Biodegradability of Atrazine, Cyanazine, and Dicamba in Wetland Soils

J.-D. Gu, D.F. Berry, R.H. Taraban, D.C. Martens, H.L. Walker, Jr., and W.J. Edmonds
Biodegradability of Atrazine, Cyanazine, and Dicamba in Wetland Soils

J.-D. Gu
D.F. Berry
R.H. Taraban
D.C. Martens
H.L. Walker, Jr.
W.J. Edmonds

Department of Crop and Soil Environmental Sciences
Virginia Polytechnic Institute and State University
This bulletin is published with funds provided in part by the U.S. Geological Survey, Department of the Interior, as authorized by Public Law 101-397.

Contents of this publication do not necessarily reflect the views and policies of the United States Department of the Interior, nor does mention of trade names or commercial products constitute their endorsement or recommendation for use by the United States Government.

William R. Walker, Director
Diana L. Weigmann, Asst. Director
Shireen I. Parsons, Editor
George V. Wills, Graphic Designer
T.W. Johnson, Typesetter

Library of Congress Catalog Number: 91-68419

Additional copies of this publication, while the supply lasts, may be obtained from the Virginia Water Resources Research Center. Single copies are provided free to persons and organizations within Virginia. For those out of state, the charge is $10 a copy.
Table of Contents

List of Figures .......................................................... v
List of Tables ............................................................ ix
Acknowledgments .......................................................... xi
Abstract ........................................................................... 1
1. Introduction .............................................................. 3
  1.1 Wetland Soils and Pesticide Transport ......................... 3
  1.2 Anaerobic Microorganisms and Biodegradative Processes ... 4
2. Methodology ............................................................... 9
  2.1 Wetland Soils and Site Description .............................. 9
  2.2 Soil Sampling and pH Measurement .............................. 9
  2.3 Chemicals ................................................................... 9
  2.4 Microcosm Setup ...................................................... 9
     2.4.1 Methanogenic Microcosms ....................................... 9
     2.4.2 Nitrate-Reducing Microcosms .................................. 10
  2.5 Enrichment Cultures ................................................. 10
  2.6 Microcosm Sampling and Analysis ............................... 10
     2.6.1 Herbicide Analysis ............................................... 10
     2.6.2 Headspace Gas Analysis ......................................... 11
     2.6.3 Nitrate Analysis ................................................... 11
3. Results and Discussion ................................................. 13
  3.1 Fate of Herbicides in Methanogenic Microcosms ............. 13
     3.1.1 Atrazine-Amended Microcosms ............................... 13
     3.1.2 Cyanazine-Amended Microcosms ............................ 13
     3.1.3 Dicamba-Amended Microcosms ............................... 14
  3.2 Fate of Herbicides in Nitrate-Reducing Microcosms ........ 15
     3.2.1 Atrazine-Amended Microcosms ............................... 15
     3.2.2 Cyanazine-Amended Microcosms ............................ 16
     3.2.3 Dicamba-Amended Microcosms ............................... 16
4. Conclusions and Recommendations .................................. 19
References ........................................................................ 21
Figures ............................................................................ 25
Tables ............................................................................... 59
List of Figures

Figure 1. 
Fate of atrazine in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 27

Figure 2. 
Fate of atrazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 28

Figure 3. 
Fate of atrazine in Levy wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 29

Figure 4. 
Fate of cyanazine in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 30

Figure 5. 
Fate of cyanazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 31

Figure 6. 
Fate of cyanazine in an enrichment culture with the inoculum originating from a methanogenic Lawnes wetland soil microcosm (25°C) ........................................ 32

Figure 7. 
High-performance liquid chromatograms of cyanazine enrichment culture filtrate at time zero and day 95 ................................................................. 33

Figure 8. 
Fate of cyanazine in Levy wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 34

Figure 9. 
Fate of dicamba in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions ................................................................. 35

Figure 10. 
Fate of dicamba in Lawnes wetland soil microcosms incubated at 25°C under methanogenic conditions ................................................................. 36

Figure 11. 
Fate of dicamba in an enrichment culture. The inoculum originated from Lawnes methanogenic wetland soil microcosms (25°C) ........................................ 37

Figure 12. 
High-performance liquid chromatograms of culture filtrate from a dicamba enrichment culture first transfer at day 1 and day 10 ................................................................. 38

Figure 13. 
Degradation of dicamba and subsequent production of methane in an enrichment culture (second transfer) ................................................................. 39
Figure 14.
Postulated pathway for degradation of dicamba under methanogenic conditions. ............................................................... 40

Figure 15.
Fate of dicamba in Levy wetland soil microcosms incubated at 25°C under methanogenic conditions. .................................................. 41

Figure 16.
Fate of atrazine in Myatt wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................ 42

Figure 17.
Nitrate concentration in atrazine-amended Myatt wetland soil microcosms. ............................................................. 43

Figure 18.
Fate of atrazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................ 44

Figure 19.
Nitrate concentration in atrazine-amended Lawnes wetland soil microcosms incubated at 15° and 25°C. ................................. 45

Figure 20.
Fate of atrazine in Levy wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................. 46

Figure 21.
Nitrate concentration in atrazine-amended Levy wetland soil microcosms. ........................................................................ 47

Figure 22.
Fate of cyanazine in Myatt wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................ 48

Figure 23.
Nitrate concentration in cyanazine-amended Myatt wetland soil microcosms. ........................................................................ 49

Figure 24.
Fate of cyanazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................ 50

Figure 25.
Nitrate concentration in cyanazine-amended Lawnes wetland soil microcosms. ........................................................................ 51

Figure 26.
Fate of cyanazine in Levy wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. ............................. 52
Figure 27.  
Nitrate concentration in cyanazine-amended Levy wetland soil microcosms .................................................. 53

Figure 28.  
Fate of dicamba in Myatt wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions .............................................................. 54

Figure 29.  
Fate of dicamba in Lawnes wetland soil microcosms incubated at 25°C under nitrate-reducing conditions. ................................. 55

Figure 30.  
Nitrate concentration in dicamba-amended Lawnes wetland soil microcosms .............................................................. 56

Figure 31.  
Fate of dicamba in Levy wetland soil microcosms incubated at 25°C under nitrate-reducing conditions. ................................. 57

Figure 32.  
Nitrate concentration in dicamba-amended Levy wetland soil microcosms .............................................................. 58
List of Tables

Table 1.
Methane production in atrazine-amended microcosms. .......... 61

Table 2.
Initial and final pH of atrazine-amended methanogenic microcosms. ................................................... 62

Table 3.
Methane production in cyanazine-amended microcosms. .......... 63

Table 4.
Initial and final pH of cyanazine-amended methanogenic microcosms. ................................................... 64

Table 5.
First-order rate constants and half-lives for cyanazine and dicamba degradation in methanogenic microcosms. ................................................... 65

Table 6.
First-order rate constants and half-lives for cyanazine and dicamba degradation in methanogenic enrichment cultures (Lawnes soil inoculum). ....................... 66

Table 7.
Methane production in dicamba-amended microcosms.............. 67

Table 8.
Initial and final pH of dicamba-amended methanogenic microcosms. ................................................... 68

Table 9.
Initial and final pH of atrazine-amended nitrate-reducing microcosms. ................................................... 69

Table 10.
Initial and final pH of cyanazine-amended nitrate-reducing microcosms. ................................................... 70

Table 11.
Initial and final pH of dicamba-amended nitrate-reducing microcosms. ................................................... 71

Table 12.
First-order rate constants and half-lives for cyanazine and dicamba degradation in nitrate-reducing microcosms. ................................................... 72
Acknowledgements

The authors would like to express their appreciation to the Water Center's William R. Walker for his administration of this project and Diana L. Weigmann for her assistance.
The members of the Project Advisory Committee—Joe Hassell, Virginia Water Control Board; Mike Flagg, Division of Soil and Water Conservation; Dave Martens, Department of Crop and Soil Environmental Sciences, Virginia Tech; Michael Barrett, Office of Pesticide Programs, US Environmental Protection Agency; John Jordan, Office of Pesticide Programs, U.S. Environmental Protection Agency; John Bishop, Department of Biology, University of Richmond; Larry Livingston, Livingston Chemicals, Inc.; Betsy Stinson, Department of Game and Inland Fisheries; Timothy Longe, Superfund Program, Department of Waste Management; and William Walls, Department of Agriculture and Consumer Services—deserve special credit and thanks for providing beneficial suggestions and comments during this research project. The successful completion of this investigation and the relevance of the Virginia Water Resources Research Center’s total research program can be partially attributed to the guidance and participation of these knowledgeable and interested members of the project advisory committee.

Diana L. Weigmann
Assistant Director
Abstract

Small amounts of applied pesticide chemicals eventually can end up in nontarget areas such as wetlands, sediments, and groundwater where anaerobic conditions often predominate. Runoff and leaching are major means by which pesticides move away from application sites. Pesticides also can find their way into nontarget areas as a result of inappropriate disposal and accidental spills. We evaluated the biodegradability of atrazine, cyanazine, and dicamba in wetland soils under nitrate-reducing and methanogenic conditions. Wetland soil samples were collected from three different sites in the Chesapeake Bay watershed region. These sites represented both tidal (Lawnes and Levy soils) and nontidal (Myatt soil) wetlands. Tidal wetlands are water-saturated throughout the year, whereas nontidal wetlands are saturated only during certain times of the year. Herbicide fate studies were conducted in wetland soil microcosms consisting of serum bottles filled with soil slurry and containing either atrazine, cyanazine, or dicamba.

Atrazine was extremely stable in wetland soil microcosms regardless of incubation temperature, redox status (nitrate-reducing versus methanogenic conditions), or soil type. Neither temperature nor redox status affected cyanazine stability in Myatt wetland soil microcosms. We observed a significant decrease in cyanazine concentration in Lawnes wetland microcosms incubated under methanogenic and nitrate-reducing conditions. Losses were more pronounced at 25°C than at 15°C. Results from enrichment culture studies suggest that cyanazine was cometabolized (i.e., cyanazine could not be used as a carbon and energy source by the microorganisms) in Lawnes soil microcosms. Dicamba was readily biodegraded in the wetland soils tested, although total mineralization was not achieved.

Key Words: Wetland soil, herbicides, degradation, denitrifying conditions, methanogenic conditions.
1. Introduction

1.1 Wetland Soils and Pesticide Transport

The Tidewater section of Virginia associated with the Eastern Shore and Chesapeake Bay has approximately 91,000 acres of brackish-water tidal wetlands, about 83,000 acres of saltwater tidal wetlands, and about 42,000 acres of freshwater tidal wetlands (Edmonds et al., 1990). Depending upon the proximity to agricultural soils, pesticides (including herbicides) can enter these environments as a result of agricultural runoff or through contaminated groundwater.

Survey studies conducted by Baker and Richards (1989) and the U.S. Environmental Protection Agency (EPA) (1990) clearly show that pesticides move away from application sites and end up in nontarget environments such as rivers and groundwater. Pereira and Rostad (1990) reported that the Mississippi River and its major tributaries contained the herbicides 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (atrazine), 2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropionitrile) (cyanazine), 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine (simazine), 2-chloro-6'-ethyl-N-(2-methoxy-1-methylethyl)acet-o-toluidide (metolachlor), and 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetonilide (alachlor). In addition, two predominant atrazine degradates, 2-chloro-4-isopropylamino-6-amino-1,3,5-triazine (desethylatrazine) and 2-chloro-4-amino-6-ethylamino-1,3,5-triazine (desisopropylatrazine) also were detected in river water in concentrations ranging from 7-82 and 8-45 ng L$^{-1}$, respectively. Glotfelty et al. (1984) monitored the Wye River, a tributary of the Chesapeake Bay that is surrounded by an agricultural watershed, for the presence of the triazine herbicides, atrazine and simazine. In a year when significant runoff occurred within two weeks of herbicide application, 2-3% of the applied atrazine moved into the Wye River.

One of the major factors that influences pesticide runoff is the time elapsed between pesticide application and a rainfall event. Trichell et al. (1968) found that runoff losses four months after application of 3,6-dichloro-2-methoxybenzoic acid (dicamba), 2,4,5-trichlorophenoxyacetate (2,4,5-T) and 4-amino-3,5,6-trichloropicolinic acid (picloram) from sod and fallow plots were less than 1% of that lost in the first 24 hours after application. Triplett et al. (1978) determined that the quantity of atrazine and simazine transported increased with the amount of rainfall and was inversely related to the length of time between application and the runoff event. In another study, Baker and Johnson (1979) found that runoff losses of O-ethyl-S-phenyl-ethylphosphonodithioate (fonofos), alachlor, atrazine, and cyanazine were dependent on the time elapsed between pesticide application and the first runoff event. They also found that the herbicides alachlor, atrazine, and cyanazine were transported more readily in the dissolved phase, while a significant amount of fonofos was transported primarily with sediment. Their study also revealed that till-plant and ridge-plant tillage systems reduced erosion and pesticide runoff loss relative to conventional tillage practices.
Movement of surface-applied pesticides through the soil surface horizon has been examined in field studies. An investigation by Wehtje et al. (1984), conducted at a field site in the Platte River Valley of central Nebraska, revealed that an average of 0.72% of the surface-applied atrazine was recovered by vacuum extractors located 1.5 m below the soil surface. They also found that groundwater samples from an observation well located at the edge of the experimental field plot and downfield in the direction of the hydraulic gradient contained an average of 0.87 µg L⁻¹ atrazine, while water samples collected from a well upfield, during this same sampling time period, contained an average 0.14 µg L⁻¹ atrazine. Experimental evidence obtained from both laboratory and field investigations clearly shows that sorption processes and water flow rates are among the primary factors controlling pesticide leaching through soil.

Within wetlands, pesticides may accumulate, be transformed to inactive forms, or be completely degraded (i.e., mineralized). If the latter occurs, wetland ecosystems may represent a natural filter or purifier in the hydrogeologic cycle. The persistence of pesticides in wetland soils depends primarily upon their chemical stability (i.e., resistance to chemical decomposition) and susceptibility to biodegradative processes. In wetland habitats, the biodegradation of organic chemicals often occurs under anaerobic conditions.

1.2 Anaerobic Microorganisms and Biodegradative Processes

Anaerobes (i.e., microorganisms that live in the absence of oxygen) dominate wetland soils and sediments of both fresh and marine habitats that are consistently oxygen deficient. Fermenters (either obligate or facultative), sulfate reducers, and methanogenic microorganisms predominate in environments where neither oxygen nor nitrate are available. Under conditions where oxygen becomes limiting and nitrate is present, many aerobic microorganisms are able to use nitrate in place of oxygen as an electron acceptor. To survive in anoxic environments, anaerobes developed the capacity to use a variety of inorganic (NO₃⁻, SO₄²⁻, CO₂) and organic compounds (pyruvate, fumarate) as electron acceptors. Microbes able to use organic compounds as electron acceptors are called fermenters. Microorganisms require electron acceptors to regenerate (i.e., oxidize) intracellular electron carriers such as cytochromes. These oxidation-reduction reactions are critical to the production of adenosine triphosphate (ATP), regardless of whether energy is generated using electron transport or substrate-level phosphorylation.

Because of the ubiquitous nature of organic matter, organic carbon (e.g., glucose) predominates as the electron donor in most anoxic environments, and is required by many anaerobes for their energy-yielding, oxidation-reduction reactions. The availability of electron acceptors will, to a large degree, control anaerobe diversity. For example, in anoxic environments that are without light and low in electron acceptors other
than CO₂, a syntrophic association involving three groups of anaerobes including fermenters, proton reducers, and methanogens (i.e., methane-producing bacteria) is required to completely mineralize an organic substrate (Berry et al., 1987). Complete mineralization of an organic substrate under methanogenic conditions, as just described, results in the formation of CO₂ and methane (CH₄), an odorless, colorless, nontoxic gas that is a common component of marsh gas. The mineralization of a nitrogen-containing organic compound under methanogenic conditions would result in the production of CO₂, CH₄, and NH₃. If sulfate predominates as the electron acceptor, as would be the case in brackish- or marine-water wetlands, then sulfate-reducing microbial populations would be expected to dominate the habitat. If nitrate is present, then nitrate-reducing organisms would predominate.

Dicamba belongs to a group of chemicals commonly referred to as the chlorinated aromatic (benzenoid) compounds. Several compounds in this group are well-known environmental contaminants, including 1,1,1-trichloro-2,2'-bis(p-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCBs), and pentachlorophenol (PCP). Many of the compounds in this group are extremely resistant to chemical decomposition or biodegradative processes. The sequence of reactions involved in degradation of halogenated aromatic compounds under anaerobic conditions is reductive dehalogenation (i.e., replacement of a halide by an H•) followed by ring cleavage (Suflita et al., 1982; Kuhn and Suflita, 1989). Under anaerobic conditions, the degradative pathway of halogenated benzenoid compounds generally proceeds through one of two pathways: 1) hydrolytic dehalogenation (i.e., replacement of a halide by a hydroxyl group) prior to ring cleavage or 2) ring cleavage followed by dehalogenation (Kuhn and Suflita, 1989).

Ide et al. (1972), who worked with PCP in anaerobic rice paddy soil, were considered the first investigators to report on the dehalogenation of an aromatic compound. They observed accumulation of stable decomposition products including: 2,3,4,5-; 2,3,5,6-; and 2,3,4,6-tetrachlorophenol; 2,4,5-; and 2,3,5-trichlorophenol; 3,4-, and 3,5-dichlorophenol; and 3-chlorophenol. Degradation of PCP was not observed in sterile autoclaved controls. In a recent investigation, Micksell and Boyd (1986) reported that PCP could be mineralized to CO₂ and CH₄ by a mixture of 2,3-, and 4-chlorophenol degrading cultures enriched from municipal sewage sludge. The proposed pathway followed the sequence PCP, 3,4,5-trichlorophenol, 3,5-dichlorophenol, 3-chlorophenol, and phenol. Phenol was readily mineralized to CO₂ and CH₄.

The highly mobile phenoxyacetate herbicides, 2,4-D (2,4-dichloro-phenoxyacetate) and 2,4,5-T also are degraded under anaerobic conditions. Gibson and Suflita (1990) observed the production of several degradates, including 2,4-D, 2,5-dichlorophenoxyacetate, 3-, and 4-chlorophenol, 2,4-, 2,5-dichlorophenol, and phenol, when 2,4,5-T was added
to methanogenic aquifer slurries. The degradative pathways, as was the case for PCP degradation, generally converged to phenol. Other studies have shown that 2,4-D could be transformed to 2,4-dichlorophenol, 4-chlorophenol, and phenol in anoxic sewage sludge, pond sediment, and methanogenic aquifer material (Mikesell and Boyd, 1985; Gibson and Sufflita, 1986). However, degradation did not occur in aquifer material under sulfate-reducing conditions. These authors reported that dehalogenation of 2,4-D and 2,4,5-T was inhibited when sulfate was added to the microcosms.

Results from early investigations suggest that aryl-reductive dehalogenation reactions are favored in highly reduced methanogenic environments (Gibson and Sufflita, 1986; Sufflita et al., 1988). In a recent investigation, Genthner et al. (1989) found that aryl-reductive dehalogenation of chloroaromatic compounds (i.e., chlorobenzoates and chlorophenols) was inhibited by the addition of sulfate to both freshwater and marine sediment microcosms. Investigations by Kuhn et al. (1990) revealed that amendments of sulfate to methanogenic aquifer slurries slowed the rate of 2,3,4,5-tetrachloroaniline degradation. The previous study, along with those of King (1988) and Kohring et al. (1989), clearly establishes that aryl-reductive dehalogenation can occur either in the presence of sulfate or under sulfate-reducing conditions. A recent investigation by Haggblom and Young (1990) established that chlorophenols can be dehalogenated and subsequently mineralized under sulfidogenic conditions, and that substrate oxidation can be coupled to sulfate reduction. While there is strong evidence to suggest that chemicals such as chlorophenols and chlorobenzoates can be mineralized under sulfidogenic conditions, there is no such proof for degradation of PCBs. Thus far, PCB degradation has been shown to occur only under aerobic (Tucker et al., 1975; Focht and Brunner, 1985; Bedard et al., 1987) and methanogenic conditions (Quensen et al., 1988; Nies and Vogel, 1990; Quensen et al., 1990).

In general, while it appears that aryl-reductive dehalogenation reactions are favored under methanogenic conditions, dehalogenation reactions also can occur under sulfidogenic and aerobic conditions (Haggblom et al., 1990; Sato and Tanaka, 1987) and under nitrate-reducing or denitrifying conditions (Genthner et al., 1989).

A survey of the literature reveals that biodegradation of dicamba under anaerobic conditions has not yet been reported. It seems reasonable to suspect that dicamba is biodegradable under anoxic conditions, since the degradation of other halosubstituted benzoate compounds, including 4-amino-3,5-dichlorobenzoate; 3-chlorobenzoate, 3,5-dichlorobenzoate; and 3,4-dichlorobenzoate, has been reported (Horowitz et al., 1983; Sufflita et al., 1982). There is some information available regarding the fate of dicamba in soil incubated under aerobic conditions. Smith (1973) amended a Regina heavy clay maintained at field capacity (40% moisture) with [14C]carboxyl-labeled dicamba. He observed a rapid
loss of the applied dicamba. Only 10% was recovered after a 5-week incubation period. As the dicamba disappeared, a degradate, 3,6-dichlorosalicylic acid, appeared. After five weeks, approximately 28% of the dicamba had been transformed to the demethylated degradate. He also found that 20% of applied radioactivity was released as $^{14}$CO$_2$ during the 5-week incubation. Demethylation reactions are also possible under anaerobic conditions (Berry et al., 1987). The anaerobe, Acetobacterium woodii, is capable of obtaining energy from demethylation of methoxylated benzenoid compounds (Bache and Pfennig, 1981).

Information regarding the biodegradability of heteroaromatic pesticides, including the triazines (i.e., atrazine and cyanazine), under anaerobic conditions is scarce (Kuhn and Suflita, 1989). Goswami and Green (1971) conducted a submerged soil study and found that only 0.005% of [U-$^{14}$C]atrazine and 1.67% [U-$^{14}$C]hydroxyatrazine (2-ethylamino-4-hydroxy-6-isopropylamino-1,3,5-triazine) could be recovered as $^{14}$CO$_2$ or $^{14}$CH$_4$. They concluded that production of hydroxyatrazine resulted from a chemical hydrolysis of atrazine. These authors postulated that, once dehalogenated, the heterocyclic ring was more susceptible to cleavage and subsequent mineralization by microbes. Earlier studies by Armstrong et al. (1967, 1968) showed that chemical hydrolysis of atrazine to hydroxyatrazine was significant in soils.

In a more recent study, Jesse et al. (1983) isolated a facultative anaerobic bacterium capable of using cyanuric acid (2,4,6-trihydroxy-1,3,5-triazine) as a sole carbon and energy source under anaerobic conditions, but not under aerobic conditions. They isolated the organism from stream sediment that had received wastewater effluent from a chemical production plant. Based on microbial growth and substrate disappearance (i.e., atrazine), they also reported that this isolate could grow using atrazine as an energy source under anaerobic conditions in a defined medium. Because no metabolites were identified, it is not known whether the organism metabolized the ring structure or just the substituent groups. One troubling aspect of the atrazine degradation experiment is that the initial concentration of atrazine (70 mg L$^{-1}$) leveled off after four days of incubation to about 40 mg L$^{-1}$ and decreased no further. It is not clear why only a small percentage of the atrazine was degraded before degradation ceased. It is also interesting to note that the water solubility of atrazine is reported to be 33 mg L$^{-1}$ at 25°C. To date, no definitive proof exists establishing that atrazine or, for that matter, any triazine herbicide, can be biodegraded under anaerobic conditions.

Adrian and Suflita (1990) showed that the brominated N-heteroaromatic herbicide, bromacil (5-bromo-3-sec-butyl-6-methyluracil), can be reductively dehalogenated in anoxic aquifer slurries. They determined that bromacil could be debrominated, producing 3-sec-butyl-6-methyluracil, under methanogenic conditions but not under denitrifying or sulfate-reducing conditions. Bromacil was not transformed in sterile
controls, indicating that the debromination resulted from microbial activity.

Little is known about the biodegradability of atrazine, cyanazine, or dicamba under nitratereducing or methanogenic conditions in wetlands. To properly assess the potential for biodegradation of pesticides in wetlands, information regarding the biodegradative capabilities of the anaerobes that inhabit these environments is needed. Information concerning the quantitative aspects of pesticide biodegradation in anoxic wetland sites is required for accurate assessment of decomposition potential.

Considering that pesticides are widely used and dispersed in the environment, it is surprising how little information is available concerning their biodegradability under anaerobic conditions. Perhaps one reason for this shortage of information is that pesticides usually are applied to well-aerated agricultural soils where aerobic microbial activity predominates. As a result, there is only limited interest in examining the biodegradability of these chemicals in anaerobic environments. Based on the pesticide mobility information discussed earlier, it is clear that pesticides move away from target sites into nontarget environments. In many cases, these nontarget environments are anaerobic.

The purpose of this study was to evaluate the degradability of atrazine, cyanazine, and dicamba in three wetland soils under nitratereducing and methanogenic conditions.
2. Methodology

2.1 Wetland Soils and Site Descriptions

Soils were collected from three sites in Virginia. Myatt soil (fine-loamy siliceous, thermic Typic Ochraquult) was collected along the edge of an agricultural field near a drainage ditch that accumulates runoff water from the field. This site, located in Suffolk County, had been used by Virginia Tech as a field-test plot for pesticide studies. The Myatt soil, saturated throughout the winter and spring months, is a nontidal wetland soil, which dries out during the late summer and early autumn, when the depth to water table can be up to 2 m (Edmonds, personal communication, 1991).

Lawnes sandy loam (fine-loamy, mixed, nonacid, thermic family of Typic Sulfaquents) was collected from Grays Creek Marsh, located along Grays Creek in Surry County. Levy sandy loam (coarse-loamy, mixed, nonacid, thermic family of Typic Hydraquents) was collected from Ken­non Marsh, located along the James River in Prince George County. In contrast to the Myatt soil, the Lawnes and Levy soils are tidal wetland soils, which remain saturated throughout the year.

2.2 Soil Sampling and pH Measurement

The Myatt soil was collected to a depth of 6 cm. Samples of the Lawnes and Levy soils were collected at depths of 0-3 cm and 3-6 cm. Soils collected at 0-3 cm were used to test the biodegradability of the herbicides under nitrate-reducing conditions, while soils collected at 3-6 cm were used to test biodegradability under methanogenic conditions. Soils were placed in a 2-liter narrow-necked bottle, capped, and transported back to the laboratory. A Sensorex (Stanton, Cal.) submersible flat-surface combination pH electrode was used on site to measure the pH of a wetland soil slurry.

2.3 Chemicals

Atrazine, cyanazine, and dicamba (purity 96-99%) were purchased from Baxter Healthcare Corp., Ill. All other chemicals were reagent grade.

2.4 Microcosm Setup

2.4.1 Methanogenic Microcosms

One liter of mineral-salts medium, consisting of 0.27 g of KH₂PO₄, 0.35 g K₂HPO₄, 0.53 g of NH₄Cl, 0.1 g MgCl₂•6H₂O, 0.073 g CaCl₂•2H₂O, 0.02 g FeCl₂•H₂O and 1 mL of trace metal solution along with 1 mL of 0.1% resazurin (redox indicator), was autoclaved for 15 minutes to remove O₂, then cooled under a positive pressure of N₂, which was passed through heated copper filings (300°C) to remove traces of O₂. After cooling, 1.2 g NaHCO₃ and 0.12 g Na₂S were added to the medium and the pH adjusted to 7.0. Wetland soil that had been passed through cheesecloth was added to the mineral-salts medium to form a wetland
soil slurry-medium (40:60, v/v) inoculum. The inoculum was stirred vigorously and sparged continuously with O₂-free N₂, while 100-mL aliquots were transferred to 160-mL serum bottles containing 9.3 µmole of herbicide (either atrazine, cyanazine, or dicamba). Following transfer, the serum bottles were sealed with thick butyl-rubber stoppers and capped with aluminum crimp seals. The pH of the soil slurry-medium was determined at the beginning and at the conclusion of the experiment. Serum-bottle microcosms were incubated stationary in the dark at either 15°C or 25°C.

Experiments were set up in triplicate. Controls consisted of one herbicide-amended autoclaved sterile control (the herbicide was added to the soil slurry following sterilization). Ammonium was excluded from the mineral-salts medium used in serum-bottle microcosms containing atrazine and cyanazine in an effort to stimulate biodegradative activity.

2.4.2 Nitrate-Reducing Microcosms

Microcosms were set up in the same manner as for the methanogenic microcosms, except that Na₂S was replaced with KNO₃ (3.9 mmol L⁻¹) and resazurin was excluded.

2.5 Enrichment Cultures

Enrichment cultures were prepared by adding 20 mL of inoculum from active wetland soil microcosms, along with 80 mL of freshly prepared mineral-salts medium, to a 160-mL serum bottle containing the appropriate herbicide. Using the enrichment culture technique, repeated transfers can be performed as often as necessary to obtain a herbicide-degrading consortium composed of only a few microbes. Enrichment cultures are used to establish whether an organic compound can serve as a carbon and energy source.

2.6 Microcosm Sampling and Analysis

2.6.1 Herbicide Analysis

Aqueous-phase samples (1 mL) were withdrawn periodically by syringe from serum-bottle microcosms. Samples were placed into glass vials and stored at 0°C until analysis. In preparation for high-performance liquid chromatography (HPLC) analysis, culture samples were thawed, mixed with methanol (1:1), centrifuged (13,000 x g), and filtered through Gelman (Ann Arbor, Mich.) 0.2-µm Acrodisc membrane filters. Methanol was used in sample preparations to ensure that herbicides would not adsorb to membranes. Samples were analyzed on an HPLC system (LDC Analytic, Riviera Beach, Fla.) consisting of a CM 3400 pump, a 7125 Rheodyne valve, and a 3100 variable-wavelength spectrometer. Separation of atrazine, cyanazine, and dicamba was accomplished by using a 25-cm Supelcosil 5-µm particle size LC-18-DB column.
Methanol and water (70:30, v/v), delivered at a flow rate of 1 mL min⁻¹, was used as the mobile phase in the HPLC analysis of atrazine and cyanazine. The mobile phase used for separation of dicamba consisted of 10:25:65 (methanol:water:acetonitrile-water-glacial acetic acid (60:39.5:0.5), v/v) at a flow rate of 1 mL min⁻¹. Quantitation of the pesticides, atrazine, cyanazine, and dicamba, was accomplished by the external standards method at wavelengths of 240, 259, and 271 nm, respectively.

2.6.2 Headspace Gas Analysis

Quantification of methane produced was accomplished by injecting 50 µL of the headspace gas into a gas chromatograph (5890 Hewlett Packard Co.) equipped with a thermal conductivity detector and fitted with a Porapak N column (1.8 m, 80/100 mesh). Column temperature was maintained at 50°C and detector temperature at 150°C. Flow rate of the carrier gas, helium, was 20 mL min⁻¹.

2.6.3 Nitrate Analysis

For those microcosms in which nitrate was monitored (i.e., atrazine and cyanazine), 1-mL samples were withdrawn periodically by syringe and placed in glass vials, which were subsequently stored at 0°C until analysis. In preparation for nitrate analysis, samples were thawed, centrifuged at 13,000 × g, and filtered through Gelman 0.2-µm Acrodisc LC PVDF membrane filters. An Orion Scientific Dual-Channel Automatic Analyzer (Scientific Instruments, N.Y.) was used to determine NO₃-N concentrations colorimetrically. This procedure uses the cadmium-reduction method outlined by the EPA (method 353.2, 1979).
3. Results and Discussion

3.1 Fate of Herbicides in Methanogenic Microcosms

3.1.1 Atrazine-Amended Microcosms

Atrazine proved to be extremely stable in microcosms maintained under methanogenic conditions, regardless of soil type or incubation temperature (Figures 1-3). Analysis of microcosm headspace gas revealed that microbial activity, as reflected by methane production, was greater for microcosms incubated at 25°C, as expected (Table 1). A ten-fold increase in temperature, which stimulated microbial activity, did not affect atrazine stability. Several of the autoclaved controls did not remain sterile, as evidenced by methane production.

One of the key factors controlling atrazine stability in soil is pH (Armstrong et al., 1967; Erickson and Lee, 1989). Microcosms were pH-buffered at 7.0 to reduce the tendency for hydrolysis. Initial and final pH measurements for atrazine-amended microcosms are shown in Table 2. In most cases, pH remained constant or increased slightly following incubation. The natural soil pH of the Lawnes and Levy soils was near neutral, measuring 6.8 and 6.6, respectively. Myatt soil pH was very acidic, measuring 4.7. It is interesting to note that, if the Lawnes and Levy soils were drained and the redox potential allowed to increase, the pH dropped 1-3 pH units due to oxidation of the reduced sulfur (Edmonds et al., 1990). The magnitude of the pH drop depends upon the amount of reduced sulfur present.

3.1.2 Cyanazine-Amended Microcosms

Cyanazine was not degraded in the Myatt or Levy wetland soil microcosms (Figures 4 and 8); however, there was evidence suggesting it could be degraded in Lawnes wetland soil (Figure 5). There was a distinguishable difference in rate of degradation based on temperature, but because autoclaved controls did not remain sterile, as determined by methane production (Table 3), we were unable to distinguish between abiotic chemical decomposition and biodegradation processes. Since the Lawnes soil was maintained at about pH 7.0 (Table 4), it is unlikely that hydrolysis played a significant role in cyanazine degradation, although interaction (i.e., protonation) with soil organic matter cannot be ruled out as a plausible mechanism. The half-life for cyanazine in the Lawnes wetland soil incubated at 25°C was 50 days (Table 5). The rate constant describing herbicide disappearance was determined by fitting herbicide concentration data to the first-order reaction equation (i.e., \(-d(H)/dt = k (H)\)) where \(k\) equals the first-order rate constant (days\(^{-1}\)) and \((H)\) equals the herbicide concentration.

To determine whether degradation of cyanazine was biologically mediated, three cyanazine enrichment cultures were prepared using inoculum from Lawnes wetland soil microcosms. Cyanazine was
degraded in the enrichment cultures with an adjusted half-life of 66 days (Figure 6 and Table 6). Cyanazine was not degraded in an autoclaved control (Fig. 6). As cyanazine disappeared, concomitant appearance of an unidentified peak (retention time 3.71 min) was observed in chromatograms of an HPLC-analyzed culture medium (Figure 7). Appearance of the unidentified degradate was not observed in the autoclaved control. These results suggest that anaerobes present in the Lawnes wetland soil were able to cometabolize or transform cyanazine, but could not use it as a sole carbon and energy source.

In soils, cyanazine has been shown to undergo hydrolysis where the chlorine is replaced by a hydroxyl group (Beynon et al., 1972). The role of microorganisms in this conversion is not clear. Beynon et al. (1972) showed that the nitrile group of cyanazine also can be converted to an amide and then to carboxylic acid. Hydration of the nitrile group to an amide was likely mediated by a nitrile hydratase. Nagasawa et al. (1988) recently isolated a bacterium (Rhodococcus rhodechrous) from soil that exhibits high activity toward conversion of 3-cyanopyridine to nicotinamide. This same microorganism also produces nitrilase, an enzyme that catalyzes the direct cleavage of nitriles to the corresponding carboxylic acids and ammonia (Nagasawa et al., 1988). These enzyme systems are not uncommon in bacteria, and are found in several species, including Pseudomonas sp. Nitrile hydratase and nitrilase activity is probable under anaerobic conditions because molecular oxygen is not required as a reactant.

3.1.3 Dicamba-Amended Microcosms

Dicamba was readily degraded in Myatt wetland soil microcosms (Figure 9). The rate of degradation was affected by temperature. The (adjusted) half-life of dicamba in soils incubated at 15°C and 25°C was 37 and 49 days, respectively (Table 5). Dicamba also was readily degraded in the Lawnes and Levy wetland soil microcosms (Figures 10 and 15). At 25°C, dicamba was degraded at a much greater rate in Lawnes and Levy wetland soil microcosms with calculated half-lives of 3.2 and 2.1 days, respectively (Table 5). The fact that dicamba was more readily degraded in the Lawnes and Levy soils than in the Myatt soil could be due to differences in microbial diversity and population densities. The nontidal Myatt soil may harbor a less-diverse and lower population density of anaerobes than the Lawnes and Levy soils, which are water-saturated throughout the year. Microbial diversity usually is considered a key factor in terms of degrading resistant organic chemicals in the soil environment.

As expected, methanogenic activity in Myatt wetland soil microcosms was greater in the soils incubated at the higher temperature (Table 7). The pH of Myatt wetland soil microcosms was maintained at 7.0 (Table 8), though dicamba is not susceptible to acid or base catalysis as are atrazine and cyanazine. Sorption of dicamba in microcosms should be
minimal, since, at pH 5.0 or greater, this herbicide would be in its anionic form and water soluble.

Enrichment culture procedures were initiated in an effort to isolate a dicamba-degrading anaerobe, starting with inoculum from Lawnes wetland soil microcosms. The pattern of substrate (i.e., dicamba) use for the first-transfer cultures (three enrichment cultures were initiated) is shown in Figure 11. Analysis of culture filtrate revealed the presence of possible metabolites, as determined by the appearance of unidentified peaks in the HPLC chromatograms (Figure 12). Dicamba-degrading activity was maintained in second-transfer enrichment cultures (Figure 13). The half-life of dicamba in the second-transfer culture was greater than that observed for the initial-transfer culture (Table 6). At the time of reporting, a total of 10 transfers had been performed. Methane production was extremely low in the second-transfer enrichment culture, indicating that complete mineralization of dicamba was not achieved. Preliminary studies showed that a dicamba-degrading methanogenic consortium was unable to degrade or dehalogenate 3-chlorobenzoate. Furthermore, we did not observe release of $^{13}$CO$_2$ (4-week incubation) when the consortium was provided with $[^{14}$C]dicamba, indicating that the benzenoid ring was not degraded. Based on information generated in this investigation, it seems probable that the initial reaction was demethylation and concomitant production of acetate (Figure 14). Production of acetate from methoxylated benzenoid compounds is a well-established fermentation reaction. The small amount of acetate produced was likely used by methanogens as a carbon and energy source. Methanogenic bacteria can use only simple organic compounds as substrate (i.e., methanol, formate, acetate). In natural anaerobic environments such as sediments, acetate is believed to be the major substrate for methanogens.

3.2 Fate of Herbicides in Nitrate-Reducing Microcosms

3.2.1 Atrazine-Amended Microcosms

As was the case for methanogenic conditions, atrazine proved to be extremely stable, regardless of soil type or incubation temperature (Figures 16, 18, and 20). Atrazine disappearance was observed in only one microcosm, and that was a control containing Lawnes soil (Figure 18).

As mentioned earlier, pH is an important factor in the stability of atrazine in the environment. Initial and final pH was between 7.0 and 8.3 for atrazine-amended microcosms (Table 9). Atrazine is stable at this pH range.

Following an initial drop within the first 90 days, nitrate concentration leveled off at about 2 mmol L$^{-1}$ for both the Myatt and Lawnes microcosms (Figures 17 and 19). Temperature appeared to exert no influence on the rate of nitrate use for either the Myatt or Lawnes soils. The initial rate of nitrate use was greater for the Levy wetland soil micro-
cosms after an initial drop to 2 mmol L\(^{-1}\) (Fig. 21) compared to the Myatt or Lawnes microcosms. In the Levy microcosms, nitrate concentration leveled off at about 1 mmol L\(^{-1}\) after 60 days of incubation. For microcosms incubated at 15°C, there was a tendency for nitrate concentration to decrease to undetectable levels after 120 days of incubation.

### 3.2.2 Cyanazine-Amended Microcosms

As was the case for methanogenic microcosms, cyanazine proved to be extremely stable in Myatt wetland soil microcosms (Figure 22), as was nitrate (Figure 23). The fate of cyanazine was difficult to interpret. There appeared to be a tendency for cyanazine to be degraded, but, because the autoclaved controls did not hold, it was not possible to distinguish between chemical decomposition, biodegradation, and sorption. Rates of cyanazine disappearance appeared to be greater for microcosms incubated at 25°C. This same situation also was observed for the cyanazine-amended methanogenic microcosms (Figure 5). Nitrate-use patterns (Figure 25), while variable, showed some parallel behavior when compared to cyanazine disappearance.

The pH of the Lawnes microcosms exceeded 8.0 for autoclaved controls. Cyanazine disappearance was most rapid in the 25°C control (Table 10), which also registered the highest pH (8.6). While it is possible that hydrolysis was a factor in cyanazine disappearance in the 25°C control, it is worth noting that only a minor amount of cyanazine was lost from the 15°C control (pH 8.5) over the same time period. These results suggest that cyanazine loss due to hydrolysis was probably not significant.

Cyanazine proved to be least stable in Levy wetland soil microcosms incubated at 15°C (Figure 26). Because the autoclaved controls did not hold, it was not possible to distinguish between chemical decomposition and biodegradation. The (adjusted) half-life of cyanazine in microcosms incubated at 15°C was 99 days (Table 12). A comparison of Figures 8 and 26 reveals that cyanazine was relatively stable in methanogenic microcosms and nitrate-reducing microcosms incubated at 25°C. Surprisingly, only the nitrate-reducing microcosms incubated at 15°C showed a decrease in cyanazine concentration. It is not likely that hydrolysis played an important role, since the pH of these microcosms was neutral (Table 10). The observation that nitrate-use patterns (Figure 27) parallel cyanazine disappearance argues in favor of biodegradation playing a prominent role. Enrichment cultures were prepared from both 15° and 25°C Levy microcosms. After 100 days of incubation, no significant losses were observed in the enrichment cultures.

### 3.2.3 Dicamba-Amended Microcosms

Dicamba was degraded in Myatt soil microcosms with (adjusted) half-lives of 41 and 137 days, corresponding to 25° and 15°C, respectively (Table 12). As expected, temperature played a role in determining the
rate of dicamba degradation, supporting biodegradation as the major degradative process. Since the autoclaved controls failed, corroborative evidence was unavailable.

Dicamba concentration in the Lawnes autoclaved control decreased rapidly after 35 days of incubation, while, in the (active) microcosms, dicamba concentrations decreased at a much slower rate (Figure 29). These results were not anticipated and were, in fact, the reverse of what was expected. Nitrate was not used to any significant extent and, thus, provided no information (Figure 30).

Degradation of dicamba in the Levy wetland soil was rapid. The half-life of dicamba was calculated to be 9.2 days (Table 12). Nitrate use was also rapid, indicating that nitrate-reducing microbes were active in these microcosms. The autoclaved sterile controls held up well, providing evidence that dicamba disappearance was due to biodegradation. The initial and final pH was near neutral for all the microcosms (Table 11).
4. Conclusions and Recommendations

Based on results of this survey study, we conclude that:

- Atrazine was not biodegraded in Myatt, Lawnes, or Levy wetland soils maintained under methanogenic or nitrate-reducing conditions.
- Cyanazine was susceptible to degradation in Lawnes wetland soil microcosms maintained under methanogenic or nitrate-reducing conditions and in Levy soil maintained under nitrate-reducing conditions.
- Temperature appears to have affected the extent to which cyanazine could be degraded.
- Enrichment culture studies provided evidence suggesting that degradation of cyanazine was probably achieved through cometabolism.
- Dicamba was readily biodegraded in Myatt, Lawnes, and Levy wetland soils maintained under methanogenic conditions and in Myatt and Levy soils maintained under nitrate-reducing conditions.
- The rate of dicamba degradation increased when soil was maintained at 25° as compared to 15°C.
- Dicamba could be used as a carbon and energy source, probably by a specific group of fermenters, as determined by enrichment culture studies.

This report provides information on the biodegradability of atrazine, cyanazine, and dicamba under nitrate-reducing and methanogenic conditions in selected wetland soils. Clearly, more information is required. To develop an adequate database for accurate assessment of biodegradation potential, research should be conducted on additional pesticides and soils incorporating aerobic and sulfidogenic test conditions.

The EPA should request information from pesticide manufacturing companies concerning the biodegradability of pesticides under strict anaerobic conditions. This information should be requisite for registration or reregistration of pesticide chemicals.
References


Figures
Figure 1.
Fate of atrazine in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 2.
Fate of atrazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 3.
Fate of atrazine in Levy wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 4.
Fate of cyanazine in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 5.
Fate of cyanazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 6.
Fate of cyanazine in an enrichment culture with the inoculum originating from a methanogenic Lawnes wetland soil microcosm (25°C). Error bars (not visible) represent 1 SD based on three replicates.
Figure 7.
High-performance liquid chromatograms of cyanazine enrichment culture filtrate at time zero (a) and day 95 (b). Cyanazine had a retention time of 4.22 minutes. The peak in chromatogram (b) with a retention time of 3.71 minutes represents an unidentified degradate.
Figure 8.
Fate of cyanazine in Levy wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 9.
Fate of dicamba in Myatt wetland soil microcosms incubated at 15° and 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.
Figure 10.
Fate of dicamba in Lawnes wetland soil microcosms incubated at 25°C under methanogenic conditions. Error bars represent 1 SD based on three replicates.

![Graph showing the fate of dicamba in Lawnes wetland soil microcosms incubated at 25°C under methanogenic conditions.](image-url)

- **LAWNES (25°C)**
  - **Active culture** (O--O)
  - **Sterile** (Δ--Δ)

DICAMBA CONCENTRATION (μmol L⁻¹) vs. TIME (DAYS)
Figure 11.
Fate of dicamba in an enrichment culture. The inoculum originated from Lawnes methanogenic wetland soil microcosms (25°C). Error bars represent 1 SD based on three replicates.
Figure 12.
High-performance liquid chromatograms of culture filtrate from a dicamba enrichment culture first transfer at day 1 (a) and day 10 (b). Peaks with retention times of 4.08 and 5.56 minutes represent possible metabolites.
Figure 13.
Degradation of dicamba and subsequent production of methane in an enrichment culture (second transfer). Error bars represent 1 SD based on three replicates.
Figure 14.
Postulated pathway for degradation of dicamba (1) under methanogenic conditions. The postulated metabolite, 2-hydroxy-3,5-dichlorobenzoic acid (2), is shown in brackets.
Figure 15.
Fate of dicamba in Levy wetland soil microcosms incubated at 25°C under methanogenic conditions. Error bars (not visible) represent 1 SD based on three replicates.
Figure 16.
Fate of atrazine in Myatt wetland soil microcosms incubated at 15° and 25°C under nitrate reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 17.
Nitrate concentration in atrazine-amended Myatt wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 18.
Fate of atrazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 19.
Nitrate concentration in atrazine-amended Lawnes wetland soil microcosms incubated at 15° and 25°C. Error bars represent 1 SD based on three replicates.
Figure 20.
Fate of atrazine in Levy wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 21.
Nitrate concentration in atrazine-amended Levy wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 22.
Fate of cyanazine in Myatt wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 23. Nitrate concentration in cyanazine-amended Myatt wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 24.
Fate of cyanazine in Lawnes wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 25.
Nitrate concentration in cyanazine-amended Lawnes wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 26.
Fate of cyanazine in Levy wetland soil microcosms incubated at 15° and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 27.
Nitrate concentration in cyanazine-amended Levy wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 28.
Fate of dicamba in Myatt wetland soil microcosms incubated at 15°C and 25°C under nitrate-reducing conditions. Error bars represent 1 SD based on three replicates.
Figure 29.
Fate of dicamba in Lawnes wetland soil microcosms incubated at 25°C under nitrate-reducing conditions. Error bars (not visible) represent 1 SD based on three replicates.
Figure 30.
Nitrate concentration in dicamba-amended Lawnes wetland soil microcosms. Error bars represent 1 SD based on three replicates.
Figure 3.1.
Fate of dicamba in Levy wetland soil microcosms incubated at 25°C under nitrate-reducing conditions. Error bars (not visible) represent 1 SD based on three replicates.
Figure 32.
Nitrate concentration in dicamba-amended Levy wetland soil microcosms. Error bars (not visible) represent 1 SD based on three replicates.

NITRATE CONCENTRATION (mmol L⁻¹)

TIME (DAYS)

DICAMBA

LEVY

Active Culture
Sterile Control
Tables
Table 1.  
Methane production in atrazine-amended microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>µmol CH₄ mL⁻¹ ± Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 117</td>
</tr>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>1.72 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>9.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>9.41</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>1.35</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>4.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 2.
Initial and final pH of atrazine-amended methanogenic microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.3</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.3</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.3</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>7.6</td>
</tr>
</tbody>
</table>
Table 3. Methane production in cyanazine-amended microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>$\mu$mol CH$_4$ mL$^{-1}$ ± Std. Dev.</th>
<th>Day 117</th>
<th>Day 236</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>2.2 ± 2.8</td>
<td>4.0 ± 4.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>12.5 ± 1.8</td>
<td>22.2 ± 6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>11.0</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>1.4 ± 1.2</td>
<td>1.3 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0.1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>5.5 ± 2.7</td>
<td>11.4 ± 3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>20.4</td>
<td>26.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>1.3 ± 0.4</td>
<td>5.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0.1</td>
<td>9.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.
Initial and final pH of cyanazine-amended methanogenic microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.3</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.0</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>7.5</td>
</tr>
</tbody>
</table>
Table 5.
First-order rate constants and half-lives for cyanazine and dicamba degradation in methanogenic microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Herbicide</th>
<th>Temp. (°C)</th>
<th>Rate constant (day^{-1})</th>
<th>r^2</th>
<th>Half-life (days)</th>
<th>Lag time (days)</th>
<th>^1Adjusted half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawnes</td>
<td>Cyanazine</td>
<td>25</td>
<td>0.014</td>
<td>0.88</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.003</td>
<td>0.99</td>
<td>231</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dicamba</td>
<td>25</td>
<td>0.22</td>
<td>0.91</td>
<td>3.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Levy</td>
<td>Dicamba</td>
<td>25</td>
<td>0.33</td>
<td>0.95</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Myatt</td>
<td>Dicamba</td>
<td>25</td>
<td>0.03</td>
<td>0.36</td>
<td>23.1</td>
<td>14</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.02</td>
<td>0.95</td>
<td>34.7</td>
<td>14</td>
<td>48.7</td>
</tr>
</tbody>
</table>

^1Adjusted half-life was calculated by adding lag time to the half-life as determined by the first order rate equation.
Table 6. First-order rate constants and half-lives for cyanazine and dicamba degradation in methanogenic enrichment cultures (Lawne's soil inoculum).

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Number of transfers</th>
<th>Rate constant (days)</th>
<th>$r^2$</th>
<th>Half-life (days)</th>
<th>Lag time (days)</th>
<th>Adjusted half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanazine</td>
<td>1</td>
<td>0.023</td>
<td>0.98</td>
<td>30</td>
<td>36</td>
<td>66</td>
</tr>
<tr>
<td>Dicamba</td>
<td>1</td>
<td>0.31</td>
<td>0.97</td>
<td>2.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.08</td>
<td>0.98</td>
<td>8.8</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted half-life was calculated by adding lag time to the half-life as determined by the first order rate equation.
Table 7.
Methane production in dicamba-amended microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>μmol CH₄ mL⁻¹ ± Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 117</td>
</tr>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>27.1 ± 21.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>0.3</td>
</tr>
<tr>
<td>Lawne</td>
<td>Culture</td>
<td>25</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>0.7</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 8. 
Initial and final pH of dicamba-amended methanogenic microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.3</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.2</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.0</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.1</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.1</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Table 9.  
Initial and final pH of atrazine-amended nitrate-reducing microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.1</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.1</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.1</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>
Table 10.
Initial and final pH of cyanazine-amended nitrate-reducing microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>8.1</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>8.5</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.4</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.4</td>
<td>7.1</td>
</tr>
</tbody>
</table>
Table 11.
Initial and final pH of dicamba-amended nitrate-reducing microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Initial pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myatt</td>
<td>Culture</td>
<td>25</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>15</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Lawnes</td>
<td>Culture</td>
<td>25</td>
<td>7.4</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Levy</td>
<td>Culture</td>
<td>25</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>7.3</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Table 12.
First-order rate constants and half-lives for cyanazine and dicamba degradation in nitrate-reducing microcosms.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Herbicide</th>
<th>Temp. (°C)</th>
<th>Rate constant (day(^{-1}))</th>
<th>(r^2)</th>
<th>Half-life (days)</th>
<th>Lag time (days)</th>
<th>(^1)Adjusted half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levy</td>
<td>Cyanazine</td>
<td>15</td>
<td>0.01</td>
<td>0.97</td>
<td>69</td>
<td>30</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>0.02</td>
<td>0.97</td>
<td>35</td>
<td>37</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
<td>0.97</td>
<td>9.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dicamba</td>
<td>25</td>
<td>0.035</td>
<td>0.99</td>
<td>20</td>
<td>21</td>
<td>41</td>
</tr>
<tr>
<td>Myatt</td>
<td>Dicamba</td>
<td>25</td>
<td>0.006</td>
<td>0.91</td>
<td>116</td>
<td>21</td>
<td>137</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted half-life was calculated by adding lag time to the half-life as determined by the first order rate equation.
The Virginia Water Resources Research Center is a federal-state organization established at Virginia Polytechnic Institute and State University in 1965 under provisions of the federal Water Resources Research Act of 1964.

Under state law, the Center’s activities are to:

- consult with the General Assembly, governmental agencies, water user groups, private industry, and other potential users of research;
- establish and administer research agreements with all universities in Virginia;
- facilitate and stimulate research that concerns policy issues facing the General Assembly, supports water resource agencies, and provides organizations with tools to increase effectiveness of water management;
- disseminate new information and facilitate application of new technology;
- serve as a liaison between Virginia and federal research funding agencies as an advocate for Virginia’s water research needs; and
- encourage the development of academic programs in water resources management in conjunction with the State Council on Higher Education.

More information on programs and activities may be obtained by writing or telephoning the Water Center.

Virginia Tech does not discriminate against employees, students, or applicants on the basis of race, sex, handicap, age, veteran status, national origin, religion, or political affiliation. The University is subject to Titles VI and VII of the Civil Rights Act of 1964, Title IX of the Education Amendments of 1972, Sections 503 and 504 of the Rehabilitation Act of 1973, the Age Discrimination in Employment Act, the Vietnam Era Veteran Readjustment Assistance Act of 1974, Federal Executive Order 11246, the governor’s State Executive Order Number One, and all other rules and regulations that are applicable. Anyone having questions concerning any of those regulations should contact the Equal Opportunity/Affirmative Action Office.