical purity of 98.6%. The triazole-labeled compound had a specific activity of 19.2% μCi/mg and a radiochemical purity of 98.4%. The first application was 43 days after planting (early boot stage, growth stage 9), and the remainder at 7-8 day intervals thereafter. Mature samples were harvested 29 days after the last application and separated into stalks, husks, and grain. Immature tops were cut either 0 days following the first application or 8 days following the second application.

Total radioactive content was determined by combustion, and sample analysis was also attempted for those samples having total residues greater than 0.05 ppm. Table 1 contains the results of

the combustion analysis. Activity distributions are similar for the phenyl- and triazole-labeled plants in all parts except the grain, in which the triazole label was much more prevalent. The petitioner suggests that this indicates cleavage of the triazole-phenyl bridge and that triazole moieties are more likely to be translocated.

Attempts were made to characterize the residues in all of the plant parts shown above. Again, there is great similarity in the distribution of phenyl- and triazole-labeled residues for all plant parts except grain, for which all of the recoverable phenyl-labeled residues were bound, while the triazole-labeled residues were found predominantly in the aqueous extract.

Table 1. TRR and Distribution of Radioactive Residues in Foliarly-Sprayed Wheat. MRID# 420900-32.

	Immature Tops (25%)	Immature Tops (50%)	Stalks	Hulls	Grain
TRR, triazole (ppm)	6.27	8.70	53.8	4.13	1.40
% organic	84.5	65.5	50.1	23.3	0.0
% aqueous	1.69	22.1	27.4	34.8	69.5
% bound	6.96	10.3	13.2	31.1	22.7
TRR, phenyl (ppm)	6.88	8.32	46.7	5.20	0.064
% organic	89.5	68.5	51.8	26.4	0.0
% aqueous	1.86	22.4	29.8	26.1	0.0
% bound	5.79	10.3	13.9	40.6	81.5

Residues were identified as follows (all percentages are of the total residues, as determined by combustion analysis (see Table 1)). **Immature tops**: both the phenyl- and triazole-label experiments showed >85% of the residues to be parent compound. **Stalks** were analyzed for **triazole**-labeled residues by first homogenizing the samples and extracting the residue with methanol:water. 85% of the activity was extracted, 15% was bound. No further attempts to release or characterize these bound residues was made. The methanolic extract was reduced to an aqueous solution and partitioned with chloroform; approximately 60% went into the organic phase and 21% into the aqueous phase. TLC analysis of the organic phase revealed that 47% of the residue was parent and 4% was CGA-205375 (see Attachment 2 for a flow chart describing the analysis of radioactivity). The aqueous phase was loaded onto an XAD column, followed by a rinse with water and elution with methanol. The remaining residue was concentrated from the methanol, dissolved in water, and applied to a Sephadex G-10 column. Three peaks eluted; the first two contained <1% of the total residue and were not further characterized, while the third contained ~10% of the total residue. This eluate was treated with cellulase, and after partitioning with ethyl acetate, 7% remained in the organic layer. After TLC

analysis, the identities of the three components comprising the residue were confirmed by HPLC/MS to be hydroxylated parent compound (1%), CGA-205375 (5%), and hydroxylated CGA-205375 (1%). **Phenyl**-labeled stalk samples were analyzed in much the same way. Initial methanolic extraction yielded 83% extractable residues. Again, the unextractable residues were not further identified. Partitioning with chloroform caused 56% of the residue to enter the organic layer (later determined to be mostly parent (50% of TRR)) and 22% into the aqueous layer. The aqueous layer was treated with cellulase and partitioned with ethyl acetate. In the resulting aqueous phase, which was analyzed by anion exchange chromatography, 5% of the activity was found in the form of "numerous metabolites", including CGA-189138 and its hydroxylated cousin. The organic layer, containing ~ 15% of the residues, was comprised of parent, CGA-205374, and the hydroxylated forms of these compounds. No further quantitation was reported.

Determination of the **triazole**-labeled residues in **grain** involved methanolic extraction, which released ~ 75% of the total residues. No attempt to release bound residues was reported. After partitioning with ethyl acetate, hexane, and chloroform, approximately 15% of the label partitioned into the organic phase (with, again, no further characterization). The aqueous phase was lyophilized, redissolved in MeOH:water, and loaded onto a Sephadex G-10 column. Only one peak, containing 43% of the total activity, was recovered when eluted with methanol:water. This material was loaded onto an anion-exchange column, and radioactive peaks were compared with analytical standards by 2D-TLC. The identified components included triazole (CGA-71019) (~ 10%) and CGA-142856 (~ 20%). **Phenyl**-labeled residues in grain were not extracted by the BD/TD process. Enzyme hydrolysis was performed as the first step in the extraction procedure. Only 35% of the total radiolabel was released into the buffer solution. Because of the low residue levels present (0.064 ppm), the petitioner could not absolutely characterize the residue, though it is thought that the material is a conjugate(s) of a phenyl ring metabolite.

MRID# 420900-33. "Uptake and Metabolism of ^{14}C-CGA-169374 by Wheat Resulting From Seed Treatment Application Under Greenhouse Conditions. Ciba-Geigy Study No. ABR-90010." Both triazole- and phenyl-labeled CGA-169374 were applied to wheat seed. Triazole-labeled parent had a specific activity of 29.0 μCi/mg, and a radiochemical purity of 97.8%. Phenyl-labeled parent had a specific activity of 30.7 μCi/mg and a radiochemical purity of 97.9%. Both labels of CGA-169374 consisted of 67% CGA-169374 3FS blank and 33% radiolabeled CGA-169374 technical. For treatment purposes, the 3FS was diluted with 7.8g H_2O/kg seed. Seeds were treated by combining the formulation and seeds inside a 16 oz. flint jar and rolling the jar to coat the seeds. Application rates were 0.25 g/kg for the phenyl seeds and 0.30 g/kg for the triazole seeds, approximately 1X the maximum proposed use (0.272 lbs ai/A).

Thirty seeds each were planted into 22 buckets (10 phenyl label, 10 triazole label, 2 controls) of loamy sand. Immature tops were harvested at 40 (25%) and 72 (50%) days. Mature plants were harvested at 236 days, and separated into stalks, hulls, and grain. Radioassays were done of treated seeds, soil, and plant parts. As shown in Table 2, residues were generally higher for triazole-labeled plants than phenyl-labeled plants, again possibly indicating a preferential uptake

and transport of triazole moieties after bridge cleavage. Note also that phenyl labeled residues seem to decrease as the plant matures, whereas the amount of triazole label remains relatively constant.

The petitioner could not definitively characterize the residues in **grain**. Elution of the aqueous phase triazole labeled activity on an ion-exchange chromatography column (Sephadex A-25) resulted in two peaks comprising 42% (Peak I) and 52% (Peak II) of the total activity. 2D-TLC analysis was inconclusive; the sample smeared and did not match any of the four standards (parent, CGA-142856, CGA-131013, and CGA-71019). Peak I did elute from the column at a time consistent with the CGA-131013 standard. Levels of phenyl-labeled residues in grain were too low to be characterized.

The petitioner experienced similar difficulties in the analysis of triazole labeled **stalk** residues. Elution of the aqueous phase activity on an A-25 column resulted in three peaks, only one of which corresponded to the pattern of an analytical standard (CGA-131013). The petitioner did not report the fraction of the TRR contained in each of the peaks. 2D-TLC analysis yielded no further clues to the identity of the residues. In addition, the organic phase activity (comprising 17% of the total residue) could not be chromatographed by TLC "due to interferences from natural products in the sample matrix combined with low levels of radioactivity". Therefore, none of the triazole-labeled activity in stalks was characterized. Phenyl-labeled residue levels were too low to be characterized.

Table 2. TRR and Distribution of Radioactive Residues in Seed-Treated Wheat. MRID# 420900-33.

	Immature Tops (25%)	Immature Tops (50%)	Stalks	Hulls	Grain
TRR, triazole (ppm)	0.148	0.010	0.069	0.141	0.183
% organic	33.3	--	16.8	--	--
% aqueous	43.3	--	71.6	96.1	80.4
% bound	15.1	--	18.3	9.8	25.3
TRR, phenyl (ppm)	0.08	0.016	0.016	0.005	0.003
% organic	70.7	--	--	--	--
% aqueous	20.2	--	--	--	--
% bound	15.3	--	--	--	--

Limited success was acheived in characterization of the **25% mature tops**. Triazole labeled organic phase residues were found (1D-TLC) to contain parent (7% of TRR) and some unidentified activity tentatively described as "[possible] carry over of some aqueous solubles into

the organic phase". Aqueous phase activity subjected to ion-exchange chromatography resolved into three peaks. The two major peaks (27% and 16% of the TRR) eluted at the same time as Peaks I and II from the grain samples and are surmised to be "probably triazole alanine and triazole acetic acid metabolites". 2D-TLC was inconclusive due to natural product interference. The third peak (4% TRR) was too small for further characterization. Similar analysis of the phenyl labeled residue indicated the presence in the organic layer of parent (8%) and CGA-205375 (23%). Aqueous phase residues eluted in 3 peaks (10%, 3%, and 2% of TRR), but could not be positively identified by 1D-TLC either before or after enzyme hydrolysis. Residues may have included aglycone-bound compounds with hydroxylated phenyl rings.

420900-34. "Uptake and Metabolism of ^{14}C-CGA-169374 By Wheat Resulting From Seed Treatment Application Under Field Conditions", CIBA-Geigy Study No. ABR-90009. Triazole-labeled and phenyl-labeled CGA-169374 were applied in separate experiments to wheat seed, which was grown under field conditions. Of the three studies, this one most closely approximates the proposed use pattern. Specific experimental details follow.

Specific Activity: 42.6 μCi/mg (phenyl), 44.4 μCi/mg (triazole)
Radiochem. Purity: 98.5% (phenyl), 98.3% (triazole)
Application Rates: 0.23 g ai/kg seed (phenyl), 0.32 g ai/kg seed (triazole)
(proposed use rate 0.272 g ai/kg seed)
Field Locations: Columbia County, NY (Northeast Station) (triazole label only)
(phenyl-labeled seed did not germinate at this location)
Champaign County, IL (Midwest Station) (phenyl and triazole labels)

Harvest Times (days):

	25% Mature	50% Mature	Mature
NY	33	62	83
IL (phenyl)	34	52	72
IL (triazole)	31	48	59

Translocation of the radiolabel into various wheat plant parts is outlined in Table 3. As shown in the above studies, triazole-labeled species appear in the grain to a much greater extent that do phenyl-labeled species. The differences in the results from the two sites is explained as a common occurence with plants grown in different locations, in different soil types and under different environmental conditions.

The petitioner attempted to characterize the residues in mature grain (triazole only), mature stalk (triazole only), and immature top (triazole and phenyl) samples. **Grains** were extracted in MeOH:H_2O (8:2); 89% of the residue was extracted and ~10% remained bound (percent of residues found in combustion analysis). The extract was reduced to water, which was loaded on an A-25 column and eluted. Two peaks were observed, and further identification by 2D-TLC showed that they were CGA-71019 (triazole) and CGA-142856 (triazole-acetic acid). Neither the relative nor absolute amounts of the two compounds were determined. **Stalk** samples were extracted similarly. An apparent 96% of the residue was extracted, with 16% remaining bound. After removal of methanol and partitioning with chloroform and ethyl acetate, approximately

14% remained in the organic phase and 85% in the aqueous phase. Attempts to analyze the organic phase were hampered by matrix interferences. The aqueous residues were subjected to column separation and 2D-TLC analysis as above, and the residues were characterized as CGA-71019 and CGA-142856 (again, no quantitation was reported). In **immature wheat tops**, approximately 89% (triazole) and 41% (phenyl) of the residues were extractable and 6% and 23%, respectively, were not. Characterization (no quantitation) of the residues showed that the phenyl activity was at least partially expressed as CGA-205375 (2-OH-3-triazole propionic acid), while triazole residues were comprised of CGA-71079 and CGA-142856.

Table 3. TRR and Distribution of Radioactive Residues in Seed-Treated Wheat. MRID# 420900-34.

	Immature Tops (25%)	Immature Tops (50%)	Stalks	Hulls	Grain
TRR, triazole, NE (ppm)	0.049	0.053	0.059	0.075	0.135
% organic	---	0.00	9.42	---	0.00
% aqueous	---	89.41	87.12	---	89.84
% bound	---	6.45	16.08	---	9.50
TRR, triazole, MW (ppm)	0.007	0.010	0.011	0.016	0.024
TRR, phenyl, MW (ppm)	0.095	0.008	0.013	0.004	0.004
% organic	0.00	---	---	---	---
% aqueous	41.57	---	---	---	---
% bound	23.39	---	---	---	---

It is seen that as in the other two studies, triazole-labeled residues are much more prevalent than phenyl-labeled residues. The petitioner has submitted a proposed metabolic pathway flowchart, shown in Appendix 2. Metabolism seems to occur at two sites: ketalization/hydroxylation of the dioxo ring, and cleavage of the triazole-phenyl bridge, with subsequent additions to the triazole ring. There is also the possibility of hydroxylation of the 4-chlorophenyl ring.

CBTS Comments. The petitioner has NOT adequately determined the nature of the residue in wheat. For our conclusions concerning the proposed seed treatment use, see the end of this section. For future tolerance requests, these following major deficiencies associated with the submitted studies shold be resolved:

1) The petitioner has been able to characterize and/or quantitate only a small fraction of the

activity in any sample, but particularly for those samples originating from seed-treated plants. In many cases, such as grain and stalk samples in MRID# 420900-33, no characterization was achieved. In other examples, such as the grain and stalk samples in MRID# 420900-34, where the activity was (partially) characterized, only rarely was the petitioner able to quantitate the activity. In addition, large portions of the activity (15% - >25%) could not be accounted for after the extraction procedures.

The petitioner must be able to characterize and quantitate the residues in wheat grain and stalks before CBTS and TOX can assess the need to regulate metabolites of CGA-169374. As the results indicate that most of the residues are not due to the parent compound, this issue is especially important.

2) In a number of cases, the petitioner did not carry out all the extraction or analysis procedures that would be expected. For example, several times no attempt was made to analyze the organic layer of grain or stalk sample separations. Also, the petitioner did not use enzymes besides β-glucosidase to release bound residues. Since significant fractions (up to 65%) of the activity were "bound", the petitioner must demonstrate that all reasonable efforts have been made to release and identify those residues.

CBTS will refer to the Metabolism Committee on the toxicological significance of metabolites once the deficiencies associated with plant metabolism have been addressed. Note that the toxicological significance of triazole-derived metabolites is related to the levels that may be present (see C. Deyrup memo of 3/28/89). A decision by CBTS concerning which residues to regulate will then follow. A tolerance on the parent only may not be appropriate; in such an instance a revised Section F and additional field studies, analytical methodology, and storage stability data may be needed.

Tomatoes. The petitioner has submitted three studies outlining the metabolism of difenoconazole in tomatoes. These studies are reviewed below.

MRID# 420900-35. "The Distribution and Characterization of Phenyl-^{14}C vs. Triazole-^{14}C-CGA-169374 and Their Metabolites in Field-Grown Tomatoes", CIBA-Geigy Study No. ABR-87033. Tomato plants were grown at CIBA-GEIGY's Western Research Station, Reedley, CA, and treated with either phenyl- or triazole-^{14}C-labeled difenoconazole. The experimental details are as follows. The specific activity of the phenyl-labeled compound was 48.6 μCi/mg, with 97% radiopurity. For the triazole-labeled compound, these values were 48.5 μCi/mg and 98% radiopurity. Each label was applied to plants growing in separate plots. Three treatments were made at a rate of ~ 100 g ai/A; the first treatment was 63 days after planting, and successive treatments followed at two week intervals. Foliage cuttings were taken just after the first application and just prior to the second application. Foliage and fruit samples were taken just prior to the third application and at maturity, 40 days after the last application.

Samples were frozen immediately and shipped to CIBA-GEIGY laboratories in Greensboro, NC, where analysis was performed. Total storage time was less than 12 months. After

homogenization in dry ice, total ^{14}C levels were determined by combustion analysis. Plant samples were extracted using the Bligh-Dyer/Ting-Dugger method, described previously in the wheat metabolism section of this review. Samples were characterized by thin-layer chromatography (one- and two-dimensional). The following compounds were used as standards: CGA-169374, CGA-205374, CGA-205375, CGA-189138, CGA-71079, and CGA-131013. Quantitation of radioactive zones was carried out by scraping each zone into a scintillation vial, adding scintillation cocktail, and radioassaying the vial contents.

Results of the analysis are summarized in Table 4. Residues of triazole-labeled difenoconazole were much higher in fruits than were phenyl-labeled residues. This pattern is consistent with the results of the wheat metabolism studies discussed above. The difference is indicative of bridge cleavage between the (4-chlorophenoxy)-phenyl and triazole moieties of the parent compound. There was little difference in the levels of the two labels in foliage.

Table 4 Residue Levels and Percent Distribution of Phenyl- and Triazole-Labeled Residues in Field Grown Tomatoes Treated With Radiolabeled Difenoconazole.

		Phenyl Label				Triazole Label			
		Residues	% Distribution			Residues	% Distribution		
Harvest	Plant Part	(PPM)	Organic	Aqueous	Nonext.	(PPM)	Organic	Aqueous	Nonext.
1	Cuttings	9.447	88.1	4.5	3.2	6.670	98.4	3.4	4.4
2	Cuttings	1.015	73.4	16.2	9.0	0.978	69.1	20.1	8.9
3	Foliage	2.127	78.1	12.9	12.7	2.943	60.5	14.9	8.0
	Fruit	0.012	...	...	...	0.114	10.3	96.9	1.3
4 (Mature)	Foliage	3.548	54.5	22.3	15.7	7.413	49.1	27.8	20.5
	Green Fruit	0.029	...	...	...	0.241	1.4	98.4	0.6
	Ripe Fruit	0.026	...	...	...	0.267	5.0	88.4	1.0

Results of characterization attempts were reported for tomato foliage (Harvests 3 and 4) only. (The petitioner has included a statement which indicates that "TLC characterization of fruit extracts will be included in the report on the identification of the corresponding metabolites".) The attempts include one- and two-dimensional TLC analysis of the organic and aqueous extracts against the standards listed above. The petitioner has included photocopies of sample chromatograms; however, the photocopies are of poor quality and are of no use to the reviewer. The petitioner should submit cleaner chromatograms to the Agency. The petitioner only successfully identified and quantitated residues in the organic phases. For both labels and both harvests, the predominant residue was the parent compound (see Table 5). Levels of CGA-205374 and CGA-205375 were also found from both labels, while CGA-189138 was found in phenyl-labeled samples. The petitioner was unable to identify or quantify any residues in the aqueous phases, apart from detecting "considerable amounts of polar material, assumedly conjugates of triazole and triazole-alanine".

Table 5. TLC Characterization of the Organic Fractions From Tomato Foliage.

	% Total ^{14}C in Foliage	
Phenyl Label	Harvest 3	Harvest 4 (Mature)
Parent	59.1	31.3
CGA-205374/CGA-205375	3.8	3.4
CGA-189138	4.3	5.2
Triazole Label		
Parent	52.1	27.8
CGA-205374/CGA-205375	3.5	4.3

MRID# 420900-38, "Metabolism of Phenyl-^{14}C CGA-169374 in Spray-Treated Tomatoes", ID# N-0964-0700, by Mark G. Schweitzer, August 15, 1990, and
MRID# 420900-39, "Metabolism of Triazole-^{14}C CGA-169374 in Spray-Treated Tomatoes", ID# N-0964-0600, by Poonam R. Velagaleti, July 5, 1990. These two studies were performed in parallel by Battelle, Columbus, OH. Tomato plants were grown under greenhouse conditions and spray treated with a 3.5 EC formulation. The specific activity of the phenyl label was 42.6 μCi/mg, with a radiochemical purity of 97.5%. The specific activity of the triazole label was 44.4 μCi/mg, with a radiochemical purity of 98.3%. Six applications were made at one week intervals for each label at a rate of ~50 g ai/A each. For each label, 20 plants were treated and 10 served as controls. Samples were taken by the following schedule: following Dose 1, prior to dose 3 (stalks only), prior to Doses 5 and 6, and 35 days after the last application, i.e. at maturity (stalks and fruit). If more than one pot was sampled per harvest (as in the last three harvests), samples were composited prior to analysis.

Collected samples were homogenized on-site in dry ice and stored at -20°C for not more than 10 months. Samples were analyzed for total ^{14}C content by combustion, with trapping of $^{14}CO_2$ in liquid scintillation cocktail and analysis by liquid scintillation counting. Two extraction procedures were used. For the triazole-label study, the Bligh-Dyer/Ting-Dugger method, as described above, was used to separate residues into organic and aqueous fractions. Employed for this purpose in the phenyl study was a method which involved homogenization of the sample in acetonitrile. The ACN extract was concentrated and the residual water partitioned with ethyl acetate, generating aqueous and organic phases. This method released 82-100% of residues in all samples (see Table 6). For each label, no further attempts were made to release bound residues remaining after the initial extraction procedure.

Several methods were utilized in separation and characterization of radioactive residues. 2D-TLC was used to separate both organic and aqueous phase activity, and 1D-TLC for conjugated derivatives and aglycones released by cellulase activity. Visualization and quantitation were

performed by computerized methods, with confirmation of the quantitation by conventional analysis of scraped zones by liquid scintillation counting. Anion exchange chromatography (A-25 resin column) was used for fractionation of aqueous fractions. For mature foliage samples from the triazole-labeled study, several peaks were desalted on either an XAD-4 column or an A-25 column run under different solvent conditions. Aqueous fractions were also subjected to enzyme hydrolysis with cellulase (from *Aspergillus niger*).

Table 8. Activity Extraction and Distribution for Phenyl and Triazole Labeled Tomato Samples.

Phenyl[1] - Foliage	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	Final Harvest[2]
Extractable	100.3%	91.2%	84.4%	82.4%	88.6%
Unextractable	1.1%	4.1%	10.3%	12.1%	5.4%
	(2.61 ppm)	(4.00 ppm)	(5.33 ppm)	(6.84 ppm)	(8.29 ppm)
Phenyl[1] - Fruit	**3rd Harv. Green**	**4th Harv. Green**	**5th Harv. Green**	**Final Harv. Green**	**Final Harv. Ripe[3]**
Extractable	85.2%	102.1%	82.3%	94.0%	98.9%
Unextractable	11.8%	5.0%	13.6%	12.3%	5.3%
	(0.20 ppm)	(0.19 ppm)	(0.22 ppm)	(0.04 ppm)	(0.17 ppm)
Triazole[1] - Foliage	**1st Harvest**	**2nd Harvest**	**3rd Harvest**	**4th Harvest**	**Final Harvest**
Organic	89.22%	98.96%	79.98%	70.75%	76.65%
Aqueous	1.34%	10.74%	13.21%	15.29%	24.45%
Unextractable	1.88%	3.49%	4.33%	13.57%	6.76%
	(3.802 ppm)	(3.451 ppm)	(6.416 ppm)	(9.734 ppm)	(7.719 ppm)
Triazole[1] - Fruit	**3rd Harv. Green**	**4th Harv. Green**	**Final Harv. Green**	**Final Int. Ripe[3]**	**Final Harv. Ripe[4]**
Organic	66.00%	52.44%	14.53%	15.62%	54.94% 61.35%
Aqueous	28.88%	40.59%	77.00%	71.94%	34.36% 35.42%
Unextractable	3.00%	1.73%	2.43%	1.57%	2.86% ---
	(0.174 ppm)	(0.151 ppm)	(0.139 ppm)	(0.128 ppm)	(0.203 ppm)

1 - Activity from phenyl samples was extracted with ACN, and that from triazole samples extracted with Bligh-Dyer/Ting Dugger technique.
2 - Final harvest foliage and fruit samples were further extracted into aqueous (H_2O) and organic (ethyl acetate) phases. Foliage: 81.6% Organic, 7.0% Aqueous. Fruit: 85.3% Organic, 14.0% Aqueous. (% of TRR)
3 - Int. Ripe = Intermediate Ripe (i.e. fruit collected before it had completely ripened).
4 - Two extractions were performed to confirm the results (see discussion below).

For the **phenyl** labeling experiment, foliage samples from the first and final harvests were analyzed, as were fruit samples (green and ripe) from the final harvest. Residues in the first harvest foliage showed that 98% of the residues were parent compound. Metabolism profiles of final harvest samples (foliage and fruit) are presented in Table 7. In both cases, the majority of residues (~65%) are in the form of the parent compound. In foliage, a significant amount of residue was released upon enzymatic hydrolysis of the aqueous layer (from 29% of residues released to 55%). Several of the metabolites released were identified as hydroxy analogs of parent and metabolites. In fruit, green and ripe fruit showed the same metabolic profile; therefore, analytical effort was directed toward the ripe fruit. Overall residues in the final harvest green fruit were anamalously low; the petitioner did not attempt to explain why this was so. Attempts to isolate and identify metabolite C-5 were unsuccessful (the petitioner did not specify which methods were tried) and further attempts were abandoned because of the low

concentration of the compound. Overall, the phenyl-labeled metabolism profile for foliage and fruit are similar; note that overall difenoconazole equivalent residue levels are much lower in the fruit (~0.166 ppm) than in the foliage (~8.25 ppm).

Final harvest samples (foliage and fruit) were analyzed in the **triazole** labeling study. Results of the fruit analyses are summarized in Table 8. The predominant residue in the organic layer was the parent compound, with small amounts of CGA-205374 and CGA-205375. The predominant residue in aqueous phase was CGA-131013, with significant amounts (total, 15.06% - 34.58%) of aqueous phase unknowns. Because of difficulties in identification of these unknowns, the petitioner attempted to further identify these residues by ion exchange chromatography. Activity partitioned into 4 peaks. Peak I cochromatographed with triazole alanine (CGA-131013); the identity of this compound was confirmed by 2D-TLC analysis of the peak fraction. Peak II may be CGA-142856, triazole acetic acid; however, confirmatory analysis by 2D-TLC was not performed on this or the other two peaks because of the "very small amounts of radioactivity in them" (p. 33 of the report).

Table 7. Metabolite Profile Summary for Phenyl-Labeled Mature Tomato Foliage and Fruit.

	Ripe Fruit		Foliage	
Compound[1]	% Initial Activity	ppm	% Initial Activity	ppm
CGA-169374	66.3	0.110	64.7	5.36
CGA-199138	ND	...	0.9	0.08
CGA-205374	1.4	0.002	3.9	0.32
CGA-205375	1.7	0.003	1.3	0.11
NP-A	2.3	0.004	1.4	0.11
NP-B	ND	...	0.8	0.07
NP-C	ND	...	0.4	0.03
Origin	13.6	0.023	10.9	0.91
C-1A	ND	...	0.6	0.05
C-1B	ND	...	0.5	0.04
C-1C	ND	...	0.5	0.04
C-2A[2]	1.3	0.002	2.1	0.17
C-2B	1.4	0.002	ND	...
C-3[3]	0.9	0.002	1.1	0.09
C-4[4]	1.5	0.003	1.8	0.15
C-5	8.4	0.014	1.6	0.13
Unextractable	5.3	0.009	5.4	1.23

1 - NP-A, NP-B, and NP-C appeared in the ethyl acetate fraction of the ACN extract. C-1A, etc. appeared upon treatment of the aqueous fraction with cellulase.
2 - Identified as CGA-205375-OH. 3 - Identified as CGA-205375. 4 - Identified as CGA-169374-OH.

Similar but more extensive analyses were performed on foliage samples. The distribution of organic phase residues, as determined by TLC analysis, in foliage was quantitatively similar to

Table 8. Metabolite Profile Summary of Triazole-Labeled Tomato Fruit Extracts.

Component	4th Harvest, Green Fruit	Final Harvest, Green Fruit	Final Harvest, Intermediate Ripe Fruit	Final Harvest, Ripe Fruit
CGA-169374[1]	46.9%	12.5%	12.6%	50.9%
CGA-205[illegible]4	0.73%	0.35%	0.21%	0.52%
CGA-205375	0.63%	0.33%	0.46%	0.74%
Origin A (unknown)	1.32%	0.99%	2.16%	2.12%
	0.54%	0.37%	0.24%	0.64%
CGA-131013 (triazole alanine)	21.7%	42.4%	39.1%	19.3%
a_1[2]	4.18%	3.47%	3.53%	0.96%
a_2	2.94%	1.93%	5.04%	3.37%
a_3	4.18%	2.77%	6.47%	1.65%
a_4	3.45%	13.6%	8.92%	6.98%
a_5	6.18%	12.8%	8.92%	2.10%
Peak I[3]	25.0%	48.2%	39.2%	18.4%
Peak II	9.74%	18.8%	21.0%	7.69%
Peak III	3.56%	1.50%	2.14%	2.68%
Peak IV	2.55%	3.01%	6.62%	3.28%
% TRR Identified	70.0%	55.6%	52.3%	71.5%
Total Residues (ppm)	0.151	0.139	0.128	0.203

1 - Organic phase residues. 2 - a_1-a_5: TLC characterization of aqueous phase residues.
3 - Peak I-IV: Ion exchange chromatography of aqueous phase residues. Peak I was identified as CGA-131013 (triazole alanine).

the distribution in fruits. In contrast, the aqueous phase distribution in foliage differs from that in fruit; for example, no evidence of CGA-131013 was found in foliage. Unfortunately, matrix interferences, poor visualization of some standards, and inadequate separation prevented identification by TLC analysis of the 6-10 components that comprised the residues. Ion exchange separation resulted in four peaks (I-IV) identical to those found in the fruit samples, though with a different relative distribution. There was also a fifth peak, which coincided with the parent compound. Peaks I, II, and III were subjected to further purification by desalting with an XAD-4 resin column and reseparated on the Sephadex A-25 column. (Peaks IV and V were deemed to be too small for further characterization.) None of the components coeluted with any standards, including Peak II, which had originally eluted at the same time as CGA-142856. Results are summarized in Table 9.

Table 9. Metabolite Profile Summary of Triazole-Labeled Tomato Foliage (Final Harvest) Extracts.[1]

Organic		Aqueous	
CGA-169374	68.00%	Peak I	6.15% (4)[2]
CGA-205374	1.63%	II	2.40% (2)
CGA-205375	1.24%	III	11.02% (3)
Origin	3.12%	IV	2.17% (-)
A (unknown)	2.66%	V	0.86% (-)

Cellulase Digestion of TLC Aqueous Phase

	Peak I	Peak II	Peak III
OH-CGA-205375	0.71%	0.37%	0.21%
CGA-205375	0.88%	0.04%	7.67%
OH-CGA-169374	0.58%	0.12%	2.19%
Unknown 1	2.07%	0.19%	0.15%
Unknown 2	0.99%	0.17%	0.40%

1 - Total recovered activity = 7.719 ppm. Total identified residue = 83.64% of TRR.
2 - Numbers in parentheses represent number of separate peaks discovered upon TLC analysis of peak.

Peaks I, II, and III were also subjected to cellulase hydrolysis and TLC separation. The experimenters were able to identify significant portions of the activity by comparison with aglycone standards supplied by the petitioner, as shown in Table 9. Total identified residues were 83.64% of the TRR; no unidentified individual metabolite was present at greater than 2.07%.

The petitioner has adequately determined the nature of the residue in tomatoes. The major metabolites in fruit are the parent compound, CGA-169374, and triazole alanine, CGA-131013.

Potatoes. MRID# 420900-36, "Metabolism of Phenyl-^{14}C CGA-169374 in Spray-Treated Potatoes", ID# N-0964-0400, by Mark G. Schweitzer, August 15, 1990, and **MRID# 420900-37**, "Metabolism of Triazole-^{14}C CGA-169374 in Spray-Treated Potatoes", ID# N-0964-0500, by Poonam R. Velagaleti, July 5, 1990. These two studies were performed in parallel by Battelle, Columbus, OH. Potato plants were grown under greenhouse conditions and spray treated with a 3.5 EC formulation. The specific activity of the phenyl label was 42.6 μCi/mg, with a radiochemical purity of 98.5%. The specific activity of the triazole label was 44.4 μCi/mg, with a radiochemical purity of 98.3%. Six applications were made at one week intervals for each label at a rate of ~50 g ai/A/application. For each label, 20 plants were treated and 10 served as controls. Samples were taken by the following schedule: following Dose 1 and prior to dose 3 (cuttings only), and prior to Doses 5 and 12 days after the last application, i.e. at maturity (cuttings and tubers). If more than one pot was sampled per harvest (as in the last three harvests), samples were composited prior to analysis.

Collected samples were homogenized on-site in dry ice and stored at -20°C for no more than 12 months. Extraction and analysis procedures used in these studies were identical to those used in the tomato metabolism studies reviewed above. Results are summarized in Table 10. Note

Table 10. Activity Extraction and Distribution for Phenyl and Triazole Labeled Potato Samples.

	1st Hvst.	2nd Hvst.	3rd Harvest		Final Harvest	
	Foliage	Foliage	Foliage	Tuber	Foliage	Tuber
Phenyl						
Organic	100.88%	86.20%	85.94%	ND[1]	80.00%	2.10%
Aqueous	1.76%	14.25%	14.45%	92.87%	21.71%	90.34%
Bound	1.48%	4.03%	4.45%	1.83%	4.56%	1.88%
Residues	2.242 ppm	3.097 ppm	5.494 ppm	0.052 ppm	9.138 ppm	0.087 ppm
Triazole						
ACN	96.3%	100.4%	90.9%	51.0%	93.9%[2]	50.2%[3]
Unextr.	1.9%	6.2%	5.9%	57.7%	9.7%	51.1%[4]
Residues	3.48 ppm	6.00 ppm	9.86 ppm	0.006 ppm	12.40 ppm	0.012 ppm

1 - Not Detected.
2 - 87.7% organic, 6.2% aqueous (after partitioning with water/ethyl acetate).
3 - 18.1% organic, 32.1% aqueous (").
4 - Subjected to enzymatic hydrolysis (see below for details).

that there was little translocation of residues from the cuttings to the tubers. Also, phenyl-labeled residues tended to collect in the aqueous layer in tubers and in the organic layer in cuttings, suggesting a different metabolic profile for the two parts of the plant. Likewise, triazole-labeled residues were much more difficult to extract with acetonitrile from tubers than from cuttings.

Phenyl-labeled samples characterized included first harvest cuttings and final harvest cuttings and tubers. First harvest residues were almost entirely parent compound, an expected result since samples were taken immediately after the first application of the radiolabeled pesticide. Residues in final harvest samples are summarized in Table 11. Major metabolites in both matrices were CGA-169374, CGA-205375 (free and bound), CGA-205374, and CGA-189138. The identities of these compounds were confirmed by mass spectrometry. For cuttings, 85% of the residues were identified, with most being the parent compound. Only one unidentified metabolite (NP-A) was present at a concentration of greater than 1.1%. In tubers, the predominant identified metabolite was CGA-205375 (free and bound). Only 30.2% of the residues were identified. The petitioner attempted to release both aqueous bound and unextractable activity with a variety of enzymes and acidic conditions, but was unable to release more than 32% of the **bound** activity (i.e. 16.3% of the total activity). Identification of the released activity from tuber samples was difficult due to the very low levels present in the samples.

For triazole-labeled samples, only the final harvest cuttings and tubers were analyzed. Results are presented in Table 12. The predominant organic-soluble residue in both matrices was the parent compound, with some small amounts of CGA-205375 and CGA-205374. However, the aqueous phase residues differed for the two matrices. In tubers, most of the activity was in the form of triazole alanine, CGA-131013, with no parent compound seen. In cuttings, some of the activity (not quantitated) cochromatographed with parent compound, while no CGA-131013 was observed. Both aqueous phase tubers and cuttings were also subjected to ion exchange chromatography. In tubers there were two peaks; most of the activity cochromatographed with CGA-131013, and the remainder with CGA-142856. Note that no activity cochromatographed with the latter in the 2D-TLC analysis. In cuttings there were five peaks. Two were identical to those observed for the tuber samples. A third peak co-eluted with the parent compound.

Table 11. Metabolite Profile Summary of Phenyl-Labeled Potato (Final Harvest) Extracts.

	Cuttings		Tubers	
Compound	Activity (%)	Residues (ppm)	Activity (%)	Residues (ppm)
CGA-169374	76.4	9.47	8.7	0.0010
CGA-189138	0.5	0.07	ND[1]	---
CGA-205374	1.1	0.14	3.1	0.0004
CGA-205375	2.2	0.27	3.0	0.0004
NP-A[2]	6.1	0.75	3.4	0.0004
NP-B	1.1	0.13	ND	---
NP-C	0.4	0.05	ND	---
NP-D	0.1	0.01	ND	---
C-1A[3]	0.2	0.03	ND	---
C-1B	0.2	0.02	ND	--
C-1C	0.2	0.02	ND	---
C-2A[4]	0.8	0.10	ND	---
C-2B	0.3	0.04	ND	---
C-3[5]	3.0	0.37	15.4	0.0018
C-4[6]	1.0	0.12	ND	---
C-5	0.5	0.06	16.5	0.0020
Unextractable	0.5	1.20	51.1[7]	0.0061

1 - Not Detected.
2 - Unknowns isolated from ACN extract.
3 - Aglycones isolated by cellulase extraction of aqueous fraction.
4 - Identified as OH-CGA-205375. 5 - Identified as CGA-205375. 6 - Identified as OH-CGA-169374.
7 - Unextractable tuber residues were subjected to enzymatic hydrolysis. %TRR released: β-glucosidase, 12.0%; cellulase, 12.8%; β-glucuronidase, 16.3%; α-amylase, 11.2%; pH 5.0 buffer alone, 9.5%; pH 6.8 buffer alone, 15.4%.

Table 12. Metabolite Profile Summary of Triazole-Labeled Potato (Final Harvest) Extracts

Component	Cuttings	Tubers
Organic Phase (2D-TLC)		
CGA-169374	71.31	1.80
CGA-205374	0.78	0.14
CGA-205375	1.85	ND[1]
Origin	2.23	0.16
A (unknown)	2.10	ND
Aqueous Phase (2D-TLC)		
a_1	---	2.80
a_2	---	4.61
a_3	---	3.97
a_4	---	ND
a_5	---	ND
CGA-131013	---	78.87
Aqueous Phase (Ion Exch.)		
Peak I[2]	12.87	70.59
II[3]	1.27	16.10
III	3.67	ND
IV	0.87	ND
V[4]	1.09	ND

1 - Not Detected.
2 - Cochromatographed with CGA-131013. 3 - Cochromatographed with CGA-142856.
4 - Cochromatographed with CGA-169374.

CBTS Comments: The petitioner has established that the primary metabolic fate of CGA-169374 in potatoes is cleavage of the phenyl-triazole bridge. Triazole-labeling studies indicate that side of the molecule becomes triazole alanine (CGA-131013), while phenyl-labeling studies indicate that side to become conjugated with a number of naturally occuring substrates. The petitioner has made reasonable attempts to identify the unknown metabolites, and no further characterization is necessary. The metabolites of concern are the parent and triazole alanine (CGA-131013).

Note: In MRID# 420900-50, "Analytical Method for the Determination of Total Residues of CGA-64250 (Propiconazole) in Crops as 1,2,4-Triazole", the petitioner states (p. 12) that interfering signals arising from natural products range from <0.10 ppm to 3.5 ppm in various plant matrices. This indicates that it is possible that naturally occuring levels of 1,2,4-triazole occur in significant quantities in plants. If this is the case, since the proposed use is a seed treatment use, which implies low residues, any triazole alanine formed in the plant from application of the pesticide would be negligible compared to naturally occuring amounts of triazole-containing crops (see PP#4F3074, A. Smith memo of 12/31/86). CBTS concludes that

the major terminal residue of regulatory concern **for this use only** is the parent compound (based on the low residues expected from seed treatment use). For future tolerance request petitions, and depending on the results of the requested metabolism studies in grains, triazole alanine may have to be regulated as triazole alanine per se, or the petitioner may demonstrate that triazole alanine is naturally occuring. CBTS will consider this issue on a case-by-case basis.

Nature of the Residue - Animals

The petitioner has carried out metabolism studies for difenoconazole in goats and poultry (2 studies for each animal). In all studies, the fate of both triazole-labeled and phenyl-labeled compounds was investigated. The petitioner has submitted GLP statements for all four studies.

- **Goats.** 1) "Metabolism of Triazole- and Phenyl-^{14}C-CGA-169374 In Lactating Goats Dosed Daily for Ten Consecutive Days." CIBA-GEIGY No. ABR-88087. MRID# 420900-44. Triazole-labeled and phenyl-labeled difenoconazole were each fed in gelatin capsules to one goat for ten days. A third goat served as a control. Average dosing per day was 5.56 ppm for the triazole-exposed goat, and 4.17 ppm for the phenyl-treated goat. (Each was fed 7.5 mg of chemical/capsule, and differences arose from different average feed consumptions.) Specific activities of the labels were 48.5 μCi/mg for ^{14}C-triazole and 48.6 μCi/mg for ^{14}C-phenyl. Urine, feces, and milk were collected daily. Blood samples were collected on Days 1, 2, 4, 6, 8, 9, and 10. The goats were sacrificed 22-23 hours after the last dose. Tissues sampled included liver, kidney, tenderloin, leg muscle, omental fat, and perirenal fat.

  All tissue samples were radioassayed by liquid scintillation counting. Feces, liver, and kidney samples were further extracted by the Bligh-Dyer/Ting-Dugger method (see **Nature of the Residue - Plants** for a description of the process). Milk samples were fractionated into fat, whey, and casein. Milk activity was also extracted sequentially with acetonitrile, hexane, and ethyl acetate. Urine activity was partially purified on an XAD-4 column and partitioned with CH_2Cl_2 at pH 9 and pH 2. Urine, feces, liver, and milk extracts were further analyzed by TLC (1D and 2D).

  As seen in Table 13, most residues were excreted in feces or urine. For each label, less than 2% of the total applied dose appeared in tissues and milk.

  In **liver**, residues totaled 0.277 ppm for the ^{14}C-triazole label, and 0.259 ppm for the ^{14}C-phenyl label. Activity was characterized for both the aqueous and organic phases. The major metabolite for both labels was CGA-205375, the alcohol

Table 13. Percent Balance of Radioactivity in Goat Tissues, Milk, and Excreta.

Triazole		Phenyl
5.56	Average Dose (ppm)	4.17
74.60	Feces	67.24
30.88	Urine	20.78
0.13	Blood	0.08
0.50	Milk	0.18
0.90	Tissues (total)	0.44

resulting from deketalization of the parent compound (triazole, 56.8%; phenyl, 57.7%; percentages are the sum of aqueous and organic phase activities). Other metabolites present at >2% were "Metabolite G", a polar metabolite that appears in both labeling experiments (triazole, 11.1%; phenyl, 4.9%), and CGA-71079, (3.2%). The parent comprised ~1% of the residues in each experiment. In total, 60.9% of the triazole residues and 61.3% of the phenyl residues were identified, 14.6% (triazole) and 8.3% (phenyl) of the residues were characterized but not identified and 8.8% (triazole) and 10.5% (phenyl) of the residues remained unknown.

Residues were not characterized for kidney (triazole, 0.094 ppm; phenyl, 0.064 ppm), omental fat (0.064 ppm; 0.025 ppm), perirenal fat (0.035 ppm; 0.022 ppm), leg muscle (0.028 ppm; 0.007 ppm), and tenderloin (0.026 ppm; 0.008 ppm).

Milk residues were 3-5 times higher for the triazole label (0.013-0.043 ppm) than the phenyl label (0.005-0.008 ppm). Fractionation into fat, whey, and casein were 18.7%, 47%, and 21.6% for the triazole label (from Day 7 sample) and 32.1%, 42.2%, and 12.4% for the phenyl label (from Day 8 sample). Residues plateaued near Day 7-8 for both labels (see Table 14). Only triazole activity was characterized: the identified metabolites were CGA-71079, 45.8% and CGA-205375, 3.3%. Also found were the polar "Metabolite D", 2.9%, and other unidentified polar metabolites, 18.2%. No parent was found.

Table 14. Time Dependence of Radioactivity in Milk From Goats Dosed Daily With ^{14}C-CGA-169374. From MRID# 420900-44. (Residue values in PPM)

Day →	1	2	3	4	5	6	7	8	9	10
Triazole	0.013	0.022	0.030	0.032	0.037	0.043	0.043	0.043	0.041	0.033
Phenyl	0.005	0.007	0.006	0.006	0.008	0.007	0.007	0.008	0.007	0.007

2) "[^{14}C]-CGA-169374 Phenyl and Triazole Label Distribution, Elimination, and Metabolism in Goats", ABR-89100, MRID# 420900-42. Four goats were fed the exaggerated rate of 100 ppm of either triazole-labeled or phenyl-labeled (two goats each) difenoconazole for three consecutive days. The doses were contained in gelatin capsules. The triazole and phenyl labels had specific activities of 13.9 and 14.0 μCi/mg, respectively. Milk samples were taken twice daily and combined into one daily sample. Excreta were collected daily. The animals were sacrificed 4-6 hours after the last dose, with the following samples taken: blood, liver, kidney, leg and tenderloin muscle, and omental fat. No mention is made of control animals or control samples used in the study.

Representative samples were processed and analyzed for total activity content (by sample combustion and liquid scintillation counting of the resulting $^{14}CO_2$) as well as for metabolite quantitation and identification (by HPLC and/or TLC). Residue distribution is summarized in Table 15, and identification of the metabolites is presented in Table 16.

In tissues, residues tended to concentrate in the **liver**. Total phenyl residues were 6.658 and 5.465 ppm for the two goats, while triazole residues were 6.533 and 8.487 ppm. Residues were extracted by homogenization of the sample with a MeOH:$CHCl_3$:H_2O (11:5:5) solution. Residues were concentrated in the organic layer (72% phenyl, 60% triazole). The major metabolite from that layer was CGA-205375. Also present were the parent compound, OH-CGA-205375, and CGA-205374. Only 3-4% of the activity was non-extractable. A significant portion of the activity (phenyl, 23%; triazole, 45%) partitioned into the aqueous phase, but the petitioner did not attempt to characterize it.

Table 15. Percent Balance of Radioactivity in Goat Tissues, Milk, and Excreta.

Triazole[1]		Phenyl[1]
100	Average Dose (ppm)	100
21.6 43.5	Feces	43.5 26.5
13.6 14.2	Urine	17.7 21.8
0.49 0.44	Blood	0.32 0.51
0.17 0.24	Milk	0.12 0.05
4.94 4.09	Tissues (total)[2]	2.55 2.97

1 - Each label was fed to two goats.
2 - Liver, kidney, muscle, and fat.

In **kidney**, total residues were 1.348 and 1.748 ppm for the phenyl label, and 1.882 and 1.745 ppm for the triazole label. Residues were extracted in a similar fashion as residues in liver and were distributed as follows: for the phenyl label, 68% was found in the aqueous phase, 42% in the organic phase, and 12% non-extractable. For the triazole label, 49% was in the aqueous phase, 58% in the organic phase, and 8% non-extractable. (Apparent residues may seem to exceed 100% because analyses of individual phases were compared to combustion analysis of a separate homogenous sample.) The major identified metabolite from HPLC analysis of both the aqueous and organic phases was CGA-205375. A small amount of parent was also present, as was OH-

Table 16. Identification and Percent Distribution of CGA-189374 Metabolites in Goat Tissues and Milk.

	Kidney[1]		Liver[2]		Milk[1]		Muscle[2]		Fat[2]	
Metabolite	P[3]	T[3]	P	T	P	T	P	T	P	T
CGA-189374	1.5	5.2	6.7	8.2	8.6	6.2	3.5	3.7	3.2	6.5
OH-CGA-189374	...	...	...	...	15.2	...	...	...	5.9	1.8
CGA-205375	30.9	51.0	52.9	49.8	21.3	34.4	68.5	43.2	73.5	75.3
OH-CGA-205375	2.0	...	6.1	...	4.4	3.0	...	2.3	3.4	1.2
CGA-71079	...	...	...	...	...	5.8	...	1.8	...	...
CGA-205374	...	...	...	...	...	...	...	1.4	...	...
CGA-189138	...	...	...	...	6.3	...	...	...	...	...
Unknown (R_t = 4.5 min)[4]	51.7	39.8	...	...	31.4	33.2	...	...	...	...

1 - Residues of organic and aqueous layer. 2 - Residues of organic layer only.
3 - P = phenyl label; T = triazole label. 4 - From MeOH/H_2O fraction.

CGA-205375. A large portion of the aqueous phase activity for each label eluted at a short retention time (4.0 min); the identity of the responsible polar chemical(s) could not be determined. Both phenyl and triazole samples show this pattern, indicating that a bridge-intact conjugate structure was formed. Hydrolysis with β-glucuronidase or aryl-sulfatase did not release any metabolites. The petitioner suggests that the activity could be due to glutathione conjugates.

Residues in **milk** samples peaked on the second day; samples from that day were analyzed. Total residues from the two goats treated with phenyl label were 0.109 and 0.163 ppm, while triazole residues were 0.428 and 0.329 ppm. Residues partitioned almost exclusively into the organic phase (phenyl 94%; triazole 82%), with some bound residues (18%; 6%) and little aqueous residues (6%; 1%). CGA-205375 is the major metabolite present at the highest levels. In total, 55.8% of the phenyl and 49.4% of the triazole residues were identified. As with the kidney, a large amount of the unidentified activity (31.4% phenyl; 33.2% triazole) eluted at low retention times and is attributed to glutathione conjugates (as it is unaffected by β-glucuronidase or aryl-sulfatase hydrolysis).

Nearly three times as much triazole activity (0.681 and 0.449 ppm) was found in **muscle** as phenyl activity (0.195 and 0.207 ppm). The majority of both labels partitioned into the organic phase, although there was a significant amount of triazole label in the aqueous phase [phenyl, 9/77/14%; triazole 37/55/14% (aqueous/organic/bound)]. Only the organic layer was characterized. CGA-205375 comprised the majority of the analyzed residue. All other residues were less than 5%.

Significant residues were also found in **fat** (phenyl, 0.503 and 0.618 ppm; triazole, 1.547 and 0.738 ppm). Over 95% of the TRR was extractable into CH_2Cl_2, with <5% bound (for both labels). Again, for both labels the predominant metabolite was CGA-205375. No other metabolite was identified to be greater than 5% of the total residue. Approximately 85% of the residues for each label were identified.

- **Poultry.** 1) "Metabolism of Triazole and Phenyl-^{14}C-CGA-169374 in Laying Hens Dosed Daily for Fourteen Consecutive Days." CIBA-GEIGY Study No. ABR-89051. MRID# 420900-43. Two hens each were treated with phenyl- or triazole-labeled difenoconazole at a daily rate of ~5 ppm (see Table 17 for details). Specific activity of the chemical was 48.5-48.6 μCi/mg, with radiochemical purity >97% for each label. Daily samples of egg yolks, egg whites, and excreta were taken. At sacrifice (22 hours after the last dose), the following tissue samples were taken: liver, kidney, blood, lean meat (dark and light), skin plus attached fat, and peritoneal fat pad. One hen served as a control for lean meat samples; appropriate predose egg and excreta samples were taken from test birds.

The radioactivity in excreta, tissues, egg whites and egg yolks was extracted with the Bligh-Dyer/Ting-Dugger method.

The distribution of activity into tissues, eggs, and excreta is shown in Table 17. At least 90%

of the residues partitioned into excreta, with much of the remainder appearing in egg white and yolk. Differences in the levels of triazole and phenyl residues appear in egg whites and lean meat. These will be discussed below.

Table 17. Percent Balance of Radioactivity in Hen Tissues, Eggs, and Excreta.

Triazole[1]		Phenyl[1]
4.66 4.91	Average Dose (ppm)	5.73 5.19
91.55 89.84	Excreta	90.39 96.77
0.677 0.802	Egg White	0.066 0.039
0.648 0.650	Egg Yolk	0.718 0.529
0.114 0.154	Blood	0.058 0.014
0.635 0.784	Tissues (total)[2]	0.336 0.166

1 - Each label was fed to two hens.
2 - Liver, kidney, lean meat, skin w/ attached fat, and peritoneal fat pad.

Residues in **egg whites** and **egg yolks** were analyzed daily. For egg whites, levels of both the triazole and phenyl labels rose quickly and plateaued by Day 5 for the former and Day 2 for the latter. Residue levels ranged from 0.101-0.155 and 0.107-0.184 ppm for the triazole label and 0.011-0.016 and 0.006-0.010 ppm for the phenyl label. Residues were characterized with respect to their partitioning into organic or aqueous phases; these results are summarized in Table 18. Further identification of the activity was not attempted. The petitioner suggests that the different distribution patterns of the triazole and phenyl labels indicates cleavage of the alkyl bridge between the two moieties.

Table 18. Partitioning of Residues in Egg Whites and Yolks. (MRID# 420900-43)

Fraction	Label	Day	Hen	Total Residues (ppm)	% Organic	% Aqueous	% Bound
White	Triazole	3	2A	0.141	11.14	80.74	12.45
		12	1A	0.155	17.88	73.14	12.33
		13	2A	0.184	13.81	77.14	18.17
	Phenyl	4	3B	0.013	81.04	<1.00	7.86
		10	3B	0.016	63.73	34.51	8.62
		11	4B	0.010	72.95	<1.00	15.22
Yolk	Triazole	3	2A	0.132	11.11	69.89	7.91
		12	1A	0.281	39.92	45.56	12.52
		13	2A	0.292	20.01	76.43	9.47
	Phenyl	3	3B	0.053	69.84	21.41	13.50
		10	3B	0.334	47.28	22.12	21.31
		11	4B	0.250	44.32	18.94	28.99

Similar extraction and characterization procedures were carried out on tissue samples, as summarized in Table 19. As in eggs, triazole residues tended to collect in the aqueous layer, regardless of the tissue (liver, kidney, or lean meat). Phenyl residues concentrated in the organic layer of liver and blood, but in the aqueous layer of kidney. The petitioner states that these differences support the idea that cleavage of the alkyl bridge connecting the phenyl and triazole moieties is the usual fate of CGA-169374 ingested by hens. Total residues were >0.1 ppm only for liver and kidney (fat and skin samples contained <0.05 ppm and were not characterized in any way).

Table 19. Partitioning of Residues in Poultry Tissues. (MRID# 420900-43)

Tissue	Label	Hen	Total Residues (ppm)	% Organic	% Aqueous	% Bound
Liver	Triazole	1A	0.118	27.79	50.83	11.23
		2A	0.134	27.98	63.50	13.59
	Phenyl	3B	0.147	51.38	13.48	23.62
		4B	0.101	53.88	7.19	31.37
Kidney	Triazole	1A	0.352	11.86	69.14	11.48
		2A	0.517	13.29	76.26	11.12
	Phenyl	3B	0.522	8.48	60.84	65.32[2]
		4B	0.449	9.59	72.60	28.51
Lean Meat[1]	Triazole	1A	0.072	8.71	82.99	9.29
		2A	0.093	7.52	86.07	7.00
Blood	Phenyl	4B	0.011	55.87	<1.0	23.64

1 - Includes dark and light meat. 2 - High value probably due to experimental error.

Excreta samples, particularly ACN/H_2O extracts, were also analyzed (TLC) for residues. For the triazole label, 22.91% of the residues were CGA-71079, 11.94% were parent compound, and 4.71% CGA-205375. A significant portion of the remainder, 35.94%, separated into at least 4 distinct zones which were not identified. The most prevalent identified phenyl-labeled residue was parent (11.63%), with 2.37% CGA-205375. Most of the phenyl residues (54.03%) were not identified. Of these residues, 18.07% shared the same chromatographic pattern as unidentified triazole-labeled residues.

2. "[^{14}C]-CGA-169374 Phenyl and Triazole Label Distribution, Elimination, and Metabolism in Hens." CIBY-GEIGY ABR-89101. MRID# 420900-41. Ten hens each were fed ~68 ppm of either triazole- or phenyl-labeled CGA-169374 daily (7.5 mg/day) for three days. The test material was applied in acetone to ground corn cobs in gelatin capsules. Each labeled compound has a specific activity of 13.9-14.0 μCi/mg and a radiochemical purity of >97.6%. Egg samples were taken daily, and the following tissue samples were taken 4-6 hours after the last

dose: blood, liver, kidney, muscle, and skin fat. One hen served as a control.

As in the previous hen study, the vast majority of the activity was recovered in excreta, as summarized in Table 20. Residues in excreta recovered on Days 1 and 2 comprised an even higher percentage of the total (89-92%) for each label. The petitioner surmises that the levels for Day 3 are lower because of the short time span between the last dose and sacrifice. Unlike the other study, very little of the residues went into egg white or egg yolk, though it can be seen that triazole residues did accumulate in eggs over the three day period. Because it takes 4-6 days for egg production in the hen, it is not surprising to see lesser radiolabeled residues in the 3-day versus the 14-day metabolism study. Triazole residue levels were also higher than phenyl residue levels in muscle and blood.

Table 20. Radioactivty Distribution in Hen Tissues, Eggs, and Excreta.

Phenyl[1]	Sample	Triazole[1]
76.1%	Excreta	78.1
<0.01% Total Day 1: 0.015 ppm Day 2: 0.023 ppm Day 3: 0.011 ppm	Egg White	0.05% Total Day 1: 0.062 ppm Day 2: 0.269 ppm Day 3: 0.413 ppm
<0.01% Total Day 1: <0.003 ppm Day 2: 0.037 ppm Day 3: 0.047 ppm	Egg Yolk	0.02% Total Day 1: 0.014 ppm Day 2: 0.132 ppm Day 3: 0.272 ppm
0.3% (0.297 ppm)	Blood	0.7% (0.649 ppm)
0.6% (4.660 ppm)	Liver	0.5% (4.259 ppm)
0.1% (2.247 ppm)	Kidney	0.1% (1.886 ppm)
0.4% (0.102 ppm)	Muscle	2.1% (0.509 ppm)
0.6% (0.454 ppm)	Skin/Fat	0.7% (0.484 ppm)

1 - Each label fed to 10 hens - results are average values. Samples taken on Day 3 unless otherwise indicated.

The metabolic fate of difencoconazole was similar for both **egg white** and **egg yolk**. Both samples were extracted with acetonitrile. Most of the ^{14}C residues, regardless of label, were extracted (whites, 116% phenyl and 90% triazole; yolks, 93% phenyl and 95% triazole). The unextractable ^{14}C residue was 31% for phenyl and 4% for triazole label in whites, and 19% for both labels in yolks. The petitioner attributes any results summing to greater than 100% (especially phenyl label for whites) as a result of sample inhomogeneity. Residues were characterized as outlined in Table 21. For both white and yolk, a major triazole-labeled metabolite is free triazole, CGA-71079. The major phenyl-labeled metabolite is CGA-205375.

Liver samples were homogenized in MeOH:$CHCl_3$:H_2O (11:5:5). The majority of the activity for both labels (72% phenyl, 74% triazole) partitioned into $CHCl_3$. (For the phenyl label, 14% of the activity was in the methanol phase, and 35% was in the solid. For triazole, those values are 16% and 31%.) This fraction was further partitioned between acetonitrile and hexane, with most of the activity (70% phenyl, 64% triazole) collecting in the ACN phase. The ACN phase was analyzed for characterization of residues, as summarized in Table 21. As for other poultry and goat tissues, the major metabolite for both labels is CGA-205375.

Residues from **kidney** samples for both labels were extracted in the same manner as liver samples. The following distribution of residues was found. For the phenyl label, 38% aqueous, 36% organic, and 82% solid; for the triazole label, 43% aqueous, 43% organic, and 52% solid. The organic phase was subsequently partitioned between ACN and hexane; 25% of the phenyl

and 40% of the triazole residues remained in the ACN phase. Also, the solid fraction from the original processing of both labels was treated with protease. Apparent residues of 90% for phenyl and 49% for triazole were released. Both the ACN and protease fractions were analyzed.

Table 21. Identification and Percent Distribution of CGA-169374 Metabolites in Hen Tissues and Eggs.

	Egg White[1,2]		Egg Yolk[1,2]		Liver[3]		Kidney[3]		Muscle[3]	
Metabolite	P[4]	T[4]	P	T	P	T	P	T	P	T
CGA-169374	2.7	4.9	0.9	0.8	4.3	...	...	1.7	...	0.9
OH-CGA-169374	12.5	1.0	12.4	4.2	2.9	...	4.8	3.2	...	...
CGA-205375	84.7	7.7	72.9	35.2	35.2	30.0	21.8	19.7	34.8	8.9
OH-CGA-205375	2.9	0.7	0.7	2.0	9.8	8.2	3.2	4.9	4.3	1.4
CGA-71079	...	67.7	...	32.3	...	5.4	...	6.9	...	4.9
CGA-205374	...	...	...	...	1.8	1.4	5.2	2.1	...	...
CGA-189138	...	...	...	...	2.7	...	...	...	...	...

1 - Percentage of residues found in eggs sampled on Day 2. 2 - Residues of ACN fraction (see text).
3 - Residues of ACN and protease fractions (see text). 4 - P = phenyl label, T = triazole label.

Results of tests of **muscle** samples showed anomalous residue distributions for both phenyl and triazole labels. The residues were extracted with an 11:5:5 MeOH:$CHCl_3$:H_2O solvent. For the phenyl label, the apparent recovery was 182% (44% aqueous, 81% organic, 97% non-extractable), and for the triazole label, 133% (76% aqueous, 32% organic, and 25% non-extractable). The petitioner claims that this is due to errors in the initial ^{14}C counting. The petitioner also notes that the normalized distribution for the phenyl label show 53% non-extractable residues, consistent with the results of other tissues. Also, the identified residue pattern in muscle was qualitatively similar to that in other tissues, so it is unlikely that the "extra" activity was due to an unusual metabolite.

The organic layer was further partitioned between ACN and hexane; 39% of the phenyl and 16% of the triazole label partitioned into ACN. The metabolites contained in the ACN phase were analyzed by HPLC and 2D-TLC.

Fat samples were extracted with CH_2Cl_2. The extractable radioactive residue was 88% for phenyl-labeled residues and 63% for triazole-labeled residues. Unextracted residues were 12% and 37%, respectively, of the TRR. The extracted residue was concentrated down and partitioned between ACN and hexane, with 84% (phenyl) and 59% (triazole) of the TRR partitioning into the ACN layer. The predominant metabolite for both labels was CGA-205375 (63.5% phenyl; 45.6% triazole). Also present were OH-CGA-205375 (8.2% phenyl, 5.7% triazole), CGA-169374 (1.6%; 1.5%), CGA-205374 (1.7%; 0.9%), and CGA-71079 (0.9%, triazole only). In total, 75% of the phenyl and 54.6% of the triazole labels were identified.

CBTS Comments: We note that the petitioner undertook little analysis of the aqueous ($MeOH/H_2O$) fraction in any sample in these studies. The only analysis of this fraction was of kidney samples taken in the second goat study and liver and milk samples in the first goat study. The petitioner claims that the similarity of the metabolism in goats and hens allows them to consider the results of the goat kidney analysis indicative of the distribution in all aqueous fractions for all animals. In that case, most of the activity eluted in the polar conjugate region (R_t = 4-5 min). There was little or no effect on the activity after treatment with sulfatase or β-glucuronidase. The petitioner suggests that the conjugates are glutathione conjugates containing bridge-intact metabolites.

CBTS agrees that, to the extent it has been elucidated, the metabolism in the two animals is qualitatively identical. The major metabolite in the organic phase of comparable tissue samples (kidney, liver, muscle, and fat) seems to be CGA-205375. We also feel that it is possible that the distribution of water-soluble metabolites will be similar in the two animals. However, we do not feel that the petitioner has adequately examined the aqueous phase activity, which constitutes up to 50% of the total radioactive residue in some tissues. Although attempts to release conjugated metabolites via enzyme hydrolysis were noted, no mention is made of the effects of acidic or basic hydrolysis on these metabolites. Also, the petitioner has not attempted to use mass spectrometry to identify the unknown metabolites.

The petitioner should consider the possibility that the polar metabolites detected by HPLC in the goat kidney and milk aqueous phases (goat study #2) are the same as Metabolites B, C, D, and G from goat study #1, which were detected by TLC. Currently, it is not possible to compare the results of these two studies because different methods are used and the labeled chromatograms provided with goat study #2 are difficult to read.

CBTS also notes inconsistencies with regards to the levels of the metabolite CGA-205375 which are reported for various goat and hen tissues as well as eggs. Since this metabolite is a bridge-intact compound, one would expect that observed residue levels would be similar for the two labeled species. However, we note that there are differences of over 20% in the observed distributions for the following samples: goat kidney and muscle, and hen muscle, egg white, and egg yolk. In particular, the egg white analysis shows that 84.7% of the phenyl-labeled species is CGA-205375, compared to only 7.7% of the triazole-labeled species. Furthermore, for that sample, the major triazole-labeled species is CGA-71079, free triazole, which implies that a large portion of the phenyl-labeled residue should also consist of a bridge-cleaved metabolite. CBTS asks the petitioner to resolve these inconsistencies.

CBTS is concerned about the unusually high recoveries observed for several samples, including hen muscle, kidney (both labels) and egg whites (phenyl label), and goat kidney (phenyl label). The sum of the aqueous, organic, and non-extractable activities in these cases totals more than 120% of the determined total radioactive residue, and in one case (hen muscle) sums to 222%. As the petitioner suggests, these aberrant values are probably due to errors in the initial ^{14}C counting. However, the magnitude of these errors suggest that additional samples should have been reanalyzed to confirm or correct the initial results.

CBTS concludes that the nature of the residue in animals is adequate for this imported crop from treated seed use. For any future tolerance request, the petitioner should take all reasonable steps to identify or release aqueous phase and conjugated metabolites which comprise up to half of the observed activity in several tissues. CGA-205375 appears to be the major organic-soluble metabolite. Unusually large recoveries were reported in the experimental results. If the petitioner has stored extra samples from these studies under frozen conditions, reanalysis of the tissues may be attempted, taking care to address the concerns listed above. In that event, the petitioner should also supply information detailing the stability of the chemical and metabolites under the storage conditions. Otherwise, the petitioner will have to reconduct these studies.

Analytical Method

The analytical method used to generate the residue data for this petition is CIBA-GEIGY Method AG-575A (MRID# 420900-52). This method is also proposed for enforcement. The method is carried out as follows. Frozen wheat samples are homogenized, and residues are extracted by boiling the samples in 8:2 methanol:concentrated ammonium hydroxide solution. The extract is diluted in water and partitioned twice with hexane. The organic layer is then partitioned twice with acetonitrile. The residues are now in the ACN phase. The ACN is evaporated and redissolved in toluene for cleanup on a silica Sep-Pak column. The toluene is evaporated, the residue dissolved in hexane, and a second cleanup is performed on a phenyl Bond-elut column. A third cleanup is then performed with a charcoal column, with toluene as the solvent. Detection is achieved by GC with a nitrogen/phosphorus detector. The petitioner notes that it may be necessary to increase the N/P element power in order to obtain sufficient peak height of the lowest calibration standard. A set of 4-6 samples can be extracted, cleaned up, and analyzed in "a 24 hour period". The method does not require use of an untreated commodity or a blank.

Recovery data was generated for the parent compound (CGA-169374). Recovery of a 0.01 ppm spike from wheat grain averaged 79% for four samples (range 70-85%). Wheat straw was spiked at 0.05 and 0.75 ppm (one sample each), with recoveries of 122% and 85%, respectively. Wheat forage was spiked at 0.05 ppm (one sample), with a recovery of 76%. Analysis of control samples (two for grain, one each for straw and forage) showed no residues above the limit of quantitiation (0.01 ppm for grain and 0.05 ppm for straw and forage).

Supplemental recovery data is provided for wheat grain, straw, and forage, and seed with the field trial data (MRID# 420900-55, p.29 and 67). Control samples were spiked at levels from 0.01-1.0 ppm (seed - 10, 400, or 500 ppm). Results are summarized in Table 22. Unless otherwise indicated, residues in control samples before application were <0.01 ppm for grain, forage, and straw, and <10 ppm for seed. Representative chromatograms show no matrix interferences. The method has been forwarded to EPA's Beltsville laboratories for a petition method validation trial (R. Lascola memo of 5/27/92).

CBTS will withhold its conclusions concerning the suitability of Method AG-575A for enforcement purposes pending completion of the method validation trial. For future domestic tolerance requests, pending satisfaction of the deficiencies associated with the plant metabolism studies, residues in addition to the parent may have to be regulated. If that is the case, additional enforcement methodology will need to be developed, or the petitioner may demonstrate the sensitivity of this method to any metabolites of concern.

Table 22. Recoveries of CGA-169374 From Wheat Grain, Straw, Forage, Seeds, and Processed Commodities. From MRID# 420900-55.

Grain		Straw		Forage		Seed	
Spike ppm	% Recovery	Spike ppm	% Recovery	Spike ppm	% Recovery	Spike ppm	% Recovery
0.01	79,89,67,88, 91	0.01	70,80,83	0.01	77,59,91	10	120,105,107, 104,109,113, 119,119
0.05	83,87,70,102, 90,100	0.05	119,83,95,79, 103,94,107, 103	0 05	96,75,88,83, 97	200	89
0 10	109	0.10	104,102,93, 81	0.10	81,82,59	250	96,114
0.20	75	0.50	98	0.20	97	400	98
1 0	97,78,100	1.0	75,129	1.0	76	500	93,95,97,112, 106,98,102, 106,105,102
AVG:	87.8 ± 12.0	AVG:	94.3 ± 15.6	2.0	88	AVG:	105.0 ± 8.7
				5.0	84		
				AVG:	82.2 ± 11.9		

Bran		Middlings		Shorts and Bran		Patent Flour	
0.01	110	0.01	68	0 01	69	0.001	76

In any event, CBTS notes the following deficiencies associated with this method. The petitioner must demonstrate that interferences from other pesticides registered for use on cereal grains will not occur with this method. The results of the method validation trial will be forwarded to the petitioner as they become available.

The petitioner has also submitted a report describing multiresidue testing of difenoconazole and its metabolites in plant and animal tissues. The method is entitled "Multiresidue Method Testing of CGA-169374 and Metabolites in Crops and Animal Tissues", CIBA-GEIGY Project No. ABR-89048, by R. K. Williams, CIBA-GEIGY Corporation, Greensboro, NC; 7/20/92; MRID# 420900-54. Compounds investigated included CGA-169374, CGA-205374, CGA-205375, and CGA-189138. The study has been sent to FDA (R. Lascola memo, 5/21/92) for comment. The

petitioner concluded that Protocols C, D, and E did not yield sufficient recoveries or responses to be useful for the detection of these chemicals. Protocol A (N-methyl carbamates) does not apply to these chemicals. Protocol B (acids and phenols) only applies to CGA-189138; however, recovery of that compound was not tested. We do not consider that compound to be of regulatory concern at this time.

Storage Stability

The petitioner has not conducted any studies on the stability of CGA-169374 in wheat matrices. Instead, studies have been carried out in tomatoes (MRID# 420900-58) and potatoes (MRID# 420900-59). Samples were fortified at 0.50 ppm, stored at -20°C, and analyzed at 1, 3, 6, 14, 18, and 24 months. Two samples were analyzed at each interval, as were a control sample and a "freshly fortified" sample (a stored control sample spiked just prior to analysis). All samples except the 24 month set were analyzed by CIBA-GEIGY using method AG-514 (see below). The last set was analyzed by BioAnalytika Laboratories of Raleigh, NC, using a slightly modified version of AG-514.

Analytical Method AG-514 is similar to Method AG-575, which was used to generate residue data. CGA-169374 is extracted from potato or tomato samples by refluxing with 8:2 (v/v) methanol:concentrated ammonium hydroxide for two hours. An aliquot of the extract is partitioned twice with 25-ml portions of hexane, then the hexane fraction is partitioned twice with acetonitrile. After removing the acetonitrile by rotary evaporation, the residue is redissolved in toluene and cleaned up with a silica Sep-Pak. Final determination is acheived by gas chromatography with N/P detection. Differences in the BioAnalytika method consisted of substitution of a 5% acetone/toluene solution in those steps where pure toluene was used by CIBA_GIEGY. Chromatograms have been provided that show that matrix interference with the CGA-169374 signal is not present. Recovery of CGA-169374 spikes ranging from 0.05 to 0.50 ppm in green tomatoes, ripe tomatoes, and potatoes averaged 97% and was not dependent on fortification level or commodity.

Study results are summarized in Table 23. Controls were less than 0.05 ppm in all cases except the 24-month samples, for which the residues were reported as <0.07 ppm. Results were corrected for procedural recoveries $<100\%$. These data indicate that CGA-169374 is stable in tomatoes and potatoes under frozen storage conditions for at least 2 years. For the purposes of this import tolerance request, CBTS will translate this data to wheat, barley, and rye grain. Should the petitioner wish to pursue a domestic registration for use of CGA-169374 on any wheat, barley, or rye commodity in the future, storage stability data on a cereal grain crop (including grain, forage, and fodder) will have to be provided. Also in that case, any conclusions concerning the adequacy of the storage stability data will be withheld until the nature of the residue in cereal grains has been determined.

Table 23. Residues of CGA-169374 In Tomatoes and Potatoes After Various Storage Intervals. (MRID# 420900-58, 59)

	Residues (ppm) after X months of storage						
	0	1	3	6	14	18	24
Tomatoes							
Freshly Fortified	--	0.59	0.58	0.52	0.60	0.54	0.60
Stored	0.45	0.59	0.56	0.50	0.57	0.53	0.55
	0.46	0.59	0.57	0.54	0.55	0.53	0.51
Potatoes							
Freshly Fortified	--	0.53	0.58	0.52	0.64	0.56	0.53
Stored	0.50	0.56	0.72	0.54	0.57	0.50	0.50
	0.54	0.54	0.56	0.57	0.55	0.52	0.51

Magnitude of the Residue

The petitioner has submitted two studies, one conducted overseas and one conducted in the US.

MRID# 420900-55, CIBA-GEIGY Reoprt No. ABR-90043. (US Trials:) Two studies are presented in this document, outlining residues in spring and winter wheat, respectively, after seed treatment with **Dividend 3FS**.

Spring wheat. Spring wheat seed was treated with either 24.4 or 51.0 g ai CGA-169374/100 kg seed (average values; approximately 0.33X or 0.8X the proposed maximum use rate of 60 g ai/100 kg seed) and planted at 6 sites (ND, WA, MT, MN, SD, and IL). The IL samples were additionally treated foliarly with up to 2 applications of either 50 or 100 g ai/A of **Dividend 3.5E**, in order to generate measurable residues in grain samples for processing. Plants were grown to maturity; forage samples were collected 54-92 days after planting, and grain and straw samples were collected 81-119 days after planting. Samples from the IL trials which had been treated foliarly were collected 0 days after the last foliar application in the case of forage, and 28 days later in the cases of grain and straw.

All samples were frozen upon collection and shipped in dry ice to the performing laboratories (processing grain, CIBA-GEIGY, Bryan, TX; all others, CIBA-GEIGY, Greensboro, NC), where they were stored frozen (TX, ~0°C; NC, ~-20°C) until analysis. Samples were stored for no longer than 10 months. Analytical method AG-575, described above, was used to analyze all samples for levels of CGA-169374. Adequate recoveries of the parent were demonstrated for all commodities.

In all trials, no residues (<0.01 ppm) were found in grain or straw as a result of seed treatment with CGA-169374 at both the 1X and 2X rates. No residues were found in forage (<0.01 ppm) in 5 of the 6 trials; only the SD trial showed any residues (0.02 ppm, 1X application rate). No residues were found in any control samples. Plants which had been treated foliarly as well as seed treated also showed no residues in the grain, and the subsequent processed commodities - bran, middlings, shorts and germ, and patent flour - also showed no residues (<0.01 ppm).

Winter wheat. (Amendment 1 to ABR-90043.) Winter wheat seed was treated at either 20.9 (1/3X) or 46.7(~0.8X) g ai/100 kg seed and planted at 6 sites (NY, AR, TX, OK, WA, KS). Spring wheat seed was treated at the same rates and planted at 2 sites (AZ, CA). Wheat was grown to maturity and grain, forage, and straw samples were harvested. Forage samples were harvested 56-67 days after planting; straw and grain samples were harvested 149-328 days after planting. Forage samples from CA were accidentally discarded and were not analyzed. Forage samples from OK had insufficiently grown, due to a lack of moisture, and were not collected. Results of analysis of forage samples from WA were considered invalid due to poor growth and contamination with mud.

All samples were shipped and stored frozen as described above for the spring wheat samples, except that the maximum storage interval was 12 months. Method AG-575 was used in the analysis.

Results of the analyses showed that residues of CGA-169374 in most samples were <0.01 ppm. The only exceptions were the following. For grain, the TX trials at both application rates had residues of 0.01 ppm. For forage, the AR trials at both rates had apparent residues of 0.02 ppm. For straw, the Ar trial at 1/3X showed residues of 0.03 ppm. Straw samples in AZ showed residues of 0.02-0.03 ppm, but were reanalyzed to show residues <0.01 ppm. All control samples had residues <0.01 ppm (again, AZ straw controls were 0.03 ppm, but were reanalyzed to show <0.01 ppm). No grain samples were prepared for processing. The exaggerated treatment of seed plus foliar treatment lead to <0.01 ppm residue of parent. Therefore, no processing study is necessary for this proposed use (assuming that the parent compound remains the only residue of concern).

MRID# 420900-56, CIBA-GEIGY Report No. ABR-91031. (European trials.) Residue trials were conducted in France (5 trials) and Germany (4 trials) using wheat, barley, or rye seed treated with CGA-169374. Wheat seeds in the French trials were treated with **Dividend 30WS** or **150FS** at a rate of 18, 36, or 60 g ai/A (0.3X, 0.6X, or 1X the maximum proposed rate), while wheat, barley, and rye seeds in the German trials were treated with **Dividend 150EW** at 60 g ai/100 kg seed.

The analytical methods used in the two trials differ from the analytical method proposed for enforcement. In France, a modified version of Method REM 07/86 was used. The method is carried out as follows. The homogenized plant material is extracted with methanol. An aliquot is evaporated, redissolved in methanol, and separated with dichloromethane. The remaining ACN layer is redissolved in dichloromethane and cleaned up by gel permeation chromatography

followed by alumina column chromatography. Determination of the parent compound is by GC with electron capture detection. Recoveries from wheat grain averaged 97 ± 21% for spike levels at a range of 0.04-0.20 ppm (see Table 24 for details). The petitioner has demonstrated that this method is adequate for detection of the parent compound in wheat (and barley and rye) grain at levels of 0.08 ppm (the recovery of the 0.04 ppm spike is too low).

In Germany, a modified version of Method AG-537 is used. This method is the method from which Method AG-575A was derived, and thus is very similar in content. A crop sample is refluxed in 8:2 methanol:concentrated ammonium hydroxide. After filtering, an aliquot of the extract is diluted with water and saturated NaCl and partitioned with hexane. The hexane fraction is partitioned with ACN and the ACN fraction is cleaned up on a silica gel Sep-Pak. The sample is then cleaned up on a charcoal:magnesium oxide:Celite column. The extract is analyzed by packed GC with electron capture detection. In field test 2030/88, a modified version of Method AG-514 was used. This method is essentially identical to Method AG-537. Recoveries averaged 105 ± 12% for 8 wheat, barley, or rye grain samples spiked at levels of 0.05-0.3 ppm (see Table ## for details). The petitioner has demonstrated that these methods are adequate for detection of CGA-169374 at levels as low as 0.05 ppm in wheat, barley, and rye grains.

Table 24. Procedural Recovery Data for CGA-169374 Residues in Wheat, Barley, and Rye Grains Using Methods REM 07/88, AG-537, and AG-514.

REM 07/88		AG-537/AG-514	
Spike (ppm)	% Recovery	Spike (ppm)	% Recovery
0.04	58	0.05	102,123,105
0.08	88	0.06	81
0.10	100,90,110	0.25	110,103,104
0.20	130,110,90	0.30	109

As noted in Table 25, no residues of CGA-169374 were found in any grain sample. Minimal residues were found in forage and straw samples.

CBTS concludes that there are no major deficiencies associated with these field trials. They indicate that residues of difenoconazole (parent only) will not exceed 0.1 ppm in wheat, barley, or rye grains or in their processed fractions after seed treatment at the proposed rate. For any future domestic registrations on cereal grains for which the petitioner cites these studies in support, we will withhold our conclusions until deficiencies associated with the determination of the nature of the residue in plants have been satisfied. If additional metabolites are determined to be of concern, the petitioner will either have to conduct new field trials to detect those compounds, or reanalyze any stored samples available from the above trials. If the latter option is chosen, appropriate storage stability data will have to be provided.

Table 25. Residues of CGA-169374 in Grain, Forage, and Straw After Seed Treatment. MRID# 420900-56.

Location	Applic. Rate (g ai/100 kg seed)	Formulation	Crop	PHI (days)	Residues (ppm)
Cussigny, FRA	60	30WS	Wheat Grain	293	<0.08
Vianne, FRA	36	30WS	Wheat Grain	288	<0.08
Aigues-Vies, FRA	18	30WS	Wheat Grain	274	<0.08
Tierce, FRA	60	150FS	Wheat Grain	277	<0.08
Cussigny, FRA	60	150FS	Wheat Grain	287	<0.08
Achern-Fautenbach, GER	60	150EW	Rye Grain	274	<0.05
			Forage	191	<0.05
			Straw	274	<0.08
Haffkrug, GER	60	150EW	Barley Grain	148	<0.05
			Forage	91	<0.05
			Straw	148	<0.06
Mecherich, GER	60	150EW	Barley Grain	177	<0.05
			Forage	88	<0.05
			Straw	177	<0.05
Osterwede, GER	60	150EW	Wheat Grain	161	<0.05
			Forage	84	<0.05
			Straw	161	<0.05

Milk, Meat, Poultry, and Eggs

The petitioner has not proposed any tolerances in Section F for CGA-169374 in/on animal commodities, nor have any animal feeding studies or an analytical enforcement method for detection of such commodities been included. The petitioner states that since no residues (i.e. ≤0.01 ppm) of CGA-169374 are found in wheat, barley, or rye grain, no animal tolerances are necessary.

CBTS notes that the analytical methods used actually had a level of quantitation of 0.08 ppm. Probably, some small level of residues would be transferred from grain to meat, milk, poultry, and eggs. However, it is also likely that the resulting residue levels would be undetectable. Therefore, CBTS agrees that tolerances for meat, milk, poultry, and eggs are not currently necessary. However, this evaluation will have to reconsidered if additional uses and tolerances are proposed for CGA-169374 on animal feed commodities. The petitioner must also assure us that this product will not be registered overseas for foliar use on cereal grains. If such a use is or will be registered, CBTS will require proposed meat (except poultry) and milk tolerances, appropriate analytical methodology, and animal feeding studies.

Other Considerations

There are no Codex, Canadian, or Mexican tolerances for difenoconazole on wheat, barley, or rye grains. Therefore, no compatibility problems are anticipated with this tolerance request.

No Craven data is associated with this petition.

Attachment 1: Codex sheet (1 page).
Attachment 2: Structures of metabolites and metabolite isolation schemes for difenoconazole (8 pages).
Attachment 3: Confidential Appendix (11 pages).

cc with all attachments: R. Lascola, PP#2E4051.
cc with Attachment 1 ONLY: RF, Circulation, D. Edwards.

H7509C:CBTS:RLascola/rjl:CM#2:Rm805B:305-7478:7/14/92
RDI: P.V.Errico:10/22/92; R.Loranger:10/20/92.
[redacted]:4051A.TXT

F. Lucas 6/4/92

Attachment:

Page 1 of 1

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL difenoconazole (CGA-169374)

CODEX NO. _____

CODEX STATUS:

(4) No Codex Proposal Step 6 or Above

Residue (if Step 8): _____

Crop(s)	Limit (mg/kg)

PROPOSED U.S. TOLERANCES:

Petition No. * 2E4051

DEB Reviewer LASCOLA

Residue: parent only *

Crop(s)	Limit (mg/kg)
wheat, barley, rye } grain	0.1

CANADIAN LIMITS:

(1) No Canadian Limit

Residue: _____

Crop(s)	Limit (mg/kg)

MEXICAN LIMITS:

(2) No Mexican Limit

Residue: _____

Crop(s)	Limit (mg/kg)

NOTES

Form Revised 1989

* 1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole.