

## DEGRADATION KINETICS ASSESSMENT FOR THE FUNGICIDE BAS 505 IN INTACT SOIL CORES VERSUS BATCH SOILS

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Field degradation rates of pesticides are often different than determined in the laboratory using homogeneous soil. This project developed an intact soil core method for determining aerobic degradation rate that is intended to address such discrepancies. The fungicide BAS 505 (phenyl-<sup>14</sup>C-labeled) [*N*-methyl-(*E*)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl)acetamide] was applied to surface 0–7.5 cm of Ruston (fine loamy, siliceous, thermic Typic Paleudults) soil cores (triplicate and duplicate series) and homogeneous (batch) soil in biometer flasks (triplicate). Recovery was measured 12 times over the 360-day incubation. Mineralization rate in cores was initially slower than in batch soil but increased to give more <sup>14</sup>CO<sub>2</sub> lost by Day 360 (11% and 8% of applied, respectively). Unextractable <sup>14</sup>C was 21% in cores, similar to that for batch soil (18%). Recovery of BAS 505 in combined MeOH and MeOH–water extracts (HPLC–LSC analysis) after 360 days averaged 36% from cores and 57% from batch soil. Degradation rate in both systems decreased over time and could be described by *N*th order kinetics but not first order. Recoveries of BAS 505 by 360 days were lower in cores, indicating faster degradation than in batch soil after long-term incubation. Lack of nutrient inputs may account for decreasing degradation rates; however, decreasing microbial activity with time was not shown by the highly variable biomass C data. Faster degradation in cores may have been due to higher microbial populations/nutrient levels in the surface soil. (Soil Science 2006;171:239–248)

**Key words:** Fungicides, degradation kinetics, intact cores.

**T**HE fate of pesticides and their susceptibility to transport may affect soil and water quality. Consequently, registration of pesticides is subject to strict environmental testing. Early fungicides were based on heavy metals (mercury, copper) or organochlorine compounds, therefore posing environmental and health risks. Since 1970, systemic fungicides such as benomyl [[1-[(butylamino)carbonyl]-1*H*-benzimidazol-2-yl] carbamic acid methyl ester] and thiabendazole

[2-(4-thiazolyl)-1*H*-benzimidazole] have been developed for control of turf, agronomic, and horticultural fungal diseases (Marsh, 1977; Jackson, 2003). Recently, BASF developed BAS 505 [*N*-methyl-(*E*)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl)acetamide] for fungal disease control in turf and cereal. Environmental fate data on BAS 505 generated for registration showed rapid dissipation in the field but slower degradation using homogeneous soil. These results are similar to the findings of several studies in which pesticide degradation was alternatively measured using homogeneous soil in batch systems and using heterogeneous, intact soil cores.

For example, studies by Moorman and Harper (1989) and Locke and Harper (1991) on metribuzin [4-amino-6-(1,1-dimethyl-ethyl)-3-(methylthio)-1,2,4-triazin-5(4)-one] degradation in homogeneous Dundee soil (fine-silty, mixed,

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thermic Aeric Ochraqualfs) found that degradation was best described using nonlinear kinetics. But a later study using intact soil cores subject to simulated rainfall suggested that degradation followed first order kinetics (Locke et al., 1994).

Similarly, Gaston et al. (1996) found that bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3)-one 2,2 dioxide] degradation in homogenous Dundee soil was nonlinear and best described as a two-compartment process with compartments corresponding to inter-aggregate (well-aerated) and intra-aggregate (poorly aerated) pore space. But degradation during unsaturated water flow through intact cores of Dundee soil was adequately described by a single first order model (Gaston and Locke, 1996) and faster than in homogeneous soil because bentazon residence was largely restricted to the better aerated, water conducting region.

In general, aeration within soil cores is poorer than biometer flasks (Bartha and Pramer, 1965) commonly used to measure pesticide degradation in soil. Aeration in the former depends on gas exchange at the soil surface, aided by infiltration of well-oxygenated water. Therefore, the ratio of surface area for gas exchange to soil volume in cores is smaller than the artificially large ratio in flasks containing 1–2 cm of soil. Thus, for compounds that are degraded faster under relatively anoxic conditions, degradation in soil cores may be more rapid. For example, since acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid] is degraded faster under anaerobic conditions (Andreoni et al., 1994), it was not surprising that Gaston and Locke (2000) found ~10 times faster degradation in soil cores than in flasks.

Such disparity of results poses a dilemma. Geometric fidelity of intact soil cores to field soil suggests that this is a better model than homogeneous soil. Not only is aeration status likely a better match with field conditions and soil solution flow possible, soil cores preserve the small-scale biological and chemical spatial variability (Gaston and Locke, 2002), which may affect pesticide fate. On the other hand, batch methods are more convenient.

Thus, the primary objectives of this project were to (1) refine experimental apparatus and protocol for the intact soil core method of studying pesticide degradation using the fungicide BAS 505 as the test compound, and (2) simplify this methodology for use as a routine complement to batch degradation studies. The extent of core replication needed for adequate

precision and the effect of BAS 505 on microbial populations were also examined.

## MATERIALS AND METHODS

### *Bulk Soil and Intact Soil Cores*

Surface 0–7.5 cm of Ruston series (fine-loamy, siliceous, thermic Typic Paleudult) soil from a mowed Bermuda grass [*Cynodon dactylon* (L.) Pers.] lawn at the LSU AgCenter Calhoun Research Station, Calhoun, Louisiana, was used. Random cores (7.5-cm diameter by 7.5-cm depth) were taken from a 2 × 2 m hill side-slope area. Soil in cores was later combined, thoroughly mixed, and stored at 4 °C pending the degradation study.

In the 2 × 2 m area, 96+ intact cores (0–7.5 cm) were taken. These were sealed in airtight wrapping and placed in padded boxes to avoid jarring during transport. Grass stems and thatch were later removed from the soil surface, soil protruding from the bottom of cores was cut flush, and cores were weighed to determine net mass of field-moist soil. Bottoms of cores were fitted with a Teflon end disk. These were re-wrapped and stored at 4 °C pending the degradation study. Three additional cores were taken with a 5-cm-diameter coring device for soil bulk density.

### *Test Compound, Reagent Chemicals, and Solvents*

Radio-labeled (phenyl-<sup>14</sup>C) BAS 505 [N-methyl-(*E*)-2-methoxyamino-2-(2-((2,5-dimethylphenoxy)methyl)phenyl)acetamide] (95% purity, total activity 96.2 MBq) was provided by BASF Corp., Research Triangle Park, North Carolina. The major impurity was the (*Z*) isomer of BAS 505. All chemicals were reagent grade and chromatography solvents were HPLC grade. Scinti-Safe Plus 50% scintillation cocktail (Fisher) was used for LSC except for combusted soil samples (Carbo Sorb/Permafluor, Perkins Elmer) and HPLC–LSC analysis (In-Flow BD, IN US Systems, Inc.).

### *Soil Characterization*

Triplicate sub-samples of bulk soil were used to determine field gravimetric water content, pH (2:1, water–soil), organic C (Nelson and Sommers, 1982), CEC (sum of basic and acidic cations; 1 N of NH<sub>4</sub>OAc and BaCl<sub>2</sub>-TEA extractions, respectively), texture (Gee and Bauder, 1986), and microbial biomass C (Vance et al., 1987). Volumetric water content at 0.75 field capacity (–33 kPa) was estimated using

measured texture and bulk density via pedo-transfer function parameters given in Wosten and van Genuchten (1988). Soil characterization data are presented in Table 1.

#### Preparation of BAS 505 Application Solution

Radio-labeled material was reconstituted in 40.0 mL of acetonitrile (ACN). Activity consistent with BASF data was confirmed using LSC (Beckman Instruments, Inc. LS Analyzer, Model LS6KLL). A portion of the reconstituted BAS 505 was diluted in more ACN to produce  $54.04 \mu\text{g mL}^{-1}$ , which if applied in 100  $\mu\text{L}$  to 20 g of soil in the biometer flasks would equal a rate of 0.28 kg a.i.  $\text{ha}^{-1}$ . This concentration assumed that 20.0 g to 7.5 cm depth of soil represented a field surface area of 1.93  $\text{cm}^2$  (based on measured bulk density).

#### Biometer Flask Experiment

Twenty grams (oven-dry equivalent mass) of soil were transferred to each of 33 biometer flasks and three 250-mL Nalgene centrifuge bottles. One hundred microliters of  $54.04 \mu\text{g mL}^{-1}$  BAS 505 was applied drop-wise to the soil, followed by sufficient water to achieve 75% of field capacity, and the soil thoroughly mixed with a stainless steel spatula. Weights of flasks with treated soil were recorded. The dosed soil in centrifuge bottles was immediately extracted as described below to determine recovery of  $^{14}\text{C}$  and BAS 505. Ten milliliters of 1 N of NaOH was added to flask sidearms, flasks stoppered and set in a dark, temperature-controlled room (23 °C).

#### Extraction Methodology and LSC for $^{14}\text{C}$

Soil in biometer flasks was removed after 2, 5, 7, 14, 30, 60, 90, 120, 180, 270, and 360 days

of incubation and transferred to 250-mL Nalgene centrifuge bottles for extraction (Day 0 samples were in centrifuge bottles from outset). Samples were extracted with MeOH ( $\sim 30 \text{ mL} \times 2$  times), followed by MeOH-water, 1:1 volume basis ( $\sim 30 \text{ mL} \times 2$  times; for recovery of more polar degradation products). Soil suspensions were shaken (325 r.p.m.) at room temperature in the dark for 1 h, centrifuged, and supernatants decanted into glass bottles (Teflon-lined caps; separate bottles for MeOH and MeOH-water extracts). Mass of extract and entrained MeOH-water solution were recorded. The latter was evaporated, dry soil removed from the centrifuge bottle and ground before analysis for unextractable  $^{14}\text{C}$  (combustion/LSC, described below). Duplicate 1-mL aliquots of MeOH and MeOH-water extracts were analyzed by LSC. The MeOH extracts were concentrated by rotary evaporation. The MeOH-water extracts were similarly concentrated to remove MeOH, then diluted with 0.01 N of  $\text{CaCl}_2$  to  $\sim 100 \text{ mL}$ , and this solution passed through conditioned  $\text{C}_{18}$  solid phase extraction (SPE) columns (MeOH followed by 0.01 N of  $\text{CaCl}_2$ ). The BAS 505 and any metabolites retained were eluted with 2.5 mL of MeOH. Duplicate 0.25-mL aliquots of concentrated extracts were analyzed by LSC for possible losses during processing. Also, 1-mL aliquots of effluent from the  $\text{C}_{18}$  columns were analyzed to verify negligible loss in this step. All concentrated extracts were analyzed by HPLC-LSC as described below.

#### Mineralization and Maintenance

One-milliliter aliquots of NaOH in biometer flask sidearms were removed at 2, 5, and 7 days and weekly thereafter for LSC analysis of evolved  $^{14}\text{CO}_2$ . The NaOH was removed, weights of flasks were measured, and water was added as needed to restore 75% field capacity. Fresh NaOH was added, flasks stoppered and incubation continued. To ensure aerobic conditions, flasks were also briefly unstoppered at midweek.

#### Intact Core Experiment, Full

A randomly selected set of 36 cores (of 96+ taken; 3 replicates for each of the 12 incubation periods) were assigned to this experiment. Each was dosed with 2.36 mL of  $54.04 \mu\text{g mL}^{-1}$  BAS 505 to give an aerial application rate of 0.28 kg  $\text{ha}^{-1}$ . Sufficient water was then applied to the surface of each core to bring its average water content to 75% field capacity and mass of cores recorded.

TABLE 1

Characterization data for the Ruston soil

Textural class	Sandy loam
% Sand	62.6
% Silt	26.8
% Clay	10.6
Bulk density ( $\text{g cm}^{-3}$ )	$1.36 \pm 0.01$
pH (1:2, soil: $\text{H}_2\text{O}$ )	$5.38 \pm 0.05$
Organic C ( $\text{g kg}^{-1}$ )	$15.1 \pm 0.8$
CEC ( $\text{cmol (+) kg}^{-1}$ )	$7.91 \pm 0.48$
Moisture (1/3 bar, $\text{g H}_2\text{O g}^{-1}$ )	0.149
Microbial biomass C ( $\mu\text{g g}^{-1}$ )	$285 \pm 54$

Measurements other than for texture were done in triplicate. Field capacity was calculated as per Wosten and van Genuchten (1988).

Thirty-three cores were then placed in a 5-L capacity airtight container into which was also placed an open vial containing 20 mL of 1 N of NaOH as a  $^{14}\text{C}$  trap. Containers were sealed and incubated in the dark at 23 °C. The remaining three cores (Day 0 replicates) were sampled as described below immediately after application of BAS 505 and water.

After prescribed incubation, cores were removed from containers and two sub-samples (1.6-cm-diameter cores to the depth of soil in cores) removed, transferred to 250-mL Nalgene centrifuge bottles, and extracted as described for biometer flasks (except larger volumes of MeOH and MeOH-water, proportional to the greater mass of soil in the sub-cores). Processing of extracts and LSC analyses were as described above. Additional core sub-samples were taken for microbial biomass C (below). Remaining soil was crumbled, mixed, and preserved for  $^{14}\text{C}$  analysis by combustion. Mineralization was tracked as described above. Cores were watered as indicated by loss of mass. Containers were briefly opened midweek for re-aeration.

#### *Microbial Biomass C and Intact Cores, Untreated Controls*

Sub-core soil for microbial biomass C was crumbled, roots or other apparent organic debris removed, material mixed thoroughly, and 10 g (moist weight) of sub-samples transferred to two 50-mL Erlenmeyer flasks for microbial biomass C analysis by the chloroform fumigation method (Vance et al., 1987).

An additional 36 randomly assigned cores were amended with 2.36 mL BAS 505-free ACN and maintained identically to the above triplicate intact core series with respect to replenishing lost water and ventilating containers twice weekly. Sub-cores were taken when treated cores were sampled and these analyzed for microbial biomass C.

#### *Intact Core Experiment, Abridged*

This parallel experiment was conducted identically to and concurrent with the triplicate intact core experiment described above; however, it included only two replicates for each sampling time (24 randomly assigned cores).

#### *Sorption/Desorption*

Five-gram (oven-dry equivalent) samples of air-dry 0–7.5 cm depth soil were placed in 50-mL Pyrex centrifuge tubes. Fifteen-milliliters of 0.1,

1, 2, 5, or 10  $\mu\text{M}$  solutions of  $^{14}\text{C}$  BAS 505 in 0.01 N of  $\text{CaCl}_2$  background (< 1% ACN) was added to centrifuge tubes in triplicate. Another triplicate set of these solutions was added to tubes without soil to measure sorption, if any, onto tubes. Suspensions and blanks were shaken 24 h. Soil solution was separated from suspension by centrifuging (10 min at 3000g). Sorption was calculated from change in solution concentration of  $^{14}\text{C}$ , corrected for blank sorption.

The reversibility of sorption was determined by measuring release of BAS 505 sorbed from 10- $\mu\text{M}$  input samples after 24 h of equilibration by sequential extractions with 0.01 N of  $\text{CaCl}_2$  for 1.5, 4.5, then 18 h of shaking times.

#### *Analyses*

Concentrated MeOH and MeOH-water extract (SPE) samples were analyzed by HPLC, with LSC detection (IN US Systems,  $\beta$ -Ram). The HPLC column used was a Phenomenex Ultracarb 5ODS (30), 250  $\times$  4.6 mm i.d. Solvents were (i) water with 0.05% formic acid and (ii) ACN with 0.05% formic acid. Each run consisted of a linear gradient from 95:5 water-ACN to 5:95 water-ACN over 60 min, followed by constant 5:95 water-ACN for 5 min, then return to 95:5 water-ACN over 10 min for 75 min of run time. The column temperature was 30 °C. Preliminary quantification was performed using  $\beta$ -Ram detector software. Background noise was subtracted and fraction of total radioactivity attributable to BAS 505 determined. In turn, this fraction was multiplied by radioactivity in the sample determined by separate LSC analysis. Radioactivity due to any other discernable peaks was similarly quantified.

Duplicate 0.3-g sub-samples of residual soil from extraction bottles, with added 0.3 g of cellulose, were oxidized (Packard Oxidizer 306, United Technologies Packard) to quantify unextractable  $^{14}\text{C}$ . Liberated  $^{14}\text{C}$  was trapped and analyzed by LSC (Davidson et al., 1970).

Total recovery of  $^{14}\text{C}$  for biometer flask soil was the sum of radioactivity in MeOH extract, MeOH-water extract, liberated on combustion, and evolved as  $^{14}\text{CO}_2$ . Total recovery for core soil was the sum of  $^{14}\text{C}$  in the extraction sub-sample (that in MeOH extract, MeOH-water extract, and liberated on combustion) + that estimated for the microbial biomass C sub-sample (assumed proportional to recovery from the extraction sub-sample) + radioactivity in remaining soil (determined by combustion of

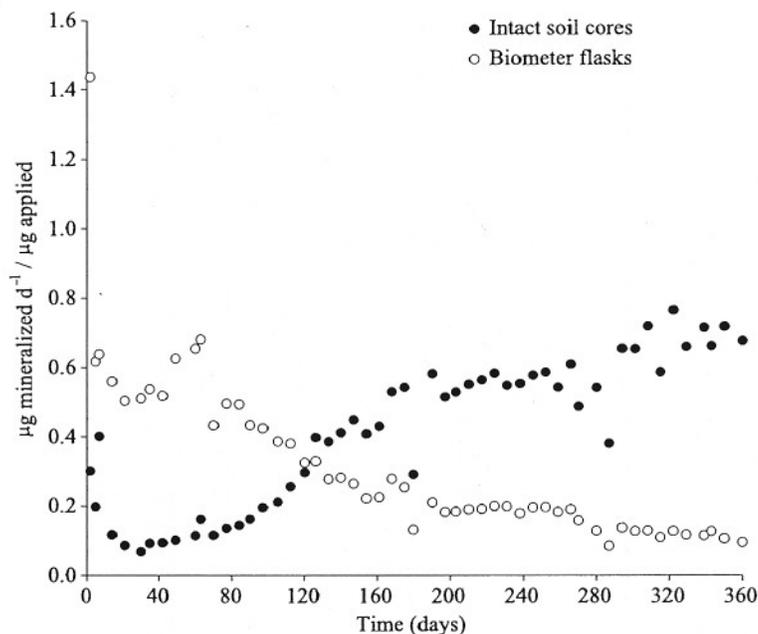


Fig. 1. Mineralization rates of BAS 505 in batch and intact soils (triplicate and duplicate series combined).

triplicate samples) + evolved  $^{14}\text{CO}_2$  trapped in NaOH.

*Modeling and Statistical Analyses*

The degradation rate of BAS 505 was modeled by first order degradation kinetics,  $M = M_0 \exp(-kt)$ , where  $M$  is mass of BAS 505 recovered ( $\mu\text{mole}$ ),  $M_0$  is mass applied,  $t$  is time (days), and  $k$  is degradation rate constant ( $\text{d}^{-1}$ ). The power rate or  $N$ th order kinetic model (Hamaker, 1972),  $M = [M_0^{(1-N)} +$

$(N - 1)kt]^{(1/(1-N))}$ , where  $N$  is order (dimensionless), was also considered.

Sorption was described with Freundlich and linear models. The Freundlich is  $S = K_f C^N$ , where  $S$  is sorbed concentration ( $\mu\text{mol kg}^{-1}$ ),  $C$  is solution concentration ( $\mu\text{mol L}^{-1}$ ), and  $K_f$  and  $N$  are constants. The linear model is  $S = K_d C$ , where  $K_d$  ( $\text{L kg}^{-1}$ ) is the sorption coefficient.

The ANOVA and curve fitting were done with SAS (SAS Institute, Inc., 1996).

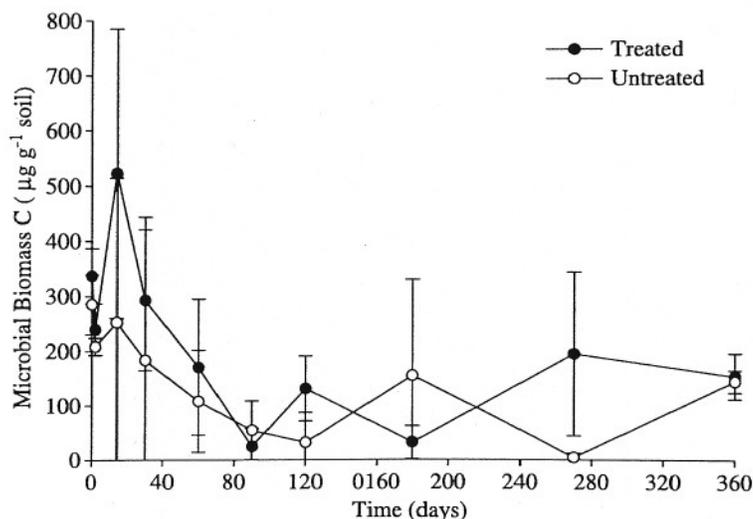


Fig. 2. Comparison of microbial biomass C in cores of Ruston surface soil with and without treatment with BAS 505 ( $0.28 \text{ kg ha}^{-1}$ ). Means for five treated and three untreated cores are shown.

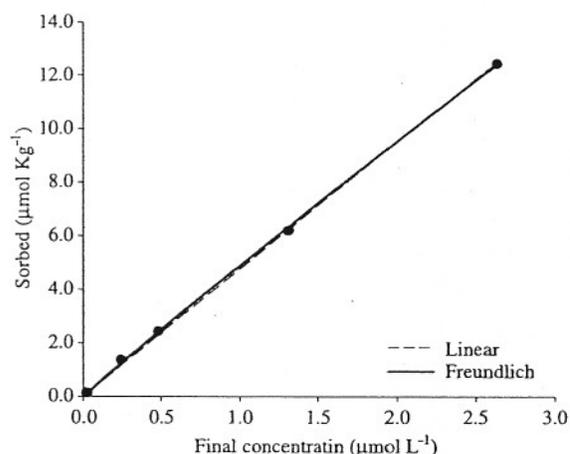


Fig. 3. BAS 505 sorption described by the Freundlich and linear models. The Freundlich model is  $S = 4.89C^{0.961}$  ( $R^2 = 0.999$ ) and the linear model is  $S = 4.74C$  ( $R^2 = 0.999$ ).

## RESULTS AND DISCUSSION

### Mineralization

Mineralization rates of BAS 505 from batch and intact soils are shown in Fig. 1. The rate of mineralization is the fraction of total applied  $^{14}\text{C}$  that was evolved as  $^{14}\text{CO}_2$  during any sampling interval divided by the length of that interval. Data are averages for all experimental units at any time (i.e., decreasing from 33 biometer flasks at Days 2–3 flasks beyond Day 270; or decreasing from 57 cores to 5 cores, the full and abridged sets combined). The initial rate over the 0–2 days of incubation for the batch soil ( $0.00144 \mu\text{g}$  mineralized  $\text{d}^{-1}/\mu\text{g}$  applied) was nearly five times that for the cores ( $0.00030 \mu\text{g}$  mineralized  $\text{d}^{-1}/\mu\text{g}$  applied). This contrasting behavior has been seen before (Gaston and Locke, 1996, 2000) and was likely due to the comparatively large surface area to mass ratio for soil in the biometer flasks compared to the soil cores. Mineralization rate for the batch soil decreased sharply at first and then gradually over the course of the study (Fig. 1), whereas that for cores decreased sharply at first but then increased to a nearly constant rate of  $^{14}\text{CO}_2$  loss that was much greater than for the batch soil. At Day 360, batch soil had a mineralization rate of  $0.00009 \text{ d}^{-1}$  compared to  $0.00068 \text{ d}^{-1}$ . However, integration of the curves (Fig. 1) over the study gave similar fractions of applied  $^{14}\text{C}$  mineralized ( $\sim 0.08$  for batch soil and  $\sim 0.11$  for cores). Inasmuch as mineralization is complete degradation, a higher rate of mineralization in cores than batch soil suggests different kinetics and more complete degradation in the cores.

### Microbial Biomass C

A reasonable hypothesis was that soil treated with BAS 505 would have significantly lower microbial biomass than untreated soil, considering its fungitoxic nature. However, variability among replicates was large and differences in biomass C were not statistically significant (Fig. 2). Also, the apparent decrease with time in microbial biomass C in the treated soil was not significant.

### Sorption Study

BAS 505 sorption data (Fig. 3) were described using a Freundlich isotherm with best-fit estimates of  $K_f = 4.89 \pm 0.08$  and  $N = 0.96 \pm 0.02$ . Thus, BAS 505 was sorbed essentially in direct proportion to its solution concentration ( $N = 1$ ). The best-fit linear model gave an  $R^2$  of 0.999 with  $K_d = 4.74 \pm 0.16$ . Such large extent of sorption might affect BAS 505 degradation rate if there is a difference in degradation rates between solution and sorbed phases, and desorption was slow (Zablotowicz et al., 2000). However, data for desorption of BAS 505 by sequential extraction with 0.01 N of  $\text{CaCl}_2$  (Table 2) showed little kinetic limitation. Desorbed BAS 505 nearly equaled that predicted assuming instantaneous equilibrium described by  $K_d$ .

### Extractable, Unextractable, Mineralized, and Total Recoveries of $^{14}\text{C}$

Total recoveries of  $^{14}\text{C}$ , based on recoveries in the various fractions (MeOH and MeOH-water extracts,  $^{14}\text{CO}_2$  mineralized, and as unextractable  $^{14}\text{C}$ ) are summarized in Fig. 4. Recoveries in the extractable and unextractable pools were determined by taking the proportions recovered as  $^{14}\text{C}$  from sub-samples to represent the situation for the whole core, then multiplying by the total  $^{14}\text{C}$  recovered, less that

TABLE 2  
Desorption of BAS 505 that was sorbed during a 24-h equilibration

Extraction No.	Time (h)	Fraction desorbed		
		Per extraction	Cumulative	Cumulative predicted
1	1.5	$0.55 \pm 0.02$	$0.55 \pm 0.02$	0.62
2	4.5	$0.28 \pm 0.01$	$0.83 \pm 0.03$	0.87
3	18.0	$0.14 \pm 0.01$	$0.97 \pm 0.03$	0.97

Stepwise and cumulative fractions desorbed by sequential extractions and cumulative predicted desorbed (linear, instantaneous equilibrium) are given.

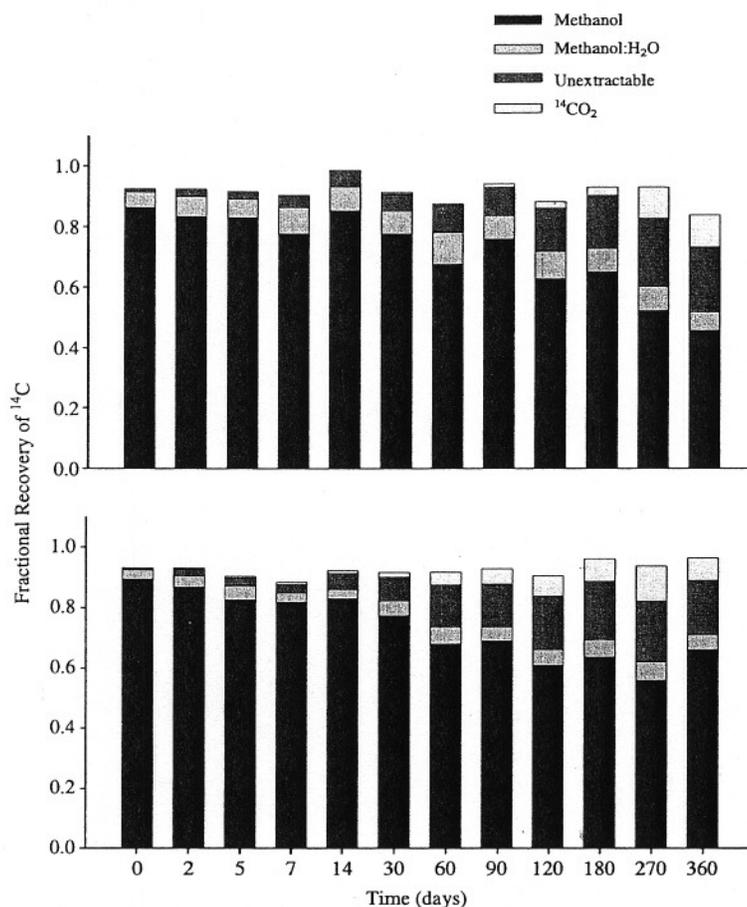


Fig. 4. Recovery of <sup>14</sup>C in indicated pools from intact cores (top) and batch soils (bottom). Combined means for triplicate and duplicate intact core series shown.

evolved as <sup>14</sup>CO<sub>2</sub>, then dividing by the total <sup>14</sup>C applied. Total recoveries of <sup>14</sup>C were acceptably high, averaging 92% and 93% for cores and

biometer flasks, respectively, with no trend toward decreasing recovery with time. Methanol extraction accounted for ~87–90% recovery of

TABLE 3  
Fractional recoveries of BAS 505 (μg recovered/applied) from cores and batch soil in MeOH and MeOH–water extracts (data combined)

Time (days)	Triplicate cores	Duplicate cores	All cores <sup>†</sup>	Batch soils
0	0.944 ± 0.092 <sup>‡</sup>	0.875 ± 0.067 <sup>a</sup>	0.917 ± 0.083 <sup>a</sup>	0.927 ± 0.018 <sup>a</sup>
2	0.934 ± 0.072 <sup>a</sup>	0.849 ± 0.014 <sup>a</sup>	0.900 ± 0.070 <sup>a</sup>	0.875 ± 0.008 <sup>a</sup>
5	0.853 ± 0.157 <sup>a</sup>	0.760 ± 0.008 <sup>a</sup>	0.816 ± 0.122 <sup>a</sup>	0.782 ± 0.007 <sup>a</sup>
7	0.765 ± 0.025 <sup>a</sup>	0.730 ± 0.002 <sup>a</sup>	0.751 ± 0.027 <sup>a</sup>	0.768 ± 0.035 <sup>a</sup>
14	0.785 ± 0.024 <sup>a</sup>	0.758 ± 0.214 <sup>a</sup>	0.774 ± 0.109 <sup>a</sup>	0.781 ± 0.006 <sup>a</sup>
30	0.665 ± 0.059 <sup>a</sup>	0.718 ± 0.045 <sup>a</sup>	0.686 ± 0.056 <sup>a</sup>	0.727 ± 0.009 <sup>a</sup>
60	0.583 ± 0.021 <sup>a</sup>	0.555 ± 0.010 <sup>a</sup>	0.572 ± 0.022 <sup>a</sup>	0.582 ± 0.015 <sup>a</sup>
90	0.637 ± 0.019 <sup>a</sup>	0.617 ± 0.034 <sup>a</sup>	0.629 ± 0.024 <sup>a</sup>	0.635 ± 0.061 <sup>a</sup>
120	0.511 ± 0.096 <sup>a</sup>	0.601 ± 0.030 <sup>a</sup>	0.556 ± 0.078 <sup>a</sup>	0.536 ± 0.016 <sup>a</sup>
180	0.510 ± 0.072 <sup>a</sup>	0.574 ± 0.043 <sup>a</sup>	0.535 ± 0.066 <sup>a</sup>	0.524 ± 0.098 <sup>a</sup>
270	0.438 ± 0.105 <sup>a</sup>	0.395 ± 0.132 <sup>a</sup>	0.421 ± 0.102 <sup>a</sup>	0.527 ± 0.046 <sup>a</sup>
360	0.366 ± 0.071 <sup>b</sup>	0.357 ± 0.022 <sup>b</sup>	0.362 ± 0.043 <sup>b</sup>	0.573 ± 0.095 <sup>a</sup>

<sup>†</sup>Combined mean for triplicate and duplicate intact core series.

<sup>‡</sup>Within a row, means followed by the same letter are not significantly different (LSD α = 0.05)

the radiolabel at Day 0 for both batch and core soils, decreasing to ~66% and ~46% for batch and cores soils, respectively, at Day 360. A subsequent methanol-water extraction accounted for an additional 3–6% for batch and 5–10% for core soils throughout the study. Upon completion of the study, mineralization was 8% for batch soils and 11% for cores. Unextractable  $^{14}\text{C}$  for intact cores increased from approximately 1–21% over the year compared to <1–18% for batch soils. Unlike patterns in mineralization rates, there was little difference between batch and intact cores in development of unextractable  $^{14}\text{C}$ .

#### Degradation of BAS 505

There was little difference in variability among replicates from the triplicate and duplicate core series in recovery of BAS 505 (Table 3). Across all cores, recovery averaged 92% on Day 0. After one year, recovery averaged 36%. For the batch soil, BAS 505 recovery decreased from 93% to 57%. Comparison of extractable  $^{14}\text{C}$  (Fig. 4) with recovered parent (Table 3) shows

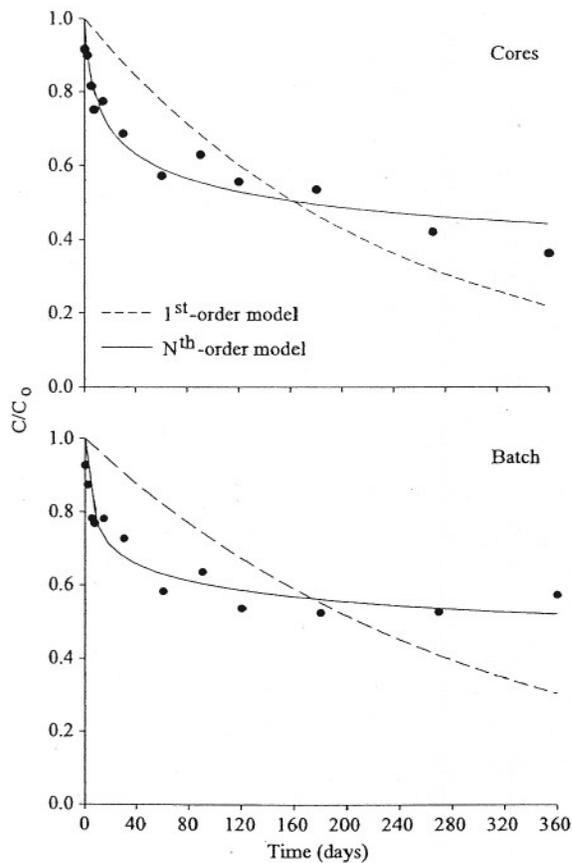


Fig. 5. Degradation of BAS 505 described by first and Nth order models for intact cores (top, triplicate, and duplicate series combined) and batch soil (bottom).

TABLE 4

First and Nth order degradation model parameters for 360 days of incubation and first order rate constants for 60 days of incubation

System	Incubation (days)	Model	$k$ ( $\text{d}^{-1}$ )	$N$
Batch	360	First order	$0.003 \pm 0.001$	—
	360	Nth order	$0.134 \pm 0.064$	$10.38 \pm 1.40$
Cores <sup>†</sup>	60	First order	$0.012 \pm 0.003$	—
	360	First order	$0.004 \pm 0.001$	—
	360	Nth order	$0.063 \pm 0.024$	$7.04 \pm 0.99$
	60	First order	$0.012 \pm 0.003$	—

<sup>†</sup>Triplicate and duplicate series combined.

that from Day 2, an increasing proportion of extractable  $^{14}\text{C}$  was due to compounds other than parent. However, distinct peaks in LSC chromatograms other than due to BAS 505 were not observed until after 120 days of incubation. Peaks included those between 29 and 32 min that correspond to retention times of BF 505-7 [(*E*)-*o*-[(2-hydroxy carbonyl-5-methyl)phenoxy-methyl]-2-methoxyimino-*N*-methylphenylacetamide] and BF 505-8 [(*E*)-*o*-[(5-hydroxycarbonyl-2-methyl)phenoxy-methyl]-2-methoxyimino-*N*-methylphenylacetamide], and at ~27–28 and ~36–37 min. The latter coelutes with methyl salicylate (which would form upon ether cleavage followed by oxidation and decarboxylation of BAS 505).

Based upon recovery data, the degradation rate of BAS 505 in cores was significantly greater than that in batch soil only by Day 360 (Table 2). Also, Fig. 5 shows that rather than first order kinetics, Nth order kinetics clearly better described BAS 505 degradation over the full course of the study for both intact core and batch soils (Table 4 gives model parameters). Data from the first 60 days, however, were consistent with those generated in registration studies. Deviation from first order kinetics/slow overall degradation and faster degradation in cores than batch soil by Day 360 warrant further consideration.

Slow desorption of the highly sorbed BAS 505 can be ruled out as possibly contributing to deviation from first order kinetics and slow overall degradation (see Table 2). Another possible explanation is decreasing microbial activity, particularly activity of BAS 505 degraders. But no trend in microbial biomass  $C$  with time could be established due to variability in the data (and organisms with a specific capacity to degrade BAS 505 were not identified

or enumerated). Nevertheless, lack of nutrient input through the soil surface with infiltrating water (absent in static systems) or to the living rhizosphere may have had a negative effect on microbial activity in these long-term laboratory incubations. On the other hand, decreasing degradation rate may simply reflect small-scale spatial variability in degradation rate (Gustafson and Holden, 1990), for which a continuum of spatially variable first order rate constants generates the type of nonlinearity seen in Fig. 5.

Regardless of why degradation of BAS 505 slowed over time, it was ultimately faster in cores than batch soil—an important result with respect to the objective of the study. Faster degradation in cores by Day 360 may be related to differences in microbial populations and organic matter substrates between cores and batch soil. Coarse organic matter (roots) was removed from the batch soil, depleting its C pool. But the physical disruption of the soil may have exposed otherwise protected substrate (Franzluebbers and Arshad, 1997). More importantly, BAS 505 was applied to the surface of cores, where both microbial populations and substrate levels were likely highest, but it was applied to homogenous 0–7.5 cm depth batch soil. Whatever advantage better aeration of the batch soil initially offered, lower average microbial populations and substrate levels in it may have limited the degradation of BAS 505 at longer times. In contrast, the highly sorbed BAS 505 remained near the soil surface in cores.

### CONCLUSIONS

In this study, an intact core methodology for measuring aerobic pesticide degradation was developed and the degradation rates of BAS 505 determined using this and conventional (batch) methodologies were compared. The extent of core replication needed for adequate precision was concurrently examined. Also, the effect of BAS 505 on microbial biomass was tracked over this yearlong project.

Based on BAS 505 recoveries, there was no difference in degradation rate between core (triplicate, duplicate, or combined) and batch systems until Day 360. Exposure of BAS 505 to likely higher microbial populations in cores (surface soil) compared with batch (homogenized 0–7.5 cm depth) soil is a possible explanation for long-term faster degradation in cores. Regardless of system, the degradation rate of BAS 505 in Ruston soil decreased with time.

This nonlinearity, described by  $n$ th order kinetics, may be an artifact of (static) incubation without nutrient re-supply to soil microorganisms. However, there was high variability in microbial biomass C data and no significant trend toward decreasing biomass with time.

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