Evaluation of Methods for Characterizing Carbofuran Hydrolysis in Soil

T. B. Parkin,* D. R. Shelton, and J. A. Robinson

ABSTRACT

The objective of this study was to develop a method that could be applied to investigations of soil/environmental factors influencing the spatial and temporal variability of carbofuran hydrolysis in field soils. Two factors were considered: (i) soil handling and pesticide application, and (ii) the method of data summarization. Three soil handling/pesticide treatments were evaluated: (i) sieved soil/sprayed pesticide application, (ii) injected pesticide application/sieved soil, and (iii) injected pesticide application/intact core incubation. This last method was developed to mimic field conditions where high localized concentrations of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) occur as a result of banded application of granular carbofuran at planting time. For all three treatments sigmoidal 14CO2 production kinetics were observed. Several mathematical models for describing sigmoidal product appearance data were evaluated and a general saturation model was found to yield the best fit. Using parameter estimates obtained from this model in statistical tests, we found that the intact core soil treatment yielded significantly longer half lives for carbofuran degradation. Additional experimental evidence suggests that soil structure of the intact cores retarded diffusion of the 14CO2 produced from carbofuran degradation and influenced the kinetic pattern observed.

Biodegradation has been thoroughly documented as a dominant fate of pesticides in soils. Biodegradation is generally considered to be desirable from both an environmental (pollution) perspective as well as an agricultural (carryover) view, as long as pesticide efficacy is not affected. In recent years, however, reduced efficacy has been reported for several pesticides including the insecticide carbofuran (2,3-dihydro-2,2dimethyl-7-benzofuranyl methylcarbamate). The underlying mechanism commonly cited as responsible for this loss of efficacy is the enhanced microbial degradation in soils that have received repeated applications of carbofuran. Such soils have been referred to as problem soils (Kaufman et al., 1985).

Carbofuran is a soil-incorporated insecticide used extensively, in the past, for control of the corn rootworm (Diabrotica sp.). Granular formulations of carbofuran are typically applied in the furrow at the time of planting and must persist for 30 to 60 d until the corn root worm larvae hatch (Felsot et al., 1985). Numerous studies including both field and laboratory incubations have documented rates of carbofuran degradation. Studies conducted prior to reports of loss of carbofuran efficacy describe apparent first-order rates of dissipation with reported half-lives ranging from 21 to 227 d, depending on soil type and incubation conditions (Caro et al., 1973; Getzin, 1973; Ou et al., 1982).

More recent studies have focused on comparisons of carbofuran degradation in problem soils and soils without a previous history of carbofuran application (nonproblem soils). In incubations of field soils, Felsot et al. (1981, 1982, 1985) observed first-order kinetics of carbofuran hydrolysis in soils with a history of carbofuran application; however, in soils without previous carbofuran exposure, a lag phase was observed prior to degradation. It was not possible to calculate half-lives in the nonhistory plots because of the apparent lag; thus, rates were summarized as DT-50%; the time required for 50% of carbofuran disappearance. Read (1983) observed an accelerated rate of degradation between the first and second carbofuran applications to a soil. In both instances, kinetics of carbofuran degradation appeared to be sigmoidal. Chapman et al. (1986) also observed an accelerated rate of carbofuran degradation in soil pretreated with carbofuran, with apparent first-order kinetics. Kaufman et al. (1985) observed a sigmoidal kinetic pattern of CO2 production resulting from the hydrolysis of carbofuran added to adapted or problem soils; however, zero-order kinetics were observed in nonproblem soils. Similar results have recently been observed by Turco and Konopka (1990).

Previous work has demonstrated that microbial growth in soil results in sigmoidal rates of degradation if physical/chemical mass transfer limitations do not control the overall rate (Focht and Shelton, 1987). Thus, the observation of sigmoidal kinetics is consistent with the hypothesis that the enhanced degradation of carbofuran observed in problem soils may be a direct result of microbial growth. Indeed, microorganisms capable of using carbofuran as a C and N source have been isolated from soil (Rajagopal et al., 1984; Karns et al., 1986; Ramanand et al., 1988; Chaudhry and Ali, 1988).

Studies of the spatial and temporal variability of carbofuran degradation activity in field soils require that a quantitative approach be adopted for the summarization of kinetic data. Mathematical modeling is a useful data summarization tool, and the first-order model has been utilized for this purpose (Ou et al., 1982). However, the first-order model is not applicable for summarizing sigmoidal kinetic data. Several mathematical relationships exist for describing sigmoidal data; however, quantitative analysis of sigmoidal carbofuran degradation data using mathematical models has only recently been applied (Scow et al., 1990).

The purpose of this study was to develop a protocol for performing large screening studies, which can be used to assess patterns of spatial and temporal variability of pesticide degradation activity in field soils. A primary requirement of such a protocol is that it must allow for the analysis of a large number of samples with minimum sample handling and preparation. Field studies are generally labor-intensive, and interpretations of field dissipation data is complicated by attenuation of kinetic data.


Abbreviations: MMF, Morgan-Mercer-Flodin; AIC, Akaike Information Criterion.
losses via other processes, such as volatilization and leaching. For this protocol development, carbofuran is used as a model compound. Since the primary pathway of carbofuran degradation is hydrolysis of the carbamate bond resulting in the formation of carbon dioxide, methylamine, and carbofuran phenol (Getzin, 1973), we chose to develop a laboratory method in which production of 14CO2 from soil samples amended with 14C-carbonyl-labeled carbofuran is monitored. This approach was designed to take advantage of an automated method for analysis of CO2 and 14CO2 described by Shelton and Parkin (1989). Two specific factors were considered in the development of this protocol: (i) the mode of soil handling and pesticide application, and (ii) the method of summarizing the kinetic data.

METHODS

Field Sampling

Surface soil (0–16 cm) was obtained from a conventional till field located at the Beltsville Agricultural Research Center, Beltsville, MD. The soil was a Hatorib silt loam (fine-loamy, mixed, nonacid, mesic Typic Fluvaquents) with 1.5% organic matter and a pH of 6.05. The site was planted to conventional till corn (Zea mays L.) during the previous 4 yr, and received banded carbofuran application (Furadan 10G, 0.18 kg/ha a.i. [1 lb/acre a.i.]) at the time of planting in each year. Thirty intact soil cores were collected between the corn rows, using a 4 cm diam. coring device in February 1989.

Soil Incubations

Initial experiments were performed to evaluate production of 14CO2 from degradation of ring- and carbonyl labeled carbofuran. Cumulative 14CO2 production from 50-g soil samples amended with 14C-carbonyl carbofuran (specific activity of 39 Bq/g soil) or amended with 14C-ring labeled carbofuran (specific activity 80 Bq/g soil) was measured. Four replicates of each treatment were used.

Carbofuran hydrolysis was monitored by measuring 14CO2 production from 14C-carbonyl-labeled carbofuran-amended soils. A saturated solution of carbofuran was prepared by dissolving commercial formulated granular material (10G Furadan) in water. The solution was filtered through glass wool to remove coarse particulate material, and radiolabeled carbofuran was then added. The resulting solution contained 400 μg carbofuran/mL at a specific activity of 5500 Bq/mL (0.15 μCi/mL). In initial experiments to assess the relative rates of CO2 production from carbonyl-carbofuran hydrolysis vs. complete mineralization of carbofuran a solution of 14C-ring-labeled carbofuran (400 μg carbofuran/mL, 9600 Bq/mL) was prepared using a similar procedure. The 14C-carbonyl and ring-labeled carbofuran was a gift from FMC Corporation (Princeton, NJ).

Soil incubations were conducted on the 7- to 9.5-cm depth segment of each core (ca. 50 g soil). These samples had an average gravimetric water content of 20%. Three methods of pesticide application/sample incubation, were evaluated using 14C-carbonyl-labeled carbofuran. These treatments are designated A, B, and C. For treatment A, intact core segments were sieved (0.5 cm mesh) and 0.35 mL of the carbofuran solution was applied using an atomizer. The samples were then mixed and placed in 125-mL flasks. For treatment B, intact soil core segments were injected with 0.35 mL of the carbofuran solution at a single location. The injected core segments were then pushed through a 0.5-cm sieve, and placed in 125-mL flasks. Thus, treatment B resulted in a more heterogeneous dispersion of pesticide. For treatment C, intact core segments were injected with 0.35 mL of the carbofuran solution. The core segments were then incubated, intact, in lengths of polycarbonate tubing, stoppered at each end with rubber stoppers. This last method most closely mimics field conditions by preserving soil structure and creating highly localized concentrations of carbofuran similar to what occurs in the field as a result of the banding of granular material at planting time. Sterile controls were prepared by adding carbofuran (Treatment A) to 50 g of autoclaved soils and incubating as described below. All soil samples were incubated at 25 °C, and carbofuran hydrolysis determined by monitoring daily 14CO2 production.

Instrumentation and Analyses

The soil samples (flasks or cores) were connected to an instrument that allowed for the semiautomated determination of total and radiolabeled CO2 evolution from soil (Shelton and Parkin, 1989). At each sampling time, the head-space of each sample was flushed for 4 min through a sparging stone immersed in a test tube containing 6 mL 0.5 M NaOH. The 14CO2 was quantified by placing 3 mL aliquots of trapping solution into 7 mL of Beckman Ready-Solv HP aqueous cocktail and counting in a Beckman model LS-6800 liquid scintillation counter. The trapping efficiency for radiolabeled 14CO2 is 100% ± 2.0% (Shelton and Parkin, 1989).

Soil Pore Space Residual Carbon Dioxide Experiments

Experiments were performed to quantify the amount of residual 14CO2 trapped in the soil pores following headspace determination of 14CO2. For these experiments, 16 additional soil cores were collected from the site. These samples had an average gravimetric water content of 15.1%. Eight cores were subjected to Treatment B (injected/sieved) and eight cores were subjected to Treatment C (injected/intact). After a 4-d incubation, 14CO2 accumulation in the headspace of each sample was determined. Immediately following measurement of 14CO2 in the headspace, the quantity of 14CO2 trapped in the soil pores (and dissolved in the liquid phase) was determined. Determination of this residual 14CO2 was accomplished by removing each core from the polycarbonate tube and immediately placing them in 1-L jars containing 100 mL water. The jars were sealed and then vigorously agitated to break up the soil cores and release the 14CO2 trapped in the soil pores. The resulting soil slurries were acidified to pH 1 with 50% H2SO4 and sparged with compressed air through 10 mL of 0.5 M NaOH to trap and quantify residual 14CO2 remaining in each core. A similar procedure was used for the sieved soil in the flasks except that 50 mL H2O was added directly to the flasks containing the soil. The resulting slurries were acidified and sparged with compressed air for 20 min.

Modeling and Statistics

Three models for summarizing the 14CO2 production data were evaluated. All the models describe cumulative 14CO2 production. The three-parameter logistic model [Eq. (1)] has been used as an empirical model for describing the growth of an organism in a closed system with finite resources. The Morgan-Mercer-Flodin (MMF) model [Eq. (2)] is a four-parameter logistic model that has been used to characterize the nutritional responses of higher organisms (Morgan et al., 1975). The third model is a modification of the mixed order model of Brunner and Focht (1984), which allows for exponential growth of microorganisms (Eq. [3]). Because the first step in the degradation of 14CO2-carbonyl carbofuran is the hydrolysis of the carbamate bond and the formation of 14
$^{14}$CO$_2$, this model has been modified in that the term that allows for endogenous $^{14}$CO$_2$ production from the soil has been deleted.

**Logistic Growth Model**

$$P = \frac{a}{1 + e^{-b-a}}$$  \[1\]

where $P$ is cumulative $^{14}$CO$_2$ formation, $a$ is the asymptote, $b$ is a location parameter, $t$ is time, and $c$ is the rate coefficient.

**Morgan-Mercer-Flodin Model**

$$P = \frac{b + at}{g + t}$$  \[2\]

where $a$ = the asymptote, $b$ is the ordinate intercept, $c$ is the apparent kinetic order of the reaction as $t$ approaches 0, and $g$ is a characteristic constant for the system.

**Brunner-Focht Model**

$$P = a[1 - \exp(-kt - E/u [\exp(ut) - 1])]$$  \[3\]

where $a$ is the initial substrate concentration, $k$ is a first-order rate coefficient, $E$ is the initial biomass, $u$ is the specific growth rate constant.

Parameter estimates were obtained for each of the models using nonlinear regression. Regression analyses were performed with the software package PCNONLIN (Statistical Consultants, Inc., Lexington, KY), using the Nelder-Mead simplex algorithm for minimizing the sum of squared residuals.

Goodness-of-fit was determined by the Akaike Information Criterion (AIC). The AIC is a discrimination criterion that has been used as a nonlinear model discrimination tool in cases where the F-test for model discrimination does not strictly apply (Robinson, 1986). Specifically, the AIC is useful in the comparison of models with different numbers of parameters (Yamaoka et al., 1978). The AIC is defined by Eq. [4].

$$\text{AIC} = N * \ln(\text{RSS}) + 2 * \text{NP}$$  \[4\]

where $N$ = total of data pairs, RSS = residual sum-of-squares, and NP = number of parameters in the model.

In a model comparison study, such as the one described here, the model that yields a lower value for the AIC for a given data set is the superior model.

Treatment differences were evaluated by performing Gabriel’s multiple comparisons test procedure (experiment wise error rate, alpha = 0.05) on parameters generated from the models (Sokal and Rohlf, 1969).

**RESULTS AND DISCUSSION**

The dominant fate of the carbonyl group of carbofuran added to the soils of this study is hydrolysis of the carbamate bond followed by rapid mineralization of the methylamine (Fig. 1). Production of $^{14}$CO$_2$ accounted for 95% of the added $^{14}$C-carbonyl-labeled carbofuran over a 13-d period. The $^{14}$CO$_2$ production from ring-labeled carbofuran exhibited linear kinetics and only 12% of the added label was released as $^{14}$CO$_2$ during a 13-d incubation period. These findings are consistent with previous work (Turco and Konopka, 1990).

Radiolabeled CO$_2$ production from all treatments exhibited sigmoidal kinetics (Fig. 2), except for three sterile samples (Fig. 2A), which exhibited little activity over the 30-d incubation period. In the nonsterile samples, the lag phase indicates growth or adaptation of the carbofuran hydrolyzing microbial population. The lag phase was followed by a period of rapid hydrolysis, which decreased until an asymptote was reached. A similar kinetic pattern of rapid carbofuran hydrolysis, following a brief lag phase, has been observed by others with soils having a history of carbofuran application (Kaufman et al., 1985; Turco and Konopka, 1990). For all three treatments the reaction was essentially complete after 14 d and recovery of $^{14}$CO$_2$ ranged from
77.6 to 100% of added labeled material. Treatment
effects of the rates of 14CO₂ production are difficult to
discern by visual inspection of Fig. 2.

The variability in 14CO₂ production curves observed
within a given treatment is likely due to variability
associated with the process of adding 14CO₂-carbofu-
rán to the individual soil samples as well as the natural
variability associated with the heterogeneous disper-
sion of carbofuran-degrading organisms in the field.
In contrast, analytical variability is quite low as in-
dicated by the small standard deviations associated
with the 14CO₂ production curves of replicate subsam-
plies form a large batch of soil that had been sprayed
with 14CO₂-carbofuran (Fig. 1).

To compare variability between treatments, the data
of Fig. 2 were summarized using nonlinear regression
modeling. Application of mathematical models is ad-
vantageous, because it allows for reducing the dimen-
sionality of the data to a single summary parameter
that can be analyzed using univariate ANOVA. This
strategy was employed by Ou et al. (1982), who used
first-order rate coefficients to predict the influence of
temperature and moisture on mineralization of ring-
labeled carbofuran. However, a first-order relationship
is clearly inappropriate for the data of Fig. 2.

Three nonlinear models were fit to the carbofuran
hydrolysis data. All the models can describe sigmoidal
product appearance data. The MMF model was se-
lected for summarizing the carbofuran hydrolysis data
generated in this study because it can describe a wide
range of kinetic patterns including sigmoidal and hy-
perbolic kinetics; thus, it is a more general model than
the three-parameter logistic equation. The modified
version of the Brunner-Focht model (Eq. [3]) is a more
mechanistic model, in that it contains a parameter for
biomass and a first-order rate constant.

All three nonlinear models yielded reasonable fits
to the 14CO₂ production data as evidenced by the high
r² values obtained for each sample (Table 1). However,
since the models have different numbers of param-
eters, goodness-of-fit cannot be determined by r² alone.
Model selection was based on the AIC calculated for
each of the models. For 24 of the 28 replicates lower
AIC values, and hence better fits were achieved for
the MMF model. The version of Model 3, which al-
lores two additional parameters. The rate at the in-
fluence of diffusion on the observed degra-
dation kinetics or the exact pathway of carbofuran de-
radation, may make interpretation of results ques-
tionable.

The MMF model (Eq. [2]) was rearranged to gen-
rate two additional parameters. The rate at the half-
life of carbofuran in soil is given by Eq. [5]. Since the term half-life often implies a first-
order reaction, the recommendation of Hamaker
(1972) is adopted, and the term, DT-50% is used to
indicate the disappearance time for 50% of the com-

Maximum Rate

$$R_i = \frac{(a-b)\gamma^c g(c-1)/(c+1)^{(c-1)/c}}{(g+g(c-1)/(c+1))^2}$$  \[5\]

Half-Life

$$DT-50\% = \frac{(a/2 - b/g)^{1/e}}{a/2}$$  \[6\]

where $R_i$ is the maximum rate, $a, b, c,$ and $g$ are the parameters of the MMF model (Eq. 2).

These two summary parameters were calculated for
each of the 14CO₂ production curves and were used to
compare treatment effects (Table 2). Degradation of
carbofuran in all three soil treatments was relatively
rapid, with DT-50% in the range of 4 to 6 d, and max-

### Table 1. Comparison of goodness-of-fit of the three models for each replicate of the three soil incubation treatments.

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<th>BF model</th>
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† All replicates except the underlined values were best described by the MMF model.
of the $^{14}$CO$_2$ production curves and, hence, may influence the model discrimination and parameter estimation. This fact has been previously recognized by Scow et al. (1986), who report that, in biometer flasks containing 50 g soil amended with $^{14}$C-bicarbonate (1 $\mu$g/kg soil), 1 h was required for 85% of the added label to diffuse from the soil into the side-arm KOH trap. In their modeling analysis this problem was addressed by deleting the first few points of their time-course data. This is not a reasonable approach for our study, as during the time course of our incubations $^{14}$CO$_2$ did not instantaneously appear (as with a $^{14}$CO$_2$-bicarbonate addition); rather, it was continuously produced. Thus, at any given point in time, total $^{14}$CO$_2$ was partitioned between the headspace and the soil.

Using the partitioning data for the mixed soil and intact-core treatments (Table 3) and the headspace $^{14}$CO$_2$ production data (Fig. 2) total $^{14}$CO$_2$ in each sample flask, at each time point was calculated. The resulting diffusion-corrected $^{14}$CO$_2$ production curves are shown in Fig. 3. It must be recognized that this correction is based on the assumption that partitioning was constant over the course of the incubations. The validity of this assumption is unknown; however, the partitioning data used in this calculation (Table 3) were obtained at the time of maximum carbofuran hydrolysis activity, and hence, maximum partitioning of $^{14}$CO$_2$ between the headspace and the soil. Thus, the data of Fig. 3 represent the maximum diffusion effect on observed $^{14}$CO$_2$ production.

For Treatments A and B the diffusion-corrected $^{14}$CO$_2$ production curves were only slightly shifted toward the origin; however, the diffusion-corrected $^{14}$CO$_2$ production curves of Treatment C were shifted

<table>
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<th>Table 2. Comparison of DT-50% and maximum rate of carbofuran hydrolysis for the three soil incubation treatments.</th>
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* Values followed by different letters in each column indicate significant difference ($P < 0.05$).
† Numbers in parentheses are coefficients of variation.

| Table 3. Residual $^{14}$CO$_2$ remaining in sieved soil samples and intact soil cores following headspace $^{14}$CO$_2$ determination ($n = 8$). |
|----------------|-----------------|-----------------|
| Sample        | Headspace       | After acid      | Total Pore space CO$_2$ |
|               | dpm             |                 | %                        |
| Flask         | 4842 (1396)*    | 2997 (1164)*    | 7820 (2303) 37.9 (8.4)*  |
| Core          | 2733 (856)      | 4276 (1128)     | 7009 (1856) 61.1 (5.2)   |

* Indicates a significant difference ($P < 0.05$) between the core and flask treatments (eight replicates each treatment).
to a greater extent, resulting in a shorter lag time. These shifts in the $^{14}$CO$_2$ production curves did not influence the results of the model discrimination. Regression analysis performed on the data of Fig. 3 revealed results similar to those obtained with the headspace $^{14}$CO$_2$ production data presented in Table 2. In 24 of the 28 samples, the MMF model yielded a lower AIC, and hence, a better fit than the other two models.

Similarly, the diffusion correction exerted only a minor influence on parameter estimates obtained from application of the MMF model (Table 4). Parameter estimates from the mixed soil treatment (Treatments A and B) were only slightly affected; however, for the intact soil cores (Treatment C) DT-50% was decreased by 11% and maximum rate increased by 26%. The resulting statistical comparison of treatment effects on summary parameters derived from the diffusion-corrected data revealed no significant treatment effects ($P > 0.10$). This result supports our hypothesis stated above; that diffusion of CO$_2$ was the primary factor responsible for the treatment effects observed with the original data.

**CONCLUSIONS**

The general saturation model proposed by Morgan et al. (1975) worked well for describing the sigmoidal CO$_2$ production patterns obtained for all three soil treatments. We chose the empirical MMF model for two reasons. A better fit was achieved with the MMF model than with either the LG model or the BF model. This better fit results in lower standard errors for the parameter estimates, which in turn results in more power when the model parameter estimates are used in hypothesis tests of treatment effects. A second reason for choosing the empirical MMF model over the Brunner-Focht model is that we were unsure of the physical effects (primarily diffusion) on the apparent kinetic pattern, and how this affects biological interpretation of model parameters with regard to biomass and first-order rate coefficient.

Statistical evaluation of the soil incubation treatments was possible using parameter estimates derived from the MMF model. Of the three soil incubation treatments tested, Treatment C best preserves "field" conditions, in that soil structure is maintained; however, the apparent experimental artifact associated with $^{14}$CO$_2$ quantification precludes its usefulness. Diffusional effects were also noted with the mixed soil treatments, yet these effects did not substantially alter the parameter estimates obtained from the regression analyses.

**REFERENCES**


