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SIMULATION OF BRANCHED SERIAL FIRST-ORDER DECAY OF ATRAZINE AND METABOLITES IN ADAPTED AND NONADAPTED SOILS

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Abstract—In the present study a branched serial first-order decay (BSFOD) model is presented and used to derive transformation rates describing the decay of a common herbicide, atrazine, and its metabolites observed in unsaturated soils adapted to previous atrazine applications and in soils with no history of atrazine applications. Calibration of BSFOD models for soils throughout the country can reduce the uncertainty, relative to that of traditional models, in predicting the fate and transport of pesticides and their metabolites and thus support improved agricultural management schemes for reducing threats to the environment. Results from application of the BSFOD model to better understand the degradation of atrazine supports two previously reported conclusions: atrazine (6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and its primary metabolites are less persistent in adapted soils than in nonadapted soils; and hydroxyatrazine was the dominant primary metabolite in most of the soils tested. In addition, a method to simulate BSFOD in a one-dimensional solute-transport unsaturated zone model is also presented. Environ. Toxicol. Chem. 2011;30:1973–1981.
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INTRODUCTION

Pesticides are applied to fields in amounts calculated to provide maximum control of the insects or weeds, while minimizing the health threats to humans and the environment. A key variable in these calculations is the half-life, or persistence, that depends on the physical and chemical properties of the pesticide and those of the soils where it will be applied. A rich literature exists describing how the persistence of a pesticide varies with temperature, moisture content, texture, pH, redox, and organic content of the soils to which it is applied [1–3]. When all environmental variables are held constant, pesticide concentrations commonly display exponential decay which plots as a line on a semi-log plot with concentration on the vertical log axis and time (days) on the linear x axis; the slope of the line is the decay rate in units of d^{-1}.

Disappearance of the pesticide, however, does not imply the disappearance of the environmental threat, as the metabolites can sometimes be more toxic than the pesticide itself [4]. Predicting the concentrations of the metabolites with time is no longer straightforward, because the concentrations vary according to the rate that the metabolites are produced and the rate that they decay.

The U.S. Geological Survey's National Water Quality Assessment (NAWQA) recently completed field observations and numerical simulations to better understand the fate and transport of agricultural chemicals in the environment [5]. During construction of the numerical models, a need was identified to reconcile production and decay rates for an assortment of pesticides and metabolites that were culled from a variety of sources. The present study focuses on the derivation of internally consistent production and decay rates of atrazine and its metabolites: deethylatrazine (DEA, 6-chloro-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine); deisopropylatrazine (DIA, 6-chloro-*N*-ethyl-[1,3,5]triazine-2,4-diamine); and hydroxyatrazine (HYA, 6-hydroxy-*N*-ethyl-*N*′-isopropyl-[1,3,5]triazine-2,4-diamine). The rates were calibrated to match variations of atrazine and DEA measured in pore water (mass/volume of water) during the NAWQA studies, and also to match variations in total concentrations (mass/mass of soil and water) of atrazine, DEA, DIA, and HYA measured for different moisture and temperature treatments for agricultural and nonagricultural soils in Mississippi and Colorado, USA.

Recent work [6–10] has shown that the half-life of atrazine in soils with no history of previous applications can be orders of magnitude longer than the half-life in soils with a history of previous applications. Soils with a history of previous applications of atrazine are referred to in the present study as adapted soils; those with no history are referred to as nonadapted soils. Microbial communities capable of using atrazine as an energy or nitrogen source become more prevalent with repeated applications [6,7].

Previous work by Kruger et al. [11] suggested that DEA was the dominant, primary metabolite of atrazine and therefore DEA was the only metabolite of atrazine included in the list of target analytes for pore waters sampled under agricultural fields during recent studies by the U.S. Geological Survey's National Water Quality Assessment Program [5]. Atrazine was found to be the most frequently detected pesticide and DEA the most frequently detected metabolite in streams and groundwater in both agricultural and urban areas [12]. Kruger et al. [11] recovered 83% of the decayed atrazine in bound residue fractions and approximately 9% in uncharacterized polar metabolites. Lerch et al. [13,14] later used mixed-mode extraction to recover 43% of bound atrazine residues from aged soils and found that 88% of those residues were of hydroxylated atrazine metabolites, with HYA accounting for the vast majority.

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The European forum for the coordination of pesticide fate models and their use (FOCUS) group ([15]; http://focus.jrc.ec.europa.eu/dk/docs/finalreportFOCDegKin04June06linked.pdf) independently recognized the importance of mass balance in developing models describing the production and degradation of multiple metabolites and provided guidance on how metabolites should be fitted to experimental data. This includes guidance on how to prepare the data for the curve fitting (handling of censured data, outliers, replicates), the optimization procedure and criteria for the acceptance of the goodness of fit. Following up on the recommendations, Schäfer et al. [16] developed the kinGUI software that contains similar mass balance constraints, systems of differential equations, and optimization routines described here. Its graphical user interface also makes it attractive to a broad user community. However, the ability to tie one parameter to another as described below is not provided, and so reasonable confidence intervals for production and degradation rates could not be obtained. Recent versions of other unsaturated zone models such as the Pesticide Root Zone Model, PRZM-3, now also incorporate branched serial first-order decay (BSFOD) dynamics [17].

The present study describes branched serial first-order decay of atrazine into its three primary metabolites: DEA, DIA, and HYA. Secondary and tertiary metabolites are accounted for as part of an unmeasured sink term. The method reduces the uncertainty in predicted concentrations and is of general use for any compound that breaks down into primary and secondary metabolites that can be modeled as BSFOD.

The present study also provides a description of the methods used by Webb et al. [18], who used the Nebraska model results to predict the leaching of atrazine and its metabolites beneath agricultural fields in Maryland where soils, land use, and agricultural management practices are similar. In that study, the Leaching Estimation and Chemistry Model (LEACHM) [19,20], which was designed to use only linear first-order decay, was modified to simulate BSFOD decay and the leaching of pesticides and metabolites to groundwater over several growing seasons. The technique used to extend LEACHM's simulation capabilities to include BSFOD, with its inherent mass balance, is also described here.

MATERIALS AND METHODS

Serial first-order decay

The decay of pesticides and their metabolites as a result of biotic and abiotic processes is often described by the same serial first-order decay model used to describe radioactive decay (radioactive decay equations presented here are modified from Keith Holbert's class notes from the University of Arizona, available at http://holbert.faculty.asu.edu/eee460/RadioactiveDecay.pdf):

$$p(t) = p(0)e^{-\lambda_p t} \tag{1}$$

where $p(0)$ is the initial concentration of pesticide, $p(t)$ is the concentration of pesticide at time t, λ_p is the decay rate of pesticide (d^{-1}), and t is the time in days.

The decay rate, λ_p, is the frequency that molecules of the pesticide degrade per unit time. For radioactive decay the rate is constant, whereas for pesticide degradation the rate is dependent on the presence of microbes, temperature, and moisture content and other chemical properties of the soil and the pesticide. The inverse of the decay rate, λ^{-1}, represents the mean lifetime of a molecule of the reactant, whereas the half-life, computed as ln(2) λ^{-1}, represents the amount of time for the reactant to degrade to one-half of its initial concentration.

The concentration of a primary metabolite that is itself reactive will vary as a balance between the rate of production from the decay of the pesticide and the rate of degradation of the primary metabolite:

$$d(t) = d(0)e^{-\lambda_d t} + \frac{p(0)\lambda_p}{\lambda_d - \lambda_p}\left[e^{-\lambda_p t} - e^{-\lambda_d t}\right] \tag{2}$$

where $d(0)$ is the initial concentration of primary metabolite, $d(t)$ is the concentration of primary metabolite at time t, and λ_d is the decay rate of this primary metabolite (d^{-1}).

The concentration of a stable secondary metabolite is described by

$$g(t) = g(0) + d(0)(1 - e^{-\lambda_d t}) + p(0)\left(1 + \frac{\lambda_d}{\lambda_p - \lambda_d}e^{-\lambda_p t} - \frac{\lambda_p}{\lambda_p - \lambda_d}e^{-\lambda_d t}\right) \tag{3}$$

where $g(0)$ is the initial concentration of secondary metabolite, and $g(t)$ is the concentration of secondary metabolite at time t.

Branched serial first-order decay

Many pesticides degrade to multiple primary metabolites with each branch undergoing serial first-order decay. For example, atrazine hydrolytically dechlorinates to HYA at the same time that it dealkylates to DEA and DIA and further to didealkylatrazine (DDA, 6-chloro-[1,3,5]triazine-2,4-diamine) (Fig. 1) [21]. The concentration of a primary metabolite will vary as a function of the rate that the pesticide degrades to that metabolite and the rate that the metabolite itself degrades.

The important constraint is that the production rates of all primary metabolites, known or unknown, must sum to the pesticide's decay rate.

$$\lambda_p = \sum_{i=1}^{n} \lambda_{pi} \tag{4}$$

where n is the number of primary metabolites, and λ_{pi} is the production rate of the ith primary metabolite.

For atrazine and its primary metabolites:

$$\lambda_{\text{atrazine_degradation}} = \lambda_{\text{HYA_production}} + \lambda_{\text{DEA_production}} + \lambda_{\text{DIA_production}} \tag{5}$$

The fraction of the pesticide degrading to primary metabolite i can be defined as

$$f_i = \lambda_{pi}/\lambda_p \tag{6}$$

such that $\sum_{i=1}^{n} f_i = 1$. Given a branched system, $f_i p(0)$ can be substituted for $p(0)$ in Equations (2) and (3) so that concentrations of primary metabolite i can be described as

$$d_i(t) = d_i(0) + A_i f_i p(0) - B_i d_i(0) \tag{7}$$

where $d_i(0)$ is the initial concentration of primary metabolite i, $d_i(t)$ is the concentration of primary metabolite i at time t, $A_i = \lambda_p(e^{-\lambda_p t} - e^{-\lambda_{di} t})/(\lambda_{di} - \lambda_p)$ where λ_{di} is the decay rate of the ith primary metolite and $B_i = 1 - e^{-\lambda_{di} t}$.

The concentration of a stable secondary metabolite resulting from the decay of primary metabolite i would be

$$g_i(t) = g_i(0) + B_i d_i(0) + C_i f_i p(0) \tag{8}$$

where $g_i(0)$ is the initial concentration of secondary metabolite through primary metabolite i, $g_i(t)$ is the concentration of

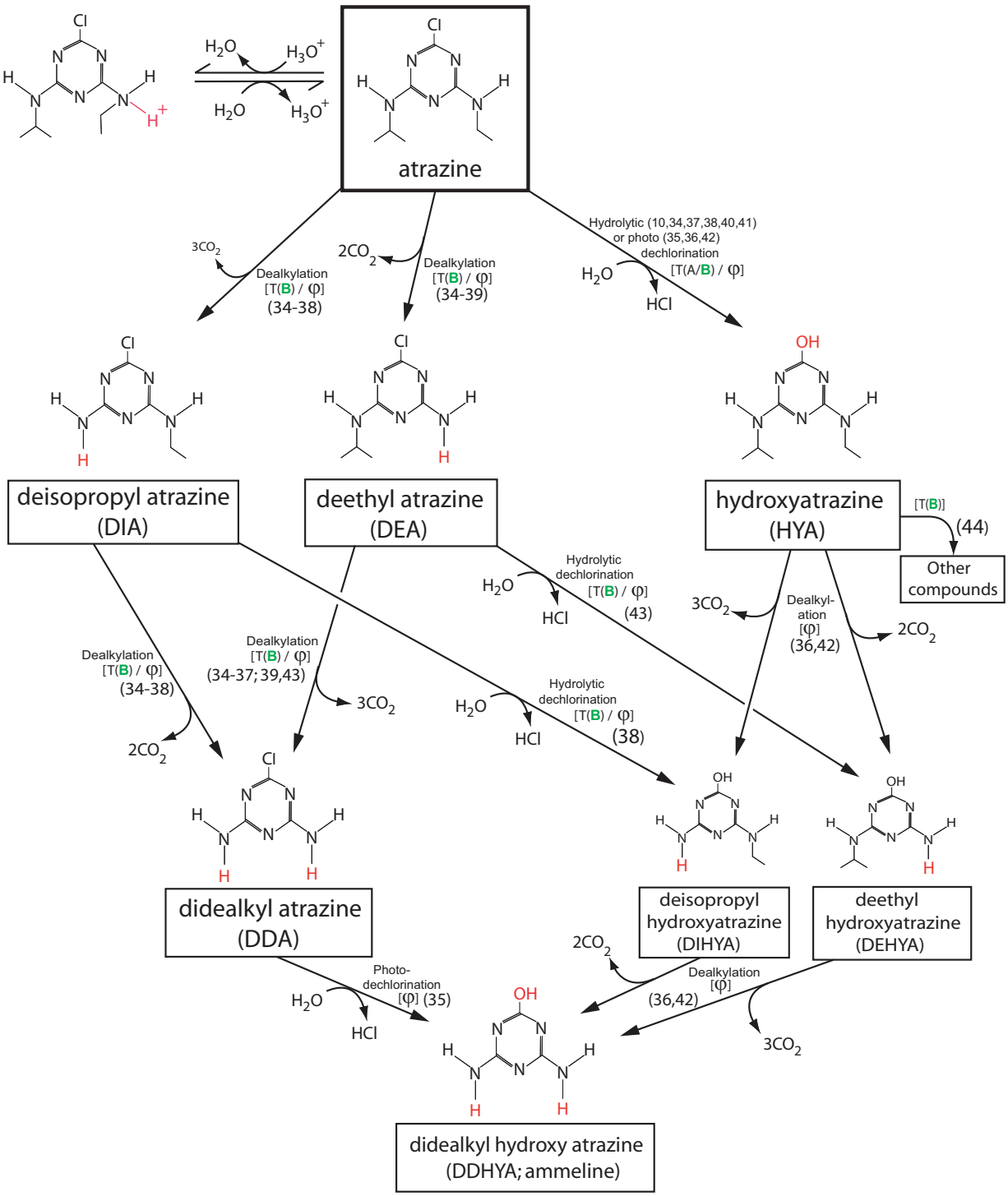

Fig. 1. Some of the reactions involving atrazine degradates that have been examined most extensively in the environment. Numbers in brackets refer to the original sources of information as follows: [34] Kaufman and Kearney, [35] Pelizzetti et al., [36] Torrents et al., [37] Beynon et al., [38] Kruger et al., [39] Rejto et al., [40] Armstrong et al., [41] Mandelbaum et al., [10] Krutz et al., [42] Hapeman-Somich, [43] Kruger et al., [44] Gao et al. Reactivity abbreviations denote thermal (nonphotochemical) reactions that are either abiotic [T(A)] or biologically mediated [T(B)]; or photochemical reactions [φ]. (J. Barbash, U.S. Geological Survey, personal communication). [Color figure can be seen in the online version of this article, available at wileyonlinelibrary.com]

secondary metabolite through primary metabolite i at time t, and

$$C_i = 1 + \frac{\lambda_{di}}{\lambda_p - \lambda_{di}} e^{-\lambda_p t} - \frac{\lambda_p}{\lambda_p - \lambda_{di}} e^{-\lambda_{di} t}$$

Laboratory observations

Samples of adapted and nonadapted soils from Colorado and Mississippi were mixed with purified atrazine to attain an initial soil concentration of 1 μg g^{-1} [10]. Adapted soils have a history of atrazine application and have developed a microbial culture that can use atrazine and its primary metabolites as an energy source. Nonadapted soils have no history of atrazine application.

Soils were incubated in the dark at a temperature of 10°C or 20°C with soil moisture maintained at 40 or 70% of field capacity through the addition of deionized water. Three

replicates of each temperature/moisture combination for each of the four soils (adapted and nonadapted for two states) were analyzed for atrazine, DEA, DIA, and HYA at 1, 2, 4, 8, 16, 32, 45, and 60 d after application, yielding 96 observations for each of combination of soil, temperature, and moisture.

Five grams of each sample were placed into a 50-ml plastic centrifuge tube and extracted with 15 ml 80:20 (v/v) methanol (MeOH)/25 mmol L^{-1} ammonium acetate adjusted to pH 8.0. The suspension was agitated on a horizontal shaker for 30 min and centrifuged at 8000*g* for 15 min and the supernatant was transferred to 50-ml plastic centrifuge tubes. The extraction procedure was repeated and supernatants combined. The supernatant was evaporated to <5 ml at 50°C with a Rapidvap, brought to 10 ml with deionized water, and concentrated on a C18 solid-phase extraction column (Thermo Electron Corp Hypersep) preconditioned with 3 ml each of MeOH, ethyl acetate, MeOH, and distilled water. The column was dried under negative pressure for 90 min and atrazine, DEA, and DIA were eluted with 2 ml ethyl acetate into 2-ml volumetric tubes. Samples were fortified with an internal standard, 10 μl of 0.1 mg ml^{-1} of butlyate dissolved in acetonitrile, brought to volume with ethyl acetate, and analyzed with gas chromatography / mass spectrometry. Subsequently, HYA was eluted from the column with 2 ml 95:5 (v/v) MeOH//0.1 N HCl into 2-ml volumetric tubes. Samples were brought to volume with MeOH and analyzed by high-performance liquid chromatography. Recovery of atrazine, DEA, DIA, and HYA from fortified soil samples was 95, 85, 90, and 80%, respectively, all with an error of ±5%. Further details on laboratory methodology can be found in Krutz et al. [10].

The limit of quantitation for all analytes was 0.007 μg g^{-1} with 335, 68, 49, and 357 observations at or above the reporting limit for atrazine, DEA, DIA, and HYA, respectively. As the BSFOD model predicts stoichiometric decay, concentrations were converted from μg g^{-1} to μmoles g^{-1} using molecular weights of 215.69, 187.63, 173.6, and 197.24 g $mole^{-1}$ for atrazine, DEA, DIA, and HYA, respectively. Values below the reporting limit were assigned a concentration of zero.

Field observations

During 2003 and 2004, local weather conditions, soil moisture and tension, and water quality of the unsaturated and saturated zones were observed on and beneath an intensively studied field subject to corn (*Zea mays* L.) / winter wheat (*Triticum aestivum* L.) / soybean (*Glycine max* L.) rotations in Nebraska [5]. On April 29, 2004, more than 10 cm of rain—the largest daily total in more than two years—fell on the fields. Runoff from the storm resulting in the ponding of water in a topographically low area near a nest of suction lysimeters installed in the downslope end rows of a cornfield (site N22) [22]. Atrazine, which had recently been applied to the land surface, mixed with the runoff and, under conditions of focused recharge in the ponded area, drained rapidly downward past the root zone. Lysimeter N22b was previously installed 7 m below the land surface in a loess (silt) layer just above a sand layer. Sand beneath loess acts as a capillary barrier and resulted in elevated soil moisture and good lysimeter recovery for four months following the event. The rapid recharge and perched water resulted in an in situ analog of the laboratory batch reaction experiments. Lysimeter samples were analyzed for atrazine and DEA using gas chromatography and mass spectrometry [5] with a method reporting limit of 0.007 and 0.006 μg L^{-1}, respectively. The ratio of the concentration of DEA to the sum of concentrations of DEA + Atrazine was computed for the seven lysimeter samples recovered from lysimeter N22b from May 14 through July 23, 2004.

A BSFOD model, implemented in a spreadsheet and optimized using a quasi-Newton method [23], was used to find a production rate of DEA that would best fit the DEA ratios described above. Constraints were those described in the *Program, optimization, and constraints* section below with some notable differences. As the literature value of 69 d for atrazine [11] was not appropriate for the adapted soils [6] that were the focus of the NAWQA study, the half-life of atrazine was fixed at 11 d, equal to $\ln(2)/\lambda_p$ where λ_p is the slope of the best fit line through the observed decline in atrazine concentrations plotted with the natural logarithm of the concentration as the ordinate and time, in days, as the abscissa. Also, the decay rates of the primary metabolites were fixed to the literature values of 48 d for DIA [11], 241 d for DEA (258 d [24,25] adjusted to 25°C), and 121 d for HYA [26] because no studies were available to suggest alternate values for half-lives of the primary metabolites in adapted soils. The reader is referred to Gilliom et al. [12] for additional information on transformation rates of common pesticides.

The resulting model predicted the fractions of atrazine decaying to each primary metabolite would be 71% HYA, 23% DEA, and 6% DIA. These production fractions were then used as parameters in LEACHM to estimate the flux of atrazine and metabolites beneath cornfields with similar soils in Maryland [18].

During the optimization run for the Nebraska site completed for the present study, the half-lives of atrazine and the primary metabolites were allowed to vary (the half-life of atrazine was fixed before) along with the initial porewater concentration of atrazine simulated for May 14, day zero of the simulation, which was also fixed at the initial concentration before. In addition, optimization was completed with the same algorithms, described below, used to fit models to the laboratory observations for the adapted and nonadapted soils from Colorado and Mississippi.

LEACHM implementation

During a 2004 workshop on exposure modeling hosted by the U.S. Environmental Protection Agency (U.S. EPA), LEACHM was selected by NAWQA as an appropriate, simple, unsaturated, zone model to estimate leaching beneath agricultural fields given its capabilities, ease of use, adequate documentation, and open architecture. Biotransformation rates in LEACHM are adjusted for temperature and moisture using the methodology of Johnsson et al [27]. Transformation rates increase using a Q10 temperature response and decrease on either side of an optimum water content. Branched serial first-order decay was enforced in LEACHM by repeating the pesticide as the head of each linear decay series and defining its disappearance rate as both a transformation rate (the production rate of the specific primary metabolite in a given degradation path) and a degradation rate (the sum of production rates for all other primary metabolites). To simulate the sensitivity of the rates to microbial activity, transformation and degradation rates were made sensitive to changes in temperature and moisture content. Standard LEACHM models are able to apply moisture and temperature corrections for transformation rates and degradation rates. If incorporated correctly, the user should be provided with identical time-varying values of the pesticide at the head of each decay chain.

Program, optimization, and constraints

Equations (1) and (7) were used to simulate the time-varying concentration of atrazine, DEA, DIA, and HYA given the initial mass and seven parameters: the decay rate of atrazine (λ_p), the percentage of atrazine that decays into each of the three primary metabolites (f_1, f_2, f_3), and the decay rate for each of the three primary metabolites (λ_{d1}, λ_{d2}, λ_{d3}). The Model-Independent Parameter Estimation and Uncertainty Analysis program, PEST [28], was used to find parameter values that minimize the sum of squares objective function,

$$\Phi = \sum_{i=1}^{n} (w_i r_i)^2 \tag{9}$$

where for n observations, wi is the weight associated with the ith observation—its value should be inversely proportional to the standard deviation of the residuals—and r_i is the ith residual describing the observed minus the predicted value.

Phi is inversely related to the goodness of fit. The measure of goodness of fit is provided by the correlation coefficient, R, as defined in Cooley and Naff [29].

$$R = \frac{\sum (w_i c_i - m)(w_i c_{oi} - m_o)}{\left[\sum (w_i c_i - m)^2 \sum (w_i c_{oi} - m_o)^2\right]^{1/2}} \tag{10}$$

where c_i is the ith observation value, c_{oi} is the model-generated counterpart to the ith observation value, m is the mean value of weighted observations, and m_o is the mean of model-generated counterparts to observations. For each set of observations, weights were assigned in a two-step process; using uniform weights first and nonuniform weights second.

The sets of observations totaled 17: eight for Colorado, eight for Mississippi, and one for Nebraska. Each of the eight sets for Colorado and Mississippi belonged to a unique group of adaptation (yes or no), temperature (10 or 20°C), and soil moisture (40 or 70% of field capacity). Each Colorado and Mississippi set consisted of 96 laboratory observations (triplicate observations of the concentration of atrazine, DEA, DIA, and HYA in solids measured at eight times after initial laboratory spike). The Nebraska set consisted of 14 field observations (single observations of the concentration of atrazine and DEA dissolved in pore water measured at seven times after initial field application).

Following the optimization using uniform weights, atrazine observations were assigned a weight of 1.0 and each primary metabolite was assigned a weight equal to the standard deviation of the atrazine residuals divided by the standard deviation of all residuals for a given primary metabolite. The weights averaged near 20 for DIA and DEA and near 5 for HYA. For the 24 observations for the nonadapted soil from Colorado at 20°C and 40% field capacity, there were only four detections of DEA at the detection level of 0.007 $\mu g\ g^{-1}$ and no detections for DIA. The spurious weight calculated for DIA exceeded 11,000 but was instead assigned a weight of 41, equal to the calculated DEA weight for that model.

The following constraints on optimized parameter values were used in all inverse modeling exercises: The half-life for atrazine or any primary metabolite was limited to the range from 0.2 d to 720 d, equivalent to decay rates of 3.5 day^{-1} and 0.001 day^{-1}, respectively. Half-lives used for the first iteration of the optimization exercise were 69 and 48 d for atrazine and DIA, respectively [11], 241 d for DEA (258 d [24,24] adjusted to 25°C), and 121 d for HYA [26]; The range for the percentage of atrazine degrading to DIA was restricted to 0.1 to 20%; previous studies [11,24,25,30] indicate DEA production is three to six times that of DIA. A multiplier of 3.91 was selected here to match the ratio used by Webb et al. [18]. Therefore, using the previous constraint, the percentage of atrazine degrading to DEA was limited to the range from 0.391 to 78.2%; The percentage of atrazine degrading to HYA was calculated as 100% minus the percentages of atrazine degrading to DEA and DIA and limited to the range, using the previous constraints, from 1.8 to 99.5%.

RESULTS AND DISCUSSION

The calibrated half-life of atrazine for adapted soils varied from 1.4 to 14 d compared to a range of 25 to 108 d for nonadapted soils (Fig. 2). For both adapted and nonadapted

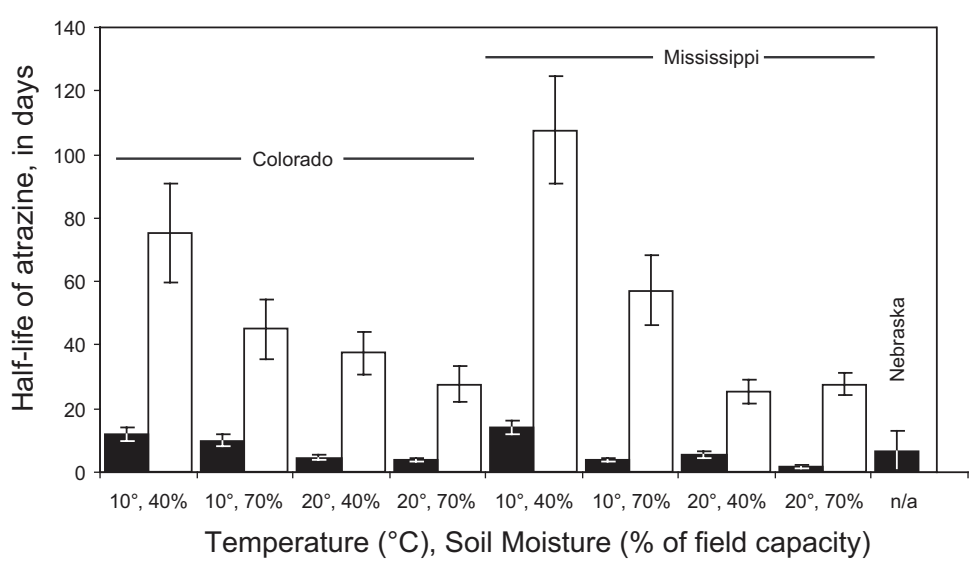

Fig. 2. Half-life of atrazine for adapted (black bars) and nonadapted (white bars) soils from Colorado, Mississippi, and Nebraska, USA. Error bars indicate 95% confidence intervals. Half-lives for Colorado and Mississippi document the disappearance of total atrazine (dissolved and sorbed) observed in the laboratory whereas the half-life for Nebraska documents the reduced concentrations of atrazine dissolved in pore water. Temperature or soil moisture data were not measured at the depth of the Nebraska lysimeter.

soils observed in the laboratory, half-lives decreased with increasing temperature and moisture. These ranges are in agreement with earlier studies [6–10] reflecting the growth of microbial communities capable of using atrazine as an energy source in soils with repeated applications of atrazine. The average half-life (over both temperature and moisture controls) fit to concentrations observed in the adapted Mississippi soils (6.2 d) was less than that fit to the concentrations in the adapted Colorado soils (7.5 d); the half-life fit to the Nebraska porewater concentrations was in between (6.6 d).

Although with greater uncertainty, the calibrated half-lives of DEA and DIA were also shorter in adapted soils than they were in nonadapted soils (Table 1). For Colorado soils, the calibrated half-life for HYA was greater (more persistent) for adapted soils compared to nonadapted soils; for Mississippi soils the opposite relation was indicated with HYA being more persistent in nonadapted soils. Adapted soils for both Colorado and Mississippi appear to show shorter HYA half-lives with increasing soil moisture. The uncertainty in the predictions of the half-lives for the metabolites will likely improve with higher frequency observations immediately after application, longer overall periods of observation, lower detection limits, and inclusion of additional site and soils characteristics in the model. Soil acidity, crop type, and average soil moisture deficit of the soils in the fields have been shown to be three important factors in determining the microbial diversity and, by extension, the availability of strains capable of accelerated atrazine decay [31,32].

Model performance

The BSFOD models described here were more sensitive to estimates of the formation fractions than they were to the estimates of the half-lives of the primary metabolites; better

Table 1. Calibrated half-lives for atrazine and primary metabolites for soils from Colorado (CO), Mississippi (MS), and Nebraska (NE), USA, for soils adapted (-A) and nonadapted (-N) to previous applications of atrazine. Observed versus simulated concentrations for rows in italics are plotted on Figure 3[a]

	Temp	Moist	Atrazine	DEA	DIA	HYA
Soil	°C	% FC	Half-life, d ± 95% confidence interval[b]			
CO-A	10	40	12.0 ± 2.1	0.2[c] ± 0.4	5.0 ± 14.1	15.7 ± 5.7
	10	*70*	*9.8 ± 1.8*	*31 ± 23*	*38 ± 64*	*1.6 ± 0.5*
	20	40	4.5 ± 0.7	0.2[c] ± 0.1	0.9 ± 1.3	42.1 ± 19.5
	20	70	3.9 ± 0.6	2.4 ± 1.8	7.5 ± 8.7	0.9 ± 0.4
CO-N	10	40	75.1 ± 15.6	24.1 ± 52.6	720[c] ± 37800	2.0 ± 0.5
	10	*70*	*45.0 ± 9.3*	*24.0 ± 14.1*	*720[c] ± 9450*	*1.6 ± 0.6*
	20	40	37.3 ± 6.8	41.7 ± 302	0.2[c] ± 30.5	1.2 ± 0.4
	20	70	27.5 ± 5.7	33.4 ± 16.2	79.2 ± 77.8	1.3 ± 0.6
MS-A	10	40	13.8 ± 2.1	0.2 ± 0.6	3.8 ± 13.2	1.3 ± 0.4
	10	*70*	*4.0 ± 0.5*	*3.5 ± 2.8*	*12.7 ± 25.6*	*0.2[c] ± 0.1*
	20	40	5.6 ± 1.1	0.2[c] ± 0.1	0.3 ± 0.3	1.6 ± 0.8
	20	70	1.4 ± 0.1	0.2[c] ± 3.9	0.2[c] ± 3.9	0.2[c] ± 0.1
MS-N	10	40	108 ± 17	166 ± 453	720[c] ± 32800	8.7 ± 2.3
	10	*70*	*57.1 ± 10.8*	*42.1 ± 42.8*	*403 ± 1050*	*5.9 ± 1.8*
	20	40	25.1 ± 3.8	0.3 ± 0.2	1.9 ± 1.0	21.2 ± 9.7
	20	70	27.6 ± 3.5	2.2 ± 0.7	4.7 ± 1.4	59.7 ± 43.4
NE-A	*NA*[d]		*6.6 ± 6.3*	*237 ± 1190*	*48 ± NA*[e]	*121 ± NA*[e]

[a] Temp = temperature, in °C; Moist = soil moisture, as percent of field capacity (FC); DEA = deethylatrzine; DIA = deisopropylatrazine; HYA = Hydroxyatrazine; NA = not available.
[b] 95% confidence interval should be viewed as an indicator of uncertainty and may extend beyond the domain of the parameter.
[c] Optimization of the half-life ended at one of the limits 0.2 or 720 d.
[d] Temperature and moisture were not monitored at the depth of the Nebraska lysimeter
[e] DIA and HYA were not included in the list of analytes for the Nebraska porewater samples. Literature values for these half-lives are reported.

Table 2. Calibrated percentage of primary metabolites produced from the observed decay of atrazine for soils from Colorado (CO), Mississippi (MS), and Nebraska (NE), USA, for soils adapted (-A) and nonadapted (-N) to previous applications of atrazine. Models are ranked (in parentheses) by their correlation coefficients (CC). The best fit model is ranked 1 and the worst is ranked 16. The Nebraska data is not ranked with the others as the units and media were unique. Observed versus simulated concentrations for rows in italics are plotted on Figure 3[a]

	Temp	Moist	DEA	DIA ± 95%CI[b]	HYA	CC(Rank)	Atrazine	DEA	DIA	HYA
Soil	°C	% FC	%Atrazine decay				%Overall phi			
CO-A	10	40	6.7	1.7 ± 3.3	91.6	0.92(12)	24.9	25.0	23.0	27.1
	10	*70*	*2.1*	*0.5 ± 0.2*	*97.4*	*0.92(13)*	*29.6*	*19.4*	*26.1*	*24.9*
	20	40	33.6	8.6 ± 2.3	57.8	0.93(9)	24.0	20.2	24.4	31.5
	20	70	3.7	1.0 ± 0.5	95.3	0.93(10)	33.4	13.2	18.5	34.9
CO-N	10	40	3.0	0.8 ± 0.9	96.2	0.94(7)	39.7	15.6	17.1	27.5
	10	*70*	*13.8*	*3.5 ± 1.3*	*82.6*	*0.91(14)*	*30.0*	*31.7*	*13.8*	*24.5*
	20	40	0.8	0.2 ± 0.3	99.0	0.94(8)	47.3	30.0	0.0	22.7
	20	70	11.7	3.0 ± 0.9	85.3	0.91(15)	26.5	26.2	24.4	22.9
MS-A	10	40	6.8	1.7 ± 4.5	91.5	0.95(6)	32.7	2.0	30.4	35.0
	10	*70*	*2.2*	*0.6 ± 0.3*	*97.3*	*0.95(5)*	*25.9*	*21.2*	*20.3*	*32.6*
	20	40	46.0	11.8 ± 4.2	42.2	0.90(16)	27.5	16.5	21.2	34.8
	20	70	2.6	0.7 ± 14.1	96.7	0.97(2)	38.0	1.0	1.0	60.1
MS-N	10	40	4.5	1.2 ± 0.7	94.3	0.99(1)	22.0	16.0	30.5	31.5
	10	*70*	*16.9*	*4.3 ± 1.9*	*78.7*	*0.93(11)*	*39.5*	*20.8*	*13.3*	*26.4*
	20	40	54.9	14.0 ± 2.1	31.0	0.95(4)	25.4	24.6	22.2	27.8
	20	70	58.9	15.1 ± 1.5	26.1	0.95(3)	34.0	16.2	20.1	29.6
NE-A	*NA*[c]		*0.9*	*0.2 ± 0.6*	*98.9*	*0.91(NA*[d]*)*	*44.9*	*55.1*	*NA*[e]	*NA*[e]

[a] Temp = temperature, in °C; Moist = soil moisture, as percent of field capacity (FC); DEA = deethylatrzine; DIA = deisopropylatrazine; HYA = hydroxyatrazine; CI = confidence interval; CC = correlation coefficient; NA = not available.
[b] 95% confidence interval should be viewed as an indicator of uncertainty and may extend beyond the domain of the parameter. Confidence intervals were calculated for the fraction of DIA only with the fractions of DEA and HYA tied as described in the text.
[c] Temperature and moisture were not monitored at the depth of the Nebraska lysimeter
[d] Not ranked because the units and media were unique.
[e] DIA and HYA were not included in the list of analytes for the Nebraska porewater samples.

analytical methods yielding lower detection levels should improve the confidence of the calibrated models. Fourteen of the 17 models predicted hydroxyatrazine to be the dominant primary metabolite, accounting for 57 to 99% of the mass of primary metabolites produced (Table 2). The two models for nonadapted soils from Mississippi soils at 20°C predicted DEA to be the major metabolite, accounting for 55 and 59% of the mass of primary metabolites produced for soils moisture of 40 and 70% of field capacity. The remaining model that predicted DEA to be the dominant metabolite, the adapted Mississippi soils at 20°C and 40% field capacity, in reality had no observations of DEA above the detection level. In that model, the worst performing model with a correlation coefficient of 0.90, fitting a spike of DIA observed in the first 8 d following the atrazine application resulting in a prediction of 46:12:42% for DEA:DIA:HYA, respectively. A model with the freedom to have the fraction of DIA greater than the fraction of DEA would produce a better model in that case. Releasing the constant ratio constraint in the kinGUI software resulted in estimates of 13:67:20% for DEA:DIA:HYA, respectively, but the estimates for DEA and DIA were not significant ($P > 0.4$). For both adapted and nonadapted soils, the fractions predicted by BSFOD for HYA decreased with temperature. Increased soil moisture increased the predicted HYA fraction in adapted soils and decreased the predicted HYA fraction in nonadapted soils.

The overall model performance as indicated by the correlation coefficient with weights varied between 0.90 and 0.99 (Table 2). Contributions to phi for the final models average approximately 30% for atrazine and HYA, and near 20% for DEA and DIA. By assigning weights inversely related to the standard deviation of the residuals produced with uniform weights, the median uncertainty (95% confidence interval) for the eight estimates of the half-lives in adapted soils (four each from Colorado and Mississippi) increased from 0.5 d to 0.9 d for atrazine; decreased from 8.2 d to 1.2 d for DEA; decreased from 1.8 d to 0.5 d for HYA; and decreased from 14.9 d to 10.9 d for DIA. The median uncertainty in the estimates of half-lives in nonadapted soils resulting from nonuniform weighting increased from 4.6 d to 8.1 d for atrazine, decreased from 123 d to 29.5 d for DEA, decreased from 6.8 d to 1.2 d for HYA, and increased slightly from 434 d to 564 d for DIA. The greater uncertainty of the long-period dynamics of DIA transformations reflected the limited observation period (60 d for the Colorado and Mississippi soils).

Atrazine concentrations predicted by the calibrated BSFOD models matched the observations with correlation coefficients of 0.70 to 0.99 (Table 3, Fig. 3). Correlation coefficients for DEA, DIA, and HYA averaged 0.60, 0.52, and 0.41, respectively. Some of the poor correlations for predicting primary

Table 3. Correlation coefficients showing fit of observed versus simulated values for atrazine and primary metabolites for soils from Colorado (CO), Mississippi (MS), and Nebraska (NE), USA, for soils adapted (-A) and nonadapted (-N) to previous applications of atrazine. Observed versus simulated concentrations for rows in italics are plotted on Figure 3[a]

			Correlation coefficients per group			
	Temp	Moist	Atrazine	DEA	DIA	HYA
Soil	°C	% FC	R[b](non-detects out of 24 observations)			
CO-A	10	40	0.96(0)	−0.27(23)	0.06(13)	0.26(0)
	10	*70*	*0.96(0)*	*0.80(9)*	*0.31(18)*	*0.79(6)*
	20	40	0.98(8)	–(24)	0.32(15)	0.60(0)
	20	70	0.97(9)	0.83(16)	0.76(18)	0.64(9)
CO-N	10	40	0.70(0)	0.68(18)	0.66(21)	0.20(0)
	10	*70*	*0.90(0)*	*0.90(9)*	*0.95(9)*	*−0.36(1)*
	20	40	0.94(0)	0.41(20)	–(24)	−0.61(0)
	20	70	0.94(0)	0.94(5)	0.90(6)	−0.61(0)
MS-A	10	40	0.98(0)	–(24)	0.03(19)	0.77(0)
	10	*70*	*0.98(1)*	*0.74(17)*	*0.56(21)*	*0.57(1)*
	20	40	0.97(7)	–(24)	0.48(18)	0.82(0)
	20	70	0.99(12)	–(24)	–(24)	0.73(1)
MS-N	10	40	0.92(0)	0.94(14)	0.44(13)	0.65(0)
	10	*70*	*0.89(0)*	*0.91(7)*	*0.89(14)*	*0.41(0)*
	20	40	0.98(0)	−0.32(12)	0.17(11)	0.82(0)
	20	70	0.99(0)	0.60(3)	0.72(6)	0.92(0)
NE-A	*NA*[c]		*0.94(0)*	*0.32(0)*	*NA*[d]	*NA*[d]

[a] Temp = temperature, in °C; Moist = soil moisture, as percent of field capacity (FC); DEA = deethylatrzine; DIA = deisopropylatrazine; HYA = hydroxyatrazine; R = correlation coefficient; – = undefined; NA = not available.
[b] R is undefined when all observations are zero.
[c] Temperature and moisture were not monitored at the depth of the Nebraska lysimeter.
[d] DIA and HYA were not included in the list of analytes for the Nebraska porewater samples.

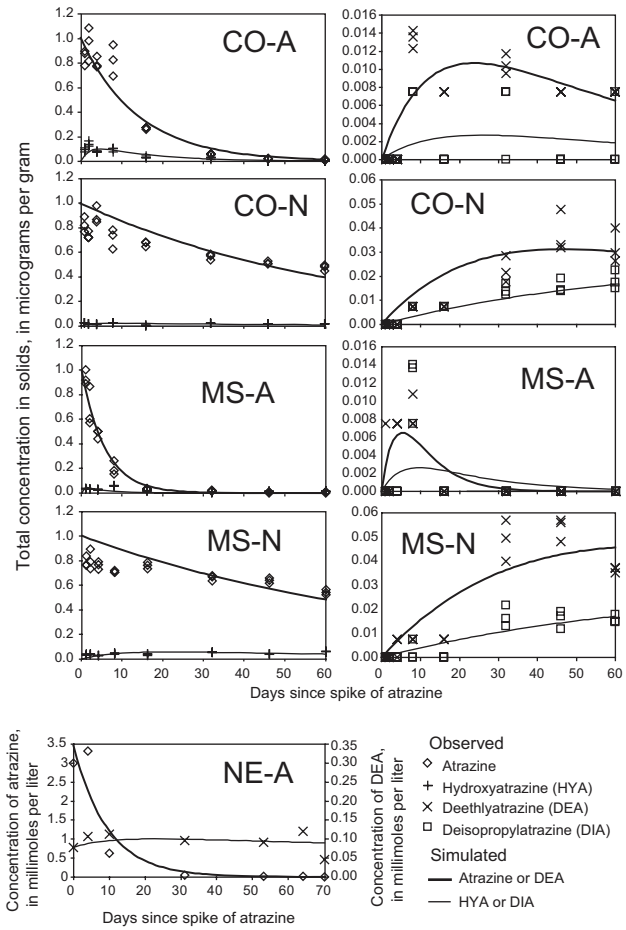

Fig. 3. Concentrations of atrazine and primary metabolites simulated using BSFOD (lines) versus observations (symbols) measured in soils from Colorado (CO), Mississippi (MS), and Nebraska (NE), USA adapted (-A) and nonadapted (-N) to regular applications of atrazine. Plots for Colorado and Mississippi show total concentrations measured in batch samples maintained at 10°C and 70% of soil moisture field capacity in the laboratory (atrazine and hydroxyatrazine are shown in the left panels, deethlyatrazine and deisopropylatrazine are shown in the right panels; note different scales). The plot for Nebraska shows dissolved concentrations of atrazine and deethlyatrazine from pore water collected beneath a cornfield in Nebraska following a spring application of atrazine in 2004. Soil temperature and moisture were not measured at the depth of the Nebraska lysimeter.

metabolite concentrations resulted from trying to match observations near or below the detection limit; some negative correlations were computed where the predicted peaks in concentrations of the primary metabolite were out of phase with the observed maxima. An objective function with more tolerance to phase mismatch may help improve the overall models [33].

CONCLUSION

The degradation of a pesticide into multiple primary metabolites can be simulated using a BSFOD model. The BSFOD model, a simplification of a myriad of processes, constrains the appearance of all individual metabolites to be consistent with the disappearance of the pesticide. The median estimate of the half-lives estimated with calibrated BSFOD models for different soil temperatures and moisture content was 5.0 d for atrazine applied to soils with a history of applications compared to 41.2 d for atrazine applied soils with no previous history of atrazine application. The median estimates for half-lives for DEA and DIA in adapted soils were similarly close to 10% of the half-lives estimated for nonadapted soils. The median predicted half-life for HYA showed less sensitivity to the suggested changes in microbial populations in adapted soils, reducing to 40% of that estimated for nonadapted soils. The dominant metabolite of atrazine applied to the adapted and nonadapted soils was HYA; the only soils where DEA significantly exceeded the yields of HYA was for warm (20°C) nonadapted soils from Mississippi. The BSFOD simulations in spreadsheets or numerical models such as LEACHM can provide well-constrained estimates of half-lives that can be used to estimate optimal application rates for pesticides. The LEACHM simulations of BSFOD of atrazine applied biannually to a field under corn/soy rotation in Maryland [22] using a half-life of 6 d showed a 13.7-fold reduction in leaching compared to the same simulations run with a half-life of 66 d.

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