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DATA EVALUATION RECORD

STUDY IDENTIFICATION:

Bade, T. R. 1990. Anaerobic Soil Metabolism of SAN-582 H: Project No. 414105. Environmental Chemistry and Toxicology Section of Sandoz Crop Protection, Des Plaines, Illinois. MRID No. 417068-01.

TYPE OF STUDY: Anaerobic Soil Metabolism (162-2)

REVIEWED BY:

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CONCLUSIONS:

1. EFGWB concludes that the study submitted is acceptable and satisfies data requirements for anaerobic soil metabolism.

2. Based on the results of the study, SAN 582 H degraded under anaerobic conditions with a half-life of 53.8 days. The material balance averages ranged from 94.6% on day 30 to 105% on day 14.

3. The major metabolite was oxalamide which reached a maximum level of 8.7% of applied radioactivity by day 93. The minor metabolite "Fr 4" reached a maximum of 3% of applied by day 58 and decreased to 2.4% by day 93. Although not identified in this study, Fr 4 has been identified (in MRID No.420348-07) as the sulfoxide of the thioglycolic acid of SAN 582 H. The metabolite "Fr 1A+B" reached a combined maximum of 3.5% of applied by day 93 and this fraction appeared to contain equal amounts of 1A and B; fraction 1A was identified as the sulfonate by TLC and confirmed by MS.

4. Mineralization to $^{14}CO_2$ increased with time even after anaerobic conditions were established. By day 30 (aerobic conditions) there was 1.53% $^{14}CO_2$ and by day 93 (63 days after establishing anaerobic conditions) there was a total of 3.27% reported.

5. The proposed metabolic pathway for SAN 582 H under anaerobic conditions (Figure 6) shows the formation of CO_2. It is requested that further information be provided as to the source of oxygen for CO_2 formation and that the metabolic pathway be discussed in more detail.

MATERIALS AND METHODS:

^{14}C SAN-582 H (3-^{14}C-thienyl)(specific activity 43.3 mCi/m mole, radiochemical purity 99.3%) was used in the study. The treatment solution used was a mixture of ^{14}C-SAN 582 H(1.873 mg; 294 uCi) with 12.8 mg of cold analytical reference standard of SAN-582 H in deionized water (resulting specific activity 44.424 dpm/ug). Kenyon loam soil (see Table II for soil characteristics) obtained from Cedar Falls, Iowa, was sieved through a 2 mm sieve. Field moist soil, equivalent to 5000 grams of dry soil, was mixed with 14.673 mg of ^{14}C SAN-582 H to yield a calculated concentration of 2.36 ug (actual concentration was 2.29 ug/g) of ^{14}C SAN-582 H per gram of dry soil. The treated soil was separated into several containers which were brought to 75% of the 0.33 bar level and incubated at 25^{0}C in the dark.

Soil samples were taken from the incubator and frozen on days 0, 1, 3, 7, 14, and 30 (these samples being equivalent to the aerobic soil metabolism samples). On day 30 four sample jars were placed in anaerobic jars, which were maintained in an anaerobic state using hydrogen plus CO_2 generator envelopes until sampled and the samples frozen. These samples were taken on days 58 and 93 (anaerobic samples). The two anaerobic jars, each containing two 100 g samples (dry equivalents) of treated soil were equipped with glass inlet and outlet tubes. Each outlet tube was connected in series to two gas washing bottles, one with 1.5 N KOH to trap CO_2 and the other to ethylene glycol to trap any volatile organic compounds.

After the soils were made anaerobic on day 30, the jars were flushed weekly with N_2 for 30 minutes. The contents of the CO_2 and organic trapping bottles were then measured and stored until being radioassayed. On days 58 and 93, one 8 oz jar of soil was removed from each anaerobic jar and soil samples frozen at -20^{0}C immediately after collection, and KOH and ethylene glycol samples were kept in a refrigerator at 4^{0}C until analyzed.

Total volatiles and soil residues were determined by LSC. Soil was extracted with methanol:water (1:1) and aliquots were radioassayed (see Figure 1 for extraction procedure). The methanol was removed and the remaining aqueous fraction was extracted with hexane, and aliquots were radioassayed. Soil which had been twice extracted with methanol:water was hydrolyzed in KOH and aliquots were radioassayed.

Radiocarbon in each of the various extraction fractions was separated and characterized by TLC using the following solvent systems: 1) toluene:ethyl acetate (1:1, v/v); 2) petroleum

ether:chloroform:ethanol (70:20:10, v/v/v), and 3) ethyl acetate:toluene:formic acid:water (87:3:5:5, v/v/v/v). The TLC quantitation of selected samples was confirmed by HPLC. After radiocarbon characterization, the soil remaining from each sampling time was extracted with methanol:water and analyzed by TLC (ethyl acetate:toluene:formic acid:water, 87:3:5:5, v/v/v/v), and autoradiographs made. Bands from these plates were collected, extracted, and further purified by HPLC. Samples were then analyzed using MS/FAB.

REPORTED RESULTS:

1. SAN-582 H degraded under anaerobic conditions with a reported half-life 53.8 days. The material balance averages ranged from 94.6% on day 30 to 105% on day 14 of the aerobic portion. The average material balance throughout the study was 98.4±7.3% (Table IV).

2. The major metabolite was oxalamide at 8.7% of the applied radioactivity by day 93 (Table IV).

3. The minor metabolite "Fr 4" reached a maximum of 3.0% of applied by day 58 and decreased to 2.4% by day 93. NMR and MS analysis were inconclusive in this study. The metabolite "Fr 1A+B" reached a combined maximum of 3.5% of applied at 93 days. This fraction appeared to contain equal amounts of 1A and B, and Fr 1A was identified as the sulfonate by TLC by TLC comparison with an authentic standard and confirmed by MS.

4. Mineralization to Co^2 increased with time even after anaerobic conditions were established. By day 30 of the aerobic portion there was 1.53% CO_2 and by day 93 (anaerobic portion) there was a total of 3.27% reported. The proposed metabolic pathway for SAN 582 H in anaerobic soil is given in Figure 6.

DISCUSSION:

1. The minor metabolite "Fr 4" was not conclusively identified by NMR and MS analysis and in this study it was reported that "Fr 4" was most probably a polar carboxylic acid derivative of SAN-582 H which is an intermediate for further degradation or incorporation in the soil biomass. However, in MRID No. 420348-07, "Fr 4" was reported as being identified as the sulfoxide of the thioglycolic acid of SAN-582 H (Figure provided for Fraction IV and 1A).

2. Fraction "Fr 1A+B" represents two compounds of similar R_f values on TLC plates and identical retention times on HPLC. MS confirmed that Fr 1A was the sulfonate.

3. Base extractable radiocarbon averaged 11.4% of applied at 0 time and increased to 16.6% of applied by day 93. Base extractable radiocarbon at zero time suggests that some SAN-582 H adheres quickly to soil but can be base released. Unextractable

radiocarbon was present at 0 time and averaged 6.1% of applied and increased to 19.2% of applied by day 93. Evolution of $^{14}CO_2$ indicates complete mineralization of SAN-582 H and the report discussion indicates that unextractable radiocarbon may very well be mineralized radiocarbon that has been incorporated into biomass before it could be volatilized as CO_2.

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