DATA EVALUATION RECORD

STUDY 4

CHEM 125620 Triticonazole §162-1

CAS No. 131983-72-7

FORMULATION–00–ACTIVE INGREDIENT

STUDY ID 448021-24

Ayliffe, J. M., and D. J. Austin. 1998. Fungicides: RPA 400727-^{14}C: Aerobic soil metabolism in three soils. Laboratory Project ID No. P91/326. Unpublished study performed and submitted by Rhône-Poulenc Agriculture Limited, Ongar, Essex, England.

CONTRACTOR

REVIEWED BY: Fernand Daussin
TITLE: Staff Scientist
SIGNATURE:
DATE:

EDITED BY: William Martin
TITLE: Senior Staff Scientist
SIGNATURE:
DATE:

EDITED BY: Kathleen Ferguson
TITLE: Senior Staff Scientist
SIGNATURE:
DATE:

ORG: Dynamac Corporation
Rockville, MD
TEL: 301/417-9800

US EPA

REVIEWED BY: Lucy Shanaman
TITLE: Chemist
ORG: ERB IV/EFED/OPP

Lucy Shanaman

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CONCLUSION:

Metabolism - Aerobic Soil

1. This study is ***acceptable***, provides useful information on the aerobic soil metabolism of triticonazole, and ***satisfies*** EPA Subdivision N Guidelines data requirements on aerobic soil metabolism. Three separate soil sets were utilized in the determination of the degradation kinetics of the parent compound and examine patterns of formation and decline of the degradates.

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2. Phenyl ring-labeled [U-^{14}C]**triticonazole**, (1RS-(E)-5-((4-chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol), in soil maintained at 75% of 0.33 bar and incubated in the dark at 22 ± 2°C for up to 1 year, degraded with calculated first order ***half-lives of 141 days*** (136 to 147 days at the 90 % confidence interval) in the clay loam soil, and ***301 days*** (265 to 350 days at the 90 % confidence interval) in the sandy loam soil. Data from an aerobic metabolism study conducted with these same two soils maintained at 10 °C for 363 days had been submitted under MRID #448021-25. Data was also submitted for Speyer 2.2 artificial loamy sand soil, but has not been considered in this review.

3. All identified aerobic degradation produced were chemical species produced through hydroxylation of the unfragmented parent molecule, triticonazole. However, unidentified bound residues could possibly include residues of toxicological concern. ***Four identified and six or seven unknown metabolites*** were monitored during this study. However, without additional data, accurate half-life estimations for these aerobic metabolism products can not be calculated. Data for these metabolites, along with data for evolved $^{14}CO_2$, non-extractable residues and [^{14}C]organic volatiles, have been tabulated below. Degradates of potential concern are: triazole, triazole lactic acid, triazole acetic acid, and triazole alanine.

Measured Residues

Metabolite	Maximum Values			
	Sandy Loam Soil		Clay Loam Soil	
	% of Applied	Day	% of Applied	Day
RPA 406341	10.6 %	56	10.4 %	112
RPA 406780	≤7.8 %	All	≤7.8 %	All
RPA 404886	≤7.8 %	All	≤7.8 %	All
RPA 407922	≤7.8 %	All	≤7.8 %	All
Six or Seven Unidentified Degradates	≤7.8 %	All	≤7.8 %	All
Non-Extractable [^{14}C]Residues	15.8 %	363	19.0 %	363
$^{14}CO_2$	12.2 %	363	25.3 %	363
[^{14}C]Volatiles	0.4 %	363	1.5 %	363

2

ABSTRACT

Metabolism - Aerobic Soil

Aerobic soil metabolism studies were conducted on sandy loam and clay loam soils that were moistened to 75% of 0.33 bar, treated with radiolabeled triticonazole, and incubated in the dark at 22 ± 2°C for up to 1 year. Data was also submitted for Speyer 2.2 artificial loamy sand soil, but has not been considered in this review. Triticonazole (1RS-(E)-5-((4-chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol) degraded with half-lives of 141 days in the clay loam soil and 301 days in the sandy loam soil. All identified aerobic degradation produced were chemical species produced through hydroxylation of the unfragmented parent molecule, triticonazole. However, unidentified bound residues accounting for up to 19% of applied radioactive residues by study termination could possibly include residues of toxicological concern. Four degradates were identified: RPA 407922 (Metabolite 6; (1RS)-E-2-(4-chloro-3-hydroxybenzilidene)-5,5-dimethyl-1-(1,2,4-triazol-1-yl-methyl)pentan-1-ol)), RPA 406341 (E-2-(4-chlorobenziliden)trans-1,3-dihydroxy-5,5-dimethyl-1-(1,2,4-triazol-1-ylmethyl) cyclopentan)), RPA 406780 (2-(4-chlorobenzylidene)-1,4-dihydroxy-5,5-dimethyl-1-(1,2,4-triazol-1-ylmethyl)cyclopentane)), and RPA 404886 (erythro-2-(4-chlorobenzylidene)-5-methyl-5-hydroxymethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1-cyclopentanol)).

Sandy loam. Phenyl ring-labeled [U-^{14}C]triticonazole, at a nominal application rate of 1.70 ppm, degraded with a calculated half-life of 301 days ($r^2 = 0.79$; 265 to 350 days at the 90% confidence interval) in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 22 ± 2°C for up to 363 days. Triticonazole was an average of 88.2-90.0% of the applied radiation at 0-14 days posttreatment, 46.9% at 224 days, and 44.65% at 363 days. RPA 406341 was a maximum average of 10.6% of the applied radiation at 56 days. RPA 406780, RPA 404886, RPA 407922, and six unidentified degradates (designated Metabolites 4, 5, 7, 8, 10, and 11), were each ≤7.8% of the applied radiation throughout the study period. At 363 days, unextracted [^{14}C]residues, $^{14}CO_2$, and [^{14}C]volatiles had reached maximums of 15.8%, 12.2%, and 0.4% of the applied radiation, respectively.

Clay loam. Phenyl ring-labeled [U-^{14}C]triticonazole, at a nominal application rate of 1.70 ppm, degraded with a calculated half-life of 141 days ($r^2 = 0.98$; 136 to 147 days at the 90 % confidence interval) in clay loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 22 ± 2°C for up to 363 days. Triticonazole was an average of 93.4-101.9% of the applied radiation at 0-7 days posttreatment, 51.8% at 140 days, and17.6% at 363 days. RPA 407922 was a maximum of 7.8% of the applied radiation at 266 days. RPA 406341 was a maximum of 10.4% of the applied radiation at 112 days. RPA 406780, RPA 404886, and seven unidentified degradates (designated Metabolites 4, 5, and 7-11) were each present at ≤7.8% of the applied radiation throughout the study period. At 363 days,

3

unextracted [^{14}C]residues, $^{14}CO_2$, and [^{14}C]volatiles had reached maximums of 19.0%, 25.3%, and 1.5% of the applied radiation, respectively.

MATERIALS AND METHODS

Subsamples of moist, sieved (2 mm) UK sandy loam (73% sand, 15% silt, 12% clay, 1.24% organic matter content, pH 6.42, CEC 8.24 meq/100 g; Table 1, p.27) and UK clay loam (45% sand, 33% silt, 22% clay, 9.76% organic matter content, pH 6.18, CEC 34.62 meq/100 g) were weighed (75 g dry weight) into glass dishes, moistened to 75% of 0.33 bar, and treated with phenyl ring-labeled [U-^{14}C]triticonazole (1RS-(E)-5-((4-chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol; radiochemical purity >98%, specific activity 32 mCi/mMol, Commissariat a l'Energie Atomique, France; pp.8-9) plus non-radiolabeled triticonazole (99.3% purity, p.8), dissolved in methanol, at a nominal application rate of 1.70 ppm. The dishes of treated soil were placed in closed glass chambers fitted with inlet and outlet ports (Fig. 1, p.33); humidified, carbon dioxide-free air was drawn through the chambers, then sequentially through trapping solutions of ethylene glycol to collect organic volatiles and through 4 M aqueous potassium hydroxide to collect $^{14}CO_2$ (p.14). The samples were maintained in the dark at 22 ± 2°C, and were weighed at regular intervals and remoistened as necessary to maintain 75% of 0.33 bar. Duplicate samples of each treated soil type were collected for analysis at 0, 1, 7, 14, 28, 56, 84, 112, 140, 168, 224, 266 and 363 days posttreatment; storage intervals and conditions were not specified. Volatile traps were collected for analysis and replaced at each sampling interval.

At each sampling interval, samples were Soxhlet-extracted with acetonitrile:water (4:1, v/v) for 3 hours, cooled, and the volume was adjusted if needed (p.15). Aliquots of the extracts were analyzed for total radioactivity using LSC; the limit of quantitation was not reported. The remaining extracts were concentrated and analyzed by reverse-phase HPLC using the following operating conditions (pp.17-18):

Column	Partisil ODS3; 4.6 x 250 mm
Injection volume	Not specified
Detector	UV at 263 nm; radioactive flow monitor

4

Mobile phase	Mobile Phase 1 Isocratic acetonitrile:water:acetic acid (40:60:2, v/v/v) containing 0.1 M ammonium acetate Mobile Phase 2 (to differentiate between RPA 404766 and RPA 406780) Solvent A: 2.5% acetic acid in water Solvent B: 2.5% acetic acid in acetonitrile Time — Gradient 0-20 min — isocratic 70:30 (A:B) 20-30 min — to 20:80 (A:B) 30-40 min — isocratic 20:80 (A:B) 40-45 min — to 70:30 (A:B) 45-50 min — isocratic 70:30 (A:B)
Flow rate	1.0 mL/minute

The samples were chromatographed and compared with reference standards of triticonazole, RPA 406780, RPA 404766, RPA 404886, RPA 407341, and RPA 406341 (Appendix A, pp.36-44). Additional aliquots of the extracts were analyzed by one-dimensional TCL on silica-gel plates developed in hexane:ethyl acetate:methanol:acetic acid (45:45:10:2, v:v:v:v; p.18). Radioactive areas were visualized using a linear analyzer; unlabeled reference standards of RPA 404886 and RPA 404766 co-chromatographed with the extracts were visualized using UV light. To confirm the identification of isolated compounds, [^{14}C]residues in select soil sample extracts (p.19) were analyzed by LC/MS under the following operating conditions (p.18):

Positive ion thermospray mass spectroscopy	
Column	Spherisorb ODS 2; 4.6 x 250 mm
Injection volume	100µL
Detector	UV at 263 nm
Mobile phase	Acetonitrile:water:acetic acid (50:50:2, v/v/v) containing 0.1 M ammonium acetate
Flow rate	1.0 mL/minute
Mass spectrometer	VG Trio 2

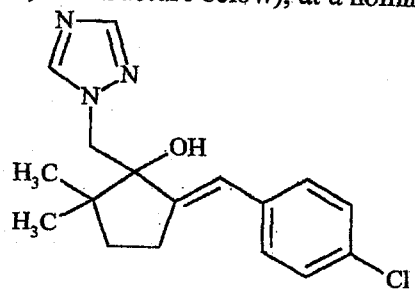
Aliquots of the ethylene glycol and 4 M potassium hydroxide trapping solutions were analyzed for total radioactivity by LSC (p.12).

The extracted soil was air-dried, and triplicate subsamples were analyzed for total radioactivity by LSC following combustion (p.15). Additional subsamples were separated into the humic acid, fulvic acid and humin fractions by extraction with 0.5 M NaOH, acidification of the resulting supernatant with concentrated HCl and redissolving of the precipitate in 0.1 M NaOH.

RESULTS/DISCUSSION

All identified aerobic degradation produced were chemical species produced through hydroxylation of the unfragmented parent molecule, triticonazole. However, unidentified bound residues accounting for up to 19% of applied radioactive residues by study termination could possibly include residues of toxicological concern.

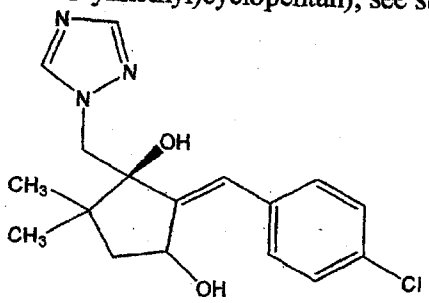
Sandy loam soil. Phenyl ring-labeled **[U-^{14}C]triticonazole** (1RS-(E)-5-((4-chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol; radiochemical purity >98%; see structure below), at a nominal application rate of 1.70 ppm,



[U-^{14}C]Triticonazole

degraded with a calculated first order half-life of 301 days ($r^2 = 0.79$; 265 to 350 days at the 90 % confidence interval) in sandy loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 22 ± 2°C for up to 363 days (p.21; Figure AV.5, p.77). Based on HPLC analysis, triticonazole was 83.8-95.9% of the applied radiation at 0-14 days posttreatment, 58.1-87.1% at 28-56 days, 38.6-55.2% at 224 days and 37.4-50.4% at 266-363 days (Table AII.2, p.141).

The degradate, **RPA 406341** (Metabolite 5; E-2-(4-chlorobenziliden)trans-1,3-dihydroxy-5,5-dimethyl-1-(1,2,4-triazol-1-ylmethyl)cyclopentan); see structure below; stereo-isomers

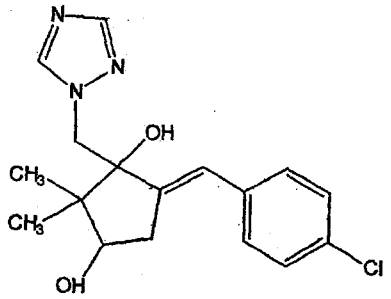


RPA 406341

6

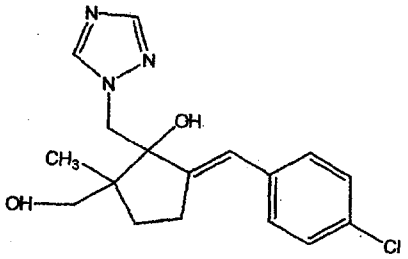
may also form), was initially detected at 1.4% of the applied radiation at 7 days posttreatment, was a maximum of 6.5-10.6% at 56 days, 5.2-9.2% at 84-168 days, 4.7-7.5% at 224-266 days and was 0-6.0% at 363 days posttreatment (Table AII.2, p.141).

The degradates, **RPA 406780** (2-(4-chlorobenzylidene)-1,4-dihydroxy-5,5-dimethyl-1-(1,2,4-triazol-1-ylmethyl)cyclopentane); see structure below; stereo-isomers may also form; Figure AI.6, p.44),



RPA 406780

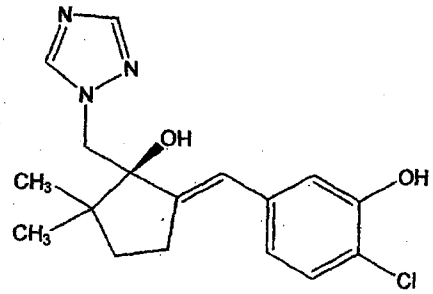
RPA 404886 (erythro-2-(4-chlorobenzylidene)-5-methyl-5-hydroxymethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-1-cyclopentanol); see structure below; stereo-isomer of RPA 405826; Figure AI.5, p.43),



RPA 404886

and **RPA 407922** (Metabolite 6; (1RS)-E-2-(4-chloro-3-hydroxybenzilidene)-5,5-dimethyl-1-(1,2,4-triazol-1-yl-methyl)pentan-1-ol); stereo-isomers may also form; see structure below; Figure AI.4, p.137), were ≤7.8%, ≤3.9%, and ≤6.3% of the applied radiation,

7



RPA 407922

respectively. Two unidentified degradates (Metabolites 5 and 7) respectively were ≤5.3% and ≤7.6% of the applied radiation at all sampling intervals; an additional four unidentified degradates (designated Metabolites 4, 8, 10, and 11), were ≤0.9, ≤1.9, ≤3.4 and ≤1.6% of the applied radiation, respectively.

Unextracted [^{14}C]residues increased from 0.3-0.4% of the applied radiation immediately posttreatment to a maximum of 13.5-15.8% at 363 days (Table AII.2, p.141). Based on organic matter fractionation analysis, [^{14}C]residues associated with fulvic acid, humic acid, and humin fractions were 4.50%, 4.42%, and 5.74% of the applied radiation, respectively, at 363 days posttreatment (Table 5, p.29). $^{14}CO_2$ and other evolved [^{14}C]volatiles (ethylene glycol trap) were a maximum of 4.7-12.2% and 0.1-0.4% of the applied radiation at 363 days, respectively.

Material balances (based on LSC analysis of individual replicates) were 82.1-97.7% of the applied radiation during the study (Table AII.2, p.141).

Clay loam soil. Phenyl ring-labeled **[U-^{14}C]triticonazole** (radiochemical purity >98%; see structure above), at a nominal application rate of 1.70 ppm, degraded with a calculated first-order half-life of 145 days (r^2 = 0.98; 136 to 147 days at the 90% confidence interval) in clay loam soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 22 ± 2°C for up to 363 days (p.21; Figure AV.4, p.76). Based on HPLC analysis, triticonazole was 88.9-97.8% of the applied radiation immediately posttreatment, 94.3-102.3% at 1-7 days, 50.7-52.9% at 140 days, and 16.8-18.5% at 363 days (Table AII.1, p.140; AV.4, p. 76).

RPA 406341 (see structure above) was initially an average of 2.50% of the applied radiation at 7 days posttreatment, 4.4-9.0% at 28-84 days, a maximum of 10.4% at 112 days, and 6.2-8.7% at 140-363 days.

RPA 407922 (see structure above) was initially an average 1.7-2.3% of the applied radiation at 56 days posttreatment, a maximum of 10.9-14.7% at 266 days, and was 11.2-

8

12.2% at 363 days. **RPA 406780** (see structure above) and **RPA 404886** (see structure above) were ≤7.8% and ≤2.0% of the applied radiation, respectively. One unidentified degradate (Metabolite 5) was ≤7.6% of the applied radiation at all sampling intervals; an additional six unidentified degradates (designated Metabolites 4, 7, 8, 9,10, and 11), were ≤0.3%, ≤1.8%, ≤0.6%, ≤1.9%,≤ 2.6%, and ≤2.7% of the applied radiation, respectively.

Unextracted [^{14}C]residues increased from 0.2% of the applied radiation immediately posttreatment to a maximum of 16.9-19.0% at 363 days (Table AII.1, p.140). Based on organic matter fractionation analysis, [^{14}C]residues associated with fulvic acid, humic acid, and humin fractions were 4.18%, 4.25%, and 9.49% of the applied radiation, respectively, at 363 days (Table 5, p.29). Evolved $^{14}CO_2$ and other evolved [^{14}C]volatiles (ethylene glycol trap) were a maximum 22.4-25.3% and 0.5-1.5% of the applied radiation at 363 days, respectively.

Material balances (based on LSC analysis of individual replicates) were 87.7-104.1% of the applied radiation during the study (Table AII.1, p.140).

COMMENTS:

1. Reported analytical results and methodology were insufficient to determine if the triazole moiety formed. No analytical standard was used for the chromatographic determination of triazole. The molecule was not radiolabeled in the proper ring to facilitate tracking the environmental fate of the triazole moiety, and the mass spectrophotometric data was not sufficiently detailed to facilitate analysis of isotopic abundance ratios.

2. A sample history detailing the storage intervals and conditions for soil samples was not provided. Storage stability studies are required for samples stored longer than 30 days prior to analysis. However, it could be determined from date stamps for May 1992 on chromatographs for the day 0 and the day 168 soil extracts (pp.50, 52, 61, 63), and from the absence of evidence that additional peaks were emerging along the chromatographic baselines, that no significant degradation occur during sample storage. The registrant should be reminded that failure to provide this data could delay the acceptance of submitted studies.

3. Two studies were conducted using the Speyer 2.2 loamy sand soil. However, artificial soils are not acceptable for meeting Subdivision N Guidelines, and neither study is reported in this study review.

4. There was no effort made to characterize/identify bound residues which exceeded 10% of the applied radiation. Nonextractable [^{14}C]residues accounted for maximum of 15.8% and 19.0% of the applied radiation at 363 days in the clay loam and sandy loam soils (Tables AII.1-2, pp.141-142).

9

5. The temperature of the test system was maintained at 22 ± 2°C except for a 5 day period (posttreatment interval not specified) during which the temperature increased to 30 ± 2°C (p.14). Subdivision N Guidelines require that the experimental temperature be held constant (± 1°C) between 18°C and 30°C. However, this deviation dose not appear to have affected the integrity of the study.

6. Limits of quantitation and detection were not reported for HPLC, TLC and LSC analysis. It is necessary that both limits of quantitation and detection be reported to allow the reviewer to evaluate the adequacy of the test method for the determination of the parent compound and its degradates.

7. The proposed metabolic pathway for triticonazole in soil is depicted in Figure 1, p.129.

8. The study authors stated that the nominal application rate of 385 g ai/ha was equivalent to a seed treatment rate of 200 g ai/100 kg seed, at a seeding rate of 180 kg/ha (pp.11, 14). The maximum label rate was not reported.

9. The soil series names were not reported. The soils tested were described as UK sandy loam, and UK clay loam (Table 1, p.27).

10. The study was conducted according to UK Principles of Good Laboratory Practice and Food Laboratory Practice in the Testing of Chemicals (OECD, Paris). A Quality Assurance Statement and a Statement of No Data Confidentiality were provided.

11. In MRID 448021-25, phenyl ring-labeled [U-^{14}C]triticonazole (radiochemical purity >98%), at a nominal application rate of 2.46 ppm, degraded with a calculated mean half-life of 349 days (r^2 = 0.77 and 0.89) in loamy sand soil adjusted to 75% of 0.33 bar moisture content and incubated in the dark at 22 ± 2°C for up to 363 days posttreatment. Triticonazole was an average of 91.5-96.3% of the applied radiation at 0-7 days posttreatment, and decreased to 42.9% at 363 days posttreatment. RPA 406341 and RPA 406780 were maximum averages of 14.8% of the applied radiation at 56 days posttreatment and 8.4% at 28 days, respectively. RPA 404886 and nine uncharacterized degradates (Metabolites 1 and 6-13), were each ≤6.2% of the applied radiation.

12. In MRID 448021-26, triazole ring-labeled [U-^{14}C]triticonazole, at a nominal application rate of 0.32 ppm, was stable in clay soil adjusted to 75 ± 5% of 0.33 bar moisture content and incubated in the dark at 25 ± 2°C for up to 365 days. Based on HPLC analysis, triticonazole was an average of 100.76% of the applied radiation immediately posttreatment, 85.63% at 50 days, 75.24% at 129 days, and was 67.26-74.93% at 189-365 days. Three degradates, RPA 406341, RPA 404766 ((E)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-((1H)-1,2,4-triazol-1-ylmethyl)-cyclopentan-1,3-diol), and RPA 406203 ((Z)-5-

(4-chlorobenzylidene)-2,2-dimethyl-1-((1H)-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol) were identified.