

Dissipation and Leachability of Formulated Chlorpyrifos and Atrazine in Organically-amended Soils

by
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(ABSTRACT)

Bioremediation was studied in soils containing high concentrations of formulated chlorpyrifos (5 mg kg⁻¹ Dursban[®] 4E) and atrazine (5 mg kg⁻¹ AAtrex[®] 4L) using amendments including lignocellulosic sorbents, microbial nutrients (vegetable oil, corn meal and fertilizers), and microbial extracts from organic media previously exposed to these pesticides (chlorpyrifos and atrazine, respectively). Radiolabeled atrazine was used to examine the various dissipation routes in contaminated soil, also amended with lignocellulosic sorbents and microbial nutrients.

Both chlorpyrifos and atrazine dissipation from contaminated soils was enhanced by organic-based material amendments. The half-lives of chlorpyrifos based on extractability for soils unamended and amended with vegetable oil and peat moss were 87 and 52 days, respectively. The half-lives of atrazine in unamended and amended soil (vegetable oil, peat moss and fertilizers) were 175 and 40 days, respectively. The leachability of chlorpyrifos from contaminated soil was dramatically reduced by 82% during the first 30 days of incubation in treatments amended with vegetable oil and peat moss while only a 28% of reduction in leachability occurred in the corresponding unamended controls. Only a slight reduction of atrazine leachability was detected in amended treatments after 120 days of incubation.

Differences were found in the leachability of chlorpyrifos and atrazine when they were applied to soil either as technical grade or formulated material. The presence of surfactants and other adjuvants in formulated chlorpyrifos (Dursban[®] 4E) reduced chlorpyrifos leachability in contaminated soil. Chlorpyrifos leachability was reduced by 43% in the formulated chlorpyrifos treatments, whereas there was a negligible decrease in technical chlorpyrifos treated soil during the first 3 days after contamination. Atrazine extractability and leachability was not affected by its formulation (AAAtrex[®] 4L).

Amendments with lignocellulosic sorbents and nutrients decreased atrazine's volatility from contaminated soils. After 16 weeks of incubation, less than 1% of ¹⁴C-atrazine was volatilized from incubated soils. Overall, after 16 weeks of incubation less than 4% of ¹⁴C-atrazine was mineralized and more radioactivity was recovered from amended treatments than unamended treatments as ¹⁴CO₂. The major portion of radioactivity (62%) was associated with physisorbed atrazine represented by the ethylacetate extract I from unamended treatments while only 28% of initial applied radioactivity was recovered in the corresponding amended treatments. Based on the sum of radioactivity in humic and fulvic acids, approximately 14% of radioactivity was incorporated or chemisorbed atrazine and its metabolites in both unamended and amended treatments. Forty-five percent of the initially applied radioactivity was associated with alkali insoluble fraction in amended treatments but only 17% of the initially applied radioactivity was detected in the corresponding unamended treatments. Less than 2 % of initial activity associated with physisorbed portions of fulvic acids and alkaline insoluble fraction indicated as the radioactivity in methylene chloride and ethylacetate extract II . Over time, more radioactivity was associated with polar atrazine hydroxylated degradation products.

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Chapter 1. Introduction

Contamination of soil with high concentrations of pesticides, resulting from accidental spills at agrochemical manufacturing, formulation, and distribution facilities, farm loading/washdown sites, or abandoned waste sites, is a serious environmental problem in many communities within the U.S. as well as other places in the world. For example, a survey conducted at agrochemical facilities in Iowa revealed that six commonly used herbicides were found in water and soil at concentrations ranging from 1000 to 270,000 ppm (Hallberg, 1986). Another investigation of agrochemical facilities in Wisconsin reported the presence of at least 17 pesticides in soil and ground water samples at concentrations as high as 37,950 ppm (Habecker, 1989).

There are a number of options available for treatment of pesticide-contaminated soils. These include physical, chemical, and biological treatments of excavated soil or excavation and disposal in an approved hazardous waste landfill or biological-based *in situ* treatments. Physical remediation technologies include incineration, air stripping, activated carbon absorption, soil flushing/washing, ion exchange, and membrane separation (Norwood and Randolph, 1990). Remedial chemical treatments include neutralization, reduction-oxidation, photolysis, and dechlorination. Biological treatments primarily include biostimulation, enhancing biodegradation through the enrichment of soil indigenous microorganisms with nutrients or organic substrate, and bioaugmentation, stimulating microbial biodegradation through the inoculation of contaminated soils with microorganisms adapted for the degradation of a particular toxic chemical (Morgan and Watkinson, 1989; Grubbs et al., 1991). Other approaches that are currently being developed include application of immobilized enzymes (Nannipieri and Bollag, 1991) and the use of plants to contain or transform pollutants or phytoremediation (Cunningham et al., 1996).

Incineration is one conventional method for treatment of contaminated soils. This method is achieved by removing contaminated soil from contaminated sites, and subsequent incineration at high temperatures (Oppelt, 1987). Incineration can be efficient

for small areas of contamination. However, the cost associated with excavation, relocation and incineration of contaminated soil can be prohibitive for small farms or businesses. Incineration costs were estimated at \$263 to \$1053 per cubic meter in 1991 (Gabriel, 1991). In addition, the natural environment might be disrupted during the process of excavation, which is not considered to be environmentally sound. On the other hand, the biological treatment of wastewater and solids presents an emerging technology for remediating contaminated sites (Bollag et al., 1994). An efficient, practical pesticide wastewater disposal technology which employs both physical absorption and biological degradation is under development by the Pesticide Disposal Research Group at Virginia Tech. The system involves sorption of pesticides onto lignocellulosic materials such as peat moss from pesticide-contaminated wastewater or rinses. Relatively high concentrations ($5000 \mu\text{g g}^{-1}$) of formulated chlorpyrifos (*O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl-phosphorothioate) and atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) were removed from aqueous solutions during this process. Only 0.3% and 0.01% atrazine and chlorpyrifos remained in aqueous solution after demulsification and sorption onto organic matrices, respectively (Mullins et al., 1992a, b). These pesticides sorbed onto contaminated organic-based materials can then be degraded in a composting environment that is rich in nutrients and produces elevated temperatures, both of which facilitate physicochemical degradation and microbial-based biodegradation and bioincorporation (Mullins et al., 1989; Berry et al., 1993a, b).

Biological solids treatment, often referred to as “bioremediation”, has been successfully used to detoxify a number of contaminated hazardous materials, especially gasoline (Gabriel, 1991; Lamar and Dietrich, 1992). Bioremediation, the detoxification of xenobiotics through microbial degradation and microbial-mediated incorporation, offers a cost-effective and environmentally acceptable alternative to clean up pesticide contaminated soils (Ritter and Scarborough, 1995). Recently, a number of papers have been published on the efficacy and safety of bioremediation of pesticides through land application and/or biostimulation. For example, alachlor dissipation from soil was accelerated by soil organic amendments, especially corn meal by using land farming and

biostimulation (Dzantor et al., 1993). Atrazine mineralization in pasture soils was increased by the application of dairy manure (Entry and Emmingham, 1995). Reduced dissipation half-lives of cyanazine and fluometuron in amended soil were observed by Wagner and Zablotowicz (1997). It was found that corn meal and ryegrass had the greatest stimulatory effect on cyanazine and fluometuron degradation in soil, respectively.

Bioremediation may be a promising technology because of its relative ease of use, low cost and environmental soundness (Bollag et al., 1994). However, there are a number of issues such as efficacy and leaching potential that must be addressed before it can be put into practical use. This study is based on the hypotheses that: 1) addition of lignocellulosic sorbent amendments and microbial nutrients will enhance microbial degradation, cometabolism, incorporation and sorption of those recalcitrant pesticides and their metabolites such as chlorpyrifos and atrazine in contaminated soils and, 2) addition of organic-based lignocellulosic materials to contaminated soil will increase the sorption capacity of pesticides, hence reducing pesticide leachability.

There were three main objectives of this study. The first objective was to examine extractability and leachability of chlorpyrifos and atrazine from contaminated soils using a variety of organic sorbent and microbial nutrients and comparing them with unamended controls. The second objective was to study the effects of surfactants and other adjuvants in chlorpyrifos and atrazine formulations on the extractability and leachability of chlorpyrifos and atrazine from contaminated soil. The third objective was to determine the dissipation routes of atrazine from contaminated soil through biostimulation, from which the overall degradative, sorption and incorporation pathways for atrazine could be determined. Two major criteria have been used to assess whether bioremediation has achieved its objectives, i.e. degree of pesticide dissipation and leaching potential from contaminated soils. Three areas of research have been conducted to meet these objectives.

1. Small scale incubation studies were conducted to monitor chlorpyrifos and atrazine extractability and leachability from soil contaminated with high concentrations of

Dursban[®] 4E and AAtrex[®] 4L. Different combinations of organic sorbent (peat moss), microbial nutrients (vegetable oil, fertilizers) and microbial extracts were used to amend the contaminated soils.

2. The effects of surfactants and other adjuvants in chlorpyrifos and atrazine formulation, i.e. Dursban[®] 4E and AAtrex[®] 4L, were examined to address leaching potential and degradability of chlorpyrifos and atrazine in their formulation.

3. Atrazine dissipation routes including volatilization, transformation, mineralization, sorption and incorporation in bioremediated soil were examined with ¹⁴C-atrazine in a closed circulation incubation system.

Chlorpyrifos is an organophosphorus insecticide commonly used for the control of a variety of pests either in agriculture (e.g. corn, cotton) or urban situations (e.g. turfgrass, indoor). Since the removal of chlordane and other chlorinated cyclodiene termiticides from the market, chlorpyrifos has also become the most widely used termiticide (Racke et al., 1994). Atrazine is extensively used to control broadleaf and grassy weeds in both crop and noncrop lands. Approximately 363 million kg was applied in the United States between 1980 and 1990 (Gianessi, 1992). Atrazine is moderately persistent in soil, with reported half-lives ranging from 4 to 57 weeks (Best and Weber, 1974; Cohen et al., 1984). Because of its relatively high water solubility and widespread usage in weed control, it is the pesticide most commonly detected in ground water in the U.S.A. (Hallberg, 1989; Funari et al., 1995). In addition to this, its concentrations in groundwater and surface water frequently exceed the 3-ppb Health Advisory Level (HAL) set by the U.S. Environmental Protection Agency (Anonymous, 1991). Based on their widespread usage in American agriculture and households, relatively long persistence in a soil environment, and potential contamination of ground water and surface water resources, chlorpyrifos and atrazine were selected as representative pesticides for this study.

Chapter 2. Literature Review

2.1. Groundwater contamination

Modern agriculture still depends heavily on the use of agrochemicals such as pesticides. In 1991, more than \$25 billion worth of agricultural pesticides were sold in the world (Ware, 1993). About 0.59 billion kg of synthetic pesticides were produced in the U.S. Some 3.79 million kg of pesticides were used in agriculture (Aspelin et al., 1992). Most of the applied pesticides did not reach the targets (Younos and Weigmann, 1988). These chemicals have the potential to contaminate our environment, especially the groundwater. From time to time, those pesticides that pollute our environment and exceed tolerance levels have been removed from the market and their use has been prohibited due to human health concerns (Osteen and Szmedra, 1989).

Two types of contamination are recognized according to their source of origin: nonpoint and point source. Point source refers to soil contamination resulting from comparatively well-defined, localized areas, often with a large number of products; Nonpoint refers to soil contamination derived from the routine application of pesticides over extensive areas (Barbash and Resek, 1996). Either of these contamination types might constitute a hazard to humans or the environment (Hallberg, 1989). Proper disposal of pesticides or the cleanup or remediation of pesticide contaminated sites currently presents a major challenge for environmental scientists.

2.1.1. Pesticide groundwater contamination in the United States

Pesticide groundwater contamination is of great and growing concern in the United States. People, especially those living in the rural areas, depend on the groundwater for drinking, and they rank the contamination of groundwater as a major issue. During a recent survey, the USEPA reported that they had detected 132 pesticides and their metabolites in the groundwater of 42 states (Cohen, 1993). Atrazine was listed as one of ten most frequently detected compounds found in the groundwater (Cohen, 1993). In New York state, 35% of wells contained detectable pesticide residues. As many as 26.5% of detected pesticides and metabolites exceeded the maximum contaminant level (MCL) and

might cause significant toxicological impacts. Based on the Cohen's 1992 data sheet, contamination of 13,696 wells out of 60,000 examined in the groundwater survey were attributed to normal agricultural use, while only 205 wells were attributed to point sources.

2.1.2. Chlorpyrifos groundwater contamination

The contamination of ground water by chlorpyrifos does not appear to be a major problem. A survey of pesticides in wells indicated that only a few contained detectable levels of chlorpyrifos (Parsons and Witt, 1988). They reported that 11 states had ground water samples in which chlorpyrifos was detected. However, the incidence was quite low; only three of 981 sampled wells contained detectable levels of chlorpyrifos (0-1 ppb) (Parsons and Witt, 1988). A summary report by Hallberg (1989) revealed no detectable chlorpyrifos in the groundwater of several states having significant chlorpyrifos use, including Wisconsin, Minnesota, and Iowa. However, chlorpyrifos and its major metabolite TCP (3,5,6-trichloro-2-pyridinol) were detected at quite low concentrations (chlorpyrifos \leq 0.1 ppb; TCP \leq 0.8 ppb) in the wells of a highly vulnerable aquifer where the water table was as low as 1.52 m (Cohen et al., 1990). On the other hand, chlorpyrifos was frequently found in the groundwater as a result of point source contamination. Long (1989) reported that 17 of 56 agrochemical facilities in Illinois had detectable levels of chlorpyrifos. The results might be attributed to the presence of these compounds in back siphonage, lack of rinsate collection, and improper disposal (Long, 1989).

2.1.3. Atrazine groundwater contamination

Atrazine is the most frequently found pesticide in the groundwater in the United States (Hallberg, 1989; Funari et al., 1995). Hallberg (1989) stated the frequency of detection for atrazine is 10 to 20-fold greater than the next most frequently detected pesticides. Atrazine has been found in the groundwater of approximately 25 states due to both point and nonpoint sources (Anonymous, 1990). In a regional survey, Koterba et al. (1993) reported that atrazine is the most commonly detected pesticide in the Delmarva Peninsula of Delaware, Maryland, and Virginia. Contamination was well correlated with the intensive use of atrazine in cornfields (Koterba et al., 1993). Not only atrazine but its

metabolites were also detected in groundwater. DeLuca (1990) detected deethylatrazine in all 32 study wells where atrazine was found and also in three wells where atrazine was not found. Deisopropylatrazine was found in 11 wells with atrazine and in two wells where atrazine was below the detection limits (Deluca, 1990).

2.2. Chlorpyrifos

2.2.1. Chlorpyrifos volatilization from soil

Soil applied pesticides volatilize from soil as a result of interaction between adsorption-desorption of the chemicals from soil particles and organic matter into the solution phase, and convection and diffusion at the atmosphere interface (Spencer et al., 1973). Chlorpyrifos has a moderately high vapor pressure ($1.8-2.0 \times 10^{-5}$ mm Hg at 25 °C, Racke, 1993). Volatilization from soil depends on a number of environmental factors such as temperature, formulation, and soil properties. The rate of chlorpyrifos volatilization from a sand and a silt loam soil was higher in the first 8 days after application, where 2.6 and 9.3% of applied chlorpyrifos were volatilized one month after application (Racke, 1993). Whang et al. (1993) investigated a number of pesticides including chlorpyrifos, fonofos, and atrazine from conventional and no-till surface soils in the field. They found that as much as one-half of the chlorpyrifos and fonofos was volatilized from no-till surface soils during 26 days.

2.2.2. Chlorpyrifos photodegradation

Once chlorpyrifos is applied, it may be exposed to photodegradative conditions either directly or indirectly. Direct photodegradation can occur from direct absorption of sunlight by chlorpyrifos itself, mostly in the ultraviolet region of the spectrum. Indirect photodegradation can occur when sunlight is absorbed by secondary reagents/substrates such as soil humic and inorganic substances. These activated reagents, in turn, are capable of reacting with chlorpyrifos (Zepp and Schlotzhauer, 1983).

2.2.2.1. Photodegradation kinetics

Photodegradation rates depend on a number of factors including the wavelength and intensity of light, the transparency of the medium, and the properties of the environment itself. Photodegradation in air has been investigated by Klisenko and Pis'mennaya (1979). They reported a half-life of 136 min for chlorpyrifos exposed to an artificial light source. A half-life of 2.2 days for chlorpyrifos on glass plate surfaces exposed to artificial sunlamps has been reported (Chen et al., 1984; Chen, 1985). However, on a dry soil surface, chlorpyrifos was quite resistant to photodegradation (Getzin, 1981b). Walia et al. (1988a) reported a half-life of 13.7 days on glass, 17.2 days on moist soil, and 52.6 days on the surface of *Polystichum setiferum* (one species of plant) upon exposure to 254 nm irradiation. In aqueous solution, Meikle et al. (1983) investigated the photolysis-hydrolysis rate of chlorpyrifos at pH 5.0, 6.9, and 8.0 aqueous buffers exposed to ultraviolet radiation. In these buffers, the half-lives after combined photolysis-hydrolysis were 11.0, 12.2, and 7.8 days, respectively, and the corresponding calculated photolysis half-lives were 13.9, 21.7 and 13.1 days, respectively.

2.2.2.2. Photodegradation pathway

The pathways of chlorpyrifos photodegradation are not clearly understood, but partial photodegradation products have been isolated and identified (Meikle et al., 1983; Walia et al., 1988b). A metabolite, 3,5,6-trichloro-2-pyridinol (TCP) has been identified as a photolysis metabolite on glass, soil, and leaf surfaces (Walia et al., 1988b). These workers determined nearly 14.5% of chlorpyrifos was converted to TCP in light, while only approximately 0.5% was converted to TCP under dark conditions. However, TCP is not considered to be a major photolysis product in aqueous solutions (Meikle et al., 1983) because of the photoinstability of TCP. Smith (1966) reported 100% photodegradation of TCP at pH 8 buffer within 24 h. Approximately 17% of applied TCP was converted to carbon dioxide, while the rest was postulated to be a series of partially dechlorinated pyridine-based diols and triols. Walia et al. (1988b) investigated the photodegradation of chlorpyrifos in hexane, methanol, and on glass, leaf surfaces and soil. They isolated and identified a range of photodegradation products that were soluble either in hexane or

methanol. Those found in hexane included: *O,O*-diethyl-*O*-(3,5-dichloro-2-pyridyl) phosphorothioate, *O,O*-diethyl-*O*-(3,6-dichloro-2-pyridyl) phosphorothioate, *O,O*-diethyl-*O*-(5,6-dichloro-2-pyridyl) phosphorothioate and *O,O*-diethyl-*O*-(monochloro-2-pyridyl) phosphorothioate. Photolysis products found in methanol extracts included chlorpyrifos oxon, and two methylated products *O,O*-diethyl-*O*-(3,6-dichloro-5-methoxy-2-pyridyl) phosphorothioate and *O,O*-diethyl-*O*-methyl phosphorothioate. They also proposed a photodegradation pathway for the various experimental conditions (Walia et al., 1988b).

2.2.3. Chlorpyrifos hydrolysis

The hydrolysis of chlorpyrifos is an important process in the degradation of organophosphorus insecticides, usually resulting in an increase in the number of polar metabolites and a reduction in acute toxicity. Two mechanisms of chlorpyrifos hydrolysis may occur due to pH effects: neutral hydrolysis and alkaline hydrolysis. Neutral hydrolysis involves nucleophilic attack of water at the ethoxy carbon, hydrolyzing chlorpyrifos to deethylchlorpyrifos and ethanol. Alkaline hydrolysis of chlorpyrifos involves in the phosphorus atom which is attacked by the nucleophilic hydroxide ion.

2.2.3.1. pH affects on chlorpyrifos hydrolysis

The hydrolysis rate of chlorpyrifos is pH dependent. At pH > 7.5-8.0, hydrolysis rates increase rapidly (Meikle and Youngson, 1978; Macalady and Wolfe, 1983). There are two hypotheses regarding the relationship between increased pH value and the rate of chlorpyrifos hydrolysis. One hypothesis suggests the rate of hydrolysis increases fairly consistently with increased pH. Meikle and Youngson (1978) reported that chlorpyrifos degrades in distilled water with half-lives of 22.8, 35.3, and 62.7 days at pH 8.1, 6.9, and 4.7, respectively. Freed et al. (1979) reported that the half-lives of chlorpyrifos degradation in water were 120 and 53 days at pH 6.1 and 7.4 at 20°C, respectively. Chapman and Cole (1982) have reported on chlorpyrifos hydrolysis in sterile 1% ethanol phosphate buffer at pH 4.5 to 8.0. They found that the half-life of chlorpyrifos in this medium was 77 days in highly acidic conditions (pH 4.5 and 5.0), but the half-life consistently decreased from 77 days to 19 days at pH 5.0 to 8.0.

The second hypothesis suggests that the hydrolysis rate of chlorpyrifos is fixed from acidic to neutral conditions, while it proceeds at an increased rate under alkaline conditions. Macalady and Wolfe (1983) investigated the hydrolysis of chlorpyrifos over the pH range of 1-13 in distilled water, aqueous buffer, 50% methanol-water, and 50% acetonitrile-water solutions. They reported a constant rate of hydrolysis from pH 1 to pH 7 at 25°C. The rate of hydrolysis increased dramatically above pH 7.5, with half-lives reduced from an average of 77.4 days over pH range 1-7.5, to 10.2 and 0.5 days at pH 9.8, and 11.1, respectively.

2.2.3.2. Temperature effects

Increased rates of chlorpyrifos degradation occur at higher temperatures. A 3.5-fold increase in hydrolysis rate has been reported for each 10 °C rise in temperature (Meikle and Youngson, 1978). The half-lives of chlorpyrifos hydrolysis at pH 7.4 were 53 and 13 days at 20°C and 37.5°C, respectively (Freed et al., 1979). Increased temperature elevates the energy of nucleophilic attack on chlorpyrifos molecules, which has an average 21.1 kcal M⁻¹ activation energy for the hydrolytic reaction (Meikle and Youngson, 1978).

2.2.3.3. Metallic ion catalyzed hydrolysis

The presence of metallic ions can have a considerable impact on both enzymatic hydrolysis and nonenzymatic hydrolysis of some organic phosphates. Mortland and Raman (1967) reported that chlorpyrifos hydrolysis was catalyzed with Cu⁺²; 2.8 ppm chlorpyrifos was completely (100%) hydrolyzed within 24 hr in aqueous methanol (50%) containing 0.1 mM Cu⁺², and 100%, 20% and 10% hydrolysis were observed by Cu-montmorillonite, and Cu-beidellite and MgCl₂ at the same conditions. No catalyzed chlorpyrifos hydrolytic activity was detected in the presence of other metal salts including CoCl₂, ZnSO₄, AlCl₃, CaCl₂, MgCl₂ and Cu-organic soil. Meikle and Youngson (1978) also reported an approximately 4-11-fold increase of pseudo first-order rate constant in the presence of 1.5 x 10⁻⁵ M CuSO₄. The catalytic effects of Cu⁺² were also dependent on its free concentration in the solution. With the increasing concentration of Cu⁺² in the

medium, the hydrolysis rate of chlorpyrifos increased until the concentration of Cu^{+2} reached $1.0 \times 10^2 \text{ M}$ (Blanchet and St-George, 1982). Upon reaching this concentration, the hydrolysis reaction was independent of the Cu^{+2} concentration. They reported that Cu^{+2} catalysis interacted with the pH of the medium, with the elevated pH, the precipitation of Cu^{+2} would follow, and alkaline hydrolysis would predominate. The significance of the copper-catalyzed hydrolysis in the environment still needs to be explored, since only approximately $2 \times 10^{-7} \text{ M}$ Cu^{+2} is found in surface water (Wetzel, 1975) and less than $1 \times 10^{-7} \text{ Cu}^{+2}$ exists in soil pore water (Lindsay, 1979).

2.2.3.4. Hydrolytic pathway

There are three bonds in the chlorpyrifos molecule that are subject to cleavage during the hydrolytic processes: two tertiary alkyl ester bonds and one phosphate ester bond. Macalady and Wolfe (1983) reported that TCP and *O,O*-diethyl phosphorothioic acid were the major metabolites of chlorpyrifos hydrolysis in aqueous buffers and aqueous/polar solvent mixtures over a pH range of 9-13. When temperatures were elevated to 70-80 °C, the major products were ethanol and desethyl chlorpyrifos, with a smaller amount of TCP and diethylthiophosphate at pH 7.68. The metabolite species formed during hydrolysis are also affected by the pH of the medium. McCall (1986) reported a relatively constant amount of TCP (13.2-14.35%) and desethyl chlorpyrifos (16.4-17.7%) formed over the pH range of 5 to 7 in buffered distilled water. However, the percentage of TCP increased up to 47.9% while that of desethyl chlorpyrifos remained at a level of 12.5% at pH 9. Thus, the alkaline pH favored TCP production, and the hydrolysis rate increased when compared with neutral or acidic conditions.

2.2.4. Microbial Degradation

Numerous studies have demonstrated the significance of soil microorganisms in the biodegradation of various kinds of xenobiotics. These living organisms can exhibit a large potential to degrade toxic chemicals such as pesticides dispersed in the environment. There are two general types of reactions which are used in degradation of these materials: catabolism and cometabolism or incidental metabolism. Catabolism results from

degradation in which microorganisms degrade or partially degrade the target compounds (xenobiotics) and gain energy or nutrients from this process which contributes to cell growth and development of the microorganisms. Incidental metabolism or cometabolism results when microorganisms gain neither energy nor nutrients for themselves; only involve these degradative processes due to their inherent metabolic activities (Racke, 1993).

Soil microorganisms play a significant role in degradation of chlorpyrifos and its metabolites. Thiels (1964) first investigated chlorpyrifos microbial transformation rates in natural and autoclaved sandy loam soil. He observed that both non-autoclaved and autoclaved soil contained TCP, a common chlorpyrifos hydrolysis product, but a relatively lower chlorpyrifos degradation rate in the autoclaved soil. Subsequently, Miles et al. (1979) reported similar results from studies comparing autoclaved and natural mineral and organic soils. Chlorpyrifos half-lives in natural and sterile mineral soil were less than 1 and 17 weeks, respectively; and 2.5 and more than 24 weeks in natural and sterile organic soil, respectively. Getzin (1981a) presented additional results which demonstrated that microorganisms enhance chlorpyrifos and TCP biodegradation in natural soil. Half-lives in sterile soils were approximately 1.7 to 2.7-fold longer than in natural soils. In addition, TCP accumulated in the sterile soil but degraded quite rapidly in the natural soil. This information indicates the importance of microbial involvement in further degrading this primary metabolite (Getzin, 1981a). Reports by Miles et al. (1983; 1984) have shown that remarkable differences can exist in chlorpyrifos persistence in sterile natural mineral (sandy loam) and organic (muck) soils when held at three different temperature and four moisture levels for a period of 24 weeks. The half-lives of chlorpyrifos in muck and sterile muck were 6 weeks and 24 weeks respectively, and the results from sandy loam were quite similar (Miles et al., 1983). Chlorpyrifos was least stable in air-dry soil (Miles et al., 1984). The rate of disappearance of chlorpyrifos increased with temperature increased from 3°C to 28°C. The authors postulated that this was correlated with the increasing microbial activities and the higher microbe populations (Miles et al., 1983).

There are reports indicating less involvement of microbes in chlorpyrifos degradation. Jones and Hasting (1981) reported no significant difference in chlorpyrifos degradation rates between sterile and natural forest soils. Yoshioka et al. (1991) found that there was no significant difference in the degradation rate between sterile and nonsterile soil containing high initial concentrations (100 ppm) of chlorpyrifos, but differences were found in the degradation rate between sterile and nonsterile soil containing lower initial concentrations (50 ppm).

Chlorpyrifos, like many other organophosphorous insecticides, is a strong cholinesterase inhibitor (Matsumura, 1985; Chambers and Levis, 1992). It also affects soil microbial activities, Pozo et al. (1995) reported that a soil aerobic dinitrogen-fixing bacterial population was initially suppressed by the addition of chlorpyrifos. However, the bacterial populations were able to recover 14 days later. Soil fungi, nitrifying bacteria, and denitrifying bacteria were not affected by the addition of chlorpyrifos (Pozo et al., 1995). Racke et al. (1994) studied chlorpyrifos degradation in soil applied at a termiticidal application rate. They found the half-lives of chlorpyrifos ranged from 175 to 1576 days at an initial concentration of $1000 \mu\text{g g}^{-1}$. One important reason for the extra long chlorpyrifos residual life was a possible chlorpyrifos inhibitory effect on the soil microbial population at high concentrations (Racke et al., 1994). Cink and Coats (1993) reported a relatively low mineralization rate of chlorpyrifos at higher concentrations in an urban Iowa soil. Soil moisture level and chlorpyrifos concentration are two major factors that dramatically influence the extent of chlorpyrifos mineralization. For example, a 17% mineralization rate was observed at 10 ppm, whereas only a 0.67 and 0.30% mineralization rates occurred at 500 and 1000 ppm respectively, at the same moisture level (0.30 bar). Somasundaram et al. (1989) suggested that TCP may have the capability of inhibiting microbial degradation of chlorpyrifos in soil because they found chlorpyrifos degradation half-life increased in soil treated with TCP before the application of chlorpyrifos.

The possibility of enhanced microbial chlorpyrifos degradation had been studied by Racke and Coats (1988, 1990) and Racke et al. (1990). Microbial populations adapted for rapid degradation of isofenphos did not change the rate of chlorpyrifos degradation. In laboratory studies, an *Arthrobacter* sp. isolated from soils with a history of isophenphos use rapidly metabolized isophenphos in pure culture but did not metabolize or cometabolize chlorpyrifos (Racke and Coats, 1988). Repeated treatments of soils with chlorpyrifos in the laboratory have not resulted in accelerated rates of chlorpyrifos degradation, and the annual field application of chlorpyrifos for 2-4 years did not alter the half-life of chlorpyrifos in the field (Racke et al., 1990). Sethunathan and Pathak (1972) reported rapid diazinon biodegradation in rice paddy water after repeated diazinon application, but not in the case of chlorpyrifos. Lal et al. (1987) studied chlorpyrifos degradation in the culture of the ciliate protozoan *Tetrahymena performs*. They found no significant chlorpyrifos metabolism by this protozoan, although they demonstrated its ability to metabolize DDT to DDD and DDE.

On the other hand, there are also reports showing that enhanced microbial chlorpyrifos degradation can occur, however, in some of these reports there is a lack of sufficient evidence. Direct microbial metabolism of chlorpyrifos in the laboratory was first reported by the Hirakoso (1969). However, twenty-seven different bacterial species (*Pseudomonas* spp., *Bacillus* spp., and others) failed to metabolize chlorpyrifos in laboratory cultures grown in peptone and glucose peptone media (Hirakoso, 1969). Significant levels of chlorpyrifos degradation have been reported by Jones and Hasting (1981) which were attributed to several species of forest soil fungi (*Trichoderma harzianum*, *Penicillium multicolor*, *Penicillium vermiculatum*, *Mucor* sp.). But chlorpyrifos loss in the control groups over the same time period was also significant. Both microbial factors and abiotic factors such as the loss through evaporation were possible, as concluded by Jones and Hasting (1981). One common yeast species (*Saccharomyces cerevisiae*) degraded chlorpyrifos rapidly in the medium, but the details of metabolism mechanism or metabolites were not provided (Lal and Lal, 1987). The wood-rotting fungus *Phanerochaete chrysosporium* was able to mineralize chlorpyrifos

(27.5%) during 18-days incubation in nitrogen-limited cultures (Bumpus et al., 1993). Their results clearly demonstrated that the chlorinated pyridinyl ring of ^{14}C -chlorpyrifos may undergo ring cleavage during biodegradation by *P. chrysosporium*.

While only the wood-rotting fungus *Phanerochaete chrysosporium*, isolated from soil, has been unequivocally shown to have the capability of chlorpyrifos degradation in the laboratory, there have been several reports indicating microbial degradation of TCP and TMP (Racke et al., 1988; Racke and Robbins, 1991; Feng et al., 1997a, b). Approximately 65-85% of the TCP applied to several soils was mineralized within 14 days (Racke et al., 1988). Racke and Robbins (1991) conducted research on the degradation of TCP in 25 different soils, and found that the addition of glucose accelerated chlorpyrifos degradation in the soils. Two of 25 soils containing microbial populations had the capability of utilizing TCP as a sole carbon source in mixed culture. Cultures isolated from soil that had 45.1% TCP mineralization were able to rapidly degrade TCP after a lag of about 7 days. However, there was little TCP degradation in the culture inoculated with the sterilized soil (Racke and Robbins, 1991). TCP was mineralized by immobilized *Pseudomonas* sp. strain M285 (Feng et al., 1997b). Overall, 75% of the mineralization rate was achieved by using this technique. Recently, they reported that they had successfully isolated a *Pseudomonas* sp. that is capable of mineralizing TCP to $^{14}\text{CO}_2$, chloride, and unidentified polar metabolites (Feng et al., 1997a). The addition of carbon sources such as glucose, maleic acid, and succinic acid stimulated this mineralization process.

2.2.5. Chlorpyrifos and TCP metabolic pathways in soil

A generalized chlorpyrifos degradation pathway which may occur in the environment has been postulated by Racke (1993) (Figure 2-1). Chlorpyrifos is thought to be hydrolyzed to TCP and subsequently converted to TMP and CO_2 as a primary degradative pathway in soil (Getzin, 1981a). Getzin (1981a) reported ^{14}C -labeled chlorpyrifos was recovered as TCP, as expired CO_2 , and as a nonextractable soil-bound residue component. As much as 29-34% TCP was detected 8 weeks after application of

chlorpyrifos in two different kinds of soil. A remarkably different mineralization rate of TCP was also reported, with 47% from clay loam versus only 8% from silt loam (Getzin, 1981a). Getzin (1981b) provided additional evidence that TCP is a primary metabolite of chlorpyrifos in soil. Between 47.9% and 67.9% of the applied chlorpyrifos was metabolized to TCP within 48 hr of soil incubation at 30 °C. In experiments with moist and air-dry soil show that from 68.5% to 100% of chlorpyrifos was converted to TCP, and 10.2 and 16.2% was further converted to TMP, respectively.

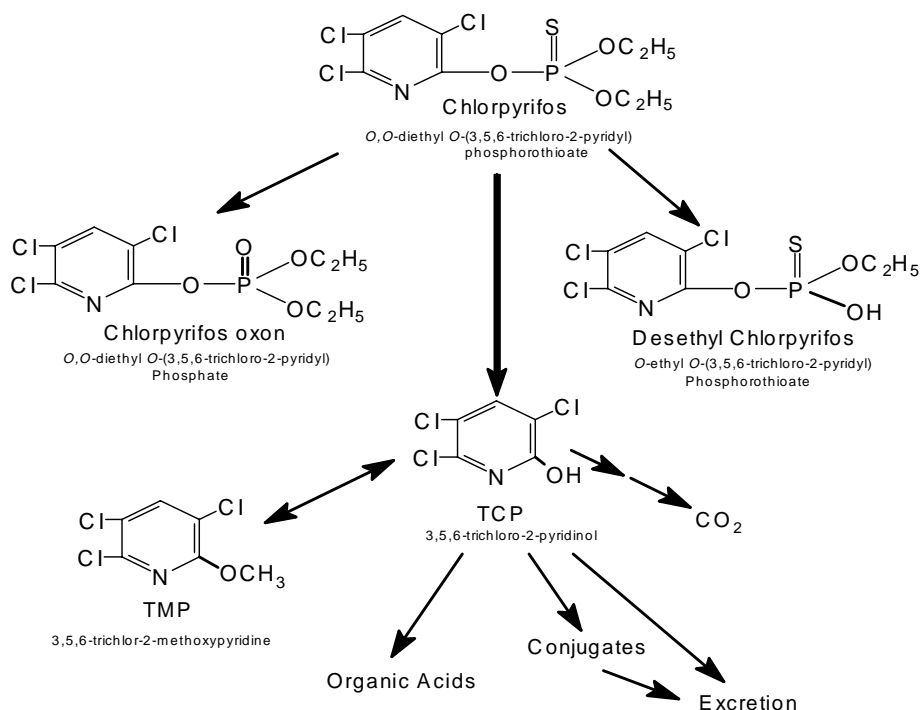


Figure 2-1. Generalized chlorpyrifos transformation pathways (Racke, 1993)

In addition to TCP and TMP, a number of other metabolites have been identified by Afifi and Kansouh (1980) and Zidan et al. (1981). Using paper chromatography (Afifi and Kansouh, 1980; Zidan et al., 1981) and thin-layer chromatography (Racke and Coats, 1988), they detected the presence of chlorpyrifos oxon, *O*-ethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphate, and desethyl chlorpyrifos. Racke et al. (1988) reported the mineralization of TCP in 10 different kinds of soils treated with 5 ppm TCP, approximately 5-80% of TCP was mineralized to carbon dioxide after 14 days of incubation. The greatest mineralization rate was associated with soil that had a previous

history of TCP use. The increased mineralization rate was possibly associated with enhanced microbial cometabolism (Racke et al., 1988). In fact, further research indicated that a mixed population of microbes isolated from soils with the capability of rapid TCP mineralization was able to grow on TCP as a sole carbon/energy source (Racke and Robbins, 1991). TCP was completely mineralized by a *Pseudomonas* sp. with a rate of 87% at a concentration of 40 mg L⁻¹ in 70h (Feng et al., 1997a). A small portion (4.1%) of initially applied TCP was incorporated to the biomass of *Pseudomonas* sp. TCP mineralization was enhanced by the addition of carbon source such as glucose and inhibited by the addition of chloramphenicol.

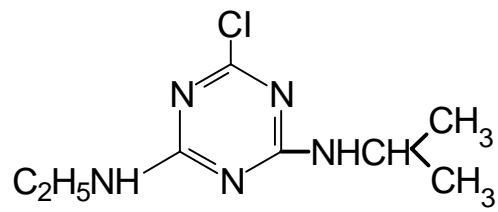
2.3. Atrazine

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is the most commonly used herbicide in corn production. It is also used as a pre- and post-emergence herbicide for the control of broadleaf and grassy weeds in many other agricultural crops. Degradation of atrazine can occur by biotic and abiotic processes. A list of atrazine and its major chemical and microbial metabolites are provided in Figure 2-2.

As was discussed previously for chlorpyrifos, atrazine dissipation in soils is generally correlated with factors favorable to microbial activity (Sheets and Shaw, 1963; Talbert et al., 1964; Burnside, 1965). Comparisons of atrazine degradation from sterile versus nonsterile soils supports this hypothesis (Burnside et al., 1961). Several species of fungi and bacteria isolated from soil have been shown to be capable of degrading atrazine in the laboratory studies. They are capable of growing in nutrient media containing atrazine as the sole source of nitrogen and carbon (Kaufman and Blake, 1970; Behki and Khan, 1986; Mandelbaum et al., 1995; Radosevich et al., 1995).

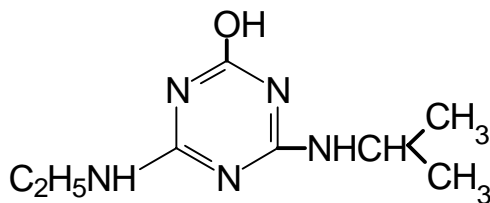
2.3.1. Microbial degradation

Soil microbes play an important role in degradation of atrazine and its metabolites to various products. Giardi et al. (1985) demonstrated the final degradative pathway of atrazine by a *Nocardia* strain through experiments on the degradation of its metabolite, 4-



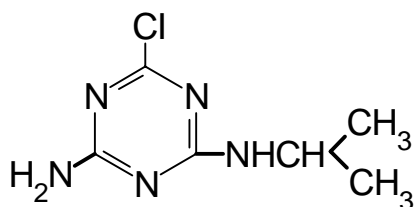
Atrazine, CIET

2-Chloro-4-ethylamino-6-isopropylamino-s-triazine



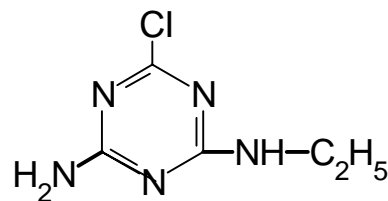
Hydroxyatrazine, IEOT

2-Hydroxy-4-ethylamino-6-isopropylamino-s-triazine



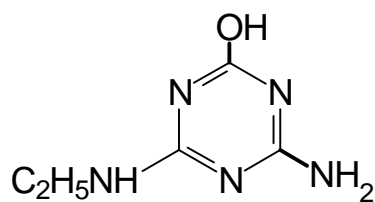
Deethylatrazine, CIAT

2-Chloro-4-amino-6-isopropylamino-s-triazine



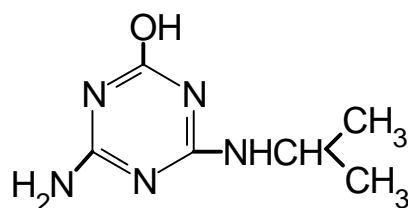
Deisopropylatrazine, CEAT

2-Chloro-4-amino-6-ethylamino-s-triazine



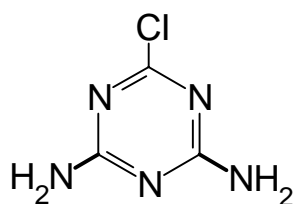
N-Ethylammeline, EAOT

2-Ethylamino-4-amino-6-hydroxy-s-triazine



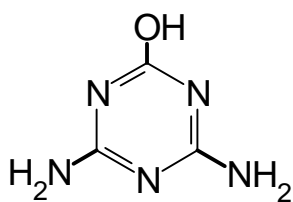
N-Isopropylammeline, IAOT

2-Isopropylamino-4-amino-6-hydroxy-s-triazine



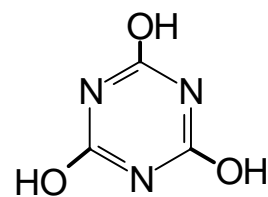
CAAT

2-Chloro-4,6-diamino-s-triazine



Ammeline, AAOT

2-Hydroxy-4,6-diamino-s-triazine



Cyanuric acid, OOOT

2,4,6-trihydroxy-s-triazine

Figure 2-2. Names, structures of atrazine and its metabolites

amino-2-chloro-6-isopropylamino-1,3,5-triazine (Figure 2-3). In bacterial medium, this compound was transformed through a microbial *N*-dealkylation into several products including one identified as 2-chloro-4,6-diamino-1,3,5-triazine (Giardina et al., 1982; Giardi et al., 1985). *N*-dealkylation is a major mechanism of atrazine degradation by a number of soil fungi (Kaufman and Blake, 1970). Behki and Khan (1986) demonstrated the *N*-dealkylation and dehalogenation of atrazine by *Pseudomonas* sp (Figure 2-4). They isolated, from soil with a long history of atrazine application, three species of *Pseudomonas* that were capable of utilizing atrazine as a sole carbon source. These *Pseudomonas* species selectively degrade atrazine to deisopropylatrazine over deethylatrazine through *N*-dealkylation. Further, dechlorination of both deisopropylatrazine and deethylatrazine may be achieved by two *Pseudomonas* species grown in glucose-supplemented mineral salts medium (Behki and Khan, 1986) (Figure 2-4). Complete mineralization of atrazine is considered to be biotic rather than abiotic. There are a number of reports that isolated mixed bacterial cultures (Mandelbaum et al., 1993; Topp et al., 1995; Zwieten and Kennedy 1995), and bacterial species (Mandelbaum et al., 1995; Radosevich et al., 1995) are able to transform atrazine to CO₂. Mandelbaum et al. (1993) were able to obtain enrichment cultures that contain atrazine at a concentration of 100 ppm as a sole nitrogen source from soils exposed to repeated spills of atrazine, and alachor, and metolachor. Atrazine was converted via hydroxyatrazine to CO₂. The half-life of atrazine degradation was found to be as short as 0.5-2 days in enrichment cultures (Mandelbaum et al., 1993). Similar results were reported by Topp et al. (1995) on alluvial sediments and enrichment cultures. In this case, atrazine was degraded within 15 d in sediment slurries incubated aerobically at 30 °C. The identified metabolites were cyanuric acid, urea and carbon dioxide. The half-life of atrazine degradation in activated sediments and enrichment culture was only approximately 1 d (Topp et al., 1995) at an initial concentration of 10 mg L⁻¹ under aerobic conditions. Rapid mineralization of atrazine ring carbon has also been documented by Zwieten and Kennedy (1995). They reported 83% of the labeled *s*-triazine ring carbons were metabolized to ¹⁴CO₂ by a mixed microbial consortia in soil that had been extensively perfused with a solution of atrazine. Three strains of *Rhodococcus* sp were able to

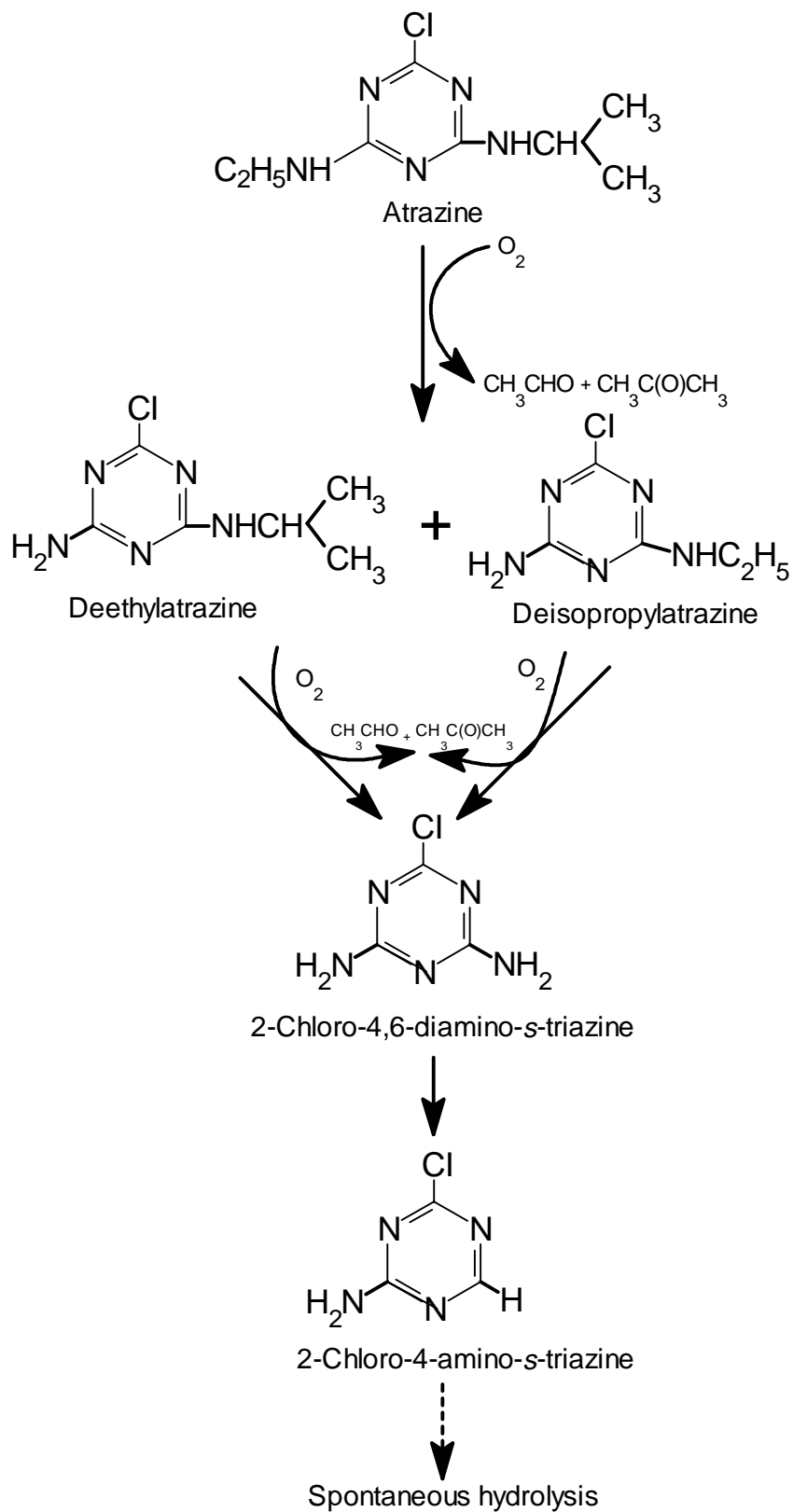


Figure 2-3. Nocardial metabolism of atrazine (Giardi et al., 1985)

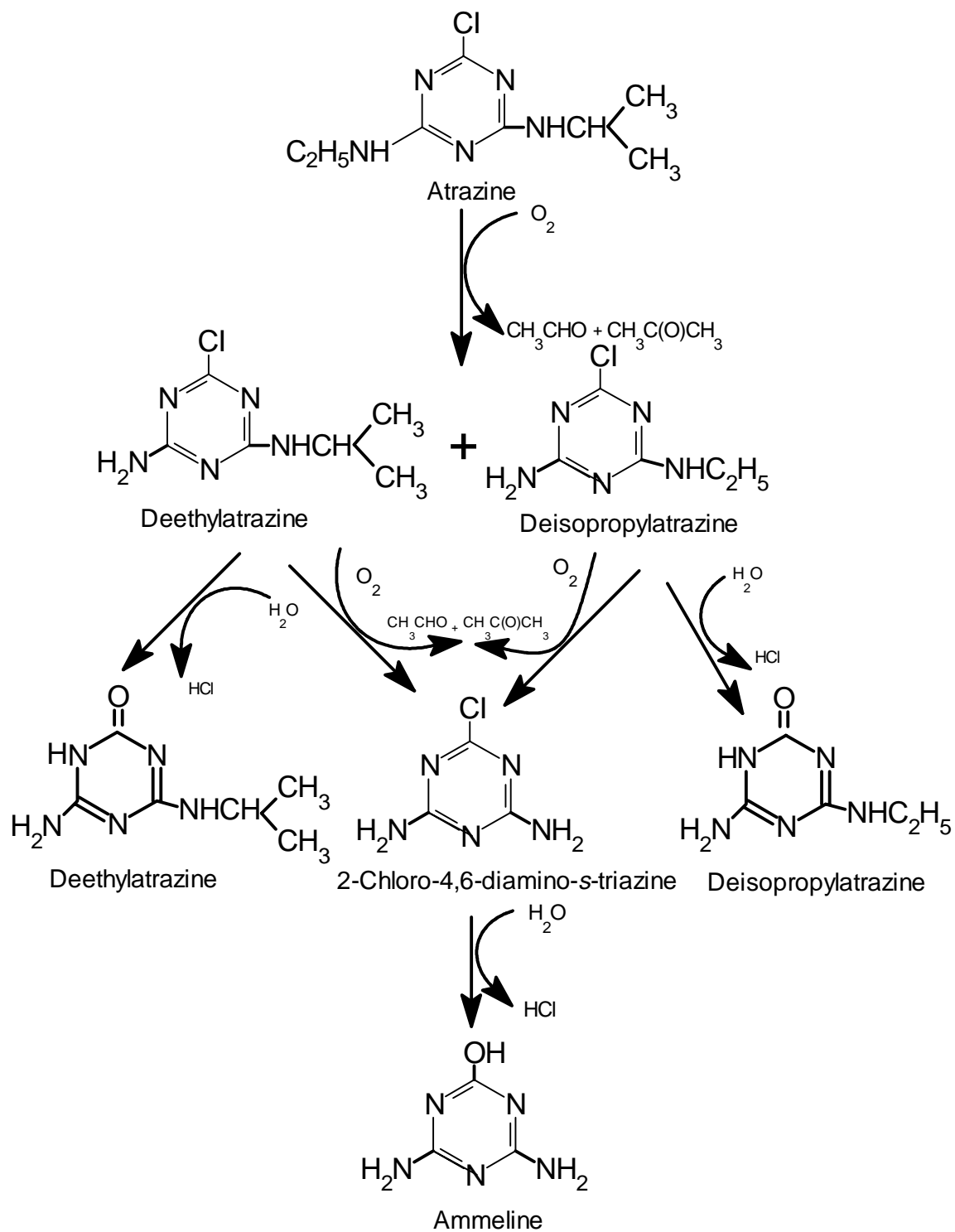


Figure 2-4. *Pseudomonas* sp. metabolism of atrazine (Behki and Khan, 1986)

metabolize atrazine to deisopropylatrazine and deethylatrazine (Behki et al., 1993; Behki and Khan, 1994; Zwieten and Kennedy, 1995) with no further degradative activities on the atrazine ring structure. *Rhodococcus* strain TE1 (Behki et al., 1993) and strain B-30 (Behki and Khan, 1994) predominantly metabolized atrazine to deethylatrazine, while *Rhodococcus* sp. NI86/21 (Zwieten and Kennedy, 1995) degraded atrazine to deisopropylatrazine as its major metabolite. A soil bacterial isolate (M91-3) has recently been isolated by the Radosevich et al. (1995). It is gram-negative and facultatively anaerobic and is capable of using atrazine under aerobic conditions as the sole source of carbon and nitrogen. Approximately 40 to 50% of ring labeled ^{14}C was metabolized to biuret and urea by M91-3. These cultures also degraded atrazine anaerobically at relatively reduced rates (Radosevich et al., 1995). Mandelbaum et al. (1995) have isolated a *Pseudomonas* sp., designated ADP, which was capable of metabolizing atrazine at very high concentrations (>1000 ppm). This species uses atrazine as its sole nitrogen source and sodium citrate as its carbon source and is able to survive in soil and increase the rate of atrazine mineralization, especially with the addition of sodium citrate (Mandelbaum et al., 1995).

Soil fungi that degrade atrazine include *Aspergillus fumigantus*, *Rhizopus stolonifer* and *Fusarium moniliforme* (Kaufman and Blake, 1970), mycorrhizal fungi (Donnelly et al., 1993) and *Phanerochaete chrysosporium* (Hickey et al., 1994; Mougín et al., 1997). All of these fungi have shown the capabilities of degrading atrazine by *N*-dealkylation of either alkylamino group, differing quantitatively and qualitatively (Figure 2-5). Two atrazine degradation products have been identified including 2-chloro-4-amino-6-isopropylamino-*s*-atrazine and 2-chloro-4-ethylamino-*s*-triazine (Kaufman and Blake, 1970). Nine mycorrhizal fungi and free-living saprophytic microorganisms were able to degrade atrazine and incorporate atrazine carbon into their tissue at concentrations of 1 mM and 4 mM (Donnelly et al., 1993). However, none of these fungi were able to mineralize atrazine (Donnelly et al., 1993). Similar results were found by Hickey et al. (1994) and Mougín et al. (1997) for the wood-rotting fungus *Phanerochaete*

chryso sporium. This organism was capable of increasing the amounts of bound atrazine residue in soil (Hickey et al., 1994).

Based on the type of chemical reaction at the triazine ring substituents, atrazine microbial metabolism can be characterized in the following sequence.

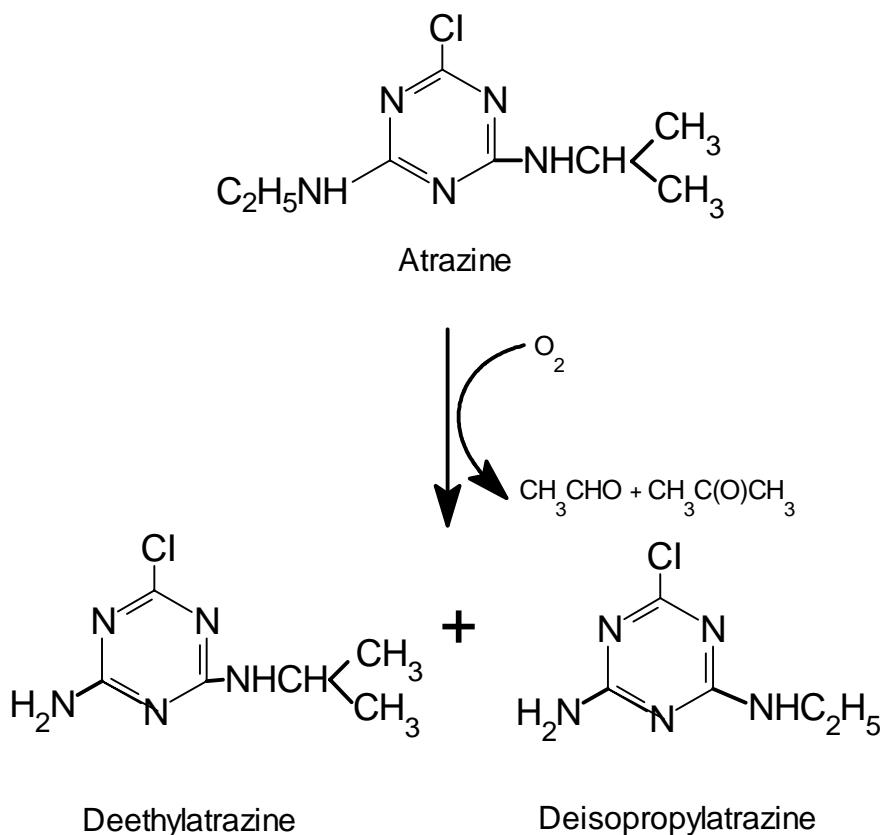


Figure 2-5. Atrazine degradation by fungi (Behki and Khan, 1986)

2.3.1.1. Dechlorination hydrolysis of atrazine

Chemical hydrolysis of atrazine to form the hydroxyl derivatives can occur in the absence of soil microorganisms (Figure 2-6). However, Jordan et al. (1970) stated that the rate of hydrolysis was ten times faster with soil than without soil. Couch et al. (1965) reported that hydroxyatrazine is the predominant metabolite when *Fusarium roseum* was incubated with ¹⁴C-ring-labeled atrazine source. In addition to effects of soil microorganisms, the chlorine at 2-position is subject to nucleophilic attack, and Fe and Al

dissolved from the soil are capable of catalyzing atrazine hydrolysis (Armstrong et al., 1967).

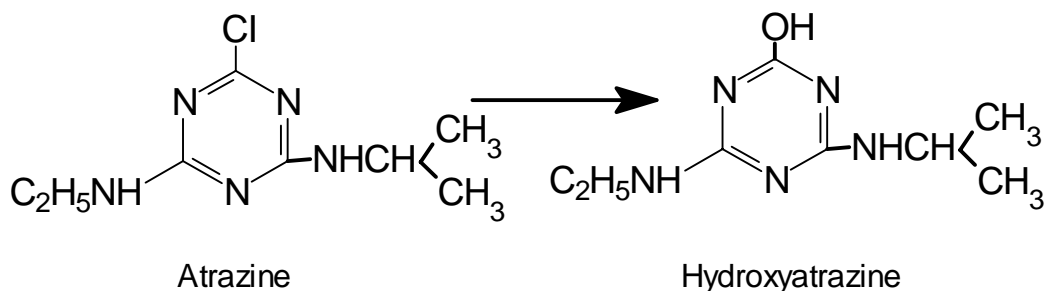


Figure 2-6. Dechlorination hydrolysis of atrazine

2.3.1.2. N-Dealkylation

Dealkylation (Figure 2-7) appears to be the first step in the microbial degradation of atrazine. Atrazine can be dealkylated to either CIAT by removing the ethyl group or to CEAT by removing the isopropyl group (Kaufman and Kearney, 1970). Under conditions where atrazine was used primarily as a source of energy, ^{14}C -labeling of the side chain generally resulted in more $^{14}\text{CO}_2$ release than that of ring-labeled carbon. The different microorganisms isolated showed a preference for removing the alkyl group on the *s*-triazine ring, i.e. some prefer removing the ethyl side chain first; others showed a preference for removing the isopropyl group first. More deisopropylated CIET was produced than CEAT in an isolated *Pseudomonas* culture (Behki and Khan, 1986). Plimmer et al. (1971) identified CEAT, CIAT, and CAAT as products of the dealkylation. The oxidative phosphorylation of side chain carbon constituents provided an energy source for atrazine degradative microorganisms (Kaufman and Kearney, 1970).

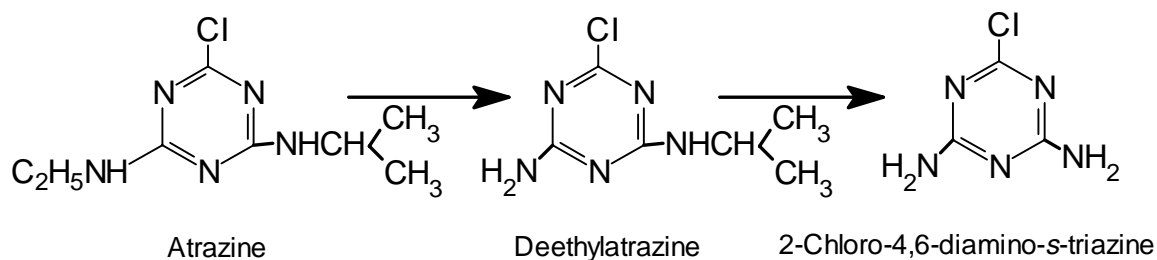


Figure 2-7. N-Dealkylation of atrazine

The importance of dealkylation was also confirmed with intact soil studies under both laboratory and field conditions. Atrazine monodealkylated derivatives i.e. CEAT and CIAT were detected after application of atrazine. Both CEAT and CIAT were subject to further microbial and chemical degradation. One of the common metabolites was OAAT.

2.3.1.3. Deamination

Atrazine amine metabolites can be degraded to their corresponding hydroxyl derivatives in soil under both laboratory and field conditions (Figure 2-8). This reaction is critical for final metabolism of atrazine. Giardi et al. (1985) studied the chemical and biological degradation of CIAT with *Nocardia*. They found CAAT was degraded by microbial attack of CIAT or CEAT, while CAAT can be deaminated further to CAHT which undergoes rapidly hydrolysis and does not accumulate in a culture medium. They proposed a scheme of biodegradation of CIET that included N-dealkylation to CAAT, formation of CAHT, and ring cleavage to form dicyanodiamidine. The biodegradation of CEAT with CEAT as a sole nitrogen source was investigated by Cook and Huetter (1984). CEAT was dechlorinated to EAOT, followed by a deamination to N-ethylammelide (EOOT). In these degradation processes, glycerol was used as a supplemental carbon source. The exponential growth of microorganisms (*Rhodococcus corallinus*) was observed with CEAT as nitrogen source and glycerol as an additional carbon and energy source.

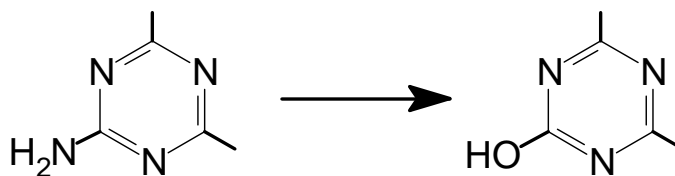


Figure 2-8. Deamination of atrazine

2.3.1.4. Triazine ring cleavage

Cleavage of the triazine ring is generally considered to be quite slow compared to that of the side chain as indicated by the amount of radioactivity of ring-labeled *s*-triazines released as $^{14}\text{CO}_2$ during soil studies (Figure 2-9). Generally, only a few percent of the

applied atrazine radioactivity was released within one to four months as $^{14}\text{CO}_2$. Soil amended with glucose showed increased biodegradation of atrazine. Wagner and Chahal (1966) reported that less than 2% of the ring-labeled carbon of atrazine evolved as $^{14}\text{CO}_2$ from glucose-amended corn soil in the period of 6 months of incubation, while less than 1% of total applied atrazine as $^{14}\text{CO}_2$ evolved from the corresponding unamended soils. However, in recent years several researchers have found rapid mineralization of atrazine in an atrazine-perfused soil (Zwieten and Kennedy, 1995), in the enrichment cultures (Mandelbaum et al., 1993, Topp et al., 1995), and in a bacterial isolate (Radosevich et al., 1995). Within 360 h, 83% of ring-labeled atrazine was mineralized to $^{14}\text{CO}_2$ by the microbial population in soil which had been perfused with atrazine for 3 years (Zwieten and Kennedy, 1995). Concentrations as high as 100 ppm atrazine were mineralized 80% or more by an enrichment culture containing atrazine as a sole nitrogen source (Mandelbaum et al., 1993). Over 200 pure cultures were isolated from enrichment cultures and tested for atrazine degradation. None of these colonies were found to individually degrade atrazine; however, when they were mixed together, their atrazine mineralizing ability was restored (Mandelbaum et al., 1993). Rapid mineralization of atrazine by enrichment culture and alluvial sediments was also reported by Topp et al. (1995). They found atrazine was mineralized aerobically, but not anaerobically, and was metabolized to carbon dioxide, cyanuric acid, urea and an unidentified hydrophilic product (Topp et al., 1995). The bacterium M91-3 was capable of partially mineralizing atrazine through dealkylation, dechlorination, deamination and ring cleavage (Radosevich et al., 1995). The final metabolites included Cl^- , NH_4^+ , CO_2 , biuret, urea, and an unidentified water-soluble metabolite (Radosevich et al., 1995). Another bacterium *Pseudomonas* sp. strain ADP, was able to mineralize 80% of 100 ppm atrazine (Mandelbaum et al., 1995). The cell extracts from *Pseudomonas* sp. ADP retained its ability to mineralize atrazine. During the incubation, atrazine was metabolized to hydroxyatrazine, an unidentified polar metabolite and carbon dioxide. A significant increase in the rate of atrazine mineralization occurred when soil has been inoculated with this organism, especially with the addition of sodium citrate as an additional carbon source (Mandelbaum et al., 1995).

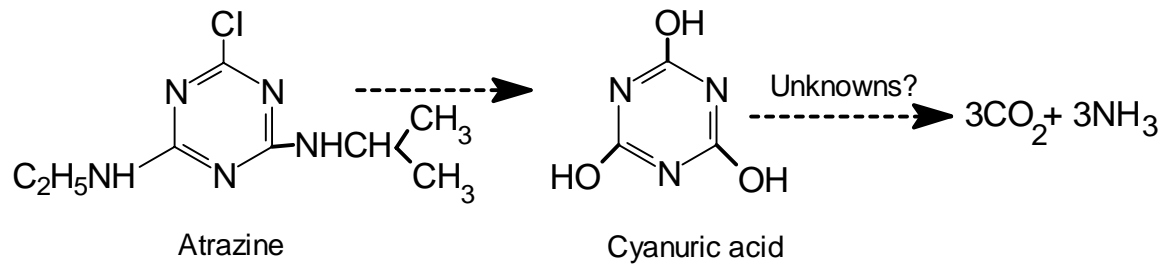


Figure 2-9. Triazine ring cleavage

Hydroxyatrazine is more readily attacked by soil microbes. Hance and Chesters (1969) reported that about 10% of ¹⁴CO₂ evolved from ring-labeled hydroxyatrazine was detected under both aerobic and anaerobic conditions in soil and lake sediments high in organic matter content. Ring cleavage of OOOT was reported by Cook et al. (1985). It can be used as a nitrogen source for microbial growth (*Pseudomonas* sp. strain D). It is first converted to biuret and carbon dioxide; biuret is then further degraded to urea, NH₄⁺ and CO₂. Ammeline degradation in soil seems to occur quite quickly. Approximately 50-80% of the ¹⁴C-labeled ammeline was converted into ¹⁴CO₂ within two or three months. This complete mineralization of *s*-triazines and related compounds was found in the two different *Pseudomonads* and a strain of *Klebsiella pneumoniae* (Cook and Hutter, 1981).

2.3.2. Chemical degradation of atrazine

Atrazine degradation is also influenced by abiotic factors. Volatilization may be an important factor influencing the persistence of some pesticides in environment but not for atrazine. Less than 0.4 μg h⁻¹ and 0.05 μg h⁻¹ of atrazine was volatilized from wet sandy soil and wet peat soil at 20 °C (Dörfler et al., 1991). As could be expected, atrazine volatilization from soil increased with temperature and decreased with soil moisture level and organic carbon content (Burt, 1974; Dörfler et al., 1991). Volatilization increased 25% when the temperature was elevated from 20 °C to 40 °C (Burt, 1974). Atrazine chemical hydrolysis to hydroxyatrazine is one of the major reactions responsible for the detoxification of atrazine in soil. In addition to atrazine hydrolysis, a number of microbial metabolites are fairly susceptible to further hydrolysis. As indicated in the previous

sections, hydrolysis products such as hydroxyatrazine are more quickly mineralized by soil microbes.

2.3.2.1. Hydrolysis

The appearance of hydroxyatrazine as a major product in sterilized soil after atrazine application is good evidence of nonbiological mechanisms contributing to this reaction (Figure 2-10). McCormick and Hiltbold (1966) and Roeth et al. (1969) reported that the degradation rate of atrazine increased two to three-fold for each 10°C temperature increase from 15 to 35°C. Atrazine is converted to hydroxyatrazine followed by the first-order kinetics in a soil-free, sterilized system (Armstrong et al., 1967). Atrazine was stable in neutral pH medium, though rapid hydrolysis occurred under highly acidic or alkaline conditions. Alkaline hydrolysis was considered as the direct nucleophilic displacement of Cl by OH, while under acidic conditions the protonation of a ring or chain N atom which increases the electron deficiency of the C-Cl bond resulting in the cleavage of the C-Cl bond by H₂O (Armstrong et al., 1967). Soil adsorption and the presence of Fe and Al were postulated as the mechanism that increased the electron deficiency of C-Cl bond, subsequently facilitating the nucleophilic attack of Cl in the atrazine molecule, and resulting in an increased rate of atrazine hydrolysis (Armstrong et al., 1967).

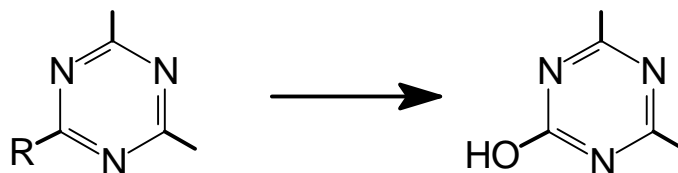


Figure 2-10. Chemical hydrolysis of atrazine

Soil microbes also participate in atrazine hydrolysis. Giardi et al. (1985) reported the microbial degradation of atrazine by a *Nocardia* strain. Atrazine was transformed to CAAT, AOIT and AOOT in a bacterial medium. The formation of AOIT and AOOT were considered to be the results of chemical dechlorination and hydrolysis (Giardi et al., 1985).

Atrazine hydrolysis increases at highly alkaline conditions. The hydrolysis, adsorption, and degradation of atrazine in soil suspension mixed with fly ash (raw material from lignite from a thermal station, pH =11.2) were studied by Albanis et al. (1989) in Greece. They reported increased degradation of atrazine by 13.3% on average in a sandy clay loam soil and 9.0% in the clay soil with the addition of fly ash up to 0.5%. The catalytic hydrolysis of atrazine at alkaline pH values were the major factors attributed to these increased loss rates (Albanis et al., 1989).

2.3.2.2. Photolysis

Photolysis of most pesticides typically produces oxidized or dechlorinated or hydroxyl-substituted metabolites which may be more readily degraded by indigenous soil microbes (Kearney et al., 1983; Kearney et al., 1984). Atrazine was photodegraded (UV λ >290 nm) to hydroxyatrazine in distilled water and artificial seawater containing humic acids (Durand et al., 1991). The rate of photodegradation in seawater was faster than in distilled water. Similar results were reported by others (Choudry, 1982; Mansour et al., 1989). Humic acids were believed to be photosensitizers which facilitated the secondary photoreactions and enhanced photochemical degradation of atrazine (Durand et al., 1991).

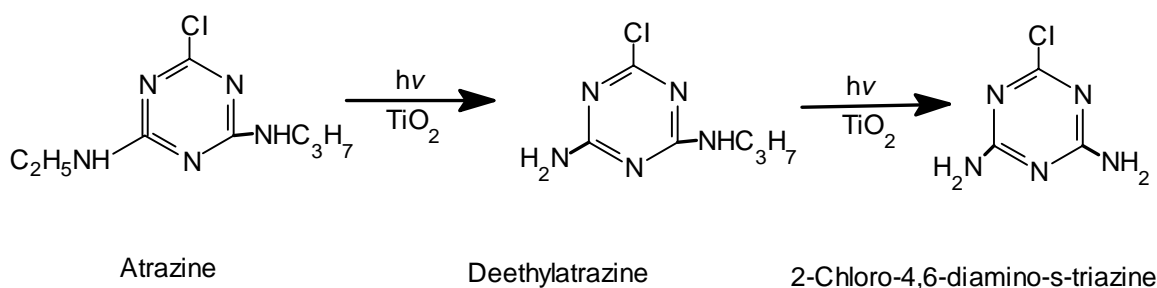


Figure 2-11. Photocatalytic degradation of atrazine with $\text{TiO}_2/h\nu$ (Hustert & Moza, 1991)

A photodegradation pathway (Figure 2-11) was proposed by Huster and Moza (1991). Atrazine and deethylatrazine were rapidly photocatalytically degraded to 2,6-diamino-4-chloro-1,3,5-triazines in aqueous titanium dioxide suspension. The highly reactive OH radicals generated by UV-irradiation of semiconductors like TiO_2 might

provide a mechanism for rapid degradation in aqueous titanium dioxide suspensions (Huster and Moza, 1991).

2.3.3. Incorporated residue of atrazine in soil

Incorporated residue, also known as bound residue, is the portion of pesticides that is nonextractable by conventional methods. However, a small portion of incorporated xenobiotics may dissolve in soluble organic fractions and are extractable with organic solvents. There are some reports indicate that soil bound pesticide residues may be available to plants (Fuhremann and Lichtension, 1978; Führ and Mittelstaedt, 1980; Khan, 1980), to earthworm (Fuhremann and Lichtenstein, 1978) and biodegraded by soil microbes (Khan and Ivarson, 1981, 1982). However, incorporated residues are generally considered to be less bioavailable, less mobile and hence less or not toxic to organisms in the environment (Bollag and Loll, 1983; Berry et al., 1993a, b). Capriel and Haisch (1983) reported that about 83% of the initially applied radioactivity of ^{14}C atrazine was recovered after nine years of application under outdoor conditions. As much as 50% of ^{14}C -atrazine of radioactivity was bound to the soil; i.e., unextractable after exhaustive solvent extraction. Among the ^{14}C -activity remaining bound in soil, both parent atrazine and metabolites were detected. The metabolites included hydroxyatrazine and the dealkylated derivatives deethylhydroxyatrazine and deisopropylhydroxyatrazine.

Winkelmann and Klaine (1991a,b) reported the bound residue of atrazine and its major metabolites by using radiolabeled compounds in a western Tennessee soil under nonirradiated and irradiated conditions. The percentages of nonextractable ^{14}C or bound residue increased with time in both nonirradiated and irradiated microcosms. Among the bound residues of several atrazine metabolites, the potential to form bound residues in the order of CAAT > CIAT \geq CEAT > IEOT, was within a range of 28 to 60% of the ^{14}C added to nonirradiated microcosm soil after 180 days incubation. Relatively high amounts of bound residue were found in the corresponding irradiated soils and reached as high as 72% of the radioactivity of the bound residue. Schiavon (1988) reported that dealkylation of atrazine increased the potential for bound atrazine derivative residues with the CAAT

being the highest amount of bound residue. On the other hand, hydrolysis of atrazine reduced the binding ability of atrazine metabolites. Although IEOT was absorbed by soil organic matter, a comparatively low amount of bound IEOT residue was detected. As much as 22% of applied atrazine was not extractable by methanol and water (Barriuso and Koskinen, 1996). The percentage of bound residue increased with time after application. Most of incorporated nonextractable atrazine residue was associated with clay particle size fraction (0.2-2 μm) (Barriuso and Koskinen, 1996).

Some information is available on the mechanisms of polymerization and incorporation of phenolic compounds and anilines which occur mainly through an oxidative coupling process through covalent bonding (Bollag and Bollag, 1990; Bollag, 1992). Covalent bonding may be catalyzed by extracellular enzymes such as laccase, peroxidase and tyrosinase; clay minerals, metal oxides, and even sometimes by autooxidation (Bollag and Bollag, 1990). Phenols and anilines can form cross-links between themselves or directly bind to the reactive groups in organic matter. These enzymes exist intracellularly in microorganisms, plants and other soil inhabiting animals which can also secrete enzymes extracellularly to the environment. These extracellular enzymes can be absorbed, entrapped and copolymerized by soil humic colloids and clay minerals. The clay- and humus-enzyme complexes actually prevent the denaturation of enzyme proteins under extreme environmental conditions and reduce the possibility of proteolytic degradation. Since entrapped enzymes or immobilized enzymes have the advantage over free enzymes, some recent researchers have focused on the development of immobilized enzyme or enzyme complex techniques for decontaminating wastewater and soil (Nannipieri and Bollag, 1991).

The polymerization and incorporation of heterocyclic aromatic compounds have not been studied as extensively as those of homocyclic aromatic compounds. Atrazine and its metabolites can be incorporated into soil humus and absorbed by soil clay and clay-metal-organic complexes by various mechanisms (Stevenson, 1982; Bollag and Loll, 1983), although little detailed information is available. Recently, Berry et al. (1993a, b)

proposed a solid-state fermentation technique for disposing of atrazine and carbofuran contaminated lignocellulosic organic matrices. They found that atrazine and carbofuran can be successfully retained on these organic materials in incubation chambers with relatively low leaching potential and low biotoxicity. Soil organic surfaces generally have numerous reactive functional groups including carboxyls, alcohols, quinones, etc. Clay minerals usually are anionic in their surface. Once atrazine enters soil, various kinds of microbial and chemical degradation activities may occur through different mechanisms (See Section 2.3.1 and 2.3.2.). Atrazine and its metabolites can form covalent bonding with these surface reactive groups (Stevenson, 1982), or be absorbed through ion-exchange, hydrogen bonding, van der Waals' force, and hydrophobic interaction depending on soil conditions (Bollag and Loll, 1983).

Soil pH may exhibit profound influence on the sorption of atrazine. When the soil pH is lower than $pK_a + 2$, atrazine exists in protonated form, and as a result, the cation exchange sorption is a primary mechanism (the pK_a of atrazine is 1.68, Lerch et al., 1995). Due to the heterogeneous nature of the soil environment, the pH of an organic matter surface might be as much as two pH units lower than that of a soil solution (Weber, 1970). That means a small percentage of atrazine could be protonated even at relatively high soil solution pH. With the increase in soil pH, other interactions between atrazine and soil organic matter will prevail. Hydrogen bonds form between unionized COOH groups and unshared electron pairs of N in substituted amines of atrazine and its metabolites. Hydrogen bonding is weaker than covalent bonding and ion exchange and stronger than Van der Waal's force, but it may be relatively easy to form, even with water (Stevenson, 1982).

Soil organic substance includes nonpolar molecules such as waxes, fats, resins and long aliphatic side chains of humic and fulvic acids. These tend to interact with atrazine and its metabolites through hydrophobic bonding. Atrazine can be "trapped" by these molecules. Van der Waal's force interacts with other sorption mechanisms to increase organic sorption capacity. The formation of covalent binding between atrazine and soil

organic matter is a very important incorporation mechanism, which could possibly explain the high percentage of bound residue found in organic fractions (Winkelmann and Klaine, 1991a,b). Covalent bonding represents a strong chemical force that cannot be broken by mild solvent extraction. Proposed possible covalent bonding mechanisms might occur with catalysis of extracellular enzymes, and reactions with soil metal oxides, etc. Atrazine and dealkylated atrazine could possibly bind to the organic surface reactive groups such as COOH, CHO, OH in various ways (Figure 2-12). Increasing soil organic sorption of *s*-triazine was found well correlated with the amount of COOH present in soil organic matter (Stevenson, 1982). Figure 2-12 illustrates a hypothetical model of covalent bonding between atrazine and soil organic matter. Atrazine has the potential of reacting with COOH, CHO, and OH, etc., by forming C-N or C=N bonds. Furthermore, atrazine and dealkylated atrazine could also bind to newly generated humus materials (Figure 2-13). Quinone, either synthesized from soil microbial activity or through the lignin degradation, could conjugate with dealkylated atrazine and form quinonimines and hence be further polymerized or condensed with other organic molecules. Carbohydrates can also possibly combine with dealkylated atrazine to N-substituted glycosylamine, and then through various fragmentation and condensation to brown fulvic acids (Figure 2-13).

2.3.4. Mixed-mode sorption of hydroxylated atrazine degradation products

Recently, Lerch et al. (1997) proposed that mixed-mode sorption of hydroxylated atrazine degradation products is a primary mechanism for bound residue in soil. They suggested that a significant proportion of atrazine bound residue was simply sorbed by two simultaneous mechanisms, i.e., cation exchange and hydrophobic interaction, which is defined as mixed-mode binding. In order to test their hypothesis, a number of cation exchange (0.5 M KCl or 0.5 M KH₂PO₄, pH 7.5), hydrophobic (25% CH₃CN), and mixed-mode (KH₂PO₄/CH₃CN or KCl/ CH₃CN) extractants were used for recovering atrazine hydroxylated degradation products from freshly spiked and aged soil. They found that significantly high percentage of hydroxylated atrazine degradation products were extracted from spiked samples with mixed-mode extractants. Cation exchange extractants generally

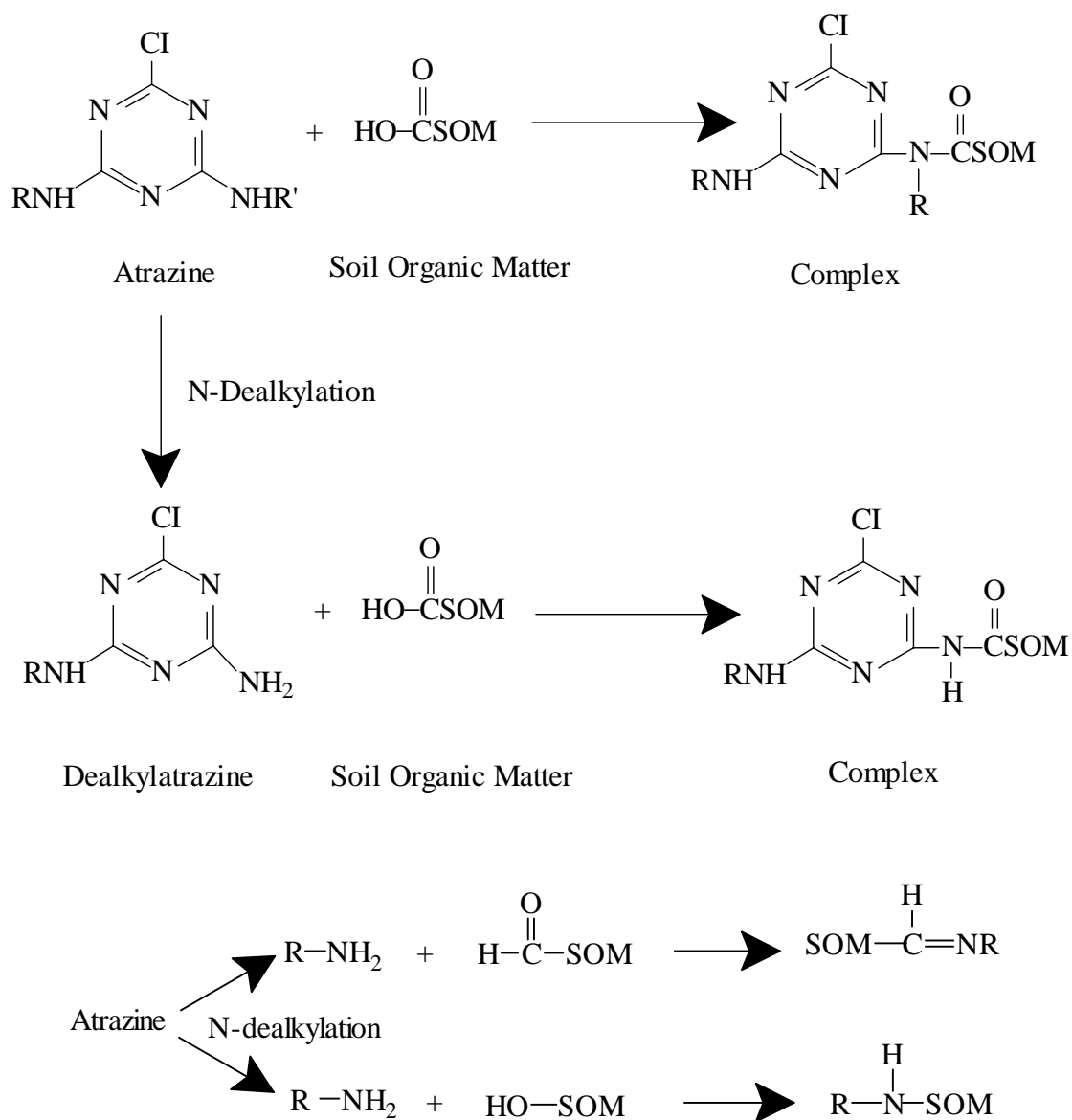


Figure 2-12. Hypothetical model of covalent bonding between atrazine and soil organic matter (Adapted from Stevenson, 1982)

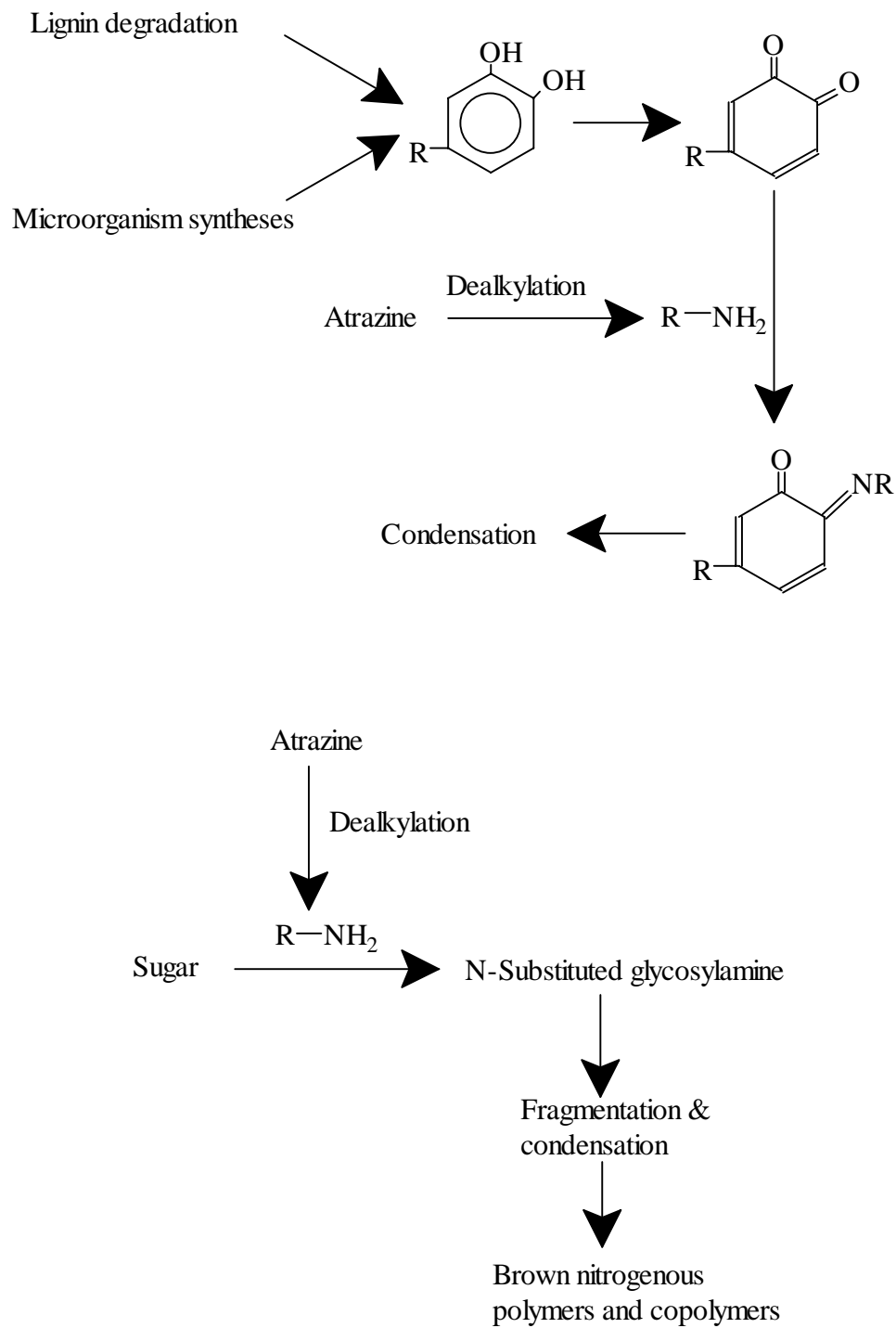


Figure 2-13. Hypothetical model of incorporation of atrazine during humification process through covalent bonding to quinone and carbohydrate (Adapted from Stevenson, 1982)

were more efficient than hydrophobic extractant alone for recovering hydroxylated atrazine at the same conditions. Furthermore, 42.8% of bound atrazine residues were recovered by mixed-mode extraction from aged soil. Therefore, they concluded that mixed-mode binding is the primary mechanism of *s*-triazines bound residue in soil.

2.3.5. Movement of atrazine

As stated previously, atrazine is one of the most frequently found contaminants in underground water (Hallberg, 1989; Koterba, 1993). It can be transported by surface water or runoff to pools, lakes and rivers. It also has the potential of diffusing directly into underground water systems (Koterba, 1993). In some cases, preferential flow through holes may be a major factor that contributes to atrazine contamination of ground water (Kruger et al., 1993). Precipitation patterns, especially during the first week or so following application, have a major influence on the extent to which a pesticide may leach or be transported in runoff water (Karickhoff and Morris, 1985). Agricultural practices, such as irrigation, also have significant impact on pesticide contamination of ground water and river water resources (Xiao et al., 1990; Xiao et al., 1991a). Once the pesticide moves below the organic rich top soil layer, its leaching hazard is significantly increased since soil below plough layer usually has less absorption capacity and less microbial degradation activity (Bowman, 1989). Soil organic matter was considered to be one of major factors that influence the movement of pesticides in soil (Helling, 1971), where greater organic matter reduced the pesticide mobility (Somasundaram et al., 1991). However, this is not always true, since water-soluble soil organic materials may also act as vehicles for transport of chemicals in soil (Madhun et al., 1986).

2.3.6. Determination of atrazine and its metabolites

Analytical methods available to quantify the amount of atrazine, its metabolites and related compounds have been reviewed by Behrens (1970), Tweedy and Kahrs (1978), Beilstein et al. (1981), and Lawrence (1982). Various techniques including HPLC, GC and HPTLC have been used to determine the concentration of atrazine and its metabolites. Some earlier indirect methods have also been used to quantify atrazine in biodegradation

studies, such as the measurement of chloride ions liberated from the transformation from atrazine to hydroxyatrazine during hydrolysis. Burchfield and Schuldt (1958) had established the colorimetric methods for the determination of atrazine, and this method has been modified subsequently by Radke et al. (1960).

Modern instrumentation improved the capability of detection and measurement of low concentration of atrazine and its metabolites in the environment. Corcia et al. (1987) have described a HPLC method capable of detecting atrazine in water at the parts per trillion level. High performance liquid chromatography (HPLC) methods have also been used to determine the atrazine level in human plasma (Pommery et al. 1990), soil residue, and plant tissue (Nelson and Khan, 1989). Beilstein et al. (1981) have used HPLC to quantitatively measure seventeen *s*-triazines and their metabolites in a fungal culture. This method was successfully employed to identify and quantify atrazine, dealkylatrazine, hydroxyatrazine, N-alkylammelins, N-alkylammelides, melamine, ammeline, ammelide, and cyanuric acid in a single analysis with good separation and a detection limit of 30-400 pmol. Potassium phosphate buffer (pH 6.7) as a mobile phase coupled with methanol elution and UV detection has been described, but requires long elution time (75 min) (Beilstein et al., 1981). Application of this method to a variety of environmental media is questionable since the method does not have any specific cleanup procedure. As a result the interference resulting from a variety of products sometimes makes the use of this method unreliable. Gas liquid chromatography (GLC) with ECD and NPD has been used quite successfully in the analysis of atrazine residue from soil (Lee and Chau, 1983; Huang and Pignatello, 1990), surface water (Potter et al., 1991) and plant tissue (Nelson and Khan, 1989). Both packed and capillary columns have been used with rapid and sensitive analyses. However, using GLC methodology for quantification of atrazine metabolites requires their derivatization which is frequently tedious and imprecise (Müir, 1980; Stoks and Schwartz, 1979). Improved identification atrazine and its derivatives has been demonstrated using GLC with a mass spectra detector (Beilstein et al., 1981; Huang and Pignatello, 1990; Potter et al., 1991). Cochromatography coupled with comparisons

of the retention times with those of authentic samples can be helpful in confirming unknown compounds (Nelson and Khan, 1989).

2.4. Soil bioremediation

As has been stated earlier, widespread, and sometimes improper pesticide usage in modern agriculture results in environmental contamination. The conventional technology for cleanup of ground water and contaminated sites is too costly and often harmful itself. For this reason, bioremediation is viewed as an alternative. The major principle of bioremediation involves uses of microorganisms such as bacteria and fungi to destroy hazardous contaminants or convert them to less harmful or mobilized forms. There are two basic types of bioremediation, intrinsic and engineered. Intrinsic bioremediation occurs without human intervention. Engineered bioremediation involves the creation of conditions which enhance degradation of hazardous compounds such as adding amendments, microbes, etc. (Anonymous, 1993).

2.4.1. Intrinsic bioremediation

For some relatively easily degraded compounds, the rate of naturally occurring biodegradation is rapid enough to slow the contaminant migration. This type of bioremediation often depends on the species and density of microbial populations on site, the types and concentration of contaminants, and the physico-chemical properties of the subsurface sites. In addition to these factors, the availability of oxygen is frequently rate limiting. Other environmental factors such as a lack of nutrients, low pH and the presence of a toxic chemical may also limit the rate of intrinsic bioremediation. Many synthetic pesticides such as atrazine and chlorpyrifos can be quite persistent in environment (Racke et al., 1994; Dousset et al., 1997). Their intrinsic bioremediation often is not fast enough to degrade these xenobiotics within a reasonable time frame.

2.4.2. Engineered bioremediation

Engineered bioremediation refers to the type of bioremediation that enhances microbial activity by adding nutrients, electron acceptors, or other biostimulants to the site

using an engineered system. It may include inoculation of a site with microorganisms capable of degrading contaminants. Since biodegradation can be a dominant mechanism of organic chemical transformation in soil and aquifers, engineered bioremediation is becoming a promising alternative for reducing pesticide contamination in soils.

Successful demonstration of engineered bioremediation has been reported. The application of dairy manure and fertilizers to pasture soils increased the total fungal and bacterial biomass and hence enhanced the mineralization of atrazine and 2,4-D in all seasons of the year (Entry and Emmingham, 1994). Fresh and aged herbicide-contaminated soils were amended with sewage sludge or corn meal (Dzantor et al., 1993). The soil dehydrogenase activity was highest in organic-material-amended soils. The organic amendment enhanced the dissipation of fresh atrazine and alachlor contaminated soils but not for aged contaminated soils. However, Dzantor et al. (1993) failed to detect any statistically significant difference between amended and unamended treatments for trifluralin in either freshly contaminated or aged contaminated soils. In a more recent study, Felsot and Dzantor (1995) reported that high alachlor concentration ($>250 \mu\text{g g}^{-1}$) inhibited soil dehydrogenase activity while this enzyme activity in corn meal amended soil surpassed the levels in corresponding no-pesticide unamended controls. Furthermore, corn meal significantly enhanced alachlor dissipation at concentration ranging from 10 to $250 \mu\text{g g}^{-1}$, compared with an unamended control. Stimulation of microbial activity by the addition of organic amendments enhances co-metabolism in high concentrations of pesticides in soil (Felsot and Dzantor, 1995).

It is preferable to use indigenous microorganisms as an inoculum resource since these "local" microorganisms frequently have become well adapted to the local environment and perhaps more capable of degrading site-specific compounds. However, the biodegradation rates of contaminant can be limited by the actual capability of naturally occurring microorganisms to degrade target contaminants. In this case, genetically engineered microorganisms showing the capabilities of degrading these recalcitrant contaminants will have an advantage over natural microorganisms. Increasingly, research

efforts have been devoted to the development of genetically engineered microorganisms to degrade environmental pollutants. Examples of engineered bioremediation are available in the literature. One example describes a situation where five different critical enzyme control genes from three distinct bacteria were combined into a single bacterium. The new bacterium was capable of degrading and growing on mixtures of chloro- and methylaromatics that were toxic even for the bacteria that could degrade the individual components of the mixtures (Rojo et al., 1987). Another example describes genetically engineered bioluminescent reporter bacterium, *Pseudomonas fluorescens* HK44, which has been successfully employed as an indicator of naphthalene and salicylate catabolism (Heitzer et al., 1994).

2.4.3. Solid state fermentation as a remediation method for contaminated soil.

The objective of hazardous material solid state fermentation, i.e. composting, is solely to convert hazardous substances into innocuous end-products. The addition of compost or other organic materials can stimulate soil microbial activity and increase both bacterial and fungal populations (Cole et al., 1994). The potential for bioremediation of contaminated environmental matrices using solid state fermentation is promising primarily because of the intensity of the microbial activity within a composting matrix. This activity is facilitated by a generally warm, moist, aerobic, and nutrient and carbon rich environment. The production of metabolic heat and the insulative properties of the physical matrix create a self-heating environment that serves to further stimulate microbial activity and turnover (Williams, 1993).

The specific contaminant destruction capabilities possessed by microorganisms in a composting mass may not differ significantly from those in soils at ambient temperature. Williams (1993) outlined several reasons that why the overall transformation potential for contaminants within a composting mass is worthy of consideration. First, elevated (thermophilic) temperatures facilitate a higher reaction rate than that generally achievable at ambient conditions. Second, the opportunity for co-metabolism (degradation of a recalcitrant compound while a microorganism is obtaining its carbon and energy from

more usable compounds) is enhanced due to the range of alternative substrates present and the high level of metabolic activity. Third, the changing physical/chemical microenvironments within a composting mass result in a diversity of microbial communities and metabolic activity in both space and time, thereby increasing the number and type of microorganisms to which a contaminant is exposed. Finally, elevated temperatures typically result in an increased contaminant solubility and higher mass transfer rates, making contaminants more available for metabolism.

As a result of these factors, the likelihood that biodegradable hazardous materials or mixtures will be degraded at a significant rate is high in an actively composting mass. Toxic contaminants may be attacked by microorganisms while readily utilizable organic matter is still available within a microsite, or after readily utilizable materials have been degraded. In either case, the large, active microbial population facilitates contaminant degradation.

Solid state fermentation has been employed to biodegrade and incorporate a number of pesticides. For example, the herbicide 2,4-dichlorophenoxyacetic (2,4-D) was actively mineralized by thermophilic microbes at a composting temperature of 60 °C (Michel et al., 1995). About half of the ¹⁴C-2,4-D was mineralized; 23% was converted to humic acid and 19.5% was unextractable after composting. Very little 2,4-D volatilization was detected during composting. Most 2, 4-D and its metabolites were absorbed by organic matter and the percentage of soluble 2, 4-D decreased over the composting interval (Michel et al., 1995).

Berry and his coworkers have investigated degradation and incorporation of a number of pesticides using solid state fermentation techniques (Berry et al., 1993a, b; Willems et al., 1996). They concluded that solvent extractable pesticides including atrazine, chlorpyrifos, carbofuran and metolachlor were significantly reduced by the process of solid state fermentation. Meanwhile, due to reduced pesticide concentrations

and increased absorption, pesticide leaching was minimal. They proposed to dispose of those persistent pesticides by increasing incorporation during solid state fermentation.

2.4.4. Some important factors which may limit the bioremediation processes during composting

2.4.4.1. Microorganisms

Microbes usually degrade organic contaminants to more polar and less toxic metabolites. Microorganisms use organic carbon as sources for building their new cell constituents (Donnelly et al., 1993) as well as obtaining energy through the electron transport (Behki, 1995; Radosevich et al., 1995). Depending on oxygen requirements during degradation processes, microbes can be divided into two types, aerobic (using oxygen) and anaerobic (not needing oxygen). Most microorganisms that participate in contaminant degradation require oxygen as an electron acceptor. Anaerobic microorganisms use nitrate (NO_3^-), sulfate (SO_4^{2-}), iron (Fe^{3+}) and manganese (Mn^{4+}) as acceptors (Anonymous, 1993). Generally speaking, there are three major types of microorganisms that play an important role in biodegradation, i.e. bacteria, actinomycetes and fungi. Bacteria represent up to 2000 different species with 10^8 - 10^9 per gram of moist compost (Biddlestone et al., 1987). However they are all quite small having a diameter of 1-8 μm . Some species form endospores during desiccation and exposure to hot environments. The actinomycetes typically have a population number in the order of 10^5 - 10^8 per gram of moist compost (Biddlestone et al., 1987). They develop quite slowly during the initial process of composting, but increase in number afterwards, contributing to degradation in the later stage of the compost cycle. Fungi are considered to be quite important in the decomposition of cellulose. They have relatively larger body sizes compared to bacteria and are sensitive to heat, dying out as temperatures exceed 55°C (Biddlestone et al., 1987). Therefore, they may be more active in their biodegradation activities as the compost mass approaches ambient temperatures (Biddlestone et al., 1987).

2.4.4.2. Nutrients

Research on the chemical composition of the cellular components of microbes have indicated the chemical constituents of typical bacterial cells are 50% carbon; 14% nitrogen; 3% phosphorus; 2% potassium; 1% sulfur; 0.2% iron; and 0.5% each of calcium, magnesium and chlorine (Alexander, 1977). The composition of carbon and nitrogen is frequently not ideal for microbial growth in compost. Other elements are relatively easily taken from the compost medium (Biddlestone et al., 1987). Since approximately 50% of carbon is evolved as carbon dioxide, the ideal C/N ratio is about 25-30. A lower or higher C/N ratio will result in a short supply of one element or excess of another element, and competition for nutrients within the microbial communities may limit the overall microbial growth and slow contaminant removal (Biddlestone et al., 1987).

2.4.4.3. Moisture content

Water acting as the primary medium for dissolving nutrients is essential for the chemical and physical processes in compost. Microbes can only absorb the nutrients from solution. Biological reactions slow down at moisture levels below 30% on fresh weight basis and may cease below 20% (Biddlestone, et al., 1987). However, that does not mean the higher the moisture content, the higher the reaction rate. Water content will affect another important factor of microbial growth, oxygen supply. Under high moisture conditions, water changes the physical properties of some substances such as corn meal, to an impervious mass which may block the oxygen supply for aerobic microorganisms, thus reducing the growth rate of microorganisms and the rate of biodegradation of contaminants (Anonymous, 1993).

2.4.4.4. Oxygen requirements

Oxygen supply is critical for aerobic microorganisms. Aerobic microbes usually obtain oxygen through diffusion from air. The voids occurring among the solid particles in a compost mass absorb air bubbles, water-air mixtures, and water. The gaseous phase exchanges continuously with outside atmospheric air: emitting carbon dioxide and

obtaining oxygen for microbial respiration simultaneously. Oxygen consumption depends on the number of microbial populations and their relative activities. Microbiological oxidation of different substrates has specific oxygen requirements. Theoretically, the more carbon per mole of contaminant, the more oxygen is required per contaminant for complete oxidation. During the depletion of oxygen supplies, anaerobic microorganism activities predominate. Instead of using oxygen, they use other substrates such as nitrate and iron as electron acceptors. Some reports have indicated they are quite productive in the degradation of recalcitrant polychloride contaminants such as PCBs through anaerobic dechlorination (Hossain and Corapcioglu, 1994). Some recalcitrant toxicants require initial anaerobic degradation, followed by aerobic metabolism. Research conducted by Nair and Schnoor (1994) indicated that atrazine ring and isopropyl side chain carbon mineralization was proportional to soil organic content and oxygen content. Furthermore, the rate of atrazine biotransformation decreased as soil oxygen level decreased.

2.4.4.5. Surfactants

Surfactants belong to a group of chemicals that reduce surface tension and usually consist of an aliphatic or aromatic hydrophobic chain attached to a hydrophilic end group which may carry a positive or negative charge. There are three types of surfactants: anionic, cationic and nonionic, according to the charge they carry (Ware, 1993). Surfactants have been used for wetting, dispersing, emulsification, solubilization and bioenhancement in pesticide formulations. Mixed results have been achieved in studies on the effects of surfactants on the biodegradation of agrochemicals. The detrimental effects of surfactants on biodegradation have been reported by Mulkins-Phillips and Stewart (1974) and Aronstein et al. (1991). The mechanisms inhibiting bioremediation include nontoxic surfactants used as preferential substrate (Mulkins-Phillips and Stewart, 1974; Laha and Luthy, 1991), and toxicity of surfactants to microorganisms at high concentration (Aronstein et al., 1991). The mechanisms of enhanced bioremediation by the addition of surfactants included increased aqueous hydrocarbon solubility (Bury and Miller, 1993), reduction of interfacial tension (Oberbremer et al., 1990), increased bioavailability by surfactant solubilization of HOCs (Guerin and Jones, 1988; Tsomides et

al., 1995), reduced interfacial area limitation by the action of emulsification (Liu, 1980), etc. On the other hand, some researchers have failed to detect any effects of surfactants on bioremediation of agrochemicals (Foght et al., 1989).

Surfactants may also affect pesticide leaching from soil. Bayer (1967) investigated the effect of a number of anionic, cationic and nonionic surfactants on leaching of substituted urea herbicides (diuron, linuron, monuron and neburon) from soil. Cationic surfactants generally reduced the leaching of substituted urea herbicides. Aliquat 204[®] almost eliminated the leaching of diuron. Anionic and nonionic surfactants increased the leaching of diuron from a soil column (Bayer, 1967). The mechanism of reduced leaching by the addition of cationic surfactants was attributed to the greater absorption of herbicides onto the soil (Smith and Bayer, 1967). Generally, the effects of cationic surfactants on pesticide leaching may vary with herbicides, surfactants, dosage, soil type, and preleaching conditions (Foy, 1992). Cationic surfactants increased the soil adsorption of picloram while anionic surfactants competed with picloram for adsorption sites on soils at pH 5 (Gaynor and Volk, 1976). For example, two cationic surfactants Adogen[®] and Monazoline[®] had no effect on bromacil leaching (Tan and Singh, 1995). High concentration of nonionic surfactants (> 500 ppm Aqua Gro[®] and Soil Penetrant[®]) decreased the adsorption and increased the mobility of lindane and diuron, but even at such a high concentration, atrazine leaching was not affected by these nonionic surfactants (Huggenberger et al., 1973). In order to reduce pesticide leaching, some alternative herbicide formulations were studied. Fleming et al. (1992) investigated the impact of starch-encapsulation and polymer additives on the leaching of atrazine. Both of these materials reduced the leaching of atrazine with starch-encapsulation having a more profound effect than the polymer additives (Fleming et al., 1992).

Chapter 3. Materials and Methods

3.1. General chemicals and equipment

General chemicals and equipment used throughout the research conducted for this dissertation are described in this section. More detailed information regarding specific experiments may be found in Sections 3.2 to 3.6.

3.1.1. Chemicals

Acetone, hexane and anhydrous sodium sulfate were analytical grade (AR) purchased from Fisher Scientific Inc. (Atlanta, GA). Hexane and acetone were redistilled and checked for impurities using gas chromatography. Anhydrous sodium sulfate was dehydrated at 105 °C for 24 h before use. One hundred mL saturated sodium sulfate was diluted to 1000 mL with distilled water to make 10% sodium sulfate solution. Keeper's solution was 1% paraffin in hexane.

3.1.2. Organic materials

Sphagnum peat moss was purchased from Fison's Western (Mississauga, Ontario Canada). It was air dried and sieved (2 mm) for amendment. Ground corn meal was sieved by 40 mesh and provided additional microbe nutrients. Two types of fertilizers (plant food and nitrogen mixture) were used as supplemental microbe growth nutrients. Plant food (Miracle Grow[®]) had a composition of 15% N, 30% potash and 15% potassium; the nitrogen mixture had a composition of 17% nitrate and 17% ammonium. Soil microbial extract was prepared by mixing 250 g chlorpyrifos contaminated compost, which had been incubated for 2 years, with 1000 mL distilled water.

3.1.3. Apparatus and equipment

Sonication (solid state ultrasonicator: Model FS-28, Fisher Scientific, USA) was used to facilitate sample extraction. Sample solvent extract volumes were reduced by a Rotavapor (Büchi, Switzerland) with a temperature adjustable water bath. Erlenmeyer flasks (250 mL or 500 mL) were used for sample collection and transfer. Sample filtration was completed with a Buchner funnel connected to a vacuum air pump. A shaking table

(Eberbach Corporation) with a maximum speed of approximately 250 rpm was used for sample extraction and the leachability study. Analytical standards were measured with an analytical balance (Mettler AE 163, Lab Tech, Inc., Model AE-163). Two types of centrifuges were used: one had a maximum speed of 4000 rpm (2800 x g) used for large volume sample separation (Marathon Model 6K, Fisher Scientific); the other had a maximum speed of 12,000 x g used for small volume sample separation (Micro-Centrifuge, Fisher Scientific, Model 59A). Sample volume was concentrated using an Analytical Evaporator (Model N-EVAP, Organomation, Associates Inc., MA) under a stream of nitrogen. After extraction, purification and volume reduction, samples were stored in 15 mL calibrated centrifuge tubes and maintained at -15 °C.

3.1.4. Soil sampling and soil properties

Soil used in the experiments collected from a corn field near Blacksburg, Virginia, and described as a Groseclose type (clayey, mixed, mesic Typic Hapludult, Harris et al., 1980) and had no pesticide usage within most recent three years. Organic matter content was 3.1% (w/w), and moisture-holding capacity was 11.4% under 15 bar testing condition. Soil pH was measured as 6.0, and with a cation exchange capacity (CEC) of 6.05. Soil physical composition was characterized as a 61.4% silt, 30.3% sand and 8.4% clay. Soil analysis was performed by Soil Testing Laboratory (Crop, Soil and Environmental Science Department, Virginia Tech). Soil was air-dried at room temperature (25 ± 2 °C), then pulverized and passed through a 40 mesh screen before use.

3.2. Extractability and leachability of chlorpyrifos in bioremediated soils

The materials and methods of "Extractability and leachability of chlorpyrifos in a bioremediated soil" are provided in this section. The results and discussion of this experiment is reported on the Chapter 4.

3.2.1. Materials

Chlorpyrifos formulated as Dursban[®] 4E (44.1% a.i.) was used in the laboratory soil incubation study. Chlorpyrifos analytical standard (purity : 99%) was provided by DowElanco (Indianapolis, IN).

3.2.2. Methodology and treatments

The experiment was conducted in an incubation chamber which was maintained at 40 °C. One kg of Groseclose soil was placed into a 4-L glass jar. Chlorpyrifos as Dursban 4E (44.1% a.i.) formulation was applied directly to the soil with a 10 mL syringe equipped with a 22 gauge needle. The final estimated chlorpyrifos concentration was approximately 5000 µg g⁻¹. The soil in the jar was constantly rotated at ca. 93 rpm for 1 h during chlorpyrifos application to ensure uniform mixing. The whole mixing procedure was conducted under a hood in order to reduce the hazard of pesticide exposure. All soil treatments were inoculated with microbial extracts obtained from chlorpyrifos contaminated soil. Spiked chlorpyrifos soils were equilibrated 24 h before the addition of sphagnum peat moss, ground corn and vegetable oil and fertilizers which were conducted in the same way as the addition of chlorpyrifos formulation. The schedule for the amendments made to the soils is presented in Table 3-1. All treatments were replicated three times. They were placed into an incubator at 40 °C for 90 days. During the incubation period, the moisture level of these treatments was constantly monitored. Whenever necessary, the amount of water was added to make them ca. 60-80% field moisture capacity. Subsamples were removed and frozen until analysis at 0, 30, 60 and 90 day intervals. Chlorpyrifos concentration was determined by organic solvent extraction, and water leachability tests were also performed on the subsamples.

3.2.3. Gas-liquid chromatography (GLC) determination of chlorpyrifos in soil

3.2.3.1. Reagents and supplies:

A stock solution of an analytical chlorpyrifos standard 99% was dissolved in acetone at 1 mg mL⁻¹. It was diluted into 0.01, 0.02, 0.05, 0.1 and 0.2 µg mL⁻¹ series of working standards.

Table 3-1. Amendments made to the chlorpyrifos-contaminated soil¹

Soil treatment	Soil (g)	Oil (g)	GC (g)	PM (g)	Fertilizers ²		INC (mL)	Water (mL)
					1 (g)	2 (g)		
Control	1000	---	---	---	---	---	---	---
Oil-treated + INC + H ₂ O	1000	100	---	---	---	---	5	200
PM + INC + H ₂ O	1000	---	---	100	---	---	5	250
Oil-treated + PM + INC + H ₂ O	1000	100	---	100	---	---	5	250
Oil-treated + PM + FZ + INC + H ₂ O	1000	100	---	100	1	9	5	250
Oil-treated + PM + FZ + GC + H ₂ O	1000	100	10	100	1	9	---	250
Oil-treated + PM + FZ + INC + GC + H ₂ O	1000	100	10	100	1	9	5	250

1)¹Control soil contains approximately 5,000 µg g⁻¹ chlorpyrifos as Dursban[®] 4E with no oil and water added.

2) PM = sphagnum peat moss; INC = microbial extract (5 mL); GC = ground corn; FZ = fertilizers.

3)²Fertilizers: 1 = plant food(15% N + 30% potash + 15% potassium); 2 = nitrogen supplement (17% nitrate and 17% ammonium).

3.2.3.2. Sample extraction

Twenty grams of soil samples were weighed into 250 mL Erlenmeyer flasks. One hundred mL of acetone was added and allowed to stand for 2 h. It was then mixed for 4 h on a shaking table and subsequently sonicated for 20 min at room temperature. After extraction, the soil acetone mixture was filtered through a Buchner funnel into a 250 mL Erlenmeyer flask under vacuum pressure. The soil matrix was rinsed 3 times with 15 mL acetone. The soil acetone extract and the rinsates were collected and transferred to a 500 mL separatory funnel. These combined extracts were mixed with 200 mL 10% sodium sulfate solution and 100 mL hexane. The solvent mixture was mixed for 5 min, and then allowed to stand 30 min for the solvent separation (aqueous/hexane). The bottom aqueous layer was drained into a flask and discarded. This procedure was repeated twice with 100 mL 10% sodium sulfate solution and 100 mL hexane. Final upper solvent layers were combined and transferred to a 500 mL Erlenmeyer flask. Three drops of Keeper's solution were added to reduce chlorpyrifos loss during evaporation. Acetone-hexane mixture extracts were evaporated to near dryness on a rotary evaporator at 30 °C under reduced atmospheric pressure. The sample extracts were rinsed immediately with hexane 5 times and transferred to 15 mL calibrated tubes. The final sample volume was adjusted to 10 mL with hexane. Sample extracts in hexane were stored at 4 °C until GLC analysis.

Chlorpyrifos soil recovery experiments followed the same procedure as above. One mL 0.2 $\mu\text{g mL}^{-1}$ chlorpyrifos standard solution was added to 20 g Groseclose soil and 10 g peat moss. One mL hexane was added to soil and peat moss for blank controls. These samples were mixed thoroughly and equilibrated overnight. All of these treatments were replicated three times. The average chlorpyrifos recoveries from soil and peat moss were $94.2 \pm 2.8\%$ and $85.4 \pm 4.5\%$, respectively.

3.2.3.3. Chlorpyrifos GLC analysis

Chlorpyrifos GLC analysis was conducted using a Tracor 540 Gas Chromatograph equipped with an electron capture detector (ECD) (Tracor Instruments Inc. Austin, TX). A 1.8 m glass column (0.6 cm I.D.) packed with Supelcoport 100-120 mesh was used. The column

stationary phase was 1.5/1.95% SP2250/2401. GC instrument temperatures were as follows: column 210 °C, injector 235 °C and detector 300 °C. Carrier gas nitrogen flow was at 45 cc/min. Chlorpyrifos eluted at approximately 4.5 min under these conditions. The method detection limit (MDL) for chlorpyrifos was 0.5 µg L⁻¹ for soil and 2 µg L⁻¹ for peat moss based on two times the minimum detector response.

3.2.4. Chlorpyrifos leachability determined by the shaking procedure

Chlorpyrifos leachability from soil amended with organic materials was evaluated by the shaking procedure developed in our laboratory. A 5 gram soil sample was placed into 250 mL flask with an addition of 20 mL distilled water, and mixed at high speed (ca. 180 rpm) for 4 hour on a shaking table. The samples were then placed into a cold room (4 °C) for 12 h after which they were centrifuged at 2800 x g for 10 min. Five mL aliquots of the samples' supernatant were centrifuged again at 11,500 x g for 5 minutes to further remove any particulates from the leachate. The supernatant from the second centrifugation was transferred into a 250 mL separatory funnel for chlorpyrifos extraction. One hundred mL hexane was added to the supernatant and mixed vigorously for 5 min and allowed to stand for 30 min. The mixture was separated into two layers. The bottom aqueous layer was transferred to another 250 mL separatory funnel. Chlorpyrifos hexane extraction procedures were repeated twice with additional 50 mL of hexane. The combined hexane extracts were placed into a 250 mL Erlenmeyer flask and passed through a 5 cm anhydrous sodium sulfate column to remove additional moisture. Three drops of Keeper's solution were added to the dehydrated hexane extract. The extracts were then evaporated to near dryness on the rotating evaporator under reduced atmospheric pressure. The samples were then rinsed with hexane three to five times and the rinsings were quantitatively transferred to 15 mL calibrated centrifuge tube. The final volume was adjusted to 10 mL with hexane using Analytical Evaporator under a nitrogen stream. These sample extracts were stored at 4°C until GC analysis. Chlorpyrifos leachability was calculated by the amount of chlorpyrifos (ug) which leached with water, based on per gram of soil matrix.

3.3. Extractability and leachability of atrazine in bioremediated soils

The materials and methods of "Extractability and leachability of atrazine in bioremediated soils" are provided in this section. The results and discussion of this experiment are reported in Chapter 5.

3.3.1. Materials

Atrazine and one of its formulations (AAtrex[®] 4L) were supplied by Ciba-Geigy Corporation (Greensboro, NC).

3.3.2. Methodology and treatments

Atrazine soil bioremediation protocol was very similar to that of chlorpyrifos. The experiment was conducted in an incubation chamber which was maintained at 30 °C. One thousand grams of Groseclose soil was placed into a 4-L glass jar. Atrazine as AAtrex[®] 4L (40.4% a.i.) formulation was applied directly to the soil with a 10 mL syringe equipped with a 22 gauge needle. The final estimated atrazine concentration was approximately 5000 µg g⁻¹. The soil in the jar was constantly rotated at ca. 93 rpm for 1 h during atrazine application to ensure uniform mixing. The whole mixing procedure was conducted under a hood in order to reduce the hazard of pesticide exposure. All soil treatments were inoculated with microbial extracts obtained from atrazine treated soil. Spiked atrazine soils were equilibrated 24 h before the addition of sphagnum peat moss, ground corn, vegetable oil and fertilizers which were conducted in the same way as the addition of atrazine formulation. The schedule for the amendments made to the soils is presented in Table 3-2. All treatments were replicated three times. They were placed into an incubator at 30 °C for 90 days. During the incubation period, the moisture level of these treatments were constantly monitored. Whenever necessary, water was added to make them ca. 60-80% field moisture capacity. Subsamples were removed and frozen until analysis at 0, 30, 60 and 90 day intervals. Atrazine concentration was determined by organic solvent extraction, and water leachability tests were also performed on the subsamples.

Table 3-2. Amendments made to the atrazine-contaminated soil¹

Soil treatment	Soil (g)	Oil (g)	GC (g)	PM (g)	Fertilizers ²		INC (mL)	Water (mL)
					1 (g)	2 (g)		
Control + INC	1000	---	---	---	---	---	10	---
PM + INC + H ₂ O	1000	---	---	100	---	---	10	200
Oil-treated + INC + H ₂ O	1000	100	---	---	---	---	10	200
Oil-treated + PM + INC + H ₂ O	1000	100	---	100	---	---	10	200
Oil-treated + PM + FZ + INC + H ₂ O	1000	100	---	100	1	9	10	200
Oil-treated + PM + FZ + GC + INC + H ₂ O	1000	100	10	100	1	9	10	200

1)¹Control soil sample containing approximately 5,000 µg g⁻¹ atrazine as AAtrex[®] 4L with no oil and water added.

2) PM = sphagnum peat moss; INC = microbial extract (5 mL); GC = ground corn; FZ = fertilizers.

3)²Fertilizers: 1 = plant food (15% N + 30% potash + 15% potassium); 2 = nitrogen supplement (17% nitrate and 17% ammonium).

3.3.3. Gas-liquid chromatography (GLC) determination of atrazine in soil

3.3.3.1. Reagents and supplies

Atrazine was quantified with Tracor 540 Gas Chromatograph (Tracor Instrument Austin, Inc.). A solid state/ultrasonic (Model FS-28, Fisher Scientific, USA) was used for atrazine extraction. Solvent extract was concentrated by Rotavapor[®] (Büchi, Switzerland) under reduced pressure. Analytical atrazine which had a purity of 99.7% was dissolved in acetone at concentration of 1 mg mL⁻¹ as a stock solution. Subsequently, it was diluted to 0.2, 0.5 and 1 µg mL⁻¹ series of working standards. Preliminary experiments indicated, atrazine has a linear response range over 0.2 µg mL⁻¹ to 2 µg mL⁻¹ with a regression coefficient index of 0.998.

3.3.3.2. Atrazine GLC analysis

Atrazine GC analysis was conducted on Tracor 540 Gas Chromatograph (Tracor Instruments Austin, Inc.) equipped with an electron capture detector (ECD) and a 1.8 m glass column (0.6 cm I.D.) packed with 1.5/1.95 OV-17/OV-210 + 100-120 mesh Chromosorb WHP. The GC instrument conditions were: oven temperature = 185°C, injector temperature = 260 °C, and detector temperature = 350 °C. Nitrogen was used as carrier gas with a flow rate of 45 cc/min. Under these chromatographic conditions, atrazine retention time was approximately 7.5 min. The minimum detection limit (MDL) for atrazine was 5 µg L⁻¹ for soil and 10 µg L⁻¹ for peat moss extracts based on two times the minimum detector response.

3.3.3.3. Sample extraction

Twenty gram soil sample was placed into a 250 mL Erlenmeyer flask soaked with 100 mL acetone: water (1:1, v/v) for at least 3 h before extraction. The sample was then agitated at a speed of 90 rpm on a shaking table for 1 hour. Afterwards, the soil sample was sonicated for 30 min at room temperature. The soil acetone mixture was filtered through a Büchner funnel into a 250 mL Erlenmeyer flask under vacuum. The soil matrix was rinsed three times with 15 mL acetone. Acetone extract and its rinsings were combined and transferred into a 250 mL separatory funnel. One hundred mL hexane

was added to the combined extracts and mixed vigorously for 5 min. The addition of a small amount of anhydrous sodium sulfate was required whenever emulsions occurred. After 30 min the sample was separated into two layers. The bottom aqueous layer was transferred to another 250 mL separatory funnel for additional extractions. The hexane extraction procedure was repeated twice with 75 mL hexane. The hexane layers were combined and passed through a 5 cm anhydrous sodium sulfate column into a 250 mL Erlenmeyer flask. The bottom layer was discarded. Three drops of Keeper's solution were added to the hexane atrazine extract which was subsequently evaporated to dryness by using a rotating evaporator at 30 °C (water bath). The concentrated sample extracts were quantitatively transferred to 15 mL graduated centrifuge tubes with three to five time hexane rinsings. The final volume was adjusted to 10 mL with hexane using Analytical Evaporator under a nitrogen stream. The atrazine sample extracts were stored at -15 °C until GC analysis.

Atrazine soil recovery experiments followed the same procedure outlined above. Twenty grams Groseclose soil and 10 g peat moss were each spiked with one mL 1.0 µg mL⁻¹ atrazine standard solution. Controls were prepared by adding 1 mL hexane instead of atrazine standards for the recovery study. These samples were mixed thoroughly and equilibrated overnight. All treatments were replicated three times. Average recoveries for soil and peat moss were 93.6 ± 2.3% and 85.5 ± 3.2%, respectively.

3.3.4. Atrazine leachability determined by the shaking procedure

Atrazine water leachability from soil amended with organic was evaluated by a shaking procedure developed in our laboratory. Five gram soil samples were placed into 250 mL flasks with an addition of 20 mL distilled water, and mixed at high speed (ca. 180 rpm) for 4 h on a shaking table. The samples were then placed into cool room (4 °C) for 12 h after which they were centrifuged at 2800 x g for 10 min. Five milliliter aliquots of the samples' supernatant were centrifuged again at 11,500 x g for 5 minutes to further remove any particulate in the leachates. The supernatant from the second centrifugation was transferred into a 250 mL separatory funnel for atrazine extraction.

One hundred mL hexane was added to the supernatant and mixed vigorously for 5 min. and was allowed to stand for 30 min. The mixture was separated into two layers, the bottom aqueous layer was transferred to another 250 mL separatory funnel. The atrazine hexane extraction procedure was repeated twice with additional 50 mL volumes of hexane. The combined hexane extracts were placed into a 250 mL Erlenmeyer flask and passed through a 5 cm anhydrous sodium sulfate column to remove additional moisture. Three drops of Keeper's solution were added to the dehydrated hexane extract. The extracts were then evaporated to near dryness on the rotating evaporator under reduced atmospheric pressure. The samples were then rinsed with hexane three to five times and the rinsings were quantitatively transferred to 15 mL calibrated centrifuge tubes. The final volume was adjusted to 10 mL with hexane. These sample extracts were stored at 4 °C until GC analysis. Atrazine leachability was calculated by the amount of atrazine (ug) which leached with water, based on per gram of soil matrix.

3.3.5. Atrazine leachability determination using the Toxicity Characteristic Leaching Procedure (TCLP)

3.3.5.1. Equipment and materials

The TCLP rotating extractor (Associated Design & Manufacturing Company, Alexandria, Virginia) was a special shaking and filtration device used for testing the leachability of substances. An Acumet[®] pH meter (Model 805 MP, Allied Fisher Scientific) was used for measuring soil pH. Extraction fluid #1 and #2 were extracting fluids used for TCLP. They had a pH within a range of 4.93 ± 0.05 and 2.88 ± 0.05 , respectively. Extraction fluid #1 was made from 11.4 mL glacial acetic acid and 128.6 mL 1 N NaOH in 1000 mL deionized H₂O. Extraction fluid #2 was an acetic acid solution, with 5.7 mL glacial acetic acid in 1000 mL deionized H₂O.

3.3.5.2. Sample preparation

Soil sample pH testing was used to determine which extractor fluid was appropriate for the testing procedure. Five grams of soil were weighed into a 250 mL beaker and 96.5 mL deionized water was added. Soil and water mixtures were mixed

thoroughly. The pH of this mixture was determined; if the pH value was less than 5, extractor fluid #1 was used for TCLP testing; if the sample pH was greater than 5.0, 3.5 mL 1N HCl was added, and mixed thoroughly at 50 °C for 10 min. After the mixture was allowed to cool down to room temperature, the mixture pH was measured again. If it was still greater than 5.0, the extraction fluid #2 was used for leaching tests, otherwise extraction fluid #1 was used.

3.3.5.3. Leachability testing using TCLP

Soil sample pH measurements were made using the aforementioned procedure, and a decision as to which extraction fluid should be used for TCLP was made. For most atrazine soil samples, extraction fluid #1 was used for leaching tests. A twenty-five gram soil sample was weighed out into a 4000 mL extractor. Five hundred mL of the appropriate extraction fluid was added to the extractor. The samples were shaken for 18-20 h at the speed of ca 120 rpm at room temperature (24 ± 2 °C). At the end of the extraction, samples were filtered under positive pressure through a 0.6-0.8 um glass fiber filter using the TCLP filtration device.

The TCLP extractor leachates were transferred to 1000 mL separatory funnels. They were extracted with 200 mL of hexane. The hexane-leachate mixtures were mixed vigorously for 5 minutes and allowed to stand for 30 min. Afterwards, the bottom aqueous layer was transferred to another separatory funnel and reextracted twice with 100 mL hexane. The hexane extracts were combined in a 500 mL Erlenmeyer flask and passed through a 5 cm column of anhydrous sodium sulfate. With the addition of 3 drops of Keeper's solution, atrazine leaching samples were evaporated down to near dryness under negative pressure. Hexane was used for rinsing samples into 15 mL graduated centrifuge tubes. The final calibrated volumes were 10 mL in hexane. The samples were ready for GC analysis or stored at -14 °C until analysis.

3.4. Effects of formulation on the extractability and leachability of chlorpyrifos from contaminated soil

The materials and methods of "Effects of formulation on the extractability and leachability of chlorpyrifos and atrazine from contaminated soil" are described in this section. The results and discussion are provided in Chapter 6.

3.4.1. Methodology

5.052 g Technical grade chlorpyrifos (99%) was weighed and dissolved into 10 mL acetone. Ten grams Groseclose surface soil (7.8% moisture level) was placed into a 20 mL scintillation vials and spiked with 100 ul 1 g mL⁻¹ chlorpyrifos acetone solution. The final chlorpyrifos spiked concentration in soil was 5 mg g⁻¹ or 5000 ppm. The experiment was sampled at 0, 3th, 7th, 28th and 56th day. Each treatment was replicated eight times; three replicates were used for the analysis of solvent extractable chlorpyrifos; another three replicates were tested for the leachability by a shaking procedure and two replicates were held in reserve.

In the case of formulated chlorpyrifos treatment, Dursban[®] 4E (44.1% a.i.) was used for the study. Ten grams Groseclose soil was placed into a 20 mL scintillation vial and spiked with 115 ul Dursban[®] 4E. The chlorpyrifos emulsion was constantly stirred by magnetic stirrer while it was pipetted into the soil sample. The calculated spiked chlorpyrifos concentration was 5 mg g⁻¹ or 5000 ppm. Soils were sampled at 0, 3, 7, 28 and 56 day intervals. Each time interval was replicated eight times; three replicates were used for the analysis of solvent extractable chlorpyrifos; three replicates were tested for the leachability by the shaking procedure and two replicates were held in reserve.

3.4.2. Chlorpyrifos extraction and GLC analysis

Ten gram Groseclose soil was used for chlorpyrifos extraction. Please refer to Sections 3.2.3.2 and 3.2.3.3. for details of chlorpyrifos extraction and analysis procedure.

3.4.3. Chlorpyrifos leachability study

A modified shaking table leachability test method was used for formulated and technical chlorpyrifos treated samples (Materials and Methods 3.2.3.4.). Chlorpyrifos leachability was determined with 10 g soil mixed with 40 mL distilled water.

3.5. Effects of formulation on the extractability and leachability of atrazine from contaminated soil

The materials and methods of "Effects of formulation on the extractability and leachability of chlorpyrifos and atrazine from contaminated soil" are described in this section. The results and discussion are provided in Chapter 6.

3.5.1. Methodology

5.052 g Technical grade atrazine (99%) was weighed and dissolved into 10 mL of acetone. Ten grams Groseclose surface soil was placed into a 20 mL scintillation vial and spiked with 100 μ l 1 g mL^{-1} atrazine acetone solution. Final atrazine spiked concentration in soil was 5 mg g^{-1} or 5000 ppm. The experiment was sampled at 0, 7th, 28th and 56th day. Each treatment was replicated eight times; three replicates were used for the analysis of solvent extractable atrazine; another three replicates were tested for the leachability by a shaking procedure and two replicates were held in reserve.

In the case of formulated chlorpyrifos treatment, AAtrex[®] 4L (40.4% a.i.) was used for the study. Ten grams Groseclose soil was placed into a 20 mL scintillation vial and spiked by 115 μ l AAtrex[®] 4L. The atrazine emulsion was constantly stirred by magnet stirrer while it was pipetted into the soil sample. The calculated spiked atrazine concentration was 5 mg g^{-1} or 5000 ppm. Soils were sampled at 0, 7, 28 and 56 day intervals. Each time interval was replicated eight times; three replicates were used for the analysis of solvent extractable chlorpyrifos; three replicates were tested for the leachability by the shaking procedure and two replicates were held in reserve.

3.5.2. Atrazine extraction and GC analysis

Ten gram Groseclose soil was used for atrazine extraction. Please refer to Section 3.3.3.2. and 3.3.3.3. for details.

3.5.3. Atrazine leachability study

A modified shaking table leachability test method was used for formulated and technical atrazine treated samples (Materials and Methods 3.3.4.). Atrazine leachability was determined with 10 g soil mixed in 40 mL distilled water.

3.6. [U-ring-¹⁴C] atrazine mineralization and incorporation in a bioremediation soil

The materials and methods of "[U-ring-¹⁴C] atrazine mineralization and incorporation in a bioremediated soil" are described in this section. The results and discussion of this experiment are provided in Chapter 7.

3.6.1. Chemicals

Analytical reference standards of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; purity: 98.8%), deethylatrazine (DEA, 2-chloro-4-[amino]-6-[isopropylamino]-1,3,5-triazine; purity: 99%), deisopropylatrazine (DIA, 2-chloro-4-[amino]-1,3,5-triazine; purity: 98%), dealkylatrazine (DAA, 2-chloro-4,6-[diamino]-1,3,5-triazine; purity: 90%), hydroxyatrazine (HYA, 2-hydroxy-4-[ethylamino]-6-[isopropylamino]-1,3,5-triazine; purity: 97%), OH-deethylatrazine (4-amino-6-[(1-methylethyl)amino]-1,3,5-triazine-2(1H)-one; purity: 97%), and OH-deisopropylatrazine (2-amino-4-ethylamino-6-hydroxy-S-triazine; purity: 95%) were provided by Ciba-Geigy Corporation (Greensboro, NC). Two hundred mCi [U-ring-¹⁴C] atrazine with a specific activity 14.6 $\mu\text{Ci mg}^{-1}$ was also contributed by Ciba-Geigy Corporation. Atrazine formulation AAtrex[®] 4L (40.4%, a.i.) was used for spiking soil.

A primary radiolabeled atrazine stock solution containing 200 mCi [U-ring-¹⁴C] atrazine (specific activity: 14.6 $\mu\text{Ci mg}^{-1}$) was prepared by dissolving it in 1000 μl acetone. Three hundred twenty microliters of the primary stock solution were mixed

thoroughly with 2400 μL AAtrex[®] 4L (40.4% a.i.) (magnetic stirrer, 200-300 rpm). The resulting secondary stock solution was then diluted by the addition of 45 mL distilled water (atrazine tertiary stock solution).

The solvents used for the separation of atrazine and its metabolites included analytical grade acetone, chloroform, benzene, propanol and butanol. Ethanolamine (AR) and 5 N KOH solution were used for trapping evolved $^{14}\text{CO}_2$.

3.6.2. Equipment

A Beckman LS 6500 Multi-Purpose Scintillation counter was used for radiolabeled sample detection. Sorvall Omni Mixer (Ivan Sorvall, Inc.) was used for solvent and alkaline extraction. Bound atrazine in soil and humic acid was combusted using a Biological Material Oxidizer (Model OX-500, R.J. Harvey Instrument Corporation). A RC-5 Superspeed Refrigerated Centrifuge (DuPont Instrument Sorvall) was used for the separation of the humic acid fraction. Matrix ethylacetate extraction separation was conducted with a non-refrigerated centrifuge (Marathon Model 6K, Fisher Scientific) with a maximum speed of 2800 x g.

3.6.3. Experimental setup and treatments

The radiolabeled experiment was conducted using a modified benchtop bioreactor system (Figure 41, in Judge, 1996) that was originally described by Petruska et al. (1985). Fifteen grams of Groseclose top soil was placed into a 100 mL incubator. The atrazine tertiary stock solution was mixed continuously while three mL of the tertiary stock solution were pipetted onto the soil contained in the remediation chamber, and the spiked remediated soil was then mixed with a stainless steel spatula for 5 min. The atrazine contaminated soil was equilibrated overnight before the amendments were added. Amendments consisted of 1.5 g peat moss, 1.5 g ground corn meal, and 22 mg fertilizer (Miracle Grow[®]) were added and mixed into the soil matrix. The final atrazine concentrations in unamended and amended treatments were approximately $5000 \mu\text{g g}^{-1}$ based on the total amount of (both radioactive and stable) atrazine in the treatments. A

low soil matrix pH was detected after the amendments of organic matrix, therefore 0.5 g of lime (in the form of $\text{Ca}(\text{OH})_2$) was added into each treatment in order to adjust the pH of the soil matrix (pH 6.5 after adjustment). Each treatment was adjusted so that the soil moisture level was approximately 80% of soil moisture holding capacity. Soil microbial extract (2 mL) prepared from atrazine compost/remediation experimental matrices from previous composting studies was also added.

Moisture-saturated air was pumped through the system 3 min per hour at the rate of 200 mL per minute. The ^{14}C -volatile organic materials evaporated from the soil matrix were trapped by polyurethane foams (PUFs) which were placed in an U-tube that received air flowing from the incubation chamber. The polyurethane foam plugs (PUFs) were extracted twice with cyclohexane and air-dried to remove impurities prior to use. $^{14}\text{CO}_2$ released from atrazine mineralization was subsequently trapped into KOH (in the form of $\text{K}_2^{14}\text{CO}_3$) as air from the incubation chamber bubbled through 3 mL 5 M KOH solution in a 50 mL buret. Polyurethane foam plugs were replaced biweekly and KOH was replaced every 4 days. PUFs were subsequently extracted and analyzed for radioactivity in the volatiles. KOH solution which contained trapped $^{14}\text{CO}_2$ was analyzed for radioactivity. At the end of incubation, the contaminated soil was removed from each of the incubation chambers and subsequently was extracted by organic solvents (ethylacetate, methylene chloride) and 0.1 NaOH solution, and fractionated into various portions. The radioactivity associated with these fractions was characterized by HPTLC and analyzed by TLC or HPTLC-LSC. A schematic diagram shows the complete fractionation of the radioactivity in atrazine contaminated soil (Figure 3-1).

The experiment was set up with two treatments: unamended and amended, and two time periods: 8 weeks and 16 weeks. Each treatment was replicated three times. In addition to this, three unamended treatments (controls) were frozen at -17°C initially and stored until extraction. Ground corn meal (0.75 g, 2 mm mesh) was added one additional time after 8 weeks incubation for 16 weeks amended treatments.

3.6.4. Determination of ^{14}C -volatile organic materials and $^{14}\text{CO}_2$

^{14}C -volatile organic materials released from the incubated soil matrix and trapped into polyurethane foam plugs were extracted by sonication twice in 30 mL methanol for 30 min (Figure 3-1). These extracts were combined and concentrated down to 5 mL by evaporation through an air stream. The total radioactivity in a 1 mL aliquot of the concentrated methanol extract was mixed with 8 mL Scintiverse BD Universal LSC cocktail, and the mixed sample was analyzed by liquid scintillation counting (LSC).

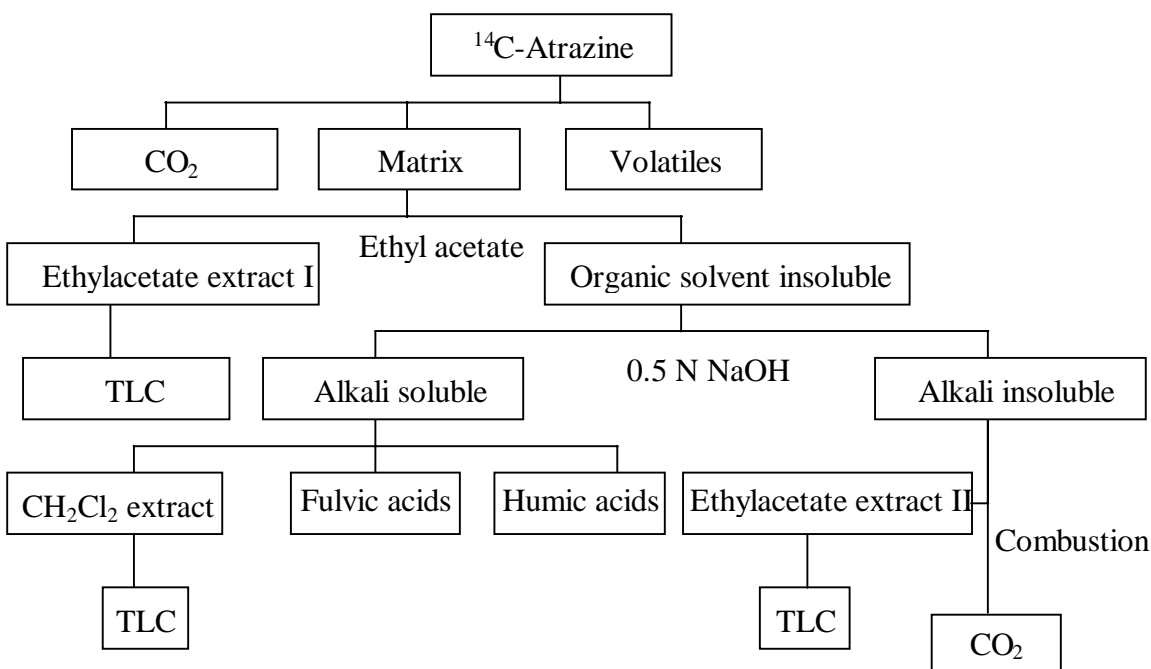


Figure 3-1. Flow chart of ^{14}C -atrazine sample extraction and analysis

$^{14}\text{CO}_2$ trapped in the 5 N KOH as $\text{K}_2^{14}\text{CO}_3$ was distilled into 1 mL 80% ethanolamine when 0.5 mL $\text{K}_2^{14}\text{CO}_3$ was reacted with 0.5 mL 25 N H_2SO_4 following the procedures described in Petruska et al. (1985). Eight mL of Scintiverse BD Universal L.S.C. cocktail was added to the $^{14}\text{CO}_2$ /ethanolamine solution and counted in a Beckman 6500 Multi-Purpose Scintillation Counter.

3.6.5. Matrix solvent and alkaline extraction

Amended and unamended soil matrices from the 8 and 16 week incubation intervals were removed from incubation chambers and placed into a 50 mL Sorvall

stainless steel homogenizer (Figure 3-1). Forty mL of ethylacetate was added and the mixture was allowed to stand for 2 hours after which it was homogenized for 3 min using a Sorvall Omni-Mixer at the medium speed setting. The sample was then sonicated for 30 min at room temperature (22 ± 1 °C). Solids were separated from the ethylacetate extract by centrifugation ($2800 \times g$, 25 min) (Marathon 6K, Fisher Scientific). This extraction procedure was repeated five more times. The 6 ethylacetate extracts were combined and concentrated to 20 mL by evaporation under a hood at room temperature. One mL aliquot was added to 8 mL Scintiverse BD Universal LSC fluid and counted.

The remaining solid soil matrices were extracted with 35 mL 0.1 N NaOH for 3 min using Sorvall Omni-Mixer, and subsequently sonicated for 20 min (Figure 3-1). Both procedures were conducted under a N_2 atmosphere. The mixture was transferred to a 50 mL Teflon centrifugation tube and centrifuged for 30 min at the speed of $11,500 \times g$ using RC-5 Superspeed Refrigerated Centrifuge (DuPont Instrument Sorvall). The supernatant was transferred to a 250 mL beaker and the solids were extracted five more times. The combined volumes from the six alkaline extractions were measured, and one mL aliquot was added to 8 mL Scintiverse BD Universal LSC fluid and counted. A forty milliliter aliquot was taken for the methylene chloride extraction, the remaining (approximately 150 mL) was used for the humic acid and fulvic acid studies. Humic acids were precipitated from the combined supernatant by adjusting the pH to 2 using 25 N H_2SO_4 . The humic acid precipitate was separated from fulvic acid solution by centrifugation ($11,500 \times g$, 30 min). The volume of fulvic acid solution was measured, and one milliliter aliquot was added to 8 mL Scintiverse BD Universal LSC fluid and counted on the LSC.

Forty milliliter aliquot of approximately 200 mL matrix alkali extracts was transferred to a 250 mL separatory funnel. One hundred milliliters of methylene chloride were added, mixed for 5 min, and allowed to stand for 30 min for complete phase separation. The methylene chloride layer was drained into a 250 mL beaker. The fulvic

acid solution was extracted two more times with 50 mL methylene chloride. The methylene chloride extracts were combined and evaporated to 10 mL in 15 mL graduated centrifuge tubes under a hood at room temperature. One milliliter aliquot of the concentrated methylene chloride extract was added to 8 mL Scintiverse BD Universal LSC fluid and counted using a Beckman 3150 LSC.

The NaOH extracted solid matrix was then reextracted with 30 mL ethylacetate three times (2 h soaking, 30 min sonication and 2800 x g centrifugation). The three ethylacetate extracts were combined and concentrated to 10 mL through evaporation under a hood at room temperature. One mL aliquot was removed and combined with 8 mL Scintiverse BD Universal LSC fluid and counted.

3.6.6. Combustion of alkali insoluble matrices and humic acids

Solid soil matrices were air dried and weighed after two separate ethylacetate extractions and one alkali extraction. Two replicates of approximately 1 g subsamples were combusted using a Harvey Biological Oxidizer. These samples were combusted at 900 °C for 3 min at the oxygen flow rate of 350 cc min⁻¹. Recoveries of ¹⁴C-spiked blank soil control and cellulose after combustion were 98.7 ± 0.5% and 99.4 ± 0.4%, respectively; based on five replicates.

The radioactivity in humic acid precipitates were determined by the same method as that for solid soil matrices. Humic acid was weighed prior to combustion. It was combusted in Biological Material Oxidizer for 3 min. and ¹⁴CO₂ released from the combustion was collected in 8 mL specialized ¹⁴C cocktail (R.J. Harvey Instruments Corp, USA). The amount of trapped radioactivity was quantified by LSC.

3.6.7. Thin Layer Chromatography (TLC)

Thin Layer Chromatography separations of atrazine and its metabolites were performed for ethylacetate extract I, ethylacetate extract II (post NaOH extraction) and methylene chloride extracts on both smaller HPTLC plates (10 cm x 10 cm, F₂₅₄) and

larger 500 μm preparative TLC Silica Gel GF plates (20 cm x 20 cm, F₂₅₄). The HPTLC plates (Merck) were activated at 105 °C for 2 h before use. The large TLC plates were prepared in our laboratory. Seventy-five grams silica gel (type GF/Fluorescent 10-40 μm , Sigma Chemical Co., USA) were mixed with 160 mL distilled water in a Waring electric blender. The mixture was immediately transferred to a TLC plater spreader (Desaga). The silica gel was then uniformly spread over 3 TLC plates (1.25 mm thickness). The plates were then air-dried for 4-5 hours followed by an activation at 110 °C for 12 h and stored in a desiccator prior to use. The TLC separation method of atrazine and its six metabolites was adapted from Judge et al. (1993). Atrazine, Cl-deethylatrazine and Cl-deisopropylatrazine standards were solubilized in acetone (1 mg mL⁻¹) and used as Mixed Standard A. Hydroxyatrazine, OH-deethylatrazine, OH-deisopropylatrazine and Cl-diaminoatrazine standards were found to be quite difficult to solubilize in most common organic solvent systems. It was determined that they were all soluble in acetonitrile:acetic acid (7:3, v/v) at concentrations of 1 mg mL⁻¹ and used as Mixed Standard B. Separation of atrazine and its other six metabolites was achieved by two, one-dimensional solvent systems. The first solvent system was chloroform:acetone (7:3, v/v), and the second solvent system consisted of benzene:propanol:butanol:glacial acetic acid:distilled water (2:2:1:1:1, v/v). The samples were developed in one-dimension, the first solvent front was allowed to migrate to 1 cm below the top of the plate. Developed plates were air-dried and examined under $\lambda=254$ nm UV light. At this time, atrazine, Cl-deethylatrazine and Cl-deisopropylatrazine were well separated. The visualized spots under UV on the plate which had the lowest R_f (Cl-deisopropyl atrazine) were marked at edge of the plate, and the second solvent system was used to separate the remaining metabolites at the origin. This solvent system was allowed to migrate to the area just beneath the Cl-deisopropylatrazine. After the development in two different polar solvent systems, atrazine and its six metabolites were well separated in TLC plates. They all had strong responses at $\lambda=254$ nm UV light. Their R_f-values were atrazine (0.89), Cl-deethylatrazine (0.70), Cl-deisopropylatrazine (0.63), OH-atrazine (0.29), OH-deethylatrazine (0.24), OH-deisopropyl atrazine (0.20) and Cl-diaminoatrazine (0.42).

The samples of the second ethylacetate extracts and methylene chloride extracts were evaporated to dryness, then redissolved in 50 μ l ethylacetate before they were spotted on HPTLC/preparative TLC plates using capillary pipettes (MicroCap). All samples were separated and examined both on the HPTLC plates and the preparative plates. The samples were spotted 1 cm apart on the HPTLC plates and 2 cm on the preparative TLC plates. One μ L aliquot of ethylacetate I, two μ L aliquots of ethylacetate II and methylene chloride extract were spotted onto HPTLC plates. The preparative TLC plates were loaded with 10 μ L ethylacetate extract I and 15 μ L of ethylacetate II and methylene chloride extract, respectively. The two mixed standard solutions were also included in each series of plates: Mixed Standard A (1 μ L for the HPTLC plates, 10 μ L for the preparative TLC plates) and Mixed Standard B (1 μ L for the HPTLC plates, 10 μ L for the preparative TLC plates), respectively. Each sample was replicated one time. Atrazine, deethylatrazine and deisopropylatrazine were analyzed on the samples covered by Mixed Standard A. Hydroxyatrazine, OH-deethylatrazine, OH-deisopropylatrazine and Cl-diaminoatrazine were analyzed from samples covered by Mixed Standard B.

3.6.8. Autoradiography of TLC plates

Developed HPTLC and preparative TLC plates were air-dried and wrapped with GLAD[®] Cling Wrap and covered with X-ray film (Kodak[®]) that was cut to the dimensions of the HPTLC/preparative TLC plates for autoradiography. A piece of black construction paper was then placed over the X-ray film in order to ensure the exclusion of ambient light exposure. This process together with other light-sensitive film processing was conducted in a dark room facility in the Entomology Department. Four of HPTLC plates with X-ray film at the top of plates were stacked together, wrapped in aluminum foil and kept in the darkness for 5 weeks. Three of the preparative plates with X-ray film as a group were stacked together and kept in the darkness for 3 weeks. After exposure, the X-ray film were developed (Kodak GBX developing solution 10 min, stop bath 1 min and fixing solution 30 min). Developed X-ray films were used to make negative contact prints using a Simmon Omega Enlarger (F-22, 45 seconds) as a light

source. Kodak F-3 bromide print paper was used for this purpose. The prints of HPTLC/preparative TLC visualized under 254 nm were also prepared for the purpose of matching detectable spots with those detection on X-ray films using Kodak T-Max 100 film. The two prints (UV-detected and X-ray detected) were mounted horizontally on to a poster board using a Technical Dry Mount Press (Englewood, N.J., 240 °C). The position of spots with radioactive materials on the HPTLC/preparative TLC plates were identified by visual inspection.

3.6.9. Determination of radioactivities in the developed TLC plates

The radioactive spots of the preparative TLC plates were marked with a pencil using information from their negative films of X-ray and UV exposure (254 nm). Silica gel containing the corresponding samples in each radioactive zone were carefully scraped, collected and placed into 20 mL scintillation vials. One mL of ethylacetate was added to solubilize the samples. After mixing by a Vortex mixer, eight mL Scintiverse BD Universal LSC fluid was added and counted using a Beckman LS 6500 Multi-Purpose Scintillation Counter. The samples matched with R_f -values of Mixed Standard A were analyzed for atrazine, Cl-deethylatrazine and Cl-deisopropylatrazine; the samples matched with R_f -values of Mixed Standard B were analyzed for hydroxyatrazine, OH-deethylatrazine, OH-deisopropylatrazine and Cl-diaminoatrazine.

Chapter 4. Extractability and leachability of chlorpyrifos in bioremediated soils

4.1 Introduction

The chlorpyrifos bioremediation study was designed to achieve two objectives relating to the use of nutrients and lignocellulosic organic amendments. They were: 1) to determine the influence of organic and nutrient amendments on the extractability rates of formulated chlorpyrifos in contaminated soil and, 2) to determine the influence of organic and nutrient amendments on the leachability rates of formulated chlorpyrifos in contaminated soil. This study was conducted in an incubation chamber which was maintained at 40 °C for 90 days. Soil contaminated with Dursban 4E (44.4% a.i.) was amended with organic materials, a microbial extract and nutrients (Materials and Methods 3.2). The extractable chlorpyrifos concentration was determined by GC as an indicator of chlorpyrifos loss (volatilization, degradation and incorporation) at 30 day intervals. The leachable chlorpyrifos concentration was measured after soil samples had been shaken vigorously with distilled water for 4 h. The estimated measurement of leaching was based on the amount of chlorpyrifos leached (μg) per gram of soil (Materials and Methods 3.2.3.4).

4.2. Degradation and incorporation of chlorpyrifos in bioremediated soils

Information presented in Table 4-1 was based on the combusted weight of the samples. This was done to allow for comparisons of the samples which had different treatments of organic materials, microbial nutrients (C, N), etc. Since combustion removes organic materials, a better comparison of chlorpyrifos-contaminated soils can be achieved based on the combusted weight of the various treatments.

Solvent extractable chlorpyrifos from contaminated soils was reduced by organic-based material amendment and biostimulation over the 90 day incubation period (Table 4-1). The initial concentrations for all of the treatments were similar, ranging from 4912

Table 4-1. Extractable chlorpyrifos after contaminated soil was amended with sorbents, nutrients and incubated at 40 °C for 90 days

Soil treatment ¹	Soil concentration $\mu\text{g g}^{-1}$; (% of original) ^{2,3}			
	Initial	30 days	60 days	90 days
Control	5239 \pm 40(100)Aa	4618 \pm 271(88)Aa	3279 \pm 58(63)Ab	2647 \pm 82(51)Ab
Oil-treated + INC + H ₂ O	4982 \pm 8.1(100)Aa	3259 \pm 188(65)Bb	2776 \pm 188(56)ABbc	2073 \pm 242(42)Bc
PM + INC + H ₂ O	5162 \pm 109(100)Aa	2966 \pm 388(57)Bb	2347 \pm 269(45)Bbc	1768 \pm 129(34)BCc
Oil-treated + PM + INC + H ₂ O	5186 \pm 113(100)Aa	2596 \pm 129(50)Bb	2385 \pm 158(45)ABb	1411 \pm 43(27)Cc
Oil-treated + PM + FZ + INC + H ₂ O	4912 \pm 69(100)Aa	2844 \pm 161(58)Bb	2155 \pm 275(44)Bbc	1520 \pm 69(31)BCc
Oil-treated + PM + FZ + GC + H ₂ O	5239 \pm 108(100)Aa	3007 \pm 297(57)Bb	2330 \pm 195(44)Bb	1465 \pm 31(28)Cc
Oil-treated + PM + FZ + INC + GC + H ₂ O	5226 \pm 70(100)Aa	3037 \pm 336(58)Bb	2081 \pm 85(40)Bc	1439 \pm 80(28)Cc

¹Control soil sample (original soil with no oil and water added); PM = sphagnum peat moss; INC = microbial extract (5 ml); GC = ground corn; FZ = fertilizer. Values are expressed as the mean \pm SE of 3 replicated samples, based on combusted weight.

²Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

³Lower case letters indicate comparison within treatments between dates. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

$\mu\text{g g}^{-1}$ (Oil-treated + PM + FZ + INC + H₂O) to 5239 $\mu\text{g g}^{-1}$ (Control and Oil-treated + PM + FZ + GC + H₂O). After 30 days of incubation, the solvent extractable chlorpyrifos concentrations in all treatments were significantly lower than their corresponding original levels ranging from 2596 $\mu\text{g g}^{-1}$ (Oil-treated + PM + INC + H₂O) to 3259 $\mu\text{g g}^{-1}$ (Oil-treated + INC + H₂O). Approximately 88% (4618 $\mu\text{g g}^{-1}$) of the all of the original solvent extractable chlorpyrifos was detected in the 30 day control samples. The Tukeys multiple range test indicated that the solvent extractable chlorpyrifos in the six treatments (2596 to 3259 $\mu\text{g g}^{-1}$) was significantly lower than that of the control (4618 $\mu\text{g g}^{-1}$). There were no significant differences among the six different amended treatments.

In the next 30 day incubation period (60 day interval, Table 4-1), the reduction of solvent extractable chlorpyrifos within treatments was only significantly different for the Oil-treated + PM + FZ + INC + GC + H₂O (2081 $\mu\text{g g}^{-1}$). There was a decline of chlorpyrifos in the control samples. The data indicates that nearly 25% of the solvent extractable chlorpyrifos in the control samples disappeared during this period. As a result, there were no statistically significant differences between the control samples and two other treatments: Oil-treated + INC + H₂O (2776 $\mu\text{g g}^{-1}$) and Oil-treated + PM + INC + H₂O (2347 $\mu\text{g g}^{-1}$). All other treatments had significantly lower levels of solvent extractable chlorpyrifos concentrations than those of the controls.

The solvent extractable chlorpyrifos concentration in all the treatments after 90 days of incubation were lower than those of the controls (2647 $\mu\text{g g}^{-1}$). After 90 days incubation, 51% of original level of solvent extractable chlorpyrifos (2647 $\mu\text{g g}^{-1}$) was recovered from the control, while approximately 27 (1411 $\mu\text{g g}^{-1}$, Oil-treated + PM + INC + H₂O) to 42% (2073 $\mu\text{g g}^{-1}$, Oil-treated + INC + H₂O) of original chlorpyrifos was detected in the various treatments. Among the treatments, the solvent extractable chlorpyrifos concentration in Oil-treated + INC + H₂O (2073 $\mu\text{g g}^{-1}$) was higher than that of the other three treatments: Oil-treated + PM + INC + H₂O (1411 $\mu\text{g g}^{-1}$), Oil-treated +

PM + FZ + GC + H₂O (1465 µg g⁻¹) and Oil-treated + PM + FZ + INC + GC + H₂O (1439 µg g⁻¹). There were no differences among the Oil-treated + INC + H₂O (2073 µg g⁻¹), PM + INC + H₂O (1768 µg g⁻¹) and Oil-treated + PM + FZ + INC + H₂O (1520 µg g⁻¹). This appears to be most likely due to the high variation among the sample replicates.

Differences between the control samples and various organic treatments, and among different treatments increased within the last 30 days incubation period (90 day interval). Approximately 26 and 28% of solvent extractable chlorpyrifos reduction was found in the treatments of Oil-treated + PM + FZ + GC + H₂O (1439 µg g⁻¹) and Oil-treated + PM + INC + H₂O (1411 µg g⁻¹). These treatments were less than the previous 60 day levels (2081 and 2385 µg g⁻¹, respectively) ($P < 0.05$). The other treatments had a lower rate of chlorpyrifos extractability.

4.3. Kinetics of chlorpyrifos extractability in bioremediated soils

The half-lives of chlorpyrifos extractability for various treatments are summarized in Table 4-2. The extractability of chlorpyrifos was fitted to the first-order kinetic model ($r > 0.96$) and k values range from 0.0079 to 0.0142 day⁻¹, and half-lives range from 48.9 to 87 days (Table 4-2). This presentation of the results was consistent with the analysis of variance with Tukeys multiple range test on the means (Table 4-1). The treatments with organic-based material amendments and microbial stimulation had approximately 50 days of extractability half-lives compared with that of the corresponding controls which had 87 days of extractability half-life. The treatment with vegetable oil alone or peat moss alone had relatively slow extractability rate with a k value of 0.009 and 0.012 day⁻¹, respectively. Their corresponding half-lives were 74.5 and 60.3 days, respectively.

4.4. Chlorpyrifos leachability from bioremediated soils

Organic-based material amendments reduced chlorpyrifos leaching from contaminated soils over time (Table 4-3). No differences in chlorpyrifos leachability were found between the unamended control (106 µg g⁻¹) and any of the treatments

Table 4-2. Chlorpyrifos extractability half-lives from bioremediated soils¹

Soil treatments	k (day ⁻¹)	Coefficient (r)	Half-life (days)
Control	0.0079	0.9865	87.0
Oil-treated + INC + H ₂ O	0.0093	0.9852	74.5
PM + INC + H ₂ O	0.0115	0.9795	60.3
Oil-treated + PM + INC + H ₂ O	0.0133	0.9634	52.1
Oil-treated + PM + FZ + INC + H ₂ O	0.0127	0.9894	54.8
Oil-treated + PM + FZ + GC + H ₂ O	0.0136	0.9911	50.9
Oil-treated + PM + FZ + INC + GC + H ₂ O	0.0142	0.9952	48.9

¹The extractability of chlorpyrifos in bioremediated soils was modeled by first-order reaction. The half-lives of chlorpyrifos extractability were calculated by the following formula (Assaf and Turco, 1994):

$$A = A_0 e^{-kt} \quad \text{or}$$

$$\ln(A/A_0) = -kt$$

where A_0 = initial substrate concentration

A = substrate concentration at time t

k = rate constant

Half-life is the t value when $A/A_0 = 1/2$.

Table 4-3. Chlorpyrifos solution concentration in leachate obtained by a shaking procedure after contaminated soil was amended with sorbents, nutrients and incubated at 40 °C for 90 days

Soil treatment ¹	Leachate concentration $\mu\text{g g}^{-1}$; (% of original) ^{2,3}			
	Initial	30 days	60 days	90 days
Control	106 ± 8(100)Aa	72 ± 16(67)Aab	49 ± 11(46)Abc	14 ± 1(13)Bc
Oil-treated + INC + H ₂ O	119 ± 3(100)Aa	69 ± 8(58)Ab	49 ± 4(41)Ab	21 ± 1(18)Ac
PM + INC + H ₂ O	110 ± 2(100)Aa	27 ± 7(24)Bb	21 ± 4(19)Bb	3 ± 0(3)Cc
Oil-treated + PM +INC + H ₂ O	103 ± 11(100)Aa	18 ± 4(18)Bb	12 ± 2(12)Bb	4 ± 1(4)Cb
Oil-treated + PM + FZ +INC +H ₂ O	99 ± 5(100)Aa	15 ± 2(15)Bb	14 ± 7(14)Bb	2 ± 0(2)Cb
Oil-treated + PM + FZ + GC + H ₂ O	110 ± 3(100)Aa	21 ± 2(19)Bb	4 ± 1(4)Bc	2 ± 1(2)Cc
Oil-treated + PM +FZ + INC + GC+H ₂ O	105 ± 1(100)Aa	17 ± 5(16)Bb	7 ± 1(7)Bbc	2 ± 1(2)Cc

¹Control soil sample (original soil with no oil and water added); PM = sphagnum peat moss; INC = microbial extract (5 ml); GC = ground corn; FZ = fertilizer. Values are expressed as the mean ± SE of 3 replicated samples, based on combusted weight.

²Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

³Lower case letters indicate comparison within treatments between dates. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

(ranging from 99 to 119 $\mu\text{g g}^{-1}$) at the initial stage of the incubation ($P > 0.05$). However, after 30 days of incubation, all the treatments had lower chlorpyrifos leachabilities than those of the initial experiment. Compared with the treatments at the 30 day incubation interval, the unamended control samples (72 $\mu\text{g g}^{-1}$) and Oil-treated + INC + H₂O (69 $\mu\text{g g}^{-1}$) had a higher chlorpyrifos leachability than the other organic-based material amended treatments (ranging from 15 to 27 $\mu\text{g g}^{-1}$). For the unamended controls, the level of chlorpyrifos leachability was down to 67% of the initial level, but there was no statistically significant difference between them.

Leachable chlorpyrifos continued to decline in the samples after 60 days of incubation, but no statistical differences were found in any treatments except Oil-treated + PM + FZ + GC + H₂O (4 $\mu\text{g g}^{-1}$) when compared with the chlorpyrifos concentration in the 30 day leachate (21 $\mu\text{g g}^{-1}$). On the other hand, the unamended control (chlorpyrifos leachate concentration) declined during this interval. Similar to the 30 day interval, the control samples (49 $\mu\text{g g}^{-1}$) and Oil-treated + INC + H₂O samples (49 $\mu\text{g g}^{-1}$) had higher chlorpyrifos leachabilities than the other organic-based material amended treatments (ranging from 4 to 21 $\mu\text{g g}^{-1}$).

Compared with the concentration level of the initial leachates of all treatments and unamended controls, only 2 to 18% of chlorpyrifos was detected in the leachates from the 90 day samples. These differences were all statistically significant (Table 4-3). After the remediated soil was incubated for 90 days, the chlorpyrifos leachate concentrations were reduced to very low levels (2 to 4 $\mu\text{g g}^{-1}$) for all treatments except Oil-treated + INC + H₂O, which contained 21 $\mu\text{g g}^{-1}$ or 18% of its initial level. The differences between Oil-treated + INC + H₂O and all other treatments were statistically significant. On the other hand, the unamended controls had a modest level of chlorpyrifos leachability (14 $\mu\text{g g}^{-1}$), significantly lower than Oil-treated + INC + H₂O (21 $\mu\text{g g}^{-1}$), but higher than all other treatments.

4.5. Discussion

4.5.1. Effects of amendments on chlorpyrifos extractability

The information obtained from this study supports the hypotheses that enhanced chlorpyrifos extractability and reduced chlorpyrifos leachability from contaminated soils can be achieved by using lignocellulosic sorbent and microbial nutrient amendments. As discussed previously in the literature review, the major chlorpyrifos dissipation pathways in soil involve both abiotic and biotic factors. The abiotic factors include volatilization, photodegradation, abiotic hydrolysis, and adsorption. The biotic factors include enzymatic hydrolysis, degradation, and incorporation into soil organic matrices. I will now discuss the abiotic factors which may have influenced the rate of extractability in this experiment. Chlorpyrifos has an intermediate level of vapor pressure (2×10^{-5} mm Hg, 25 °C, Racke, 1993). Based on this vapor pressure, it is unlikely that volatility was a major factor influencing chlorpyrifos extractability from the lignocellulosic sorbents-amended soils. The amount of chlorpyrifos loss through volatilization is dependent upon soil matrix-water interactions and other factors such as temperature, soil moisture level, air movement rate at soil surface, etc. The lignocellulosic sorbent amendments may act to reduce the amount of chlorpyrifos exposed to soil particle surfaces. Therefore, it is likely that chlorpyrifos volatility may have been slightly higher from unamended soil than from lignocellulosic material-amended soil. However, for the unamended controls, losses through volatilization could reach a higher level especially at the initial stages of the experiment or when soil moisture decreased to a low level. Since the chlorpyrifos contaminated soils were maintained in darkness throughout this experiment, the effects of photodegradation on chlorpyrifos extractability were probably negligible.

Abiotic chlorpyrifos hydrolysis is influenced by the pH of the matrices, temperature and elevated concentrations of some metallic ions such as Cu^{+2} (Macalady and Wolfe, 1983). The pH of the amended Groseclose soil samples used in this study ranged between 5.2 to 5.7 (not reported in the results); the unamended Groseclose was 6.0. Since the rate of alkaline hydrolysis has been reported to increase when pH exceeds 7.5 (Meikle and Youngson, 1978; Macalady and Wolfe, 1983), degradation from alkaline hydrolysis probably was not a significant factor contributing to the enhanced chlorpyrifos extractability in the amended soils. However,

chlorpyrifos hydrolysis may occur under neutral pH conditions (Chapman and Cole, 1982). Under these conditions, these workers found that chlorpyrifos is hydrolyzed to deethylchlorpyrifos and ethanol by the nucleophilic attack of water at the carbon of the ethoxy groups. Elevated temperature facilitates the nucleophilic attack of chlorpyrifos molecule, hence increases its hydrolysis rate (Meikle and Youngson, 1978). Because of the relatively low concentration of Cu^{+2} in soil, metallic ions-catalyzed hydrolysis in soil is unlikely to make significant contribution to chlorpyrifos dissipation in soil.

Biotic factors probably had a greater influence in the extractability rate observed in this study because nutrient materials were added to enhance their activities. Microbial cometabolism most likely was promoted by biostimulation with microbial nutrients and microbial-mediated chlorpyrifos hydrolysis and degradation and contributed significantly to its dissipation in amended treatments. Results from this study indicate that organic amendments reduced the solvent-extractable chlorpyrifos from the contaminated soils. After 90 days of incubation, only 27 to 42% of chlorpyrifos was recovered from various treatments amended with lignocellulosic materials and microbial nutrients (corn meal, vegetable oil and fertilizers). The corresponding unamended controls contained 51% of solvent extractable chlorpyrifos after the same incubation period. These results are similar to the findings of Berry et al. (1993a) who reported that chlorpyrifos disappearance from contaminated compost was stimulated by the addition of corn meal. They suggested that both enhanced degradation and incorporation might be attributed to the decrease of solvent extractable chlorpyrifos. Amendments with corn meal were found to enhance the dissipation of a number of pesticides including alachlor, trifluralin and atrazine (Dzantor et al., 1993).

In this study, bacterial and fungal populations probably increased when microbial nutrients such as vegetable oil and corn meal were used as amendments. The increased microbial activities most likely enhanced the enzymatic hydrolysis rates of chlorpyrifos in organic amended treatments. There is considerable evidence indicating that the availability of organic-based material amendments promotes soil microbial activities which may enhance enzymatic-mediated chlorpyrifos degradation. For example, Somasundaram et al. (1987)

reported 15-and 44-fold increases in bacterial populations at manure loading rates ranging from 3 and 30 tons per acre when compared with unamended soil. Fungal populations also increased by 6-and 9-fold at the two loading rates, respectively (Somasundaram et al, 1987). Furthermore, soil fungi (*Phanerochaete chrysosporium*) can completely mineralize chlorpyrifos (Bumpus et al., 1993). Chlorpyrifos hydrolysis could be catalyzed by enzymes produced by *Pseudomonas diminuta* (Havens and Rase, 1991).

Because many lignocellulosic materials are relatively low in energy as compared with other nutrient materials used as amendments, their role may have contributed to other aspects (other than nutrition) which enhanced dissipation. One of these factors might involve the reduction of the “effective” concentration of pesticides in the microbial environment. There is evidence that high concentrations of chlorpyrifos and its metabolites might have negative impacts on some soil microbial populations. For example, Racke et al. (1994) reported that the half-life of chlorpyrifos extractability in soil was as high as 1576 days at an initial concentration of 1000 $\mu\text{g g}^{-1}$. They speculated that extended half-life of chlorpyrifos extractability might be caused by the inhibiting effects of TCP and chlorpyrifos on soil microbial populations. Pozo et al. (1995) also observed inhibitory effects of chlorpyrifos on soil aerobic dinitrogen-fixing bacterial populations but not on fungal microflora. Since TCP, which is a major metabolite of chlorpyrifos, may accumulate in soil, it may inhibit soil bacterial and fungal activities (Racke et al. 1988; Somasundaram et al. 1990). Since chlorpyrifos contaminated soils amended with peat moss and microbial nutrients resulted in a decrease in extractable chlorpyrifos in most of the treatments in this study, it appears that chlorpyrifos and its degradative products did not substantially affect the overall activities of soil microbial communities during 90 days of incubation because significant rates of chlorpyrifos extractability were observed (Table 4-1 & 4-2). This phenomenon might be related to a “spatial” function of lignocellulosic materials and organic nutrient amendments. The lignocellulosic materials have relatively large adsorption surface areas, and as a result, have increased absorption capacity and potential sites in which microbes could inhabit. As a result, soil amended with lignocellulosic materials have increased absorption capacity for contaminants and provide additional spatial arrangements which

possibly reduce the negative impact of high concentrations of chlorpyrifos on microbial populations.

There was an interaction between the vegetable oil nutrient and lignocellulosic sorbent materials. Vegetable oil alone did not appear to enhance chlorpyrifos dissipation. The solvent extractable chlorpyrifos was about 42% from vegetable oil amended treatment after 90 days of incubation, which was the second highest concentration after the controls (Table 4-1). Without the presence of lignocellulosic sorbents, soil microbial activity may have been inhibited by the high concentrations of chlorpyrifos and TCP as discussed in previous section. In fact, the results from this experiment indicate that vegetable oil treated with peat moss had the effect of reducing chlorpyrifos recovery from treatments. Only 27% chlorpyrifos was recovered from Oil-treated + PM + INC + H₂O which exhibited the highest level of extractability. In this case the oil treated with peat moss may have served to provide increased surface area and a lipid or hydrophobic environment where some of the chlorpyrifos could be sequestered prior to degradation as well as providing additional energy sources for soil microbial populations.

Microbial mediated dissipation includes the possibility of metabolite incorporation into soil matrices. Some incorporation is likely to have occurred in the amended/unamended treatments. Incorporated pesticides are generally considered to be less bioavailable and either less or non-ecotoxic (Bollag and Loll, 1983; Freitag et al., 1984). Incorporation of ¹⁴C-chlorpyrifos into lignocellulosic materials has been investigated by Judge (1996). He found that less than 5% of ¹⁴C-chlorpyrifos was associated with soil organic matrices after 16 weeks of incubation. Unlike the phenolics, which readily polymerize with other phenolics in enzymatic reactions, the pyridinol rings in chlorpyrifos and TCP substituted with the three electron-withdrawing chlorine atoms usually existing in the keto form but do not possess the same reactivity of the phenolic compounds (Berry and Boyd, 1984, 1985). Hence, they have quite limited potential of covalently bound to soil organic matrices (Judge, 1996). Most chlorpyrifos absorbed by soil organic matrices appear to be physically absorbed and are readily extractable by organic solvents.

4.5.2. Effects of amendments on chlorpyrifos leachability

The results strongly indicate that lignocellulosic sorbent and nutrient amendments reduced chlorpyrifos leachability from contaminated soil. Chlorpyrifos is quite water insoluble (< 2 ppm), due to its nonpolar molecular characteristics. The low leachability ($99-119 \mu\text{g g}^{-1}$) observed in this study is consistent with its low water solubility from soil even at high concentrations (4912 to $5239 \mu\text{g g}^{-1}$). As discussed previously, since lignocellulosic materials increased the sorption capacity of amended soils, it would be expected that more chlorpyrifos would be absorbed by soil organic matrices and less would be available for leaching. The leachability data from this study are consistent with this information except for the initial samples. It should be noted that there was no difference among amended and unamended treatments in chlorpyrifos leaching from contaminated soils in samples taken 24 h after the pesticide contamination and immediately following amendment addition. There are two possible explanations for this. First, it is known that oil-treated lignocellulosic sorbents have an increased sorption capacity for hydrophobic pesticides over lignocellulosic sorbents without oil amendment (Mullins et al., 1992c). However, in this study, lignocellulosic sorbent and oil amendments were made sequentially versus adding oil-treated lignocellulosic sorbent directly; the initial samples were taken immediately after amendments were made. The adsorption-desorption of chlorpyrifos by soil organic matter might take a few days to achieve an equilibrium between the chlorpyrifos, soil, oil and lignocellulosic materials. Therefore, only a small amount of leachability reduction which might be attributable to the lignocellulosic sorbent amendments was observed at the initial time interval. The second possibility might be the chlorpyrifos leaching was also affected by the surfactants and other adjuvants in chlorpyrifos emulsion. Judge (1996) suggested the surfactants in Dursban[®] 4E could act as a barrier for pesticide sorption. A few days after pesticide application, these surfactants might be separated from pesticide emulsion micelles through volatilization and diffusion. Thus, less leachable chlorpyrifos was detected as the surfactant effects became less influential and sorption to the soil and contaminated matrices increased. In order to further study the effects of surfactants and other adjuvants in pesticide formulations on the extractability and leachability of chlorpyrifos, the experiment described in Chapter 6 was conducted.

4.5.3. Relationship between leachability and extractability

It should be apparent that chlorpyrifos leachability from contaminated soils is related to its availability represented by its solvent extractability. As chlorpyrifos is subjected to the various routes of dissipation which have been discussed, it is reasonable to expect that chlorpyrifos leaching from soil should decrease as the level of solvent extractable chlorpyrifos decreases. However, differences in extractability and leachability rates observed are evident and important. This can be seen by comparing chlorpyrifos extractability and leachability data from Tables 4-1 & 4-3. The amount of chlorpyrifos in lignocellulosic sorbent amended treatments ranged from 28 to 34% (Control: 51%) and the leachability ranged from 2 to 4% (Control: 13%) of their initial levels after 90 days of incubation. Because the results for the extractability and leachability rates obtained from lignocellulosic sorbent amended treatments were similar, all five treatments with lignocellulosic sorbent were grouped as “PM-treated”, and were compared to the controls and vegetable oil amended treatments (Oil-only-treated). These comparisons are presented in Figures 4-1 & 4-2. Based on the results in Figure 4-1, PM-treated and Oil-only-treated had higher extractability rates, especially after the first 30 days of incubation. Nearly 44 and 35% of chlorpyrifos was dissipated in PM-treated and Oil-only-treated, respectively, while only 12% of chlorpyrifos was unrecoverable in the controls during this interval. Overall, the difference in chlorpyrifos extractability rate could be interpreted as representing a reduction of potential environmental hazards due to leaching that can be achieved by using lignocellulosic sorbent and nutrient amendments. For example, there is a 21% difference in chlorpyrifos extractability rate between the controls and PM-treated after 90 days of incubation.

Of particular interest is the observation that amendments with lignocellulosic sorbent substantially reduced chlorpyrifos leachability within the first 30 day incubation period. Leachability was reduced to 18% of the corresponding level in PM-treated compared with 58 and 67% in Oil-only-treated and the controls, respectively. This is significant since the results indicated environmental hazards associated with chlorpyrifos leaching away from the contaminated site can be reduced by lignocellulosic sorbent amendment within a relatively short time period.

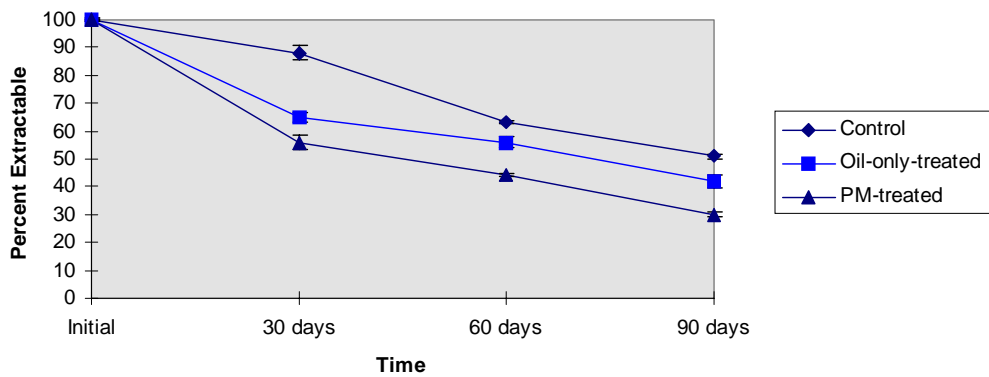


Figure 4-1. Comparison of chlorpyrifos extractability rates in contaminated soil

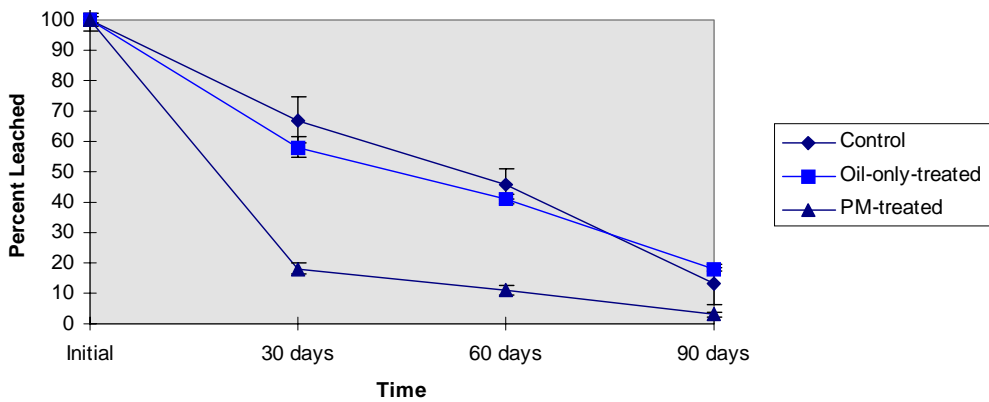


Figure 4-2. Comparison of chlorpyrifos leachability rates in contaminated soil

In summary, it appears that a combination of lignocellulosic sorbent and vegetable oil amendments are the most effective means for stimulating chlorpyrifos transformation and reducing its leachability in contaminated soils. Organic-based material amendments reduced the leachability of formulated chlorpyrifos from contaminated soil within a short time period, although there was no significant difference from the control at the initial stage. Chlorpyrifos dissipated from amended soils at rates relatively slower than the corresponding decrease in leaching potential.

Chapter 5. Extractability and leachability of atrazine in bioremediated soils

5.1. Introduction

This study is similar to the chlorpyrifos bioremediation study. It was designed to achieve two objectives: 1) to determine the influence of organic and nutrient amendments on the extractability rates of formulated atrazine in contaminated soil and, 2) to determine the influence of organic and nutrient amendments on the leachability rates of formulated atrazine in contaminated soil. Groseclose soil contaminated with formulated atrazine (AAtrex 4L, 40.8% a.i.) was amended with organic materials, microbial extract and nutrients and incubated in an incubation chamber at 30 °C for 120 days (Materials and Methods 3.3.). Atrazine concentrations were determined by GC analysis and were used as an indicator of atrazine degradation, sorption and incorporation. Atrazine leachability was tested using two different approaches: a shaking procedure developed in our laboratory and a Toxicity Characteristic Leaching Procedure (TCLP) (Materials and Methods 3.3.4 & 3.3.5.). This experiment was designed to examine extractability rates of atrazine in soil amended with organic materials. More specific information with regard to atrazine dissipation (mineralization, degradation, sorption and incorporation, etc.) in amended soil is provided by the experiment conducted with radiolabeled [U-ring-¹⁴C] atrazine (Chapter 7).

5.2. Degradation and incorporation of atrazine in bioremediated soils

Solvent extractable atrazine from the contaminated Groseclose soil was reduced by organic-based material amendment and biostimulation over a 120 day incubation period (Table 5-1). The initial concentration of amended and unamended samples ranged from 4501 (Oil-treated + PM + INC + H₂O) to 4984 μg g⁻¹ (Oil-treated + PM + FZ + GC + INC + H₂O) based on combusted weight. There were no differences among the initial concentrations of all treatments and the controls ($P > 0.05$). After incubation for 90 days, solvent extractable atrazine was reduced significantly in all treatments except Oil-treated + INC + H₂O (3493 μg g⁻¹). The percentage of atrazine in these treatments ranged from 17% (Oil-treated + PM + FZ + INC + H₂O, 730 μg g⁻¹) to 77% (Oil-treated + INC + H₂O, 3493 μg g⁻¹) of the initial concentration during this period. Amended treatments containing peat moss and vegetable oil treated plus

Table 5-1. Extractable atrazine after contaminated soil was amended with sorbents, nutrients and incubated at 30 °C for 120 days

Soil treatment ¹	Soil concentration $\mu\text{g g}^{-1}$; (% of original) ^{2,3}		
	Initial	90 days	120 days
Control + INC	4723 \pm 23(100)Aa	3515 \pm 278(74)Ab	2863 \pm 191(61)Ab
PM + INC + H ₂ O	4932 \pm 283(100)Aa	2805 \pm 279(57)ABb	1881 \pm 230(38)Bc
Oil-treated + INC + H ₂ O	4511 \pm 232(100)Aa	3493 \pm 322(77)Aa	2310 \pm 306(51)ABb
Oil-treated + PM + INC + H ₂ O	4501 \pm 498(100)Aa	1918 \pm 388(43)Bb	1585 \pm 206(35)Bb
Oil-treated + PM + FZ + INC + H ₂ O	4397 \pm 252(100)Aa	730 \pm 14(17)Cb	609 \pm 43(14)Cb
Oil-treated + PM + FZ + GC + INC + H ₂ O	4984 \pm 369(100)Aa	890 \pm 38(18)Cb	749 \pm 129(15)Cb

¹Control soil sample contained approximately 5,000 $\mu\text{g g}^{-1}$ atrazine as AAtrex[®] 4L with no oil and water added.

PM = sphagnum peat moss; INC = microbial extract (5 ml); GC = ground corn; FZ = fertilizers. Values are expressed as the mean \pm SE of 3 replicated samples, based on combusted weights.

²Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

³Lower case letters indicate comparison within treatments between dates. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

fertilizer or fertilizer with ground cornmeal had a significant effect on extractable atrazine; only 17 to 18% of atrazine was recovered from these treatments compared with others having concentrations ranging from 43 to 77% ($P < 0.05$, Table 5-1). Extractable atrazine in the controls was 74% of the initial level ($3515 \mu\text{g g}^{-1}$). The differences between the treatments and the controls were significant for Oil-treated + PM + INC + H₂O ($1918 \mu\text{g g}^{-1}$), Oil-treated + PM + FZ + INC + H₂O ($730 \mu\text{g g}^{-1}$) and Oil-treated + PM + FZ + GC + INC + H₂O ($890 \mu\text{g g}^{-1}$) but not significant for PM + INC + H₂O ($2805 \mu\text{g g}^{-1}$) and Oil-treated + INC + H₂O ($3493 \mu\text{g g}^{-1}$).

Among the treatments, differences between the solvent extractable atrazine were observed between the 90 day remediated samples: Vegetable oil or peat moss treated alone (PM + INC + H₂O and Oil-treated + INC + H₂O) had higher solvent extractable atrazine than the other three treatments. The peat moss combined with vegetable oil contained an intermediate level of solvent extractable atrazine (Oil-treated + PM + INC + H₂O, $1918 \mu\text{g g}^{-1}$). Treatments containing additional nutrient amendments (fertilizers and ground corn meal) had the lowest levels of solvent extractable atrazine. These were Oil-treated + PM + FZ + INC + H₂O ($730 \mu\text{g g}^{-1}$) and Oil-treated + PM + FZ + GC + INC + H₂O ($890 \mu\text{g g}^{-1}$), respectively ($P < 0.05$).

Incubation of samples for 120 days provided similar results to those observed from the 90 day incubation interval. However, the solvent extractable atrazine was reduced in nutrient-amended treatments (fertilizers and corn meal) but the peat moss treated or vegetable oil treated showed a greater decline in the solvent extractable atrazine. The atrazine concentrations in PM + INC + H₂O ($1881 \mu\text{g g}^{-1}$) and Oil-treated + INC + H₂O ($2310 \mu\text{g g}^{-1}$) were lower at 120 days than at 90 days ($P < 0.05$). However, the nutrients amended treatments i.e. Oil-treated + PM + FZ + INC + H₂O ($609 \mu\text{g g}^{-1}$) and Oil-treated + PM + FZ + GC + INC + H₂O ($749 \mu\text{g g}^{-1}$) still had the lowest level of solvent extractable atrazine after 120 days of incubation (Table 5-1).

5.3. Kinetics of atrazine extractability in bioremediated soils

The same formula used for chlorpyrifos extractability half-life calculation was used for the calculation of atrazine extractability (Results and Discussion 4.3.). The half-lives of atrazine extractability for various treatments are provided in Table 5-2.

Table 5-2. Atrazine extractability half-lives from bioremediated soils¹

Soil treatment	k(day-1)	Coefficient(r)	Half-life (days)
Control	0.004	0.984	175
PM + INC + H ₂ O	0.008	0.983	91
Oil-treated + INC + H ₂ O	0.005	0.915	140
Oil-treated + PM + INC + H ₂ O	0.009	0.998	78
Oil-treated + PM + FZ + INC + H ₂ O	0.017	0.987	40
Oil-treated + PM + FZ + INC + GC + H ₂ O	0.017	0.987	42

¹The extractability of chlorpyrifos in bioremediated soils was modeled by first-order reaction. The half-lives of chlorpyrifos extractability were calculated by the following formula (Assaf and Turco, 1994):

$$A = A_0 e^{-kt} \quad \text{or}$$

$$\ln(A/A_0) = -kt$$

where A_0 = initial substrate concentration

A = substrate concentration at time t

k = rate constant

Half-life is the t value when $A/A_0 = 1/2$.

The extractability of atrazine was fitted to the first-order kinetic model ($r > 0.91$) and k values ranged from 0.004 to 0.017 day^{-1} , and the half-lives ranged from 40 to 175 days (Table 5-2). The results were consistent with the analysis of variance with Tukeys multiple range test on the means (Table 5-1). The treatments with organic-based materials and microbial nutrients had only approximately 40 days of extractability half-life compared with the corresponding controls which had 174 days. Treatments with vegetable oil or peat moss alone had relatively slower extractability rates with a half-lives of 91 and 140 days, respectively, while treatments with both vegetable oil and peat moss had a extractability half-life of 78 days. With additional nutrient sources such as fertilizers and corn meal, atrazine extractability half-life decreased to approximately 40 days (Table 5-2).

5.4. Atrazine leachability from bioremediated soils

Organic-based material amendments combined with biostimulation reduced atrazine leachability over 120 days incubation period, based on the 4 h shaking method (Table 5-3). Initial atrazine leachabilities ranged from 118 to 157 $\mu\text{g g}^{-1}$ for amended treatments and unamended controls. The controls had the highest leachability (157 $\mu\text{g g}^{-1}$). Oil-treated + PM + FZ + INC + H₂O had the lowest level of leachability (118 $\mu\text{g g}^{-1}$), but these leachate concentration differences were not statistically significant ($P > 0.05$, Table 5-3). After 120 days of incubation, the leachabilities in the controls as well as in peat moss treated or vegetable treated treatments (PM + INC + H₂O and Oil-treated + INC + H₂O) were not significantly different ($P > 0.05$, Table 5-3), even though their level of leachability was approximately 80% of initial leachate concentration. Significant declines of atrazine leachate concentration in Oil-treated + PM + H₂O, Oil-treated + PM + FZ + INC + H₂O and Oil-treated + PM + FZ + GC + INC + H₂O samples were found at both 90 days and 120 days compared with that of initial samples ($P < 0.05$). The atrazine concentrations in leachate were significantly lower in Oil-treated + PM + FZ + INC + H₂O (90 and 120 days) and Oil-treated + PM + FZ + GC + INC + H₂O (90 days) than in the controls. No significant difference was found between the leachability of Oil-treated + PM + FZ + GC + INC + H₂O and that of controls. It appears that this might be attributed to the high amount of variability among the replicates.

Table 5-3. Atrazine solution concentration in leachate obtained by a shaking procedure after contaminated soil was amended with sorbents, nutrients and incubated at 30 °C for 120 days

Soil treatment ¹	Leachate concentration $\mu\text{g g}^{-1}$; (% of original) ^{2,3}		
	Initial	90 days	120 days
Control + INC	157 \pm 10(100)Aa	136 \pm 9(86)Aa	124 \pm 20(79)Aa
PM + INC + H ₂ O	143 \pm 11(100)Aa	117 \pm 7(82)ABa	112 \pm 11(79)ABa
Oil-treated + INC + H ₂ O	136 \pm 5(100)Aa	118 \pm 3(87)ABa	115 \pm 4(85)ABa
Oil-treated + PM + INC + H ₂ O	121 \pm 10(100)Aa	107 \pm 2(88)ABab	99 \pm 4(81)ABb
Oil-treated + PM + FZ + INC + H ₂ O	118 \pm 15(100)Aa	102 \pm 14(86)Ba	84 \pm 3(71)Bb
Oil-treated + PM + FZ + GC + INC + H ₂ O	122 \pm 12(100)Aa	104 \pm 9(85)Bab	89 \pm 6(73)ABb

¹Control soil sample contained approximately 5,000 $\mu\text{g g}^{-1}$ atrazine as AAtrex[®] 4L with no oil and water added.

PM = sphagnum peat moss; INC = microbial extract (5 ml); GC = ground corn; FZ = fertilizers. Values are expressed as the mean \pm SE of 3 replicated samples, based on combusted weights.

²Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

³Lower case letters indicate comparison within treatments between dates. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; $P > 0.05$).

Although the concentrations of atrazine released by the TCLP method were higher than those using the 4 h shaking method (Table 5-4), both methods provided similar results for all samples during 120 day incubation period. The initial concentrations of atrazine in these TCLP leachates ranged from 146 to 172 $\mu\text{g g}^{-1}$, but there were no significant differences between the treatments and the controls. After 90 days of incubation, the atrazine leachate concentrations were lowered to approximately 80% of the initial levels but there were no statistically significant differences between the controls and the treatments. However, after 120 days of incubation, all treatments except for the Oil-treated + INC + H₂O samples (129 $\mu\text{g g}^{-1}$) were different from the controls. Among the treatments, Oil-treated + PM + FZ + INC + H₂O had the lowest level of leachable atrazine (103 $\mu\text{g g}^{-1}$), and Oil-treated + INC + H₂O had the highest level of leachable atrazine (129 $\mu\text{g g}^{-1}$). Atrazine concentration in leachate was approximately 70 % of its initial level for these four treatments after 120 days incubation at 30 °C.

5.5. Discussion

5.5.1. Effects of amendments on atrazine extractability

The results from this study support the hypothesis that lignocellulosic sorbent and microbial nutrient amendments decreased atrazine extractability in contaminated soils. However, there was no, and only a slight reduction of atrazine leachability observed in the 4 h and TCLP method, respectively. Atrazine is a moderately persistent herbicide which is subject to various routes of abiotic and biotic dissipation in the environment which are discussed in the literature review. Some of the abiotic factors are discussed here. The losses from volatilization probably were not significant due to the relatively low vapor pressure of atrazine. Abiotic atrazine hydrolysis was most likely a factor which attributed to the overall atrazine dissipation in the contaminated Groseclose soil. There is evidence that atrazine can be hydrolyzed to hydroxyatrazine abiotically in a sterilized soil (Burnside et al., 1961). Since the atrazine chemical hydrolysis rate is faster at high pH than at low pH, and organic amendments (peat moss, vegetable oil) reduced soil pH to a relatively small degree (less than 2 pH units as indicated by the testing from this experiment; not reported in the results), it appears that chemical hydrolysis

Table 5-4. Atrazine solution concentration in leachate obtained by TCLP after contaminated soil was amended with sorbents, nutrients and incubated at 30 °C for 120 days

Soil treatment ¹	Leachate concentration $\mu\text{g g}^{-1}$; (% of original) ^{2,3}		
	Initial	90 days	120 days
Control + INC	172 ± 14(100)Aa	149 ± 17(87)Aa	138 ± 7(80)Aa
PM + INC + H ₂ O	155 ± 4(100)Aa	122 ± 8(79)Ab	105 ± 10(68)BCb
Oil-treated + INC + H ₂ O	153 ± 6(100)Aa	147 ± 13(96)Aa	129 ± 3(84)ABa
Oil-treated + PM + INC + H ₂ O	156 ± 8(100)Aa	136 ± 9(87)Aa	106 ± 7(68)BCb
Oil-treated + PM + FZ + INC + H ₂ O	146 ± 12(100)Aa	127 ± 15(87)Aab	103 ± 3(71)Cb
Oil-treated + PM + FZ + GC + INC + H ₂ O	151 ± 9(100)Aa	122 ± 7(81)Ab	104 ± 2(69)BCb

¹Control soil sample contained approximately 5,000 $\mu\text{g g}^{-1}$ atrazine as AAtrex[®] 4L with no oil and water added.

PM = sphagnum peat moss; INC = microbial extract (5 ml); GC = ground corn; FZ = fertilizers. Values are expressed as the mean ± SE of 3 replicated samples, based on combusted weights.

²Upper case letters indicate comparison within columns between treatments. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

³Lower case letters indicate comparison within treatments between dates. Concentrations with the same letter are not significantly different; analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

of atrazine might not have been increased by lignocellulosic sorbent and nutrient amendments.

Several biotic or microbially-mediated activities, including atrazine dealkylation, hydrolysis, degradation and incorporation, probably were more important in dissipation of atrazine than chemical hydrolysis. Data obtained from this study indicated that the addition of microbial nutrients significantly enhanced atrazine dissipation in amended soils. Over 85% of atrazine was degraded or incorporated into soil organic matrices after 120 days of incubation in two of the treatments. These results were more dramatic than those reported by Dzantor et al. (1993) who found that atrazine degradation was stimulated by corn meal amendment in field plots after 100 days (59% reduction with high variation). Enhanced atrazine dissipation from soil by microbial nutrient amendments has also been observed by a number of other researchers (Wagner and Chahal, 1966; Entry and Emmingham, 1995; Mandelbaum et al., 1995). However, the use of amendments for contaminated soil combining microbial nutrients and lignocellulosic sorbents have not yet been reported.

The enhanced atrazine losses observed in this study are likely associated with enhanced microbial cometabolism of atrazine in amended soils. As discussed in the previous chapter, soil microbial activities, which can be enhanced by biostimulation with microbial nutrients, may influence atrazine enzymatic dealkylation, hydrolysis, etc. In addition to this, it is also possible that substantial biotransformation/mineralization might be attributed to atrazine-degrading microorganisms in nutrient-amended soils. Microbial extracts from previous pesticide disposal bioreactors where atrazine had been degraded were used in this study. Therefore, the possibility exists that microorganisms from these sources were able to degrade atrazine and its metabolites at increased rates. There are a number of researchers who have recently isolated bacteria or bacterial cultures that were shown capable of mineralizing atrazine as a sole carbon or nitrogen source at a significant rate (Mandelbaum et al., 1995; Radosevich et al., 1995). However, these bacteria were obtained from enrichment cultures or soil which had been

perfused with atrazine for extended periods. Nevertheless, soil microbial populations with enhanced atrazine degradative capability may have become more dominant as a result of preferential selection from naturally-occurring genetic alterations by atrazine selection pressure invoked by the experimental protocol. Unfortunately, due to limited resources and time, no effort was made to isolate or identify these microorganisms.

Not all atrazine absorbed by soil organic matter is physisorbed, atrazine may become covalently bound to organic matrices. Covalent bonding could be enhanced by microbes which may secrete extracellular enzymes that might catalyze incorporation processes or may catalyze reactions causing “internal” incorporation into their biomass, which will contribute directly to the soil xenobiotic-incorporated biomass after upon their death. Information provided in the literature review discusses evidence that large amounts of atrazine could be incorporated into soil organic matrices (Capriel et al., 1985; Winkelmann and Klaine, 1990a; Judge 1996). For example, in an organically-based compost environment, more than 50 and 60% of the ¹⁴C-atrazine radioactivity was associated with alkali-soluble and insoluble fractions of soil matrices after 16 weeks of solid state fermentation in both nutrient-amended and unamended treatments, respectively (Judge, 1996).

Assuming that nutrient amendments are capable of enhancing atrazine metabolism and its incorporation to soil matrices, there are differences in these activities based on the specific combinations of these amendments. The potential effects resulting from nutrient amendments and their interactions will now be discussed. It appears that vegetable oil alone (Oil-treated + INC + H₂O) did not provide a significant increase in atrazine extractability. Similar to high concentrations of chlorpyrifos (discussed in the previous chapter), microbial activities in treatments containing no lignocellulosic amendments might be inhibited to some extent by high concentrations of atrazine and its metabolites. The results appear to support this since solvent extractable atrazine from treatments with vegetable oil only was the highest among all the treatments except for the controls. On the other hand, soil amended with lignocellulosic sorbents had a medium

level of atrazine extractability after 120 days of incubation. Lignocellulosic sorbents increase the sorption capacity of amended soil in addition to providing a relatively small amount of nutrients for soil microbes. Soil amended with lignocellulosic sorbents may have increased atrazine incorporation and, to a small extent, enhanced transformation. However, due to the limited nutrient source in lignocellulosic materials, microbial activity in soils amended with lignocellulosic materials was probably low. Hence, the microbial transformations were probably limited. The combination of a lignocellulosic sorbent and vegetable oil may provide both sorption capacity for increased binding/incorporation and enhancement of microbial activity for biotransformation. This possibility is supported by the results which indicate that the treatment with oil and peat moss had a relatively lower solvent extractable atrazine than either treatment with oil or treatment with peat moss. Addition of fertilizers appeared to balance the C/N ratio and provided additional micronutrients for soil microbes enhancing soil microbial activity and microbial-mediated biotransformations. The ideal C/N ratio is about 25-30 for soil microbes (Biddlestone et al., 1987). This enhancement activity is supported by the data obtained from the treatment with fertilizer (Oil-treated + PM + FZ + INC + H₂O) which had a significantly lower level of atrazine extractability than the treatment without fertilizer (Oil-treated + PM + INC + H₂O). Further, addition of corn meal did not appear to increase atrazine extractability over these oil and fertilizer-amended treatments. This indicates that soil microbes may have had sufficient nutrients for their degradative activities.

5.5.2. Effects of amendments on atrazine leachability

Atrazine leachability from contaminated soils was not reduced (the 4 h shaking method) and was slightly reduced (the TCLP method) by lignocellulosic sorbent and microbial nutrient amendments after 120 days of incubation. Lignocellulosic material amendments and nutrient fortification may provide additional sorption area and enhanced microbial degradation of atrazine and its metabolites through increasing overall microbial activities, as discussed in the previous sections. However, only a slightly lower leachability was detected in organically-based material amended

treatments based on the TCLP method. The results may relate to relatively high atrazine solubility (33 mg kg^{-1} , $20 \text{ }^\circ\text{C}$) and physicochemical properties of atrazine formulation (AAAtrex[®] 4L) used in this study. The effects of formulated atrazine on its extractability and leachability were examined in the experiment described in Chapter 6.

The two different leachability testing procedures provided similar results. The TCLP method is an acidic and more rigorous extraction procedure (a larger volume of extraction solution and a longer shaking time) which has the potential of increasing the amount of atrazine leached from soil. The atrazine leachability data obtained from the TCLP method showed a similar pattern as that obtained by the 4 h shaking procedure with distilled water. The only difference between them was that more atrazine was leached by the TCLP extraction. This is as expected due to the nature of the TCLP method compared to that of the 4 h shaking procedure. Nevertheless, the strong acidic medium itself might not result in high leachability for atrazine since more atrazine is protonated in acidic conditions; as a consequence, more atrazine is sorbed near to its pKa (Weber et al., 1969). Based on Tukey's multiple range test, the TCLP method yielded more significant differences between amended and unamended treatments than the 4 h shaking method for the samples after 120 days of incubation (Table 5-4 and 5-5). These results suggest that amending with organic sorbents and nutrients has the potential of sustaining more severe leaching conditions.

5.5.3. Relationship between leachability and extractability

Atrazine leachability from contaminated soil during the bioremediation experiment can be correlated with its solvent extractability. Comparison of atrazine extractability and leachability rates based on the 4-h shaking method are presented as percentages of initial rates in Figures 5-1 and 5-2 (data from Tables 5-1 and 5-3), respectively. The overall trend observed was that the greater the solvent extractable atrazine, the greater the atrazine leachability. Atrazine leachability using the 4-h shaking procedure was based on its solubility in water while solvent extractable atrazine was based on solubilization and extractability by organic solvents. Atrazine absorbed by

lignocellulosic materials and soil organic matter can be divided into two fractions: one is loosely physisorbed; the other is chemisorbed to soil organic matrices. The physisorbed nonpolar materials are most likely extractable by organic solvents. The chemisorbed materials are much more stable and less extractable by organic solvents. Physisorbed atrazine could be extractable and leachable at the same time. However, only a portion of physisorbed atrazine may be leachable by distilled water.

Comparisons of percent extractable and percent leachable atrazine indicate the relatively higher percentage of atrazine was leachable from treatments under low solvent extractable atrazine concentration (120 days of amended and unamended treatments) (Figures 5-1 & 5-2). Thus, a significant portion of atrazine could be leachable even under the low concentrations produced after a considerable amount of dissipation had occurred. This result might relate to a higher water solubility of atrazine (33 mg kg^{-1} at $25 \text{ }^\circ\text{C}$). In addition to this, atrazine may be sorbed or bound to water soluble organic substances and hence present in the leachate. Similar results were reported by Madhun et al. (1986). These workers found that a number of herbicides including simazine, bromacil, chlorotoluron, etc. could bind to water-soluble organic materials from soil.

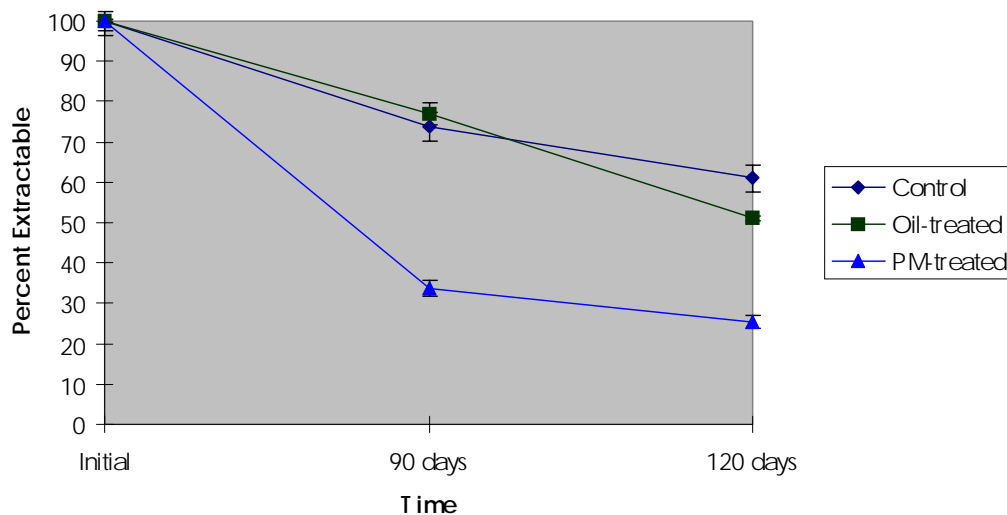


Figure 5-1. Comparison of atrazine extractability rates in contaminated soil

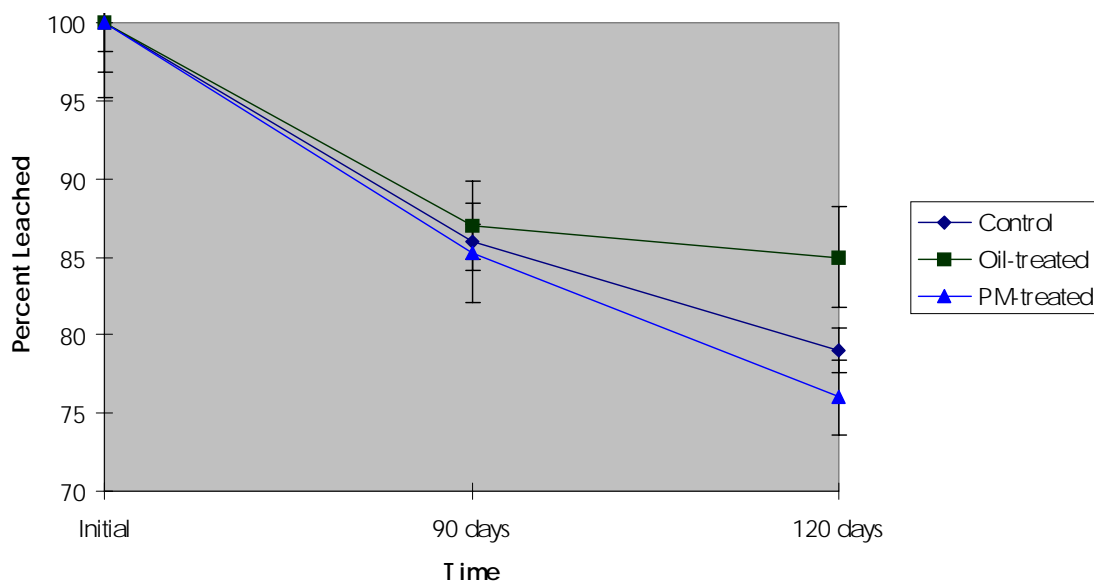


Figure 5-2. Comparison of atrazine leachability rates in contaminated soil

Atrazine is more leachable than chlorpyrifos when present at similar concentrations and in soils with similar organic amendments. After 120 days of incubation, there was only about a 30% reduction of atrazine leachability from amended treatments, compared with an approximately 95% reduction chlorpyrifos leachability from amended treatments after 90 days of incubation. Therefore, atrazine as AAtrex® 4L could represent a more serious leaching hazard with regard to movement from the amended soils treated atrazine spill sites than from similarly treated chlorpyrifos spill sites.

In summary, atrazine dissipation can be enhanced by biostimulation. Microbial degradation might play a more important role for atrazine dissipation than for chlorpyrifos dissipation. Soil microbes appear to have not only enhanced atrazine degradation but also possibly increased incorporation of atrazine into soil organic matrices. Due to a higher water solubility, atrazine is more mobile than chlorpyrifos. Its leachability depends on the actual concentration of atrazine in contaminated soil, and the respective atrazine binding relationships. Two different leachability test methods

provided similar results with TCLP having a slightly higher concentration in the leachates. Both enhanced containment with lignocellulosic sorbent and enhanced microbial transformation by biostimulation appear to be crucial for reducing atrazine hazard in the environment. Some specific aspects about atrazine dissipation, metabolism and incorporation are examined in Chapter 7.

Chapter 6 Effects of formulation on the extractability and leachability of chlorpyrifos and atrazine from contaminated soils

6.1. Introduction

Possible effects on chlorpyrifos and atrazine extractability and leachability resulting from their respective formulations were observed in Chapter 4 and 5. Because of the complex abiotic/biotic interactions occurring in the studies described in the previous chapters, it was difficult to determine the influence of the surfactants and other adjuvants in commercial formulations of chlorpyrifos (Dursban[®] 4E) and atrazine (AAtrex[®] 4L). This experiment was done to determine whether the leachability and extractability of chlorpyrifos and atrazine are influenced by their respective formulations under conditions where biotic activity was limited. This study compares extractability and leaching rates of formulated chlorpyrifos and atrazine with their technical forms from Groseclose soil applied at similar concentration levels. Undiluted formulations of chlorpyrifos (Dursban[®] 4E) and atrazine (AAtrex[®] 4L) were applied directly to the top of soil samples without mixing. Technical grade chlorpyrifos and atrazine were mixed with acetone and applied at similar concentrations as their formulated forms (Materials and Methods 3.4 and 3.5).

6.2. Chlorpyrifos

Although major efforts were made to deliver comparable initial concentrations of formulated and technical chlorpyrifos to the soil samples, there were slight differences in the two experimental groups. There was a trend for reduction in the concentrations of solvent extractable chlorpyrifos (CSEC) (85% of the initial extractable after 56 days, Table 6-1) found in soil treated with technical grade chlorpyrifos, but these differences were not significant. Most of CSEC that was lost or unavailable (ca. 10%) occurred during the first three days after technical grade chlorpyrifos was added to Groseclose soil. The CSEC declined by an additional 5% during the 53 days that followed, but not at a significant level. Significant differences in extractability or unavailability of chlorpyrifos were found in soil treated with formulated chlorpyrifos (Dursban 4E) during

Table 6-1. Concentration of solvent extractable chlorpyrifos and atrazine 56 days after Groseclose soil had been contaminated either with technical or formulated chlorpyrifos and atrazine

Soil treatment ²	Solvent Extractable Pesticides $\mu\text{g g}^{-1}$; (% of original) ¹				
	Initial	3 days	7 days	28 days	56 days
Technical Chlorpyrifos	5034 \pm 590(100)A	4574 \pm 199(91)A	4474 \pm 152(89)A	4326 \pm 107(86)A	4300 \pm 174(85)A
Formulated Chlorpyrifos	6381 \pm 517(100)A	5366 \pm 567(84)B	4866 \pm 198(76)B	4695 \pm 22(74)B	4643 \pm 89(73)B
Technical Atrazine	3236 \pm 201(100)A	**	3190 \pm 319(99)A	3111 \pm 300(96)A	3275 \pm 103(101)A
Formulated Atrazine	2437 \pm 99(100)A	**	2557 \pm 230(105)A	2597 \pm 225(107)A	2576 \pm 173(106)A

¹The results were expressed as the mean \pm standard error of three replicated samples. Technical chlorpyrifos (99.8%) and technical atrazine (99.8%) were solubilized in acetone prior to soil application. Formulated chlorpyrifos as Dursban[®] 4E and atrazine as AAtrex[®] 4L were applied directly to soil.

²Upper case letters indicate comparison within row between days after treatment. Concentrations with the same letter are not significantly different. Analysis through analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

**Data were not available for atrazine after 3 days contamination.

Table 6-2. Chlorpyrifos and atrazine concentration in leachates 56 days after Groseclose soil had been contaminated either with technical or formulated chlorpyrifos and atrazine

Soil treatment ²	Pesticides in leachate $\mu\text{g g}^{-1}$; (% of original) ¹				
	Initial	3 days	7 days	28 days	56 days
Technical Chlorpyrifos	4.39 \pm 1.1(100)A	4.37 \pm 1.2(99)A	4.44 \pm 0.3(101)A	3.73 \pm 1.2(85)A	3.58 \pm 0.5(82)A
Formulated Chlorpyrifos	4.41 \pm 0.3(100)A	2.51 \pm 0.6(57)B	2.45 \pm 1.1(56)B	2.45 \pm 0.6(56)B	2.54 \pm 0.3(58)B
Technical Atrazine	148.5 \pm 34(100)A	**	151.3 \pm 9(102)A	147.6 \pm 31(99)A	154.4 \pm 4(104)A
Formulated Atrazine	86.6 \pm 7(100)A	**	84.7 \pm 14(98)A	80 \pm 8(92)A	76 \pm 7(88)A

¹The results were expressed as the mean \pm standard error of three replicated samples. Technical chlorpyrifos (99.8%) and technical atrazine (99.8%) were solubilized in acetone prior to soil application. Formulated chlorpyrifos as Dursban[®] 4E and atrazine as AAtrex[®] 4L were applied directly to soil.

²Upper case letters indicate comparison within row between days after treatment. Concentrations with the same letter are not significantly different. Analysis through analysis of variance with Tukeys multiple range test on the means (Anonymous, 1985; P > 0.05).

**Data were not available for atrazine after 3 days contamination.

the same period. The major difference in extractability occurred the first three days after treatment (ca. 16%). Overall, approximately 27% of CSEC was unrecoverable by routine extraction after 56 days (Table 6-1).

A relatively small amount (less than 0.1%) of chlorpyrifos leached into water from soil samples treated with either technical grade or formulated chlorpyrifos after 4 h shaking (Table 6-2). There were no significant differences in the chlorpyrifos leachability measured over the 56 day incubation interval in the technical grade chlorpyrifos treated soil. However, the mean leaching rate was lower by approximately 18% during the incubation interval. On the other hand, significant differences were detected in chlorpyrifos leachabilities from formulated chlorpyrifos treated soil samples over the same time period. About 43% of chlorpyrifos leachability observed at the initial time was reduced during the first three days in this treatment, but remained at approximately the same level after that time.

6.3. Atrazine

At high concentrations, atrazine both in AAtrex[®] 4L and in acetone was difficult to transfer onto soil because it tended to leave a residue on the pipette surfaces. Therefore, during the transfer process, a small portion remained on the pipette surfaces, resulting in relatively lower initial atrazine concentrations than were intended. However, the delivery was done in a consistent manner across the replicated treatments reducing sample variability. The initial concentration of atrazine as AAtrex[®] 4L in soil was 2500 $\mu\text{g g}^{-1}$; and that for technical grade atrazine in the treated soil was about 3200 $\mu\text{g g}^{-1}$. Over the 56 day interval, there were no significant differences found in comparisons of the concentration of solvent extractable atrazine (CSEA) in either technical or formulated atrazine treated soil samples (Table 6-1).

Nearly 5% of the atrazine applied initially was leachable in both technical and formulated atrazine treated soils (Table 6-2). The initial atrazine leaching rate from soil treated with technical atrazine was approximately 150 $\mu\text{g g}^{-1}$, whereas the initial atrazine

leaching rate resulting from soil treated with AAtrex[®] 4L was 87 µg g⁻¹. The differences between these two rates are likely to be related to the actual amount of material that was applied to the soil samples (Table 6-1: 3236 vs. 2437 ug g⁻¹, respectively). Over 56 days, the leachability rates for both technical and formulated atrazine treated soils did not change (P > 0.5). However, the leaching rate declined by approximately 12% in the formulated atrazine treated soil.

6.4. Discussion

The information obtained from formulated (Dursban[®] 4E) and technical grade chlorpyrifos studies indicate that the surfactants and other adjuvants contained in commercial formulations can influence its extractability and leachability. However, this is not the case for formulated (AAtrex[®] 4L) and technical grade atrazine in terms of extractability and leachability. Since this experiment was conducted with relatively low soil moisture and undiluted formulated chlorpyrifos and atrazine, biotic degradation was minimized. Because of this, this study allowed for more precision in addressing the influence of the surfactants and other adjuvants in their formulations on chlorpyrifos and atrazine adsorption, extractability and leachability.

6.4.1. Effects of formulation on extractability of chlorpyrifos and atrazine

Based on the results from this study, less formulated chlorpyrifos was recoverable than formulated atrazine over an identical time period. Most unrecoverable chlorpyrifos might be considered to be the result of abiotic degradation, volatilization and binding/incorporation with the possibility of a high percentage of chlorpyrifos sorbed or incorporated into soil in formulation treated samples. Abiotic degradation probably was not important because the soil moisture was low (McGilvery, 1970). An increased amount of unrecoverable chlorpyrifos due to the effects of the surfactants and other adjuvants on volatilization in Dursban[®] 4E is unlikely to have occurred since formulation design protocol would include a plan to reduce volatility during and post application. Nevertheless, increased sorption/incorporation seems to provide a more appropriate explanation since some adjuvants in Dursban[®] 4E might increase its

absorption by soil minerals and organic materials and result in a relatively low solvent extractable chlorpyrifos.

There was little change with regard to the solvent extractable atrazine for treatments fortified either with technical atrazine or formulated atrazine (AAAtrex[®] 4L). The results are quite different with that obtained for chlorpyrifos. The difference most likely can be explained on the basis of the differences between the formulations used. The formulated chlorpyrifos (Dursban[®]4E) is an emulsion while atrazine formulation (AAAtrex[®] 4L) is a liquid solution which in this case has characteristics similar to that of a flowable (personal communication, Novartis). Once these formulations were applied to soil, there was a dynamic adsorption-desorption equilibria between the formulated compounds (chlorpyrifos and atrazine) and soil matrices (soil minerals, organic matter, lignocellulosic materials, etc.). In the case of chlorpyrifos, the equilibria should have favored adsorption to the soil matrices due to its relatively lipophilic properties. Furthermore, the surfactants in emulsions may facilitate chlorpyrifos molecules to partition into hydrophobic sites in soil organic matter or micropores in soil minerals. Afterwards these emulsions were broken by soil minerals, hence chlorpyrifos was strongly sorbed by soil matrix. However, the equilibria resulting from atrazine presentation as a particulate did not favor soil solid particulate phase. As a result, less residue would be sorbed to soil matrices, and would be extracted by organic solvents more easily.

6.4.2. Effects of formulation on leachability of chlorpyrifos and atrazine

The results indicated the surfactants and other adjuvants in chlorpyrifos emulsified concentrate had a significant influence on the reduction of chlorpyrifos leachability from contaminated soil. A similar trend was found in formulated atrazine-treated soil samples although it was not statistically significant. Surfactants are generally considered to be agents that are capable of increasing the suspensibility of hydrophobic compounds and therefore increase their leaching potential from soils (Aronstein and Alexander, 1992). That was not the case in this study. The reason may relate to the

specific design or difference of the formulations used for this study. In addition to the surfactants, other adjuvants have been added into these commercial products to decrease leachability, and to increase stability, etc. Some additives such as acrylic polymers and starch were able to effectively reduce atrazine leaching (Fleming et al., 1990). Therefore, those polymer additives, if they exist, would tend to counteract the effects of the surfactants in the Dursban[®] 4E and AAtrex[®] 4L.

Similar to the discussion presented in Section 6.4.1., the difference of chlorpyrifos and atrazine leachability might be explained by the difference in their adsorption by soil matrices. Chlorpyrifos was more strongly sorbed to soil matrices after the disruption of its emulsion while atrazine was only loosely sorbed to soil matrices. Hence, it appears that chlorpyrifos leachability was substantially decreased a few days after its application, but atrazine leachability was not because it was more easily displaced by the leaching test. The overall reduction in leachability appears to have been primarily associated with the dissipation of the atrazine concentration in the soil.

6.4.3. Relationship between extractability and leachability

There are some interesting interrelationships between the extractability and leachability of chlorpyrifos and atrazine, which need to be discussed. Comparisons of extractability and leachability rates of chlorpyrifos and atrazine are presented as percentages of initial rates in Figures 6-1 and 6-2, respectively. Results from Figure 6-1 indicate that the treatments with technical chlorpyrifos (extractability and leachability) had slightly decreased (but not significantly) after 56 days while the treatments with formulated compound had declined during the same time period. The leachability had a higher reduction rate than the corresponding extractability, especially within the first 3 days after the experiment initiated. The chlorpyrifos leachability was only 57% of the original level compared with 84% of the original level for chlorpyrifos extractability. The results are consistent with that obtained from the study described in Chapter 4 and provide additional evidence that the surfactants and other adjuvants in chlorpyrifos

formulation may account for the sharp decrease in its leachability after formulated chlorpyrifos was applied /spilled to soils.

The information from this atrazine extractability and leachability study indicated that no significant effects of the adjuvants in atrazine formulation had been observed based on the extractability and leachability rate (Figure 6-2). This result is also in conformity with that obtained from the study described in Chapter 5. Atrazine formulation has only a slight influence on its leachability from contaminated soil, moreover, this effect might take a relatively long time to be observed. Leachability was reduced by 12% over a 56 day experimental time period. The extractability may undergo a little change without enhanced microbial-mediated degradation and sorption/incorporated by organic-based material amendment such as that described in Chapter 5.

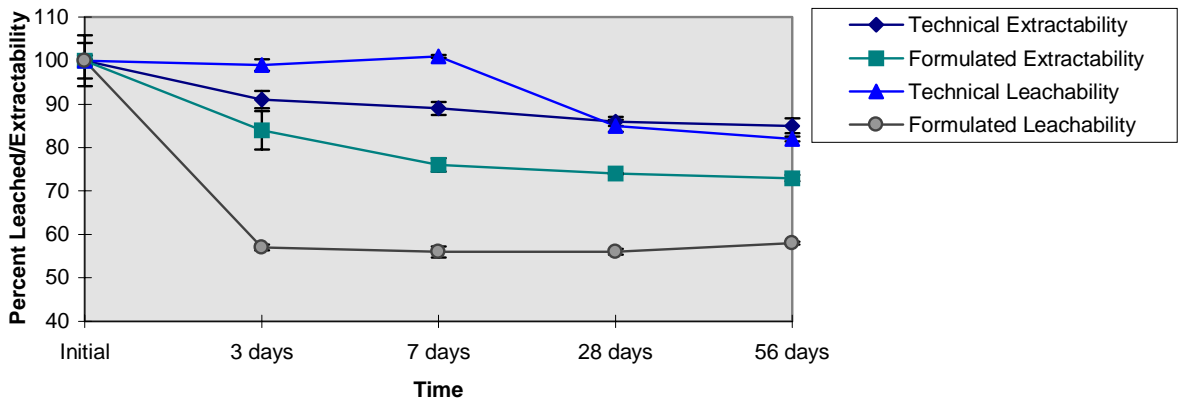


Figure 6-1. Comparison of chlorpyrifos extractability and leachability rates in contaminated soil

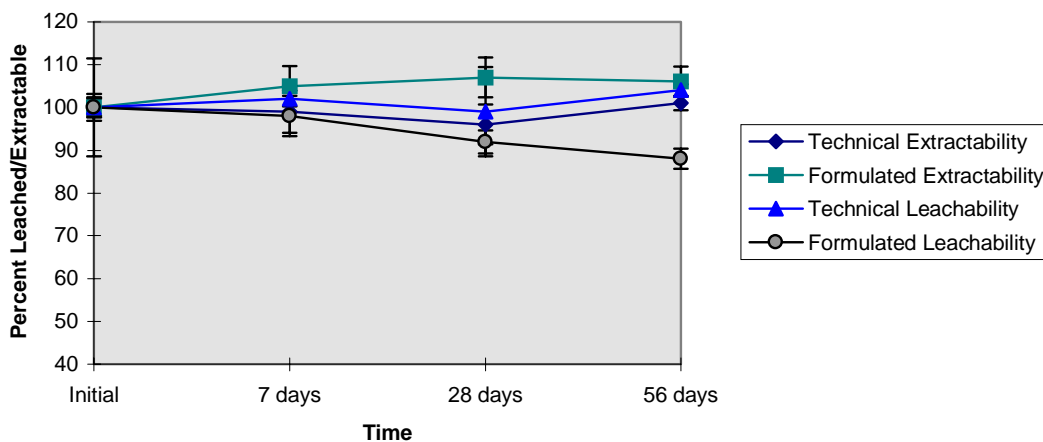


Figure 6-2. Comparison of atrazine extractability and leachability rates in contaminated soil

In summary, the results provided by this experiment support those provided in the studies described in Chapter 4 and 5. The adjuvants in Dursban[®] 4E had significant effects on chlorpyrifos leachability and extractability in a short time period. Chlorpyrifos leachability decreased at a faster rate than the corresponding extractability. Both technical grade atrazine and formulated atrazine were stable at high concentrations with little biodegradation and incorporation. Its leachability was slightly reduced by the influence of its formulation, presumably associated with its particulate form as AAtrex[®] 4L.