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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: PP#4F3007/PP#4F3074: Tilt on Pecans and Small Grains Amendment of September 29, 1986 (Accession No. 265190; RCB#1484)

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THRU: Charles L. Trichilo, Ph.D., Chief
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TO: Lois Rossi, PM#21
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and

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CIBA-GEIGY Corporation has submitted an amendment in response to RD's letter of March 10, 1986 (H.M. Jacoby, PM#21) and RCB's memo of 1/9/86 (A. Smith, PP#4F3007). These memoes requested the submission of data showing that the crop backgrounds are due to triazole related compounds and any additional evidence that triazolealanine is a naturally occurring compound in crops.

No new CIBA-Geigy studies are submitted. A data Summary Table and the analytical method used to collect the data are submitted. The Summary Table contained results of analyses for triazolealanine of untreated cereal grains from various countries. Also included are a copy of a letter to the Journal of Antibiotics "Occurrence of 1, 2, 4-Triazole Ring in Actinomycetes") and Residue Data from crops treated with propiconazole. The submissions are discussed below.

A Summary Table was submitted (Mobay Report RA-225/6531, "Triazole-alanine: Residue Situation in Cereal Grains") in which untreated cereal grain (barley, wheat, rye) samples from various countries (Germany, Yugoslavia, Norway, Sweden, France, Australia, America) were examined for residues of triazolealanine. The soils in which the grains were grown had no history of treatment with triazole ring-containing compounds. The triazolealanine was determined as the N-heptafluorobutyryl isobutyl ester using gas chromatography with an electron capture detection system (ECGC).

The residues so determined were confirmed by a gas chromatography and mass spectrometry system (GC/MS).

The triazolealanine residue levels in 15 samples were 0.01-0.05 mg/kg (ECGC) and 0.01-0.03 mg/kg (GC/MS). One barley sample from Norway had levels of 0.17 mg/kg (ECGC) and 0.08 mg/kg (GC/MS).

The analytical method was developed for the determination of triazolealanine in grains. A sample is macerated with water and methanol and filtered. The filtrate is cleaned up on a cation exchange resin, and the heptafluoroester derivative is formed by treatment with isobutanol and heptafluorobutyric anhydride. The ester is cleaned up on a polystyrene gel and silica gel column and determined by gas chromatography.

The residues of triazolealanine in the grain are quantitated by comparison with a triazolealanine standard curve.

Control grain samples were fortified with triazolealanine at levels of 0.02-1.0 mg/kg and analyzed. Recoveries averaged 65-91%.

The next study was previously reviewed in our memo of May 15, 1984 (A. Smith, PP#4F3007, p. 20).

In order to determine if the crop background is wholly or partially due to triazolealanine, an isotope dilution study was performed with peanut kernel and radiolabelled C^{14}-triazolealanine (Report No. ABR-83047, "Structure Elucidation of the Triazole Background in Control Peanut Kernels"). A sample of untreated (control) peanut kernels was fortified with a low level of C^{14}-triazolealanine (as a tracer) in order to follow any triazolealanine through the method and determine the recovery of the tracer.

The residue method involved the extraction, cleanup, and formation of the n-buty ester/N-trifluoroacetyl derivative. Following analysis, a level of 0.029 ppm triazolealanine-equivalent residues were determined. This level reflected a 9% recovery of the tracer. The mass spectrometric analysis confirmed the presence of the n-butyl ester/N-trifluoroacetyl derivative of triazolealanine.

The report states that the 0.029 ppm level is equivalent to 0.063 ppm parent compound CGA-64250 (propiconazole). The crop background level found with the triazole residue method (Report No. AG-357) was 0.66 ppm CGA-64250 equivalent residues. The results show that triazolealanine contributed only about 10 percent to the background level. The report concludes that triazole residues are therefore due to other compounds. As a result, the crop background found with the residue method (AG-357) probably results from the presence of triazole-containing compounds.

These conclusions reasonably reflect the results of this study. RCB concludes that triazole-containing compounds occur naturally in peanuts. However, the conjugated triazole, 1,2,4-triazolealanine (a metabolite of CGA-64250), has not been shown to occur naturally in peanuts. This conclusion relates to triazole type compounds as determined by Method AG-357.

The analytical method AG-357 is used for the determination of total residues of propiconazole in crops as the triazole. Analyses with this method have produced variable background levels of residue believed to be due to components which contained the triazole ring (i.e., triazole-like compounds). The method has been evaluated in previous reviews (PP#4F3007/PP#4F3074, cf. memo of 6/20/86, A. Smith). The residue data for pecan meats show levels of triazole-like compounds as high as 12 ppm.

Because of the high and variable background levels of triazole-like compounds, this method would not be suitable for determining only residues due to treatments with propiconazole. However, the method does appear to be adequate for the determination of total triazole residues (i.e., triazoles due to crop background plus any triazoles contributed by propiconazole treatments).

A copy of a letter to the editor (Imamura, N. et. al., J. Antibiotics, vol. 38, No. 8, p. 1110, 1985) on the formation of 1,2,4-triazolealanine by soil bacteria was submitted ("Occurrence of 1,2,4-triazole Ring in Actinomycetes"). The soil bacteria, actinomycetes was found to produce 1,2,4-triazole-3-alanine upon incubation with an artificial medium containing wheat bran, meat extract, and soybean meal. Following the incubation period, the 1,2,4-triazole-3-alanine was isolated from the culture and identified.

Identification techniques included a variety of physical and chemical tests: elemental analysis; melting point determination; molecular weight determination; specific optical rotation; circular dichroism; mass spectrometry; ultra-violet and infra-red spectroscopy; ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy. The structure of the isolated compound was reported as 1,2,4-triazole-3-alanine.

Residue Data

Residue data for triazoles were obtained from field samples of small grains grown in Nebraska, North Dakota, California, Arkansas, Illinois, Kansas, Mississippi, and Louisiana. The samples had been previously analyzed using method AG-415 (method determines residues of propiconazole and its metabolites containing the 2,4 dichlorophenyl ring as the 2,4 dichlorobenzoic acid), and were reviewed in our memo of 7/12/84 (A. Smith, PP#4F3074).

The crops had received a single foliar application at 50 gm act/A or 125 gm act/A, or a split application of 75 gm act/A plus 75 gm act/A.

Reserved samples of summer barley, winter barley, winter wheat, and rice were analyzed for triazole residues using method No. AG-357 "Analytical Method for the Determination of Total Residues of CGA-64250 in Crops as 1,2,4-triazole. "This method determines the parent compound plus metabolites containing the 1,2,4-triazole moiety as methyldibromotriazole (MDBT) in crops.

The samples which contained MDBT were also analyzed using GC/MS to confirm the presence of triazole residues. The presence of triazoles were determined by comparison of peak retention times of sample and standard MDBT; determination of MDBT by capillary GC using a nitrogen specific detector; mass spectral comparison of isotope ratio for isotope cluster characteristic of standard MDBT with that of sample MDBT. The residues were reported as propiconazole-equivalent residue.

The untreated (control) grain samples had propiconazole-equivalent residues as follows: barley (0.32-0.59 ppm); rice (0.10-0.24 ppm); wheat (0.23-0.35 ppm).

For the treated samples, the barley grain had 0.34-1.5 ppm at 39-43 days after treatment of 50 gm act/A. The rice grain had 0.09-0.55 ppm at 67-80 days after 125 gm act/A, and 0.07-0.40 ppm at 53-66 days after the last of the split application (75 plus 75 gm act/A). The wheat grain had 0.16-0.67 ppm at 49-69 days after treatment at 50 gm act/A.

The residue data show that the grains contain triazole-containing components in untreated and treated samples. These data further support the presence of components containing the triazole moiety in small grains.