

US EPA ARCHIVE DOCUMENT

in a silt loam soil that was incubated in the dark at $20 \pm 2^\circ\text{C}$ maintained at 75% of field capacity for 360 days. Acibenzolar decreased from 58.05% of applied at 0 days posttreatment to 2.51% at day 1, 2.37% at day 3, 1.47% at day 7, 1.19% at day 14, 0.6% at day 28 and remained at that concentration until day 360 at 0.58%. The major degradate from the hydrolysis of the ester to the acid is CGA-210007. The calculated half-life of CGA-210007 is 16.5 days. CGA-210007 was present at 38.37% of applied at day 0, increased to a maximum of 82.57% of applied by day 1, decreased to 77.86% of applied at day 3, was at 69.5% by day 7, 55.1% by day 14, 39.59% by day 28, 7.09% by day 56, 3.77% by day 90, 0.68% by day 120, 0.37% by day 182, and was at 0.435 by day 360.

METHODOLOGY:

Sieved (2 mm) silt loam soil (31.2% sand, 54.9% silt, 13.9% clay, 2.7% organic carbon, pH 7.00, CEC 17.7 mmol/100g) was added to (200g) Steilbrust flasks and inoculated with 2 ppm CGA-245704 [$^{14}\text{C}(\text{u})$]benzo[1,2,3]thiadiazole-7carbothioic acid S-methyl ester; (radiochemical purity 99%, specific activity 2.08 MBq/mg = 56.22 uCi/mg, Ciba-Geigy), dissolved in acetonitrile and mixed thoroughly. The soil moisture was then adjusted to 75% of the field capacity. The flasks of the treated soil were attached to a open gas-flow system; air was continuously drawn (60 ml/min) sequentially through distilled water, ethylene glycol, sulfuric acid and sodium hydroxide to trap organic volatiles and evolved CO_2 (Figure 1). The treated soil was incubated in darkness at approximately $20 \pm 2^\circ\text{C}$. During incubation the soil moisture was measured and adjusted (method not described). Single soil samples were taken at day 0, 1, 3, 7, 14, 28, 56, 90, 120, 182, and 360.

After a 28 day aerobic incubation period, another set of soils that were treated equally as the above soils and maintained under similar aerobic conditions were flooded with a water layer of 2-3 cm (200 ml) and ventilated with nitrogen four times daily for 15 minutes at 60 ml/min to maintain anaerobic conditions. Samples were taken up to 120 days.

Another experiment was carried out using sterile autoclaved soils. The soil (200g) dry weight soil was treated with 1 ml of the application solution (2 ppm), adjusted to 75% of field capacity and connected to the flow through apparatus with ultramembrane filters to maintain sterile conditions. The incubation flasks were maintained under conditions equal to those from the nonsterile soils. Samples were taken up to 90 days.

Soil samples were extracted with acetone/water 80/20 (v:v) three times, the filtrates combined and analyzed by LSC. Soil sediments were combined and extracted with a Soxhlet apparatus for 5 hours and the radioactivity was determined by LSC. Day 360 sampling intervals were wetted with 0.01 M CaCl_2 for 2 hours then extracted with acetonitrile/water 80/20 v/v by ultrasonic treatment for 20 minutes then refluxed for 2 hours. Then the soil was extracted

with refluxing acetonitrile/water 80/20 v/v adjusted to pH 2 with 1N HCL, then the soil was washed with distilled water for 1 hour and extracted with refluxing acetonitrile/water 80/20 adjusted to pH 10 with 1N NaOH. The radioactivity of the extracts were determined by LSC. Samples with radioactivity more than 4% of the applied were analyzed by TLC.

DATA SUMMARY:

[¹⁴C(u)]benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; (radiochemical purity 99%, specific activity 2.08 MBq/mg = 56.22 uCi/mg, Ciba-Geigy), at 2 ppm, degraded with a registrant calculated first order ($r^2 = 0.99$) half-life (DT_{50}) of 0.22 days (approx. 5 hrs.) in a silt loam soil that was incubated in the dark at $20 \pm 2^\circ\text{C}$ in a silt loam soil maintained at 75% of field capacity for 360 days. The DT_{90} calculated from $\ln 10/k$ was equal to 0.73 days. Acibenzolar decreased from 58.05% of applied at 0 days posttreatment to 2.51% at day 1, 2.37% at day 3, 1.47% at day 7, 1.19% at day 14, 0.6% at day 28 and remained at that concentration until day 360 at 0.58%. The major degradate from the hydrolysis of the ester to the acid is CGA-210007. The calculated half-life of CGA-210007 is 16.5 days. CGA-210007 was present at 38.37% of applied at day 0, increased to a maximum of 82.57% of applied by day 1, decreased to 77.86% of applied at day 3, was at 69.5% by day 7, 55.1% by day 14, 39.59% by day 28, 7.09% by day 56, 3.77% by day 90, 0.68% by day 120, 0.37% by day 182, and was at 0.435 by day 360.

Five unknown metabolites (named A-E) were produced in addition to CGA-210007. The highest concentration was for the transient metabolite A which amounted to 4.25% of applied after 14 days of aerobic incubation. These metabolites were not identified due to their low concentrations and short resident times.

After 28 days of incubation about 44% of the applied radioactivity is bound to soil as determined by combustion and after 56 days a maximum of 60% bound residues is achieved and decreasing to 53.39% of applied by day 360. Mineralization was considerable after 360 days as 39.37% of applied radioactivity accounted for volatile products that were trapped by NaOH and precipitated out by barium chloride as CO_2 . Under aerobic conditions extractable radioactivity steadily decreased from about 85.08% to 4.65% of the applied after 360 days.

Under anaerobic conditions installed in a subset of soil samples after 4 weeks of aerobic incubation the production of volatiles ceased indicating the mineralization of extractable and non extractable fractions is dominated by the aerobic process.

Under sterile aerobic soil conditions the degradation of acibenzolar was very slow. A half-life of 344 days was calculated under those conditions. The acid metabolite was observed in these soils at a maximum of 8.74% of applied by day 90. Indicating

microbial hydrolysis is the main process occurring as opposed to abiotic hydrolysis.

The recoveries ranged from 101.89 to 85.45% (96.00 ± 5.03 , $n = 11$), 92.88 to 95.45% (93.75 ± 1.47 , $n = 3$) and 101.99 to 105.90 (104.01 ± 2.03 , $n = 4$) of the dose applied for the aerobic, aerobic/anaerobic and aerobic/sterile experiments, respectively. Values in brackets are mean values.

DISCUSSION:

1. It appears that only one soil flask was sampled at each individual sampling interval for the duration of the study. The results of analysis of a series of replicate samples can reveal directly something about the magnitude of random error. It is preferable to analyze two to three replicates at each sampling interval to delineate inherent experimental variability. Possibly the data presented could have been an average of two replicates that were collected at each sampling interval. If so, the results from analysis of each soil or soil:water sample should have been presented to provide the maximum concentrations of parent, degradates and unidentified [^{14}C] compounds that were detected at each sampling interval, and to assess the variability between the replicates.

The study authors state that the kinetics of the parent decomposition and the quantitation of metabolites were based on TLC due to the lower sensitivity of the LC system. The results obtained by the TLC methods could be confirmed by HPLC only for the main metabolites. The minor metabolites could not be seen due to the lower sensitivity of this system.

2. The Les Evouettes soil was not adequately compared to those soils of the continental United States.

3. Degradation rates of the parent molecule were calculated assuming first order kinetics and completing a linear plot of each individual component present at % of applied radioactivity vs. time.

4. The analytical method was incomplete: Description of extraction efficiency as percent applied individually for parent and degradates was not submitted.

The limit of detection of ^{14}C material by TLC analysis was calculated to be 0.4 ppb with respect to the soil matrix. The limit of detection based on HPLC was 16.0 ppb with respect to the soil matrix.

5. The time from sampling to analysis was not reported. According to section 2.4 Sample Preparation and Extraction Procedure it appears that analysis immediately followed the sampling and extraction.

6. The study authors state that the maximum recommended field rate of acibenzolar is 500 g a.i./ha.

7. Soils used in the sterile aerobic soil metabolism experiment were steamed autoclaved which may have affected the chemical and physical properties of the soil.