Soil metabolism of [^{14}C]acetaminophen in two soil types using various soil aliquot sizes

S. McLaughlin, A. Dean, D. Koch, R. Brackett and K. Malekani

Smithers Viscient, Wareham, MA 02571, USA

Abstract

The OECD 307 Guideline 'Aerobic and Anaerobic Transformation in Soil' and the EPA Guideline OCSPP 835.4100 'Aerobic Soil Metabolism' define the degradation rate of an organic chemical in soil. These guidelines and preceding guidelines like them have been used successfully for years, but questions often still remain concerning the optimum soil size to use in the study and its effect on microbial biomass and the final degradation rate of the test substance.

A soil metabolism study design based on the above guidelines was modified in this experiment by setting up treated samples with the pharmaceutical [^{14}C]acetaminophen using 3 different soil aliquot sizes with two soil types. Comparisons of the rate of degradation, rate of mineralization and maintenance of microbial biomass have been made between the different soil aliquot sizes among the two different soil types. These results provide justification of the optimum soil aliquot size to be used in future soil metabolism studies.

Methods: Conduct of the Soil Metabolism Test

- **Test Design**: conduct of the soil metabolism study was based on a modified version of the OECD 307 Soil Metabolism Guideline as well as the OCSPP 835.4100 Soil Metabolism Guideline using [^{14}C]acetominophen as the test substance at 1 mg/Kg. Three soil sample sizes (10g, 50g and 100g) were used to evaluate the rate of [^{14}C]acetaminophen degradation and the population size of the microbial biomass in each of the sample sizes.
- **Soil**: Two soils characterized as a Sandy Loam (named MSL) and a Loamy Sand (named PD) were used and further characteristics are presented below.
- **Volatile and CO_2 Trapping solutions**: Ethylene glycol used for volatile organics and 1 N KOH trapping solution used for CO_2.
- **Aeration**: Hydrated air delivered under negative pressure at approximately 1 bubble per second.
- **Temperature**: 20 +/- 2ºC
- **Sampling and Analysis**: Duplicate soil samples per soil, sacrificed at time zero and on approximately days 3, 14, 30, 60 and 120. Soils were extracted twice with acetonitrile:water (90:10, v:v) and again with acetonitile:water:formic acid (90:10:0.1, v:v:v) at a volume 1.5 to 2.0 times the weight of the soil sample. Extracts were quantified by liquid scintillation counting (LSC) and profiled by high-performance liquid chromatography with radiometric detection (HPLC-RAM). Non-extractable residues (NER) were quantified by combustion followed by LSC.
- **NER extractions**: Additional extractions were conducted with less polar tetrahydrofuran (THF) and non-polar hexane as recommended by EPA guidance for treatment of bound residues.
- **Microbial biomass**: Quantification of the soils' microbial biomass was conducted at the beginning, middle and end of the test. The fumigation/extraction technique (F/E) was used to quantify the microbial biomass in each instance.
- **Kinetics**: single, first-order (SFO) kinetics using the computer software CAKE is generally used to determine the rate constants, however since biodegradation of acetaminophen was rapid, it was empirically reported as a "less than" value.

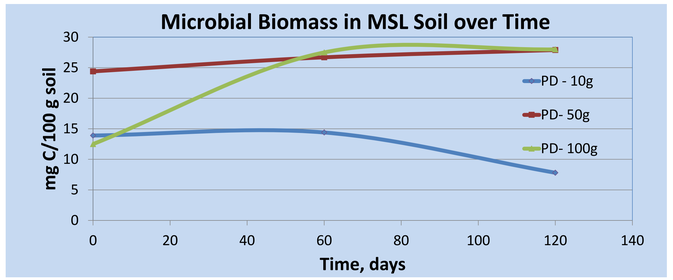
Soil ID	Soil Type	pH	%Sand	%Silt	%Clay	%OC
MSL	Sandy Loam	6.5-7.0	68	16	16	1.9
PD	Loamy Sand	4.8-5.3	82	12	6	0.5

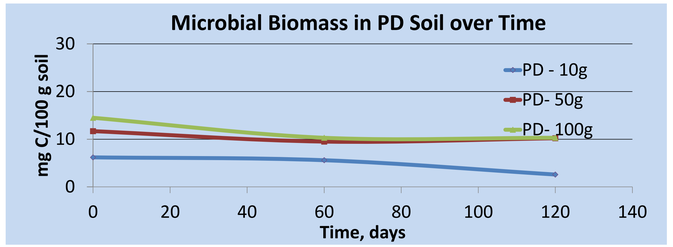
Microbial biomass, as %OC

Soil Name	Soil Type	Start 10g	Middle 10g	End 10g	Start 50g	Middle 50g	End 50g	Start 100g	Middle 100g	End 100g
MSL	sandy loam	0.7	0.8	0.5	1.3	1.4	1.7	0.7	1.4	1.6
PD	loamy sand	1.1	1.0	0.5	2.2	1.8	1.9	2.7	1.9	1.7

Microbial biomass, as mg Carbon/100 g soil

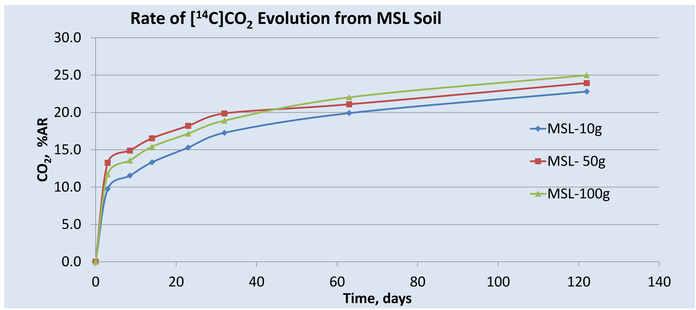
Soil Name	Soil Type	Start 10g	Middle 10g	End 10g	Start 50g	Middle 50g	End 50g	Start 100g	Middle 100g	End 100g
MSL	sandy loam	13.9	14.4	7.6	24.4	26.7	27.9	12.5	27.5	28.0
PD	loamy sand	6.2	5.6	2.6	11.7	9.5	10.2	14.5	10.3	10.3





Rate of [^{14}C]CO_2 Evolution, %AR			
MSL- soil	10g	50g	100g
Day	CO_2	CO_2	CO_2
0	NA	NA	NA
4	9.7	13.2	11.7
14	13.3	16.5	15.4
32	17.3	19.9	18.9
63	19.9	21.1	22.0
122	22.8	23.9	25.0

Rate of [^{14}C]CO_2 Evolution, %AR			
PD- soil	10g	50g	100g
Day	CO_2	CO_2	CO_2
0	NA	NA	NA
4	16.9	16.5	14.4
15	18.2	19.5	17.4
32	22.9	23.3	20.7
63	26.7	26.8	24.9
122	29.5	28.2	28.1



Results and Discussion

- Material balance was maintained near or within 90% to 100% for both soils at all three soil sizes. Extractability was poor given the extremely fast uptake of the pharmaceutical into the microbial biomass as evident of the high $^{14}CO_2$ production. Additional non-polar solvents provided little to no additional extractable radioactivity.
- $^{14}CO_2$ evolution from both soil-types in all aliquot sizes was greater than 20% and approached 30% in the PD loamy sand soil which may have provided better contact between the test substance and the soil microbial biomass.
- The major metabolite of acetaminophen was $^{14}CO_2$.
- The microbial biomass declined significantly ($p < 0.05$) in both soils in the 10 gram sample. Microbial biomass was maintained throughout the study in the 50 and 100 gram sample sizes for both soil-types.
  - Interestingly, the overall ultimate biodegradation rates (i.e., conversion to CO_2) were similar for all three soil sizes for both soil types. The 10 gram soil sized showed a slightly slower mineralization rate in the MSL soil but a slightly faster rate in the PD soil.
  - There was little to no difference between the overall biodegradation DT50 rates of acetaminophen for any of the sample sizes which in part may be due to the rapid degradability of acetaminophen. Since there was little to no acetaminophen measured on day 4, the DT50 value of acetaminophen is reported as <4 Days.

Conclusions

- Based on the results of this study, a soil selection of 50 or 100 grams for a soil metabolism study should have no real difference on the microbial biomass, rate of primary biodegradation or rate of CO_2 evolution of a pesticide provided the soils are kept at their targeted moisture content of pF 2.5 throughout the study.
- A soil selection below 50 grams should be avoided for definitive testing due to the decline of microbial biomass. However the overall mineralization rate to $^{14}CO_2$ in the smallest aliquot size was still similar to the other soil aliquot sizes.
- Microbial biomass remained relatively high (near or above 1% OC) for both the 50 and 100 gram soil sample sizes through the end of the 122 day study.
- Reasons for the decline of microbial biomass in the 10-g samples are not completely clear but may be due to less mass of natural soil nutrients or increased surface area for potential moisture loss.
- Based on the biodegradation results of this study, microbial speciation may be as important or more important than overall microbial biomass quantity since biodegradation results are similar across soil aliquot sizes.

Reference

Vance et al., 1987. An extraction for measuring soil microbial biomass C. Soil Biol Biochem. 19 (1987) 703-707.

Acknowledgments

The authors would like to acknowledge Hope Aubin for assisting in the development of the poster layout and printing.