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DATA EVALUATION RECORD

STUDY 7

CHEM 128997 Terbuconazole 165-1

FORMULATION--12--EMULSIFIABLE CONCENTRATE (EC)

STUDY ID 40700964

Leimkuehler, W., C. Lenz, and J. Delk. 1988. Radioactive residues of ¹⁴C-Folicur in rotational crops. Laboratory Project ID 95638. Unpublished study performed and submitted by Mobay Corporation, Stilwell, KS.

DIRECT REVIEW TIME - 10

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CONCLUSIONS:

Confined Accumulation - Rotational Crops

1. This study cannot be used to fulfill data requirements. Because of the deficiencies listed below, it is uncertain whether it provides enough information to allow development of a suitable protocol for a field study.

- 1) [¹⁴C]residues in the crops were not adequately characterized (the investigator assumed that day 120 samples represented all time periods);
- 2) storage stability data were not provided for the plant and soil substrates;
- 3) total radioactivity in the soil was not determined prior to the soil surface treatment and at the time of harvest of the rotational crops;

- 4) [^{14}C]residues in the soil were quantified but not identified immediately posttreatment; the test substance was not analytical grade or purer;
 - 5) the analytical methodology was not adequately described.
2. For this study to fulfill the accumulation in confined rotational crops data requirement, the registrant must do the following: characterize organosoluble and water-soluble [^{14}C]residues in all crops from all three rotations; provide storage stability data for the plant and soil substrates; if samples are still available, quantify [^{14}C]residues in the soil prior to the soil surface treatment and at the time of harvest of the rotational crops and characterize [^{14}C]residues from those two intervals plus [^{14}C]residues in the soil immediately after the soil surface application; and provide additional details about the analytical methodology and the plant growing conditions (refer to Comments 6 and 8).
 3. [^{14}C]Terbuconazole residues accumulated in kale, beets, and wheat planted 29, 122, and 273 days after sandy loam soil was treated with terbuconazole at 500 g ai/ha. In general, accumulation was greatest in crops from the 122-day rotation and least in the crops from the 29-day rotation. Residues in the kale ranged from 0.3 to 2.7 ppm; in beets from 0.2 to 1.3 ppm (tops and roots); and in wheat from 3.8 to 35.4 ppm (grain) and 1.1 to 4.2 ppm (straw). [^{14}C]Residues extracted from the crops included terbuconazole, terbuconazole-t-butyl-hydroxy, triazole, triazolylalanine, triazolylacetic acid, and triazolyl-lactic acid. In the soil, terbuconazole (the only extractable [^{14}C]compound) decreased from 0.43 to 0.02 ppm between the 29- and 273-day posttreatment plantings; total residues decreased from 0.52 to 0.16 ppm during the same period.

METHODOLOGY:

Triazole ring-labeled [^{14}C]terbuconazole (radiochemical purity 98.8%, specific activity 17.42 mCi/mmol, Mobay) was mixed with unlabeled terbuconazole (Folicur, 22.5% dry flowable), "22.5% dry flowable formulation blank", and water; the formulated test substance contained 3.1% radioactive ai. The formulated test substance was applied as a foliar spray at 500 g ai/ha (equivalent to ≈ 1.1 lb ai/A) to wheat (boot stage) growing on sandy loam soil (70% sand, 26% silt, 4% clay, 2.8% organic matter, pH 5.2, CEC 21 meq/100 g) contained in one galvanized tub (8 x 2.5 x 2.5 feet) in a greenhouse. At 50 days posttreatment, the wheat was harvested and the formulated test substance was applied again at 500 g ai/ha directly to the soil surface. The soil was cultivated to a depth of 1 inch and allowed to age for 29 days.

At 29 days following the application of terbuconazole to the soil surface, the soil was planted to kale, beets and wheat; each crop covered one-third of the soil (Table II). Kale and beets were harvested at maturity (58 days postplanting); wheat was harvested when immature (41 days postplanting) and at maturity (93 days postplanting). At 122 days posttreatment, the soil used for the 29-day rotation was again planted to kale, beets, and wheat. Kale and beets were harvested at maturity (85 days post-

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planting); wheat was harvested when immature (43 days postplanting) and at maturity (85 days postplanting). At 273 days posttreatment, the soil used for the 29- and 122-day rotations was again planted to kale, beets, and wheat. Kale and beets were harvested at maturity (60 and 107 days postplanting, respectively); wheat was harvested when immature (30 days postplanting) and at maturity (99 days postplanting). Crops were separated into their various parts, homogenized with dry ice (except for wheat grain), and stored frozen (-10 C) until analysis; wheat grain was stored intact. Soil samples (6-inch cores) were taken immediately following the application of terbuconazole to the soil surface (second application) and at each planting interval; storage conditions for the soil samples were not described.

Plant samples, except for wheat grain, were extracted twice with methanol:water (1:1) (Figure 2). Extracts were filtered, combined, concentrated, and partitioned twice with methylene chloride:acetonitrile (2:1). For the 29-day plant samples, the organic phase was analyzed by reverse-phase radio-HPLC with UV detection and GC/MS; the aqueous phase was not analyzed. For the 122-day plant samples, the organic phase was not analyzed and the aqueous phase was applied to a cation exchange column; radioactivity that was not initially retained on the column was collected. Radioactivity remaining on the column was eluted with a 100 mM to 1 M linear gradient of sodium chloride; fractions containing radioactivity were pooled, concentrated, then derivatized with 3 N hydrochloric acid in n-butanol and heptafluorobutyric anhydride. Following the derivatization, the sample was evaporated to dryness, dissolved in acetonitrile, and analyzed by HPLC and GC/MS as described above. Radioactivity that was not initially retained by the cation exchange column was adjusted to pH 4-7, applied to an anion exchange column, eluted with the sodium chloride gradient, derivatized with n-butanolic hydrochloric acid, then analyzed by HPLC and GC/MS.

Wheat grain was ground to a fine powder, then extracted with methanol followed by a 2-hour reflux with 1 N hydrochloric acid (Figure 3). Extracts were filtered, combined, concentrated, applied to a cation exchange column, eluted with the linear sodium chloride gradient, derivatized, and analyzed by HPLC and GC/MS as described above.

Soil samples were analyzed for total radioactivity by LSC following combustion. Additional samples of the soil were extracted for 2 hours with methanol. The extract was filtered, concentrated, and analyzed by TLC (type of plates unspecified) using acetonitrile:methanol:acetic acid (90:10:1). Unlabeled terbuconazole was cochromatographed with the samples. Following development, radioactive areas were located using autoradiography.

DATA SUMMARY:

[¹⁴C]Terbuconazole residues accumulated in kale, beets, and wheat planted 29, 122, and 273 days after the second of two 500 g ai/ha applications of formulated triazole ring-labeled [¹⁴C]terbuconazole (radiochemical purity

98.8%); the first application was to wheat growing in a tub of sandy loam soil and the second application, 50 days later, was directly to the sandy loam soil surface. The concentration of [^{14}C]residues in crops from the 122-day rotation was ≈ 4 to $9\times$ greater than the concentration in crops from the 29-day rotation; the concentration of [^{14}C]residues in crops from the 273-day rotation was generally ≈ 2 - $4\times$ greater than the concentration in crops from the 29-day rotation.

In crops planted at 29 days posttreatment, [^{14}C]residues at harvest were 0.3 ppm in kale, 0.2 ppm in beet tops and roots, and 3.8 and 1.1 ppm in wheat grain and straw. Organosoluble residues ranged from 0.4 to 22.9% of the recovered radioactivity, water-soluble residues ranged from 51.1 to 88.6%, and unextractable residues ranged from 5.8 to 29.7%. In the organosoluble fraction,

terbuconazole -- comprised 20.7% of the total radioactivity in kale; 15.2 and 5.6% in beet tops and roots, 22.9% in immature wheat, and 5.4% in mature wheat straw; terbuconazole was not detected in the wheat grain. In addition, in the mature wheat straw,

terbuconazole-t-butyl hydroxy -- comprised 9.3% of the recovered radioactivity.

Five unknowns (0.4-1.6%) were detected. Water-soluble [^{14}C]residues were not characterized.

In crops planted at 122 days posttreatment, [^{14}C]residues at harvest were 2.7 ppm in kale; 1.3 and 0.8 ppm in beet tops and roots; and 35.4, 4.2, and 15.0 ppm in wheat grain, straw, and chaff. Organosoluble residues ranged from 0.6 to 8.1% of the recovered radioactivity, water-soluble residues ranged from 85.5 to 100%, and unextractable residues ranged from 0 to 13%. In the water-soluble fraction, the primary degradate in all crops was

triazolylalanine -- detected at 1.1 ppm in kale, 0.16 and 0.3 ppm in beet tops and roots, 0.7 ppm in immature wheat, and 0.51 and 12.7 ppm in mature wheat straw and grain. Another degradate found in all crops was

triazolylacetic acid -- detected at 0.04 ppm in kale, 0.05 and 0.03 ppm in beet tops and roots, 1.5 ppm in immature wheat, and 0.4 and 3.1 ppm in mature wheat straw and grain.

Triazolyl-lactic acid -- detected in beet tops (0.37 ppm) and roots (0.01 ppm) and wheat straw (0.8 ppm), and

triazole -- detected in beet roots (0.02 ppm).

Organosoluble [^{14}C]residues were not characterized.

In crops planted at 273 days posttreatment, [^{14}C]residues at harvest were 2.0 ppm in kale; 1.0 and 0.9 ppm in beet tops and roots; and 7.6, 2.6, and 6.0 ppm in wheat grain, straw, and chaff. Organosoluble residues ranged from 0.2 to 2.8% of the recovered radioactivity, water-soluble residues ranged from 79.0 to 96.4%, and unextractable residues ranged from 2.7 to 20.0%. Organosoluble and water-soluble [^{14}C]residues were not characterized.

In the 0- to 6-inch soil depth, total [^{14}C]residues were 1.5 ppm immediately following application of formulated [^{14}C]terbuconazole to the soil surface, 0.52 ppm at 29 days posttreatment, 0.29 ppm at 122 days posttreatment, and 0.16 ppm at 273 days posttreatment. Between 29 and 273 days posttreatment, extractable [^{14}C]residues decreased from 84 to 14% of the total radioactivity; terbuconazole was the only compound detected in extracts from the 29- and 122-day soil samples (quantitative data were not provided).

COMMENTS:

1. [^{14}C]Residues in the crops were not adequately characterized. Although the relative amounts of organosoluble, water-soluble, and unextractable residues in all plant parts was determined, only the organosoluble fraction from the 29-day rotational crops and the water-soluble fraction from the 120-day rotational crops were analyzed for specific [^{14}C]compounds. The study author assumed that the composition of the organosoluble and water-soluble fractions were identical for the three rotational intervals.
2. Freezer storage stability data were not provided for the plant substrates; it was also not specified how long samples were stored frozen prior to analysis. Storage conditions for the soil samples were not described. The freezer storage data for soil supplied in Study 6 (NRID 40700962) was unacceptable.
3. Although it was stated that the soil was sampled when the rotational crops were harvested, no data were provided. For the 29- and 122-day rotations, the date of final harvest is near to the date of planting for the next rotation, so the planting date concentrations should be valid; however, data for the 273-day rotation harvest are needed.

Also, no soil samples were collected after the wheat treatment or before the application to the soil surface. The contribution of the first application to the "time 0" concentration in the soil (the sampling immediately after the soil surface was treated) could not be determined. The concentration in the 0- to 6-inch soil depth at time 0, 1.5 ppm, was much higher than the expected 0.55 ppm (assuming an application of 1.1 lb ai/A and 1 acre containing 2 million pounds of soil).

4. It was reported that only terbuconazole was detected following TLC of the 29- and 122-day soil extracts; however, quantitative data were not

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provided. [¹⁴C]Residues in the 0- and 273-day soil sample were not characterized. Time 0 should have been analyzed because [¹⁴C]residues may have remained from the application to the wheat. The study author stated that insufficient extractable material from the 273-day interval was available for analysis.

5. The test substance was formulated (final purity unspecified) and, therefore, was not analytical grade or purer.
6. The analytical methodology was not adequately described; a) the type of TLC plate used was not specified, b) it was not specified how unlabeled terbuconazole was detected following TLC, c) it was not always clear what compounds were being derivatized to, and d) it was not clear at what stage of the methodology the plant extracts were analyzed for free triazole (which apparently required a separate derivatization step). Recovery efficiencies of terbuconazole and degradates from fortified soil and plant samples were not provided.
7. Immature kale and beets were not analyzed.
8. A description of the growing conditions, such as watering schedule, air temperatures, and relative humidity, was not reported.
9. The experimental design was not typical. It could not be determined why terbuconazole was applied first to a growing crop, then to the soil surface. Also, the same tubs of soil are generally not used for all three rotation intervals, rather soil that has been unvegetated for the entire rotation interval is used.
10. A confined accumulation rotational crop study using phenyl ring-labeled [¹⁴C]terbuconazole may be required.
11. The study author refers to 30-, 120-, and 270-day rotations in the text and tables. In fact, the rotations were 29, 122, and 273 days.

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