

Abstract

The OECD 307 Guideline 'Aerobic and Anaerobic Transformation in Soil' and the EPA Guideline OCSPP 835.4100 'Aerobic Soil Metabolism' outline the procedures to 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 aliquot 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 [¹⁴C]atrazine 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 [¹⁴C]atrazine as the test substance at 1 mg/Kg. Three soil sample aliquot sizes (10g, 50g and 100g) were used to evaluate the rate of [¹⁴C]atrazine degradation and the population size of the microbial biomass estimates in each of the sample sizes.

Soil: Two soils characterized as a Clay Loam (named DU) and a Loamy Sand (named RMN) were used and further characteristics are presented below.



Volatile and CO₂ Trapping solutions: Ethylene glycol used for volatile organics and 1 N KOH trapping solution used for CO₂.

Aeration: Hydrated air delivered under negative pressure at approximately 1 bubble per second.

Temperature: 22 +/- 2°C

Sampling and Analysis: Duplicate soil samples per soil, sacrificed at time zero and on approximately days 14, 30, 60 and 120. Soils were extracted twice with acetonitrile:water (90:10, v:v) and again with acetonitrile: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 (with the NAFTA option) was used to determine the rate constants.

Soil ID	Soil Type	pH	%Sand	%Silt	%Clay	%OC
DU	Clay Loam	5.1-5.4	44	26	30	3
RMN	Loamy Sand	4.8-5.3	86	6	8	0.8

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
DU	clay loam	0.6	0.5	0.2	1.2	1.0	0.8	1.4	1.0	0.8
RMN	loamy sand	0.9	0.3	0.3	0.8	1.0	1.0	0.8	1.1	1.1

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
DU	clay loam	18.8	13.8	5.5	36.8	26.2	21.4	41.4	27.5	22.6
RMN	loamy sand	7.8	2.8	2.4	6.5	8.8	8.4	6.5	9.7	9.4

Rate of [¹⁴ C]atrazine decline, %AR			
DU- soil	10g	50g	100g
Day	atrazine	atrazine	atrazine
0	89.6	97.0	92.4
14	68.4	71.7	74.4
32	48.5	55.2	52.6
63	45.0	29.3	35.5
122	20.1	17.5	20.3
SFO DT50	56.1	41.6	47.2

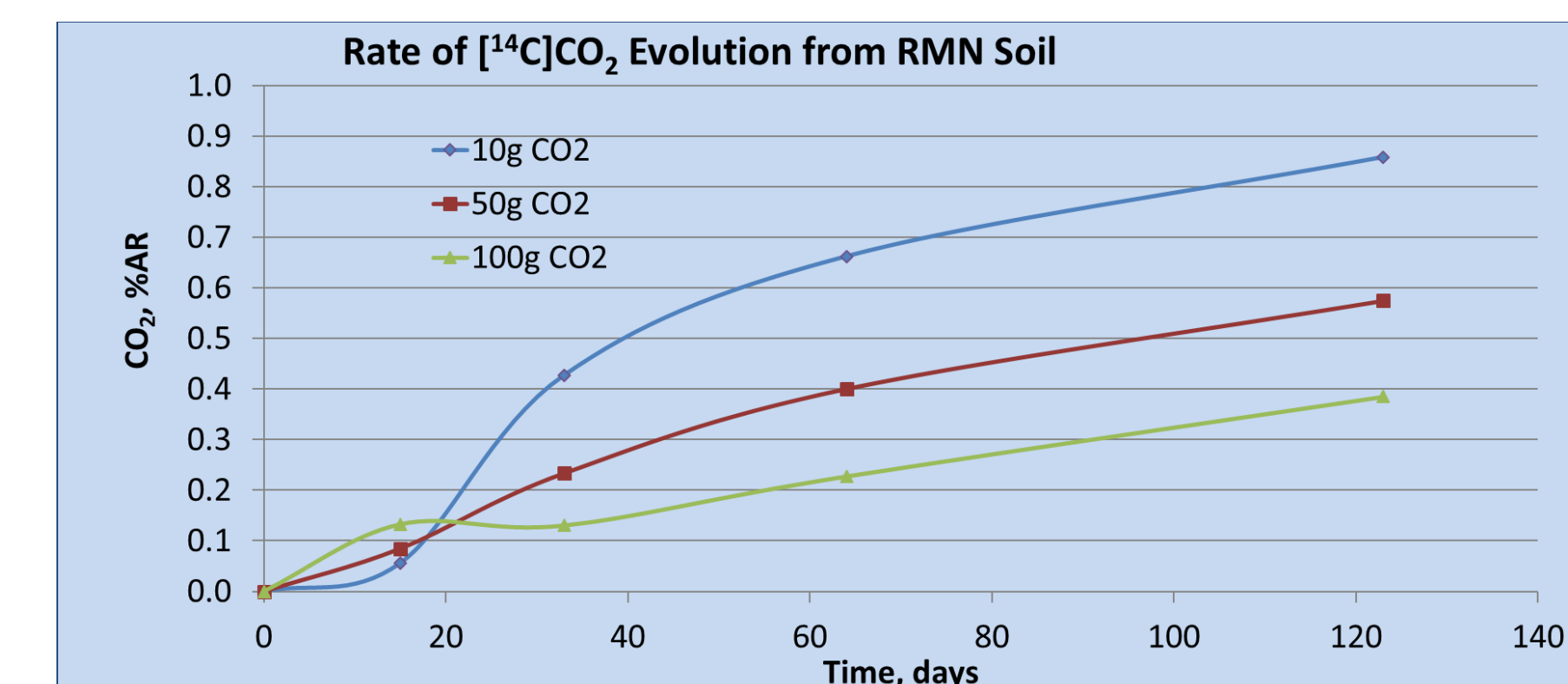
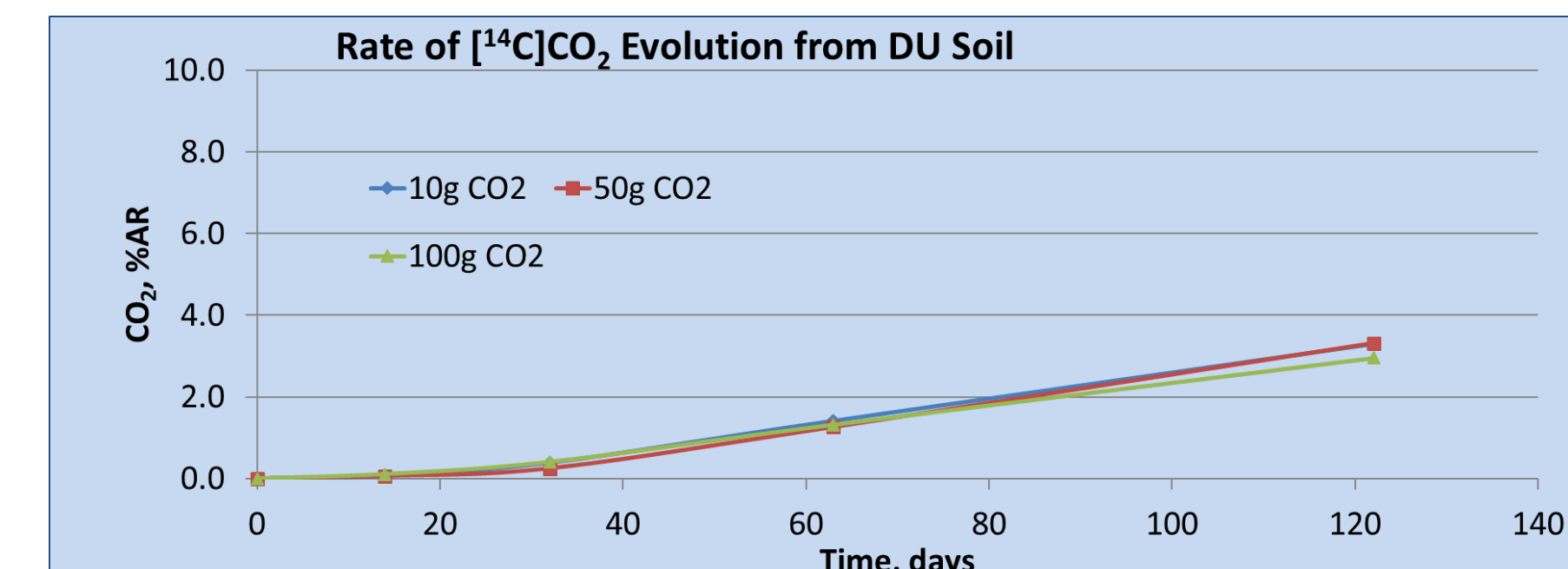
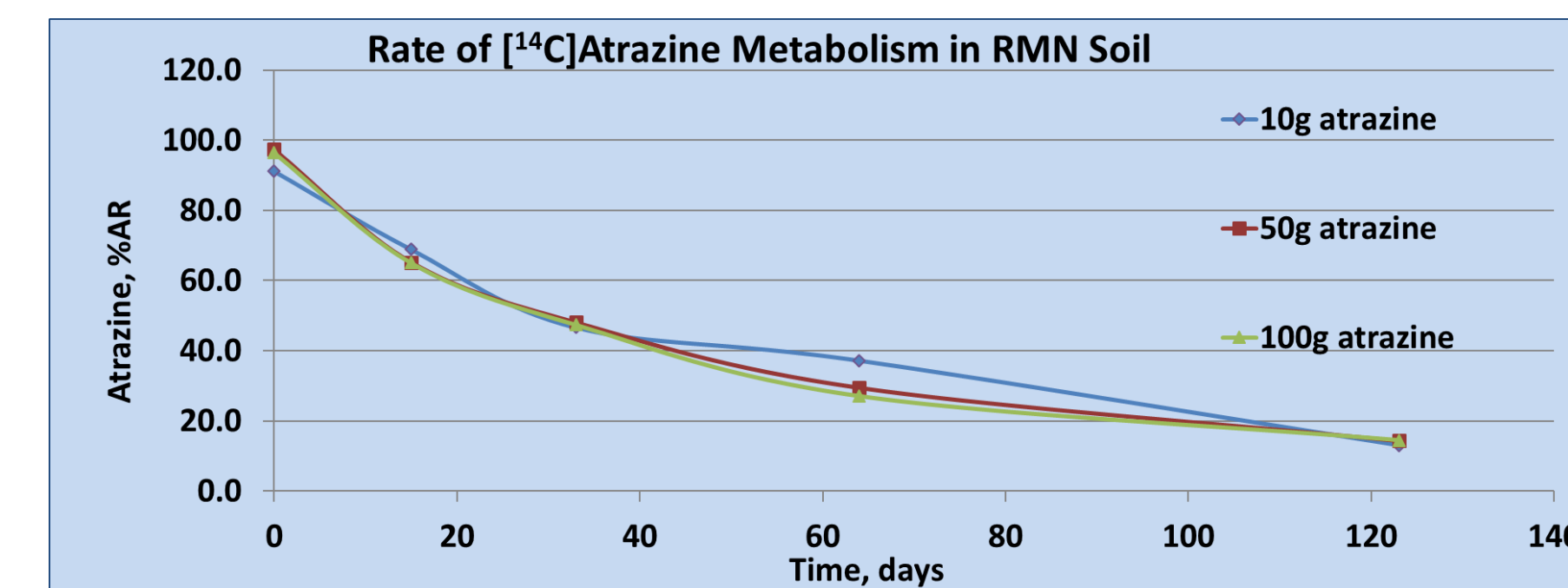
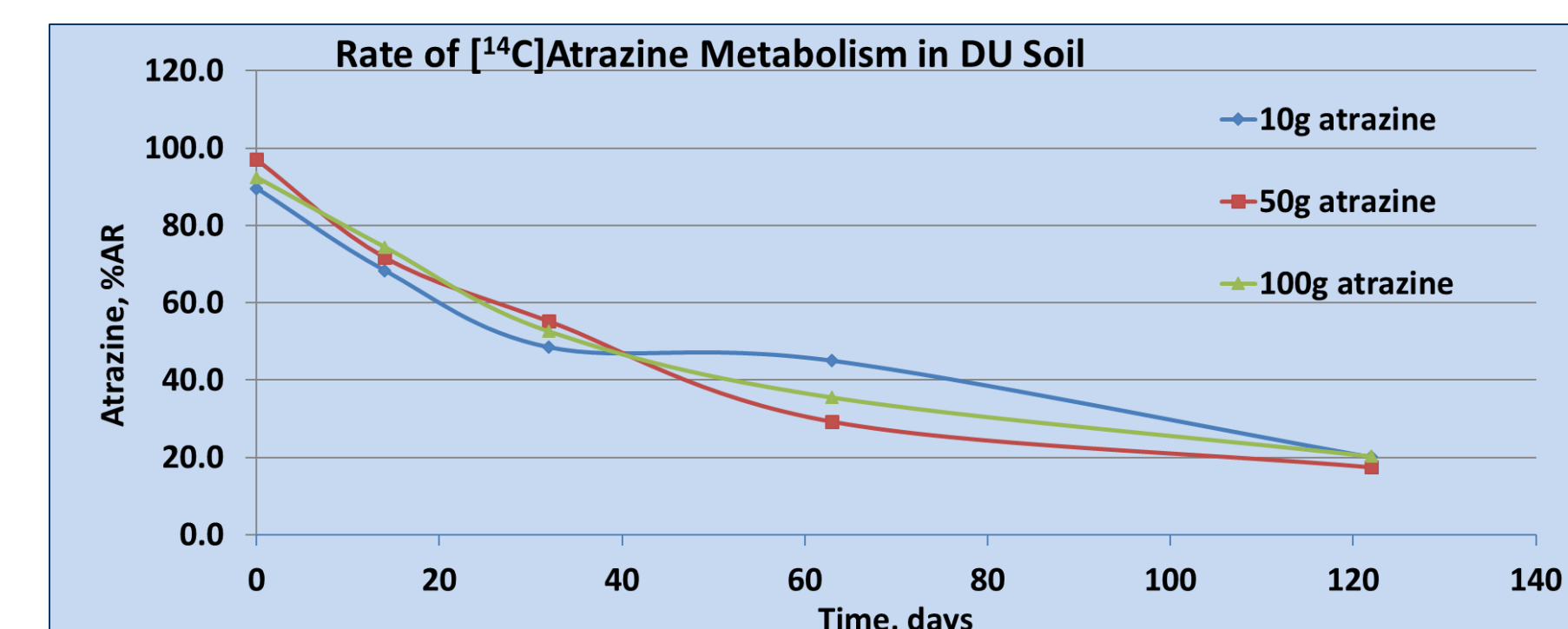
Rate of [¹⁴ C]atrazine decline, %AR			
RMN- soil	10g	50g	100g
Day	atrazine	atrazine	atrazine
0	91.2	97.4	96.5
15	68.9	65.2	65.0
33	46.6	48.1	47.4
64	37.2	29.5	27.1
123	13.0	14.3	14.5
SFO DT50	44.2	36.6	35.4

Rate of [¹⁴ C]CO ₂ Evolution, %AR			
DU- soil	10g	50g	100g
Day	CO ₂	CO ₂	CO ₂
0	NA	NA	NA
14	0.1	0.1	0.1
32	0.4	0.3	0.4
63	1.4	1.3	1.3
122	3.3	3.3	3.0

Rate of [¹⁴ C]CO ₂ Evolution, %AR			
RMN- soil	10g	50g	100g
Day	CO ₂	CO ₂	CO ₂
0	NA	NA	NA
15	0.1	0.1	0.1
33	0.4	0.2	0.1
64	0.7	0.4	0.2
123	0.9	0.6	0.4

Results and Discussion

- Material balance was maintained between 93% and 103% for both soils at all three soil sizes. Extractability was approximately 50% and was relatively independent of sample size. Additional non-polar solvents provided little to no additional extractable radioactivity.
- Although not a major metabolite, CO₂ in the DU soil with higher organic carbon content and overall higher microbial biomass based on mass than the other soil, produced a higher percentage of CO₂ evolution over the RMN soil which has a lower organic carbon content and lower microbial biomass.
- The major metabolite of atrazine was a polar metabolite with a retention time of 3 minutes and is still under investigation.
- The end 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 biodegradation rates were similar for all three soil sizes for both soil types. The 10 gram soil sized showed a slow down in atrazine degradation in the middle of the study, but then appeared to catch-up by the end of the study. There was little to no difference between the rates of atrazine degradation for the 50 and 100 gram sample sizes.
 - Although a minor formation in this study, the rate of CO₂ evolution was equal to or higher in the 10 gram sample than the 50 or 100 gram sample sizes.



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 CO₂ 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. However the overall half-life results based on CAKE kinetic software (with the SFO model) showed the 10 gram soil size were within approximately 20% of the other soil sizes.
- Microbial biomass remained relatively high (near or above 1% OC) for both the 50 and 100 gram soil sample sizes.

Reference

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

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