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Uptake and Metabolism of Atrazine by Poplar Trees

JOEL G. BURKEN† AND JERALD L. SCHNOOR*,‡

Department of Civil Engineering, 204 Carlton-Butler Hall, 1870 Miner Circle, University of Missouri−Rolla, Rolla, Missouri 65409-0030, and Department of Civil & Environmental Engineering, 116 Engineering Research Facility, The University of Iowa, Iowa City, Iowa 52242

Hybrid poplar trees can uptake, hydrolyze, and dealkylate atrazine to less toxic metabolites. In whole plant studies, the parent compound atrazine and ^{14}C ring-labeled metabolites were extracted from poplar tissues and analyzed via high-pressure liquid chromatography (HPLC) with UV and radiochromatographic detectors in series. The concurrent separation and identification of these metabolites has not been previously reported in higher plants for phytoremediation applications. Unidentified metabolites were also detected. Metabolism of atrazine occurred in poplar roots, stems, and leaves and became more complete with increased residence time in tissues. In poplar cuttings exposed to atrazine for 50 days, the parent compound comprised only 21% of the ^{14}C label in the leaves, while it constituted 59% of ^{14}C activity remaining in the soil. After 80 days, the parent compound remaining in the leaves had decreased to only 10% of the ^{14}C label recovered in the leaves. Preferred metabolic pathways were suggested by relative rates of reaction, and a mathematical model was developed to estimate rate constants for the proposed degradation mechanism. This research provides evidence for vegetative detoxification of contaminants and suggests that phytoremediation of atrazine-contaminated soils may be feasible.

Introduction

Phytoremediation is an emerging technology that has the potential for cleanup of contaminated sites. Four mechanisms are involved in phytoremediation of organic pollutants: direct uptake and accumulation of contaminants and metabolism in plant tissues; transpiration of volatile organic hydrocarbons through the leaves; release of exudates that stimulate microbial activity and biochemical transformations in the soil; and enhancement of mineralization at the root−soil interface that is attributed to mycorrhizal fungi and the microbial consortia associated with the root surface (*1*). Direct uptake of organic compounds has been examined in previous studies (*2*−*4*). Researchers have determined many of the chemical properties that govern uptake into vascular plants (*5*−*7*). Briggs et al. (*8*) found that translocation of organic compounds is governed by lipophilic properties such as the octanol−water partition coefficient, K_{ow}. For compounds of similar structure and molecular weight, they found that uptake and translocation are greatest for compounds with log K_{ow} values ranging from 1 to 3. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] has a log K_{ow} of 2.56 at 25 °C (*9*), and it has been shown to be readily taken up by terrestrial vegetation (*4*, *10*). One study found that 91% of atrazine applied to poplars grown in silica sand can be assimilated in less than 10 days (*10*).

Once organic compounds have been assimilated into plant tissues in a phytoremediation treatment system, the chemical structure and ultimate fate of the pollutant is of paramount concern. The majority of earlier studies did not establish whether contaminants that accumulated in above-ground portions of the vegetation were present as parent compound, bound residues, or metabolites (*11*−*14*). As Nellessen and Fletcher (*15*) point out, uptake/accumulation, translocation, adhesion, and biotransformation of xenobiotics are environmentally important because these influence the amount and nature of food chain contamination. The study presented here addresses the issue of whether plant biomass grown at hazardous waste sites contaminated with atrazine could itself be a hazardous waste and pose a health threat.

Numerous studies have established metabolism of organic compounds occurring in plant cell cultures (*16*, *17*) and in whole plant experiments with selected species (*3*, *6*, *18*, *19*). The majority of these studies established that metabolism occurred by analyzing for the presence of radiolabeled compounds, other than the parent compound, in plant tissues. Metabolites were generally categorized by their polarity and solubility, but specific metabolites were not identified in plant tissues (*3*, *6*, *16*, *17*, *19*, *20*). However, in a study utilizing corn, atrazine metabolism was reported to occur via 2-hydroxylation and N-dealkylation pathways, and glutathione and cysteine conjugates were identified (*21*). Atrazine metabolism to unidentified metabolites has also been reported in the literature (*19*, *20*, *22*).

In studies investigating the uptake of organic xenobiotic compounds, the formation of bound residue, covalently bound compounds that are non-extractable, is often an appreciable sink for compounds that enter plant tissues. Komossa et al. (*23*) give a good review of the formation of bound residues for many xenobiotics. The formation of bound residues from atrazine is considered to be a detoxification mechanism. Studies performed with atrazine found that plant-bound residues were excreted nearly quantitatively in feces, by rats within 1 day and by sheep within 2 days (*24*). Khan and Dupont (*25*) found that a major portion of bound residues is associated with lignin. In a review of pesticide-bound residue studies, they concluded that feeding plant materials containing bound residues to monogastric animals may be considered to be of little toxicological concern.

Plant metabolism of several organic xenobiotic compounds is well documented; however, the plant species studied were primarily annual agricultural crops and not woody perennials such as poplars that are proposed for remediation of shallow contaminated soils at hazardous waste sites (*1*). Recently, work by Strand et al. (*18*) demonstrated that poplar trees have the capability to uptake and degrade the chlorinated solvent TCE to aerobic degradation products: 2,2,2-trichloroethanol, trichloroacetic acid, and dichloroacetic acid. In that study, dichloroacetic acid was the most prevalent metabolite detected in leaf tissues. For phytoremediation to become an accepted treatment option, either in conjunction with traditional technologies or on its own, identification of metabolites must be addressed to ensure that there are no toxic products. This paper shows that hybrid poplar trees, a vigorously growing hearty perennial with proven phytoremediation potential (*1*), have the capability to degrade atrazine that has been taken up from contaminated water and soil.

* Corresponding author phone: 319-335-5649; fax: 319-335-5585; e-mail: jschnoor@cgrer.uiowa.edu.
† University of Missouri−Rolla.
‡ The University of Iowa.

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Materials and Methods

Uptake Studies. Uptake studies were conducted either in an Iowa agricultural silt-loam soil (Nodeway-Ely series) taken from an uncontaminated field site with a fraction organic matter, f_{om}, of 0.025 or in washed silica sand media (f_{om} = 0.001). Soil was air-dried, pulverized, and passed through a 2-mm sieve. Sand or soil was then homogenized, and 800 g was placed in each 1-L bioreactor (Fisher).

One poplar tree cutting (*Populus deltoides x nigra* DN34, Imperial Carolina), about 25 cm (length) by 7 mm (stem diameter), was grown in each bioreactor. Cuttings were rooted in hydroponic solution, one-quarter Hoagland's solution (*26*), until a vascular root system appeared and were then transplanted to the soil or sand reactors. In order to mimic field conditions, the cuttings utilized in this work were clones taken from 2−4-year-old trees growing at an uncontaminated field site. Starting poplar trees from cuttings is the same method used in full-scale phytoremediation projects. Bioreactors were treated with doses of 48.3 μg (2.47 μCi) of ^{14}C uniformly ring-labeled atrazine (specific activity = 10.9 mCi/mM, Sigma Chemical). This is approximately three times field application rates of 4−6 lb/acre. Atrazine chemical purity was 97.1%, and radiochemical purity was 99.5%. Bioreactors were maintained in a laboratory growth chamber under artificial growth lights at 27 °C and were watered daily with 0.5 strength Hoagland's solution to maintain reactors at 70% field saturation. Photon flux at the leaf surface was 300−350 μmol s^{-1} m^{-1} in the photoactive range. The growth chamber is described elsewhere (*10*).

After 13 days, any ^{14}C activity remaining in the root zone of the sand bioreactors was removed. This was performed for all sand-filled bioreactors regardless of total time of incubation. The sand in the bioreactors was repeatedly rinsed with deionized H_2O and drained, until <1% of the added ^{14}C activity was detected in the rinse water removed from the bioreactors. By removing all ^{14}C atrazine or metabolites and curtailing further uptake, it was ensured that any further degradation was occurring within poplar tissues after uptake and not prior to transport through poplar tissues. Bioreactors not sacrificed at 13 days were placed into the growth chamber again for the remainder of their growth period. Washing procedures were done only on the sand reactors.

At predetermined time intervals, total ^{14}C uptake was quantified in poplar root, stem, and leaf samples via oxidation to CO_2 and subsequent liquid scintillation counting (LSC), performed on a Beckman LS6000IC. This oxidation and LSC method exhibited an efficiency of >96% and was calibrated with pre- and post-analysis efficiency testing each time it was employed. Efficiency testing was completed by oxidizing a know amount of ^{14}C activity, and LSC determination of the captured ^{14}C activity. Remaining poplar biomass samples were dried at 103 °C for 24 h, homogenized, and stored at 4 °C until further analysis was performed. The oxidation procedure is explained elsewhere (*10*).

Extraction Procedure. Stored samples from uptake studies were placed in 10−80 mL of 80% aqueous methanol (Optima grade, Fisher Scientific) in 120-mL vials fitted with Teflon-lined caps. Vials were placed in an Orbit heated shaker bath for 48 h at 70 °C and 100 rpm. Upon removal, vials were centrifuged, and the supernatants were filtered (Whatman GF-C). For dilute samples, the filtrate was evaporated to near dryness under a pure N_2 stream at 60 °C. The residue was then re-suspended in 4 mL of methanol:water. The extraction procedure was also performed on 50 g of sand and soil samples, wet weight. The filter residue was stored at 4 °C until it could be oxidized to quantify the formation of bound residue.

Metabolite Analysis. From the extracts, 100-μL samples were analyzed via HPLC equipped with a Gilson Spectra 100 variable wavelength detector and a Packard Radio-Chromatographic A500 detector in series. Metabolites were separated on a C-8 reverse phase column (Econosphere C8 3U, 100 mm × 4.6 mm, Alltech) with a mobile phase gradient of deionized H_2O (eluent A) and methanol:water 90:10 and 50 mM ammonium acetate, pH 7.4 (eluent B). The gradient started at 2% B, ramped linearly to 8% at 4 min, 45% at 10 min, 55% at 16 min, 85% at 23 min, and holding at 85% B throughout the 27-min run. In accordance with soil analysis techniques developed by Rustum et al. (*27*), identification of individual radioactive peaks was conducted by comparing the retention times of ^{14}C peaks with peak retention times of non-radioactive standards (Chem Service) obtained at a wavelength of 220 nm. Quantification of individual metabolites in the poplar extracts was accomplished by measuring ^{14}C activity, detected with the radiochromatographic detector. Peak measurement using ^{14}C activity assured that metabolites emanated from the [^{14}C]atrazine. The radiochromatographic detector utilized a 0.5-mL flow-through liquid cell with a scintillation cocktail (Ultima-Flo M, Packard) flow of 3 mL/min. This yielded a 3:1 cocktail to HPLC flow ratio. Output of the detector was subject to an automatic 20 disintegration/min (DPM) background subtraction. For peak search and integration programming, a minimum peak area of 40 DPM was selected.

Results and Discussion

Uptake Studies. Total uptake, determined as percent of ^{14}C applied, equaled 27.8% and 29.2% for the trees grown in soil and harvested at 52 and 80 days, respectively. Uptake by poplar cuttings grown for 52 and 80 days was not significantly different when subject to the Student's *t*-test. The uptake of the [^{14}C]atrazine from soil-filled reactors is covered in-depth elsewhere (*10*). After 13 days, the poplar cuttings grown in sand had assimilated an average of 71 ± 8% of the applied ^{14}C label. Data for all cuttings grown in sand were grouped for the uptake calculations listed for 13 days, regardless of the total growth period. Uptake by poplars grown for longer periods was not different than those sacrificed at 13 days. All uptake measurements were determined via oxidation and LSC analysis of poplar tissues. More information on the uptake of atrazine is also presented elsewhere (*10*).

Metabolite Analysis. Extraction methods proved to be effective at extracting ^{14}C compounds. Greater than 80% of ^{14}C activity present in plant tissues and soils was recovered in extracts. The ^{14}C activity remaining in plant tissues and soils was determined to be non-extractable bound residue as defined by Klein (*28*). Bound residue ranged from 8.4 ± 1.0% of applied ^{14}C label in poplars harvested from sand bioreactors at 13 days up to 15.8 ± 2.9% in cuttings exposed for 48 days. The percentage of ^{14}C activity identified as bound residue is in agreement with the range of values found in earlier studies on the fate of atrazine (*23*). A more aggressive extraction such as supercritical fluid extraction would likely have recovered a greater percentage of the labeled compounds from the soils and tissues. The method used allowed for a large number of samples to be processed simultaneously, and the greater than 80% extraction was deemed to be satisfactory for this study. For this experimental arrangement, total mass balances accounted for greater than 86% of the applied label in all subjects tested, and closure of the mass balance was generally greater than 94% (*10*).

Extraction procedures and analytical methods were developed to separate, detect, and quantify the following atrazine metabolites: hydroxyatrazine (HA), deethylatrazine (DEA), deisopropylatrazine (DIA), deethylhydroxyatrazine (DEHA), and didealkylatedatrazine (DDA), utilizing the UV and radiochromatographic detectors in series. The molecular structure of these compounds can be seen in Figure 1. Ammeline (2-hydroxy- 4, 6-diamino-*s*-triazine) was also one of the compounds analyzed in this study. Ammeline was the most polar and the least retarded of analytes with a retention time of 1.80 min. The pinnacle of the first peak often preceded

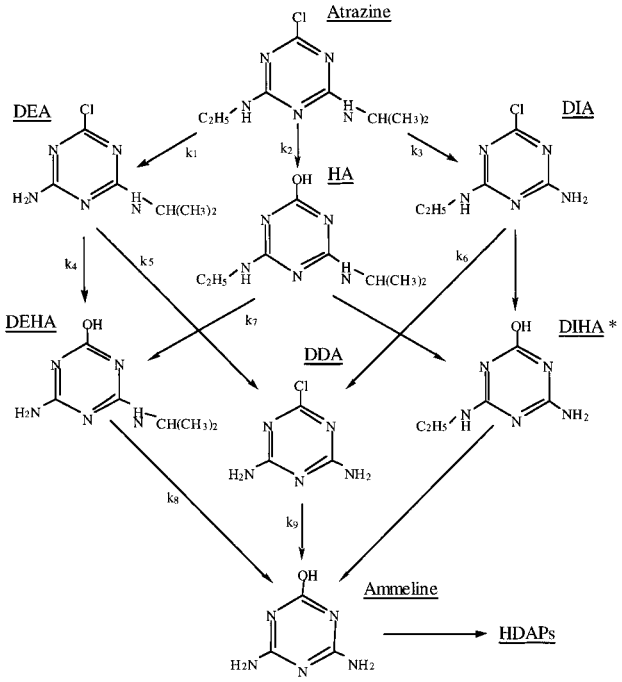

FIGURE 1. Proposed mechanism for atrazine degradation in poplar tissues. An asterisk (*) indicates that DIHA was not detected. DEA, deethylatrazine; HA, hydroxyatrazine; DIA, deisopropylatrazine; DEHA, deethylhydroxyatrazine; DDA, dideal kylatedatrazine; HDAP, hydroxylated dealkylated products.

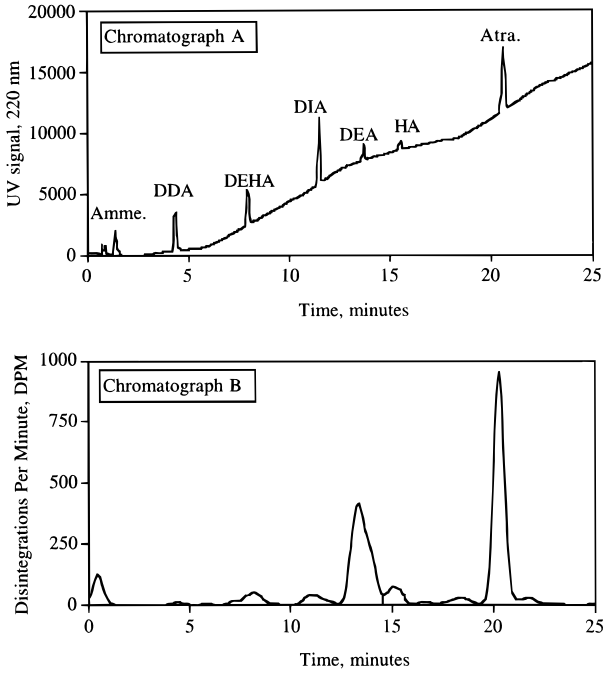

FIGURE 2. Sample chromatographs of (A) UV detector output for metabolite standards and (B) radiochromatograph detector output for leaf extract. The sloping tendancy of the baseline in chromatograph A is a manifestation of the methanol gradient method as methanol absorbs light at $\lambda = 220$ nm.

the retention time of ammeline by 0.2−0.6 min. The retention times of the unidentified peaks and the progression of their appearance in the sampling scheme are implications that ammeline may have been further degraded to the hydrophilic compounds ammelide (2,4-hydroxy-6-amino-*s*-triazine), to cyanuric acid (2,4,6-hydroxy-*s*-triazine), or to ring cleavage products. It is presumed that the peaks were hydroxylated de-alkylated products, termed HDAP, that were produced in the plant tissues and co-eluted with ammeline in the HPLC analysis, thus causing the peak to elute in the range of 1.2−1.8 min. Efforts to separate and identify these possible products via HPLC methods were unsuccessful. These potential metabolic products with a retention time of 1.2−1.8 min are summed with ammeline and reported as HDAP for the remainder of this paper.

Identification by a single chromatographic procedure such as HPLC is not absolute. The ^{14}C associated with the chromatographic peaks does confirm that the separated compounds originated from the introduced [^{14}C]atrazine. Further analytical procedures (MS, NMR) would have been necessary to unequivocally state that the detected metabolites were the compounds listed above. The small sample size, ^{14}C activity, and the extracted plant constituents precluded the use of MS or NMR analysis. The presence of ^{14}C activity known to emanate from [^{14}C]atrazine and the similarities in retention times of the detected metabolites to known atrazine metabolites strongly suggest that the detected metabolites from the poplar extracts were the atrazine metabolites listed above.

The parent compound atrazine and metabolic products were detected in all samples analyzed. Sample chromatograms of a standard and a leaf tissue extract are presented in Figure 2. The baseline drift in the UV signal presented in Figure 2 was a manifestation of the methanol:water gradient used to separate the metabolites. Methanol absorbed light at the wavelength of 220 nm used to determine retention times of atrazine and metabolites. Of the listed metabolites, DEA and HA were most frequently detected. In all cases, the metabolites, along with HDAP, accounted for over 95% of the extracted label. In a limited number of HPLC samples, small peaks of ^{14}C activity could not be identified, as is evident in Figure 2. These peaks generally accounted for less than 2% of the extracted radioisotopic compounds.

Atrazine degradation in the soil was not affected by the presence of the poplar cutting. In 80-day soil controls without plants, the parent atrazine constituted 56.25 ± 0.03% of the ^{14}C-labeled compounds (data not shown) as opposed to 58.53 ± 0.16% in the planted bioreactors (Figure 3). Of the six metabolites studied, none were statistically different in concentration between the planted and non-planted controls. In the pore water removed from the sand bioreactors at 13 days, the parent compound atrazine constituted 73% of the total ^{14}C activity, and HA and DEA were the only metabolites detected (Figure 4), suggesting that fewer microbial transformations occurred in the sand reactors as compared to the soil reactors. Results are presented as normalized concentrations, i.e., the percent of ^{14}C activity that was extracted from tissue samples or in aqueous samples. Presentation in terms of normalized concentrations emphasizes the breakdown of the atrazine within the poplar tissues independent of other factors. Presentation of results on a total mass basis would obscure the metabolite transformation reactions that are the subject of this paper. Research investigating the total uptake is detailed elsewhere (*10*).

Transformation reactions increased along the translocation pathway as labeled compounds moved into the roots from the soil, through the stem, and to the leaves. A greater normalized concentration of metabolites occurred in the poplar tissues than in the soil, and the parent atrazine decreased as a percentage of ^{14}C-labeled compounds in every plant sample as compared to the soil or sand in which the cutting grew. However, degradation occurring in the soil bioreactors and degradation within the plant after uptake could not be distinguished. Normalized concentrations of atrazine and metabolites along the translocation pathway of poplars grown in soil with an 80-day exposure period are shown in Figure 3. The normalized concentration of atrazine was 58.5 ± 0.2% in the soil, decreased to 39.0 ± 5.0% in roots, and further decreased to only 9.6 ± 3.4% of the ^{14}C-labeled

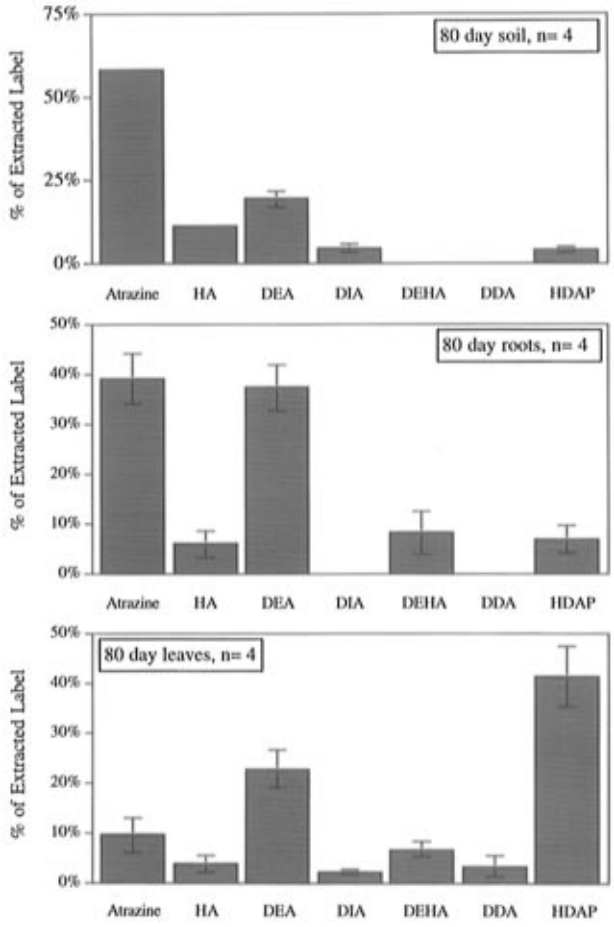

FIGURE 3. Atrazine degradation along the translocation pathway of poplar cuttings grown in Nodeway silt-loam soil for 80 days. Error bars represent ± 1 SD.

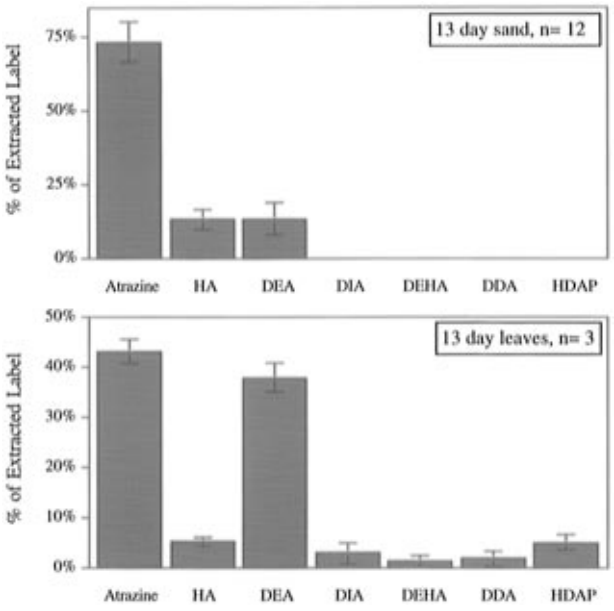

FIGURE 4. Atrazine degradation along the translocation pathway of poplars grown in silica sand for 13 days. Error bars represent ± 1 SD.

compounds in the leaves. Detection of HDAP and ammeline was a strong indication that atrazine metabolism occurred in poplar tissues as these metabolites were fully dealkylated and hydrolyzed. In the 80-day experiment conducted in soil, the HDAP comprised 4% in the soil, 7% in the roots, and 42% of the ^{14}C activity in the leaves. Ammeline and related

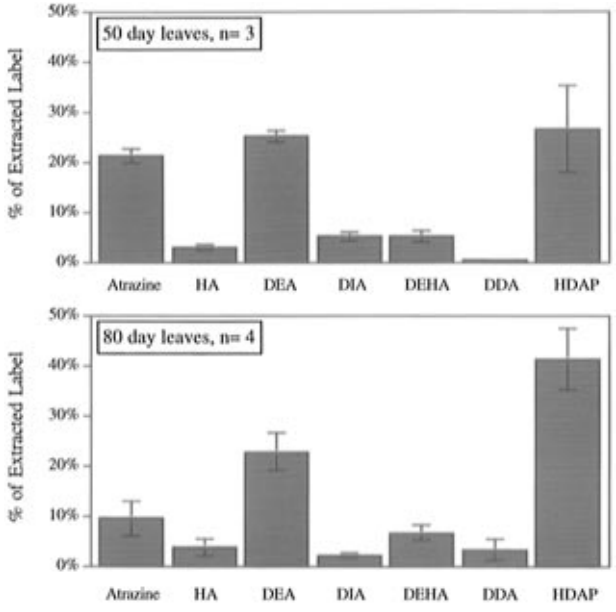

FIGURE 5. Normalized concentrations of atrazine and metabolites extracted from leaves vs time of exposure. Poplar trees were grown in soil. Error bars represent ± 1 SD.

compounds were the predominant metabolites detected in the leaves. Similar results were observed for poplars grown in sand-filled bioreactors. After poplars were incubated for 48 days in sand, the normalized concentration of HDAP was 8% in the roots and 19% in the leaves, and atrazine concomitantly decreased from 75% in the roots to 29% in the leaves.

The decline in parent atrazine along the translocation pathway was most pronounced in long growth period experiments (>15 days), implying that the transformation of atrazine increased with residence time in poplar tissues. In Figures 5 and 6, the time series of metabolites in poplar leaf extracts are displayed. Atrazine along with the mono-dealkylated metabolites DEA and DIA all decreased in normalized concentrations as growth periods increased. Over the same growth periods HDAP, DEHA, and DDA all increased, and HDAP showed the greatest increase. This demonstrates that atrazine was assimilated into poplar tissues, and it gradually degraded over time in poplar tissues.

In Figures 2−6, it can be observed that the preferential N-dealkylation product was DEA as opposed to DIA. DEA concentrations were 9.0 ± 2.6 times higher than DIA concentrations. This demonstrates that, for atrazine in poplar tissues, dealkylation of the ethyl side chain (deethylation) was the preferential dealkylation mechanism as opposed to deisopropylation. DEA was the predominant dealkylated species before any didealkylated metabolites were accumulated. This fact rules out the hypothesis that deisopropylation occurs at a similar rate with subsequent rapid degradation of DIA to other metabolites. A proposed schematic highlighting the preferential pathway for atrazine metabolism and fate in a poplar phytoremediation system is shown in Figure 7.

Similar findings were discovered for N-dealkylation reactions in the unsaturated zone of soil profiles and concluded that the deethylation rate of atrazine is approximately 2−3 times greater than the rate of deisopropylation (*29*). Our findings were similar for degradation occurring within the soil as DEA was 4.4 ± 0.8 times more abundant than DIA. Other research has shown DEA to be the predominant initial biotic degradation product of atrazine in surface water environments (*30, 31*) and in deeper soil and groundwater where denitrifying conditions prevail (*32*). Comparison of the findings presented here with research performed in soil

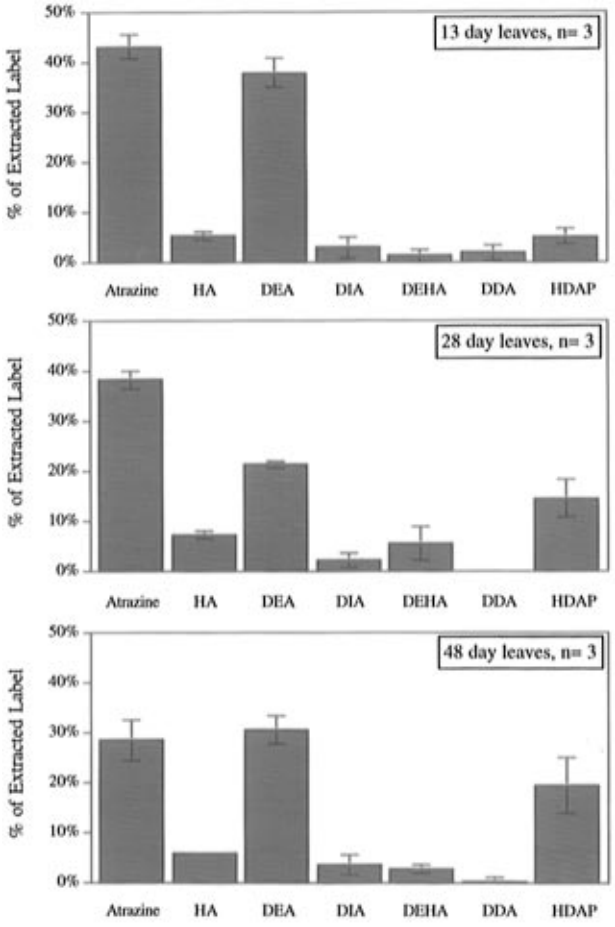

FIGURE 6. Normalized concentrations of atrazine and metabolites extracted from leaves vs time. Uptake was curtailed at 13 days by clearing the reactors of all atrazine and metabolites. Subsequent metabolites had to emanate from plant reactions and not microbial transformations. Poplar trees were grown in sand. Error bars represent ± 1 SD.

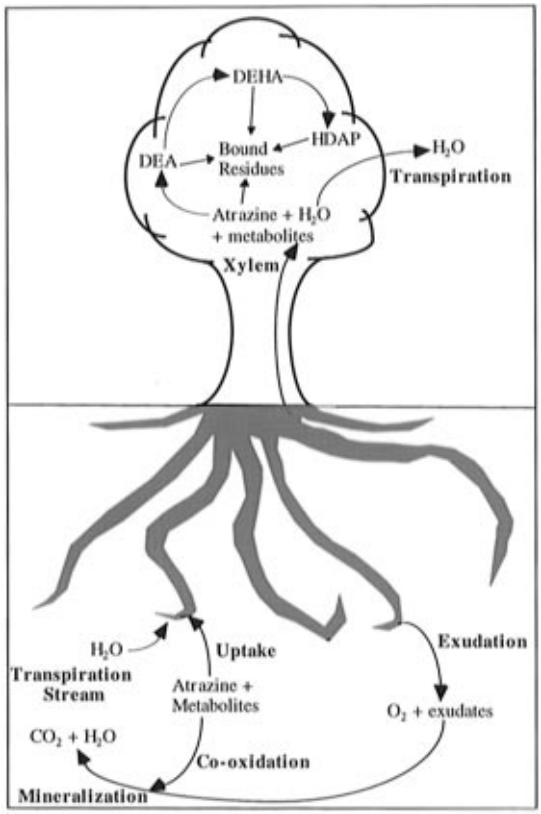

FIGURE 7. Atrazine fate in poplar cuttings: uptake, metabolism, and bound residues. Volatilization from leaves was not important. Mineralization of atrazine by microbes in the soil is also important (*2, 10*).

and aqueous environments implies that similarities exist between the metabolic pathways in poplar tissues and in aerobic biological mechanisms that occur in surface waters and soil.

After uptake, atrazine and the ensuing metabolites are confined to the poplar biomass and no longer in a mobile state, and the potential health threat is thought to be decreased. It has been shown that these compounds were not transpired through the leaves, thus eliminating any potential airborne health threat (*33*). The formation of hydroxylated atrazine degradation products is generally thought to detoxify atrazine. Jones and Winchell (*34*) found that HA showed no photosynthetic inhibition to four atrazine sensitive aquatic plants at the concentrations tested (0.5−1.5 ppm), and HA, although acutely toxic to rats at >3 g kg^{-1}, is not mutagenic or teratogenic (*31*). DEA and DIA have also been shown to be less phytotoxic than the parent atrazine with exhibited photosynthetic inhibition, usually less than half that of atrazine. Other research indicates that DEA and DIA might be of lesser concern as an environmental contaminant in soil or surface water, represented by increased mineralization rates and decreased half-lives as compared to the parent compound atrazine (*29, 35*). Furthermore, the formation of glutathione and cysteine conjugates (*19*) or the formation of other bound residues (*23*) are likely to be the ultimate fate of these metabolized compounds. Studies performed with atrazine found that plant-bound residues were excreted nearly quantitatively in feces, by rats within 1 day and by sheep within 2 days (*24*). Khan and Dupont (*25*) found that a major portion of bound residues are associated with lignin. In a review of pesticide-bound residue studies, they concluded that feeding plant materials containing bound residues of pesticides to monogastric animals may be considered to be of little toxicological concern. In brief, these metabolites in poplar tissues appear to be less toxic than the parent compound. However, the overall impact of atrazine metabolites and residues on terrestrial or aquatic ecosystems has not been fully assessed.

Model Development

A dynamic mathematical model was developed to gain a better understanding of the processes that metabolize atrazine within plant tissues in this laboratory experiment. This model is not intended to serve as a general model for all systems, but rather it is specialized to simulate these experiments. The model considers the following specific processes: metabolism in the bulk solution, transfer into roots with the transpiration stream, translocation within the plant via the transpiration stream, metabolism in the root and leaf tissues. The resulting model then describes the uptake, distribution, metabolism, and accumulation of atrazine and its metabolites in plant compartments. In order to focus on the metabolic processes within the plant, results from the sand/poplar bioreactors were modeled. In the sand bioreactors, mass transfer limitations were deemed to be negligible, and the reactors were assumed to be well mixed and homogeneous. (Assumptions of this nature were not applicable to soil reactors.) The stem was also considered to have a short residence time and was treated as a conservative transport pathway between the roots and the stem. Previous research found the stem to act as an inefficient chromatography column, acting only to slow the transport of compounds (*36*). The model incorporated degradation in the sand prior to uptake. Degradation was assumed to be first order and went

TABLE 1. Log K_{ow}, TSCF, and RCF Values for Atrazine and Metabolites

compd	log K_{ow}	TSCF[a]	RCF[a]
atrazine	2.56	0.6110	3.64
DEA	1.24[b]	0.6957	1.09
HA	1.94[b]	0.7758	1.76
DIA	0.60[b]	0.4431	0.907
DEHA	0.56[b]	0.4260	0.901
DDA	−0.6[b]	0.0769	0.830
ammeline	−1.2[b]	0.0206	0.823

[a] Calculated as described by Briggs (*5*). [b] Calculated by the method developed by Lyman (*38*).

only to HA and DEA, which were the only metabolites found in the sand. All metabolic reactions within the plant were also modeled as first-order reactions.

Atrazine uptake has been demonstrated to be a linear function of estimated soil solution concentration and transpiration (*4*). The linear model presented here employs first-order metabolic kinetics and transport similar to the model developed by Trapp et al. (*37, 38*). Sample equations are presented in eqs 1 and 2. In eq 2, uptake is a linear function of transpiration, concentration, and the transpiration stream concentration factor (TSCF). The TSCF is the ratio of a compound's concentration in the transpiration stream to the concentration in the bulk solution (*8*). A relation of the TSCF for a compound to the log K_{ow} of a compound was developed. The root concentration factor (RCF) was also used to estimate the sorption of the contaminants to the root tissues. It is defined as the ratio of the concentration in the roots to the concentration in the bulk solution. In order to obtain the TSCF and RCF values for atrazine and the metabolites, log K_{ow} values were calculated by methods reported by Lyman (*39*). The estimated log K_{ow}, TSCF, and RCF values are listed in Table 1. The predictive uptake and metabolic equations were then combined to mathematically predict the concentration of atrazine and subsequent metabolites in the pore water of the reactor and in plant tissues. Example model equations for atrazine concentration in bulk pore water solution and in leaf tissues are

$$\left(\frac{d[\text{Atra}]_W}{dt}\right) = -k_{1W}[\text{Atra}]_W - k_{2W}[\text{Atra}]_W - k_{3W}[\text{Atra}]_W - \text{TSCF}_{\text{Atra}}\frac{T[\text{Atra}]_W}{V_W} - k_S\left([\text{Atra}]_W - \frac{[\text{Atra}]_{RS}}{RCF}\right) \quad (1)$$

and

$$\left(\frac{d[\text{Atra}]_L}{dt}\right) = \frac{T[\text{Atra}]_R}{V_L} - k_{1L}[\text{Atra}]_L - k_{2L}[\text{Atra}]_L - k_{3L}[\text{Atra}]_L \quad (2)$$

Here the subscripts are defined as follows: W, pore water/bulk solution; R, root xylem; RS, root surface; L, leaf. Time, *t*, is measured in days.

A full list of the parameters used in the modeling analysis is presented in Table 2, and a schematic of the model is presented in Figure 8. The set of ordinary differential equations were solved using the modeling program STELLA II, which utilized a Runga−Kutta fourth-order numerical integration routine. The calculated TSCF and RCF values, measured transpiration rates, and measured water volumes were used in the model. First-order rate constants were adjusted to fit the data for each sampling period. While adjusting the rate constants, certain constraints were observed. For example, the rate of deethylation was maintained at approximately 5-fold the rate of the deisopropylation as was previously discussed. The model was constrained by laboratory measurements of metabolite concentrations, transpiration volumes, and uptake rates. The resulting values for all of the rate constants are tabulated in Table 2.

TABLE 2. List of Model Parameters

parameter	description	value	units
T	transpiration	23	mL/day
k_{1W}	dealkylation of atrazine ethyl side chain, water	0.012	d^{-1}
k_{2W}	chemical hydrolysis of atrazine, water	0.012	d^{-1}
k_{1R}	dealkylation of atrazine ethyl side chain, roots	1.6	d^{-1}
k_{2R}	hydrolysis of atrazine, roots	0.001	d^{-1}
k_{3R}	dealkylation of atrazine isopropyl side chain, roots	0.3	d^{-1}
k_{4R}	hydrolysis of DEA, roots	0.04	d^{-1}
k_{5R}	dealkylation of DEA isopropyl side chain, roots	0.06	d^{-1}
k_{6R}	dealkylation of DIA ethyl side chain, roots	0.3	d^{-1}
k_{7R}	dealkylation of HA ethyl side chain, roots	1.3	d^{-1}
k_{8R}	dealkylation of DEHA isopropyl side chain, roots	3.0	d^{-1}
k_{9R}	hydrolysis of DDA, roots	1.2	d^{-1}
k_{1L}	dealkylation of atrazine ethyl side chain, leaves	0.010	d^{-1}
k_{2L}	hydrolysis of atrazine, leaves	0.003	d^{-1}
k_{3L}	dealkylation of atrazine isopropyl side chain, leaves	0.002	d^{-1}
k_{4L}	hydrolysis of DEA, leaves	0.008	d^{-1}
k_{5L}	dealkylation of DEA isopropyl side chain, leaves	0.008	d^{-1}
k_{6L}	dealkylation of DIA ethyl side chain, leaves	0.010	d^{-1}
k_{7L}	dealkylation of HA ethyl side chain, leaves	0.004	d^{-1}
k_{8L}	dealkylation of DEHA isopropyl side chain, leaves	0.06	d^{-1}
k_{9L}	hydrolysis of DDA, leaves	0.08	d^{-1}
k_S	sorption to root surface	0.5	d^{-1}
V_W	average volume of H_2O in reactor	170	mL
V_R	average volume of H_2O in poplar roots	2	mL
V_L	average volume of H_2O in leaves	5	mL

Model-generated data and experimentally acquired data for the 48-day poplar cuttings are compared in Figure 9. The model overestimated DDA and HA at 48 days. Some model overestimation was unavoidable as the sum of measured metabolites did not equal 100% of the uptake due to the presence of bound residues (about 15 %) that were not extractable from the leaf tissue. (This model does not include the formation of bound residues in the plant tissues because it was not possible to estimate the rates at which the individual metabolites were conjugated to form bound residue.) It is also likely that metabolic activities of the root tissues were overestimated as metabolism by the microbial community associated with the root tissues were not measured separately. The model results were generally within one standard deviation (SD) of the lab data. The two state variables of primary interest, atrazine and HDAP in the leaf tissues, were plotted versus time (Figure 10). The simulation is considered a test of the mass balance model to evaluate rate constants. It is not considered as a final verification of the model.

From the model, information concerning the reaction rates was inferred. The root tissues had higher N-dealkylation reaction rates than the leaf tissues; rates in the roots were over 10-fold the rates in the leaves. Hydrolysis rates were much lower in the plant tissues than in the bulk solution prior to uptake. The estimated hydrolysis rates were lower in the poplar tissues than had been measured in agricultural crops such as corn (*21*). Deethylation predominated as the first step of the parent compound metabolism.

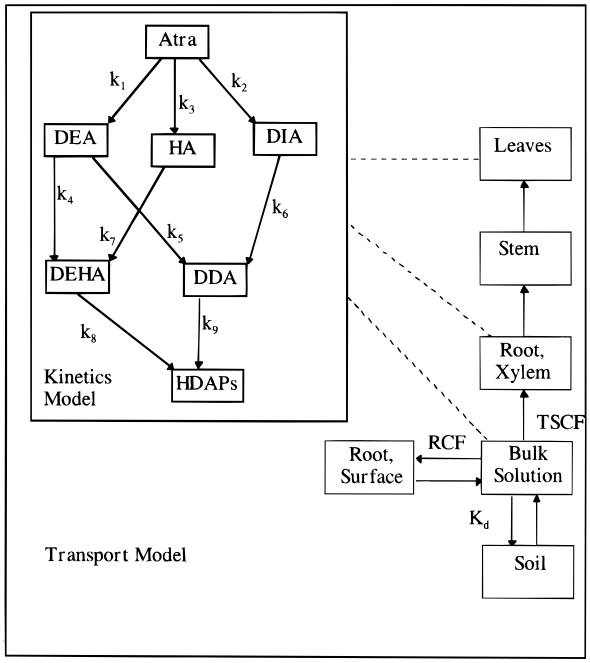

FIGURE 8. Compartmentalized model schematic. The transport model describes movement of atrazine and metabolites from the different compartments of this system. The kinetics model explains the metabolism of atrazine and metabolites in the bulk solution, root xylem, and leaves.

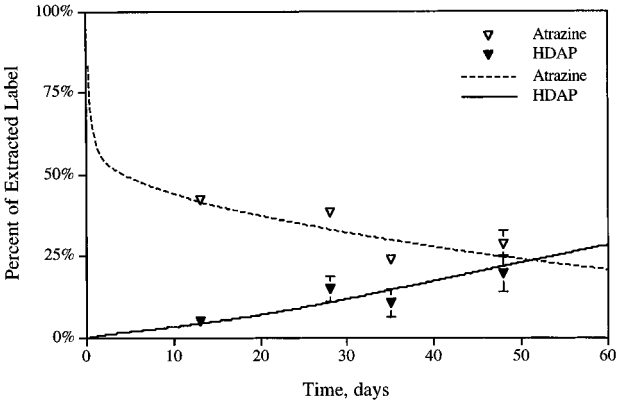

FIGURE 9. Normalized concentrations of atrazine and hydroxylated dealkylated products (HDAP) extracted from poplar leaves as a function of time compared with model results. Poplar trees were grown in sand.

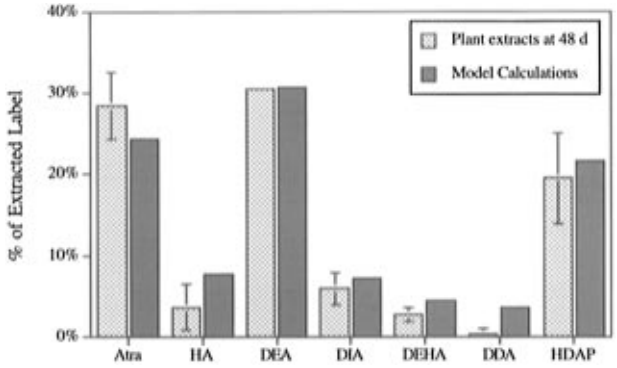

FIGURE 10. Comparison of actual and model calculated distribution of metabolites for poplar cuttings grown for 48 days.

Hybrid poplar trees were able to rapidly degrade assimilated atrazine to potentially innocuous metabolites. In this study, a high mass fraction of applied ^{14}C-labeled atrazine was taken up as ^{14}C-labeled atrazine. Subsequent extraction and analysis of biomass revealed that degradation, including complete dealkylation and hydrolysis, occurred in whole-plant experiments utilizing hybrid poplar trees grown in sand or soil. Degradation mechanisms were elucidated with respect to N-dealkylation rates and with comparison to established biotic degradative mechanisms.

If phytoremediation is to be a viable technology, the end products must be elucidated and identified. This research provides evidence for vegetative detoxification of atrazine to less toxic products and for the utility of phytoremediation. It demonstrates that hybrid poplar, a plant shown to offer other distinct advantages for treatment of organic contaminants (*1*), has the capability to remove, hydrolyze, and dealkylate atrazine from contaminated soils. Phytoremediation technology has the potential to provide an aesthetically pleasing, cost-effective treatment option for the *in-situ* cleanup of atrazine-contaminated sites.

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Literature Cited

(1) Schnoor, J. L.; Licht, L. A.; McCutcheon, S. C.; Wolfe, N. L.; Carriera, L. H. *Environ. Sci. Technol.* **1995**, *29*, 318A−323A.
(2) Nair, D. R.; Burken, J. G.; Licht, L. A.; Schnoor, J. L. *ASCE J. Environ. Eng.* **1993**, *119*, 842−854.
(3) McFarlane, J. C.; Nolt, C.; Wickliff, C.; Pfleeger, T.; Shimabuku, R.; McDowell, M. *J. Environ. Toxicol. Chem.* **1987**, *6*, 847−856.
(4) Walker, A. *Pestic. Sci.* **1972**, *3*, 139−148.
(5) Briggs, G. G.; Bromilow, R. H.; Evans, A. A.; Williams, M. *Pestic. Sci.* **1983**, *14*, 492−500.
(6) McFarlane, J. C.; Pfleeger, T.; Fletcher, J. S. *J. Environ. Toxicol. Chem.* **1990**, *9*, 513−520.
(7) Topp, E.; Scheunert, I.; Attar, A.; Korte, F. *Ecotoxicol. Environ. Saf.* **1986**, *11*, 219−228.
(8) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. *Pestic. Sci.* **1982**, *13*, 495−504.
(9) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. In *Environmental Organic Chemistry*; John Wiley & Sons, Inc.: New York, 1993.
(10) Burken, J. G.; Schnoor, J. L. *ASCE J. Environ. Eng.* **1996**, *122*, 958−963.
(11) Wang, M. J.; Jones, K. C. *Environ. Sci. Technol.* **1994**, *28*, 1260−1267.
(12) Anderson, T. A.; Walton, B. T. *Environ. Toxicol. Chem.* **1995**, *14*, 2041−2047.
(13) Ferro, A. M.; Sims, R. C.; Bugbee, B. *J. Environ. Qual.* **1994**, *23*, 272−279.
(14) McFarlane, J. C.; Pfleeger, T.; Fletcher, J. *J. Environ. Qual.* **1987**, *16*, 372−376.
(15) Nellesen, J. E.; Fletcher, J. S. *Environ. Toxicol. Chem.* **1993**, *12*, 2045−2052.
(16) Wilken, A.; Bock, C.; Bokern, M.; Harms, H. *J. Environ. Toxicol. Chem.* **1995**, *14*, 2017−2022.
(17) Harms, H.; Langbartels, C. *Plant Sci.* **1986**, *45*, 157−165.
(18) Strand, S. E.; Newman, L.; Ruszaj, M.; Wilmoth, J.; Shurtleff, B.; Brandt, M.; Choe, N.; Ekuan, G.; Duffy, J.; Massman, J. W.; Heilman, P. E.; Gordon, M. P. presented at ASCE Conference Proceedings, Pittsburg, PA, 1995.
(19) Benyon, K.; Stoydin, G.; Wright, A. N. *Pestic. Biochem. Physiol.* **1972**, *2*, 153−161.
(20) Montgomery, M. L.; Freed, V. H. *J. Agric. Food Chem.* **1964**, *12*, 11−14.
(21) Lamoureux, G. L.; Shimabukuro, R. H.; Swanson, H. R.; Frear, D. S. *J. Agric. Food Chem.* **1970**, *18*, 81−87.
(22) Trapp, S.; Matthies, M.; Schunert, I.; Topp, E. M. *Environ. Sci. Technol.* **1990**, *24*, 1246−1252.
(23) Komossa, D.; Langbartels, C.; Sandermann, H. J. In *Plant Contamination: Modeling and Simulation of Organic Chemical Processes*; Trapp, S., McFarlane, J. C., Eds.; CRC Press Inc.: Boca Raton, FL, 1995.
(24) Bakke, J. E.; Shimabukuro, R. H.; Davison, K. L.; Lamoureux, G. L. *Chemosphere* **1972**, *1*, 21.

(25) Khan, S. U.; DuPont, S. Presented at Sixth International Congress of Pesticide Chemistry, Ottawa, Canada, 1986.
(26) Licht, L. A. Ph.D. Dissertation, University of Iowa, 1990.
(27) Rustum, A. M.; Ash, S.; Saxena, A.; Balu, K. *J. Chromatogr.* **1990**, *514*, 209−218.
(28) Klein, W.; Scheunert, I. International Atomic Energy Agency Report IEIA-SM-263/38, 1982.
(29) Mills, M. S.; Thurman, E. M. *Environ. Sci. Technol.* **1994**, *28*, 600−605.
(30) Kolpin, D. W.; Kalkhoff, S. J. *Environ. Sci. Technol.* **1993**, *27*, 134−139.
(31) Lerch, R. N.; Donald, W. M.; Li, Y. X.; Alberts, E. E. *Environ. Sci. Technol.* **1995**, *29*, 2759−2768.
(32) Nair, D. R.; Schnoor, J. L. *Environ. Sci. Technol.* **1992**, *26*, 2298−2300.
(33) Schwartz, P. Masters Thesis, University of Iowa, 1991.
(34) Jones, T. W.; Winchell, L. *J. Environ. Qual.* **1984**, *13*, 243.
(35) Coats, J.; Kruger, E.; Anderson, T.; Chaplin, J.; Kanwar, R. *Iowa Groundwater Q.* **1995**, *6*, 28.
(36) Uchida, M. *Pestic. Biochem. Physiol.* **1980**, *14*, 249−255.
(37) Trapp, S. In *Plant Contamination: Modeling and Simulation of Organic Chemical Processes*; Trapp, S., McFarlane, J. C., Eds.; CRC Press Inc.: Boca Raton, FL, 1995.
(38) Trapp, S.; Matthies, M. *Environ. Sci. Technol.* **1995**, *29*, 2333−2338.
(39) Lyman, W. J. In *Handbook of Chemical Property Estimation Methods*; Lyman, W. J., Reehl, W. F., Rosenblatt, D. H., Eds.; McGraw-Hill: New York, 1982.

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