3-11-2002

DATA EVALUATION RECORD

STUDY 5

CHEM 120603	Tetraconazole	§161-3

CAS No. 112281-77-3
FORMULATION--00-ACTIVE INGREDIENT

STUDY ID 44367004
Pizzingrilli, G., A. Scacchi, and F. Castoldi. 1995. Aerobic soil metabolism of [^{14}C-U-triazole]tetraconazole (under sunlight). Laboratory Project ID: R/ABT.94.10. Unpublished study performed by Isagro Ricerca S.r.l., Novara, ITALY; and submitted by Sostram Corporation, c/o Landis International, Inc., Valdosta, GA.

DIRECT REVIEW TIME = 45 Hours

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General:
This study was submitted to fulfil the aerobic soil metabolism guideline (162-1), however, its design was inadequate to assess tetraconazole metabolism in soil. The study was reviewed under the photodegradation in soil guideline (163-1).

CONCLUSIONS

Degradation - Photodegradation on soil

1. This study is valid and provides marginally supplemental information on the photodegradation of tetraconazole on Italian silt loam soil.

2. Uniformly triazole ring-labeled [^{14}C]tetraconazole, at a nominal concentration equivalent to 250 g/ha, dissipated with a EFED-calculated half-life of 106 days ($r^2 = 0.84$) in Italian silt loam soil adjusted to 100% of 0.33 bar moisture content and maintained at 3°-50°C while irradiated under natural light in Novara, Italy, for up to 112 days. This is a non-guideline study in which dark control was not studied, temperature was not constant, natural light was not characterized, and moisture content was 100% of 0.33 bar. Because this study was conducted using soil with a high microbial activity and exposed to sunlight, it is unclear whether the major route of dissipation is dependent on photodegradation and/or microbial-mediated degradation. Because the soil metabolism study (supplemental study, MIRD 44367005) showed that tetraconazole was microbially stable in sandy loam soil under aerobic conditions it is likely that the tetraconazole dissipation was due to photodegradation, not microbially-mediated processes.

   This study was conducted in compliance with the GLP Regulations of the Italian Ministry of Health - DL n° 120-01.27.1992 and not in accordance with Good Laboratory Practices (GLP) as required by FIFRA.

3. Six degradates were detected: M14360-dihydro-isoquinoline-triazole (SML-1), M14360-alcohol (SML-2), 1,2,4-triazole (SML-3), M14360-acid (SML-4), M14360-difluoroacetic acid (SML-5), and triazolylacetic acid (TAA, SML-6) which reached the maximum of 0.91% (at 0-112 days posttreatment (PTT)), 4.3% (30 days PPT), 3.7% (112 days PPT), 8.9% (112 days PPT), 6.1% (30 days PTT), and 4.9% (112 PTT), respectively. Bound [^{14}C]residues were 18.1-22.0% of the applied radioactivity at 60-112 days posttreatment.

4. This study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on photodegradation in soil for the following reasons:

   (i) The dark control samples were not utilized; therefore, it could not be confirmed that the observed degradation was actually photodegradation.

(ii) The incubation temperature was not held constant (± 1°C) or maintained between 18 and 30°C throughout the incubation period;

(iii) The intensity of the natural sunlight was not reported;

(iv) The hours of sunlight per day, latitude, and atmospheric cover were not reported; and

(v) The soil moisture content was not maintained at 75% of 0.33 bar.

As the registrant submits the natural light information (i.e., intensity, number of hrs of day-sunlight, latitude), the study may be upgraded to supplemental toward the fulfilment of the data requirement for tetraconazole soil photodegradation.

5. The test soil in this study was the same as the soil used in the soil photodegradation pilot study (MRID 45080901) in which test samples were maintained in different light, temperature, and soil moisture conditions. In the pilot study, the longest half-life of approximately 71 days was obtained for sunlight irradiated soil in an uncovered vessel, maintained at 15°-30°C, and moisture content of 41%. Two major degradates, ^{14}C-M14630 acid and unknown compound (R_f = 0.01) were a maximum of 13.2% and 5.9%, respectively, at 60 days posttreatment. Two minor degredates ^{14}C-Triazole and M-14630 alcohol were a maximum of 2.1% and 3.4%, respectively. The bound radioactivity also reached a maximum 20% (60 days PTT). Although this soil photodegradation study does not meet Subdivision N Guidelines, both studies data indicate a rather slow soil photodegradation rate.

6. No further information is needed on the soil photodegradation of tetraconazole at the present time because a new study probably would not provide substantially new information.

METHODOLOGY

Samples (10 g) of Italian silt loam soil (from Italy; 18% sand, 66% silt, 16% clay, 1.8% organic matter, pH 8.2, CEC 17.3 meq/100 g; Table 1, p. 37) were sieved (2 mm) and weighed into glass trays (area 44 cm^2); the soil was placed on the tray to form a 4-mm thick layer (pp. 14, 17). Samples were treated (dropwise) with uniformly triazole ring-labeled [^{14}C]tetraconazole {M14360; (±)-2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propyl 1,1,2,2-tetrafluoroethyl ether; radiochemical purity 98.1%, specific activity 136.6 μCi/mg; p. 13}, dissolved in acetonitrile, at a nominal application rate equivalent to 250 g/ha and adjusted to 0.33 bar moisture content (pp. 16, 17; see Comment #4). The sample trays were placed under natural light in Novara, Italy, from July to November 1994 (diagram not provided; see Comment #3); the temperature ranged from 14 to 50°C and from 3 to 22°C during the day and night, respectively.

The total light intensity of the natural sunlight was not report8.6ed. The soil moisture level was determined 1-3 times per day by weighing the trays and adjusted as necessary. Duplicate samples were removed for analysis at 0, 15, 30, 60, 90, and 112 days posttreatment.

At each sampling interval, duplicate samples were extracted by shaking twice with acetone, twice with methanol:water (1:1, v:v), and once with methanol:0.1 *N* HCl (1:1, v:v; p. 17). Following each extraction, the samples were centrifuged, and the supernatants were decanted and diluted with the extraction solvent (p. 1); duplicate aliquots of each extract were analyzed for total radioactivity by LSC (Figure 1, p. 45). The limit of detection for LSC was 0.043% of the total applied radioactivity (p. 19). Aliquots of each of the five soil extracts were combined into one sample used to characterize degradates (p. 20).

To characterize degradates, aliquots of the combined soil extracts were concentrated by evaporation and analyzed by LSC (p. 22) and TLC on Kielsegel 60 F_{254} plates developed with ethyl acetate (p. 20; Figure 2, p. 46). Samples were analyzed alone and were co-chromatographed with radiolabeled reference standards of the parent or the potential degradates M14360-alcohol, M14360-acid, triazole, and TTA (pp. 20, 22); radiolabeled compounds were detected and quantified by radioimage scanning (p. 20). The combined extracts were partitioned three times with n-hexane; aliquots of the organic phase were analyzed by LSC, two-dimensional TLC as previously described (except plates were developed with ethyl acetate, two runs; p. 22), and HPLC (SUPELCO LC-18 column) using a mobile phase gradient of 82% methanol plus 18% acetonitrile:99.8% water plus 0.2% CF_3COOH (60:40 to 78:22, v:v) with radioactive flow detection (p. 23). HPLC samples were analyzed alone or were co-chromatographed with radiolabeled reference standards of the parent or the potential degradates M14360-alcohol, M14360-acid, and TTA. Aliquots of the aqueous phase were analyzed by LSC (p. 22) and TLC as previously described (except plates were developed with chloroform:methanol:water; 55:40:5, v:v:v; p. 20). The aqueous phase was then acidified with 12 *N* HCl and partitioned three times with ethyl acetate; aliquots of the acidified aqueous phase were analyzed by LSC (p. 22) and TLC as previously described for the aqueous phase. The acidified aqueous phase of the ethyl acetate extraction was evaporated to dryness and redissolved in methanol (p. 21). The methanol samples were applied to a preparatory TLC plate developed with chloroform:methanol:water (55:40:5, v:v:v); radioactivity was visualized by radioimage scanning, and radioactive areas were scraped and extracted with methanol. Aliquots of the methanol extract were analyzed by LSC (p. 22), TLC on Kielsegel plates as previously described (except plates were developed with chloroform:methanol:water; 55:40:5, v:v:v), and HPLC as previously described (except with an isocratic mobile phase of 82% methanol plus 18% acetonitrile:99.8% water plus 0.2% CF_3COOH; 50:50, v:v; p. 23). The methanol extract was evaporated to dryness, redissolved in isobutanol, and derivatized by bubbling with gaseous HCl (p. 21). The isobutylated samples were then evaporated to dryness, redissolved in water, and partitioned three times with n-hexane. Samples were analyzed by LSC (p. 22), TLC as previously described above for the n-hexane extracts, and HPLC as previously described with the isocratic mobile phase (p. 23). The organic phase of the ethyl acetate extract was evaporated to dryness (p. 21); the residue was redissolved in $CHCl_3$:CH_3OH (90:10, v:v) and analyzed by liquid chromatography (preconditioned silica gel

column) eluted with a gradient of $CHCl_3$:CH_3OH (90:10 to 0:100, v:v; solvent ratio intervals not specified). The eluate was collected in fractions and analyzed by LSC; four eluate fractions (designated D-1 to D-4) containing radioactivity were evaporated to dryness and redissolved in methanol. Aliquots of fraction D-1 were analyzed by LSC (p. 22), two-dimensional TLC on Kieselgel plates as previously described (except plates were developed with ethyl acetate and chloroform:methanol; 7:3, v:v, two runs), and HPLC as previously described (except using a mobile phase gradient of 82% methanol plus 18% acetonitrile:99.8% water plus 0.2% CF_3COOH; 50:50 to 78:22, v:v; p. 23), and GC/MS (p. 24).[1] Aliquots of fraction D-2 were analyzed by LSC (p. 22) and two-dimensional TLC on Kieselgel plates as previously described (except developed with chloroform:methanol; 7:3, v:v). Aliquots of fraction D-3 were analyzed by LSC, TLC, LC/MS, and HPLC (systems not specified; Figure 2, p. 46; see Comment #15). Aliquots of fraction D-4 were analyzed by LSC (p. 22), TLC on Kieselgel plates as previously described (except developed with chloroform:methanol:water; 55:40:5, v:v:v), and HPLC as previously described for fraction D-1 (p. 23). Aliquots of fractions D-3 and D-4 were evaporated to dryness, redissolved in isobutanol, and derivatized by bubbling with gaseous HCl (p. 21). Isobutylated fractions D-3 and D-4 were evaporated to dryness, redissolved in distilled water, and partitioned three times with n-hexane. Aliquots of the isobutylated fraction D-3 were analyzed by TLC (system not specified; Figure 2, p. 46), GC/MS (capillary J & W DB-5ms column) and LC/MS (Merck LiChrosopher 100 RP-18 column; pp. 24, 25). Aliquots of the isobutylated fraction D-4 were analyzed by GC/MS (capillary SPB-5 column; p. 24) and TLC on Kieselgel plates as previously described (except developed with ethyl acetate; p. 22).

Post-extracted soil samples were air dried and analyzed for total radioactivity by LSC following combustion (p. 18). Data were corrected for combustion efficiency (90%); the limit of detection was 0.004% of the total applied radioactivity (p. 19).

To determine soil viability prior to the incubation, soil subsamples were mixed with distilled water and diluted. Aliquots of each dilution were added to Plate Count Agar and incubated at 30°C for 48 hours (p. 14); results indicated that the soil was viable (Table 1, p. 37). Soil viability was not determined at the end of the incubation (see Comment #4).

DATA SUMMARY

Uniformly triazole ring-labeled [^{14}C]tetraconazole (radiochemical purity 98.1%), at a nominal concentration equivalent to 250 g/ha, degraded with a registrant-calculated half-life (reported as a DT_{50}) of 72 days (r^2 not reported; see Comment #7) in Italian silt loam soil adjusted to 100% of 0.33 bar moisture content and maintained at 3°C-50°C while irradiated under natural light in Novara, Italy, for up to 112 days (p. 27; Figure 10, p. 54). Dark control samples were not used; therefore, it could not be confirmed that the observed degradation was actually photodegradation. The parent compound was initially 97.7% of the applied radioactivity, decreased to 59.2% of the applied by 30 days posttreatment, was 48.5-50.3% of the applied at 60-90 days posttreatment, and was 40.6% of the applied at 112 days posttreatment (Table 7, p. 43). The minor degradate 9-

chloro-5,6-dihydro-6-(1,1,2,2-tetrafluoroethoxy)-methyl-[1,2,4]triazolo[5,1-a]isoquinoline (SLM-1; p. 35) was 0.57-0.91% of the applied radioactivity at 0-112 days posttreatment. The minor degradate 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)-1-propanol (SLM-2) was initially 3.8% of the applied radioactivity at 15 days posttreatment, increased to a maximum of 4.3% of the applied by 30 days posttreatment, and decreased with variability to 1.5% of the applied by 112 days posttreatment. The minor degradate 1,2,4-triazole (SLM-3) was first detected at 1.6% of the applied radioactivity at 15 days posttreatment and increased with variability to a maximum of 3.7% of the applied by 112 days posttreatment. The minor degradate 2-(2,4-dichlorophenyl)-3-(1H-1,2,4-triazol-1-yl)propionic acid (SLM-4) was initially present at 1.4% of the applied radioactivity at 15 days posttreatment and increased with variability to a maximum of 8.9% of the applied by 112 days posttreatment. The minor degradate 5-(2,4-dichlorophenyl)-2,2-difluoro-6-(1H-1,2,4-triazol-1-yl)-3-oxahexanoic acid (SLM-5) was initially 4.7% of the applied radioactivity at 15 days posttreatment, increased to a maximum of 6.1% of the applied by 30 days posttreatment, and was 3.2% of the applied at 112 days posttreatment. The minor degradate (1H-1,2,4-triazol-1-yl)acetic acid (SLM-6) was initially 1.7% of the applied radioactivity at 15 days posttreatment and increased with variability to a maximum of 4.9% of the applied by 112 days posttreatment. Unidentified radioactivity comprised of at least five minor degradates was initially 3.4% of the applied radioactivity at 15 days posttreatment and increased to a maximum of 10.7% of the applied by 112 days posttreatment (see Comment #11). Nonextractable [^{14}C]residues were initially (day 0) 0.23% of the applied radioactivity, increased to 11.5% the applied radioactivity by 30 days posttreatment and were 18.1-22.0% of the applied at 60-112 days posttreatment; radioactivity associated with the humic acid, fulvic acid, and humin fractions was not determined.

Material balances (based on LSC analysis of individual replicates) were 94.7-100.0% of the applied radioactivity throughout the study (Table 2, p. 38).

COMMENTS

1. Dark control samples were not utilized in the study. Without dark control data, the reviewer was unable to determine whether the degradation observed in the study was actually due to photolysis of the parent compound or/and soil microbial degradation. Subdivision N Guidelines require that dark control samples be prepared and maintained in the same manner as the irradiated samples only in darkness. However, tetraconazole was metabolically stable in sandy loam soil maintained at 25 ± 1°C for one year (MRID 44367005) which suggests that tetracinazole dissipation was likely due to photodegradation not microbil-mediated processes.

2. The experimental temperature ranged from 14 to 50°C and from 3 to 22°C during the day and night, respectively (p. 17). Raw temperature data were not submitted; therefore; the reviewer could not determine the frequency and magnitude of the temperature

fluctuations. Subdivision N Guidelines require that the experimental temperature be held constant (± 1°C) between 18°C and 30°C throughout the incubation period.

3. The natural sunlight was not adequately characterized. The total light intensity, hours of sunlight per day, latitude of the experiment site, and atmospheric cover during the study were not reported. The reviewer noted that the testing facility was located in Novara, Italy (p. 12). Subdivision N Guidelines require that latitude, hours of sunlight per day, and light intensity data be provided for natural light sources.

4. The soil moisture content was not adjusted to and maintained at 75% of 0.33 bar during the incubation. The study authors stated that the soil was adjusted to "moisture field capacity" (41%; p. 17); samples were maintained at that moisture level (100% of 0.33 bar) throughout the study. Subdivision N Guidelines require that the soil moisture content be maintained at 75% of 0.33 bar to allow for sufficient aeration to ensure soil viability during the incubation. Additionally, soil viability, which is affected by soil moisture content, was not tested at the conclusion of the incubation.

5. Nonextractable [^{14}C]residues were considerably high, at 18.1-22.0% of the applied radioactivity at 60-112 days posttreatment (Table 7, p. 43). Under Subdivision N Guidelines, the registrant must demonstrate that a reasonable attempt was made to characterize [^{14}C]residues present at >10% of the applied radioactivity. The reviewer notes that the soils were extracted five times, with three different solvent systems (p. 17); however, no harsh extractions were performed.

6. The reviewer could not confirm whether the soil utilized in this study was the same type of soil used in an aerobic soil metabolism study. In a submitted aerobic soil metabolism study (MRID 44367005), a sandy loam soil collected from Donalsonville, GA, was utilized.

7. The half-life (72 days) of the parent compound was reported as a DT_{50} by the study author. The study authors stated that the DT_{50} was determined by running a 1.5 order regression on the parent data (pp. 26, 27; Figure 10, p. 54). The reviewer-calculated half-life was 106.6 days (r^2 = 0.84) using a first-order regression of the parent data.

8. The study was conducted at an exaggerated rate of twice the label rate. The study authors stated that the parent compound was applied at twice the maximum label rate to aid in the determination of the decline of the test substance and formation and decline of its degradates (p. 16). The maximum label rate for tetraconazole was reported as 125 g/ha; the study was conducted at an application rate equivalent to 250 g/ha.

9. The study authors submitted this study under Subdivision N Guideline 162-1 (Aerobic soil metabolism); however, the study was reviewed under Subdivision N Guidelines 161-3 (photodegradation on soil).

10. The study was conducted using uniformly triazole ring-labeled [^{14}C]tetraconazole (p. 13). A study was not conducted using [^{14}C]tetraconazole radiolabeled in the phenyl ring position.

11. Unidentified radioactivity comprised of at least five minor degradates was detected throughout the study (Table 7, p. 43). The degradates were a combined maximum of 10.7% of the total applied radioactivity at 112 days posttreatment. The study authors stated that each degradate accounted for ≤4% of the applied radioactivity (p. 32).

12. The application rate was not reported in terms of test sample concentration (e.g., ppm), but rather as the equivalent to a field application rate (250 g/ha; p. 16). Additionally, the results data were reported only as percentages of the applied radioactivity. In future studies submitted to the EPA, it is preferable that the application rate and results data also be reported in units of concentration (e.g., ppm).

13. Method detection limits were reported for LSC, but not TLC, HPLC, GC/MS, or LC/MS analyses. Both limits of detection and quantitation should be reported for each method utilized to allow the reviewer to evaluate the adequacy of the methods for the determination of parent and degradates in the test system.

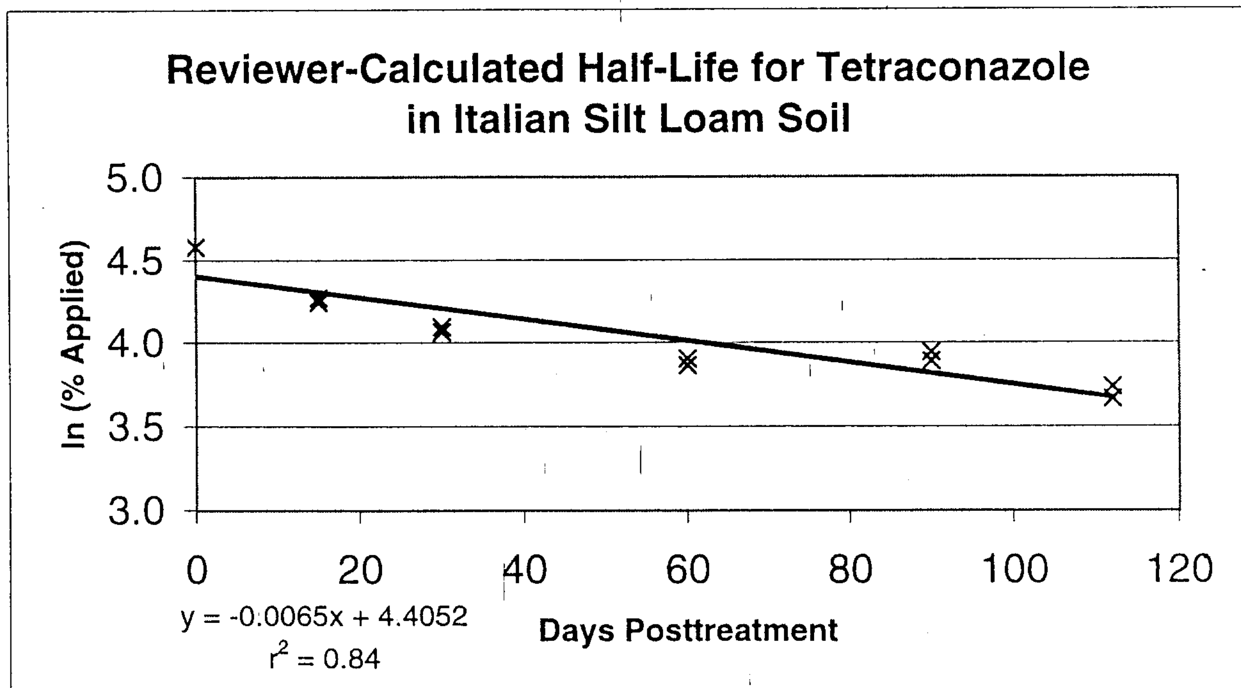
14. The proposed degradation pathway of tetraconazole was presented on page 34.

15. The reviewer noted that some analyses appeared in the flow chart for the methodology (Figure 2, p. 46), but were not described in methodology section of the text (pp. 20-25). The reviewer reported the analyses presented in the flow chart, but was unable to determine whether the flow chart was correct. In future studies submitted to the EPA, it is necessary that the details of the study be adequately, accurately, and clearly reported.

Italian Silt Loam Soil

Day	% Applied Radioactivity as Parent	ln (% Applied)	Mean % Applied
0	97.6	4.6	
0	97.7	4.6	97.7
15	71.7	4.3	
15	69.7	4.2	70.7
30	58.3	4.1	
30	60.1	4.1	59.2
60	47.7	3.9	
60	49.6	3.9	48.5
90	49.0	3.9	
90	51.7	3.9	50.3
112	42.0	3.7	
112	39.1	3.7	40.6

Half-life = 106.6 days



ATTACHMENT 1
Data Critical to the Study Interpretation

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY SEE THE FILE COPY

Page ______ is not included in this copy.

Pages 11 through 21 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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✓ FIFRA registration data.

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