Evaluating the Hazard of Land Applying Composted Diazinon Waste Using Earthworm Biomonitoring

by

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Abstract

A process for disposing of pesticide rinsewater generated from the rinsing of application equipment is being developed at Virginia Polytechnic Institute and State University. This process involves the sorption of pesticides onto an organic matrix followed by degradation in a composting environment. We are now evaluating the hazards that might be associated with land-applying composted pesticide waste. Diazinon was the first pesticide selected for evaluation, which consisted of two studies. The first used the earthworm species *Eisenia foetida* to evaluate the toxicity of soil amended with composted diazinon waste. The second study determined the bioavailability of D-2-^{14}C-diazinon and its degradation products to *E. foetida* in soil amended with composted D-2-^{14}C-diazinon.

Results from the first study indicate that uncomposted diazinon sorbent and 30-day composted diazinon sorbent were toxic to *E. foetida* at sublethal and lethal levels. However, *E. foetida* exposed 60-day composted diazinon sorbent did not experience mortality or demostrate sublethal effects commonly associated with acetylcholinesterase inhibition.

Earthworms exposed to diazinon that was uncomposted or composted for 30 days in the radiolabelled study experienced higher mortality than in the field study. After 30 and 60 days of composting ^{14}C-diazinon became unextractably incorporated into organic matter and very little was mineralized. Earthworms were shown to accumulate radioactivity when exposed to soil amended with 60- day composted D-2-^{14}C-diazinon. The majority of this radioactivity was unextractably bound to earthworm tissue and that which was extractable contained only trace levels of D-2-^{14}C-diazinon. Based on the absence of toxicity in the field study and the low levels of ^{14}C-diazinon present in earthworm tissues, 60 days of composting appears to greatly reduce the hazard that diazinon rinsate poses to *E. foetida*.

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1. Introduction

Pesticide applicators can generate large volumes of pesticide-contaminated water from the rinsing of spray equipment. This material will almost certainly contain pesticide concentrations that exceed maximum allowable concentrations for disposal into a water body based on the Clean Water Act. Legal options for disposal of this material include this material respraying at field label rates, on label-approved crops, or disposal as hazardous waste. Because of accidental releases and improper management of pesticide waste, frequent high contamination of soil and groundwater at and around pesticide mixing-and-loading facilities continues to be a problem (Barbash and Resek, 1996). Therefore, there continues to be a need for systems to contain pesticides spilled during the mixing-and-loading process, as well as inexpensive, efficient processes for disposing of rinse water from pesticide equipment.

A process for the disposal of rinsewater generated from the clean-up of pesticide application equipment is being developed at Virginia Tech. This process uses organic sorption, followed by biological and chemical degradation (composting) to sorb and degrade pesticides contained in rinsewater. It has been tested on several formulated pesticides and shown to be effective at removing pesticides from rinsewater and subsequently degrading these sorbed residues (Hetzel et al., unpublished data). The treated pesticide rinsewater typically contains concentrations of pesticide in the low μg/mL to ng/mL, thereby representing an approximate thousand-fold reduction in the concentration of the original rinsate. The concentration of this treated rinsewater is likely to be lower than what would be found following the third rinse of pesticide spray equipment. Options for disposal of this treated rinsewater are relatively clear. If concentrations of pesticide are low enough, an National Pollution Discharge Elimination System (NPDES) permit could be obtained to discharge the treated rinsewater. However, because the concentrations of pesticides required for such a permit are typically below 1 ng/mL, the treated rinsewater would likely need to be re-used to rinse subsequent application equipment or resprayed at label rates on label-approved crops.

Options for disposal of the composted pesticide waste present a more difficult problem. These options include; reusing sorbent materials in the rinsewater treatment process, incinerating sorbent material or burning it on site, or land applying the sorbent material. We are now in the process of evaluating options for disposing of the composted pesticide waste and we have examined the possibility of reusing this material as sorbent for subsequent pesticide rinsates. This reused material, however, blocked filters during the process due to its relatively fine particle size. Although this is a technical problem that could likely be remedied, at some point this material would need to be disposed of in an appropriate manner. Incinerating the material would be a safe option. The advantage of sorbing the pesticide rinsate onto an organic matrix before transporting it to incineration facilities would be the added safety in transport. This option, however, would likely not be cost effective. The material could be burned on site rather than transporting it to a specialized incineration facility. Inefficient combustion of the material, however, may result in volatilization of a pesticide or its metabolites, as well as a residue in the ash. The most likely option for disposal is to land apply the composted pesticide waste. Before this could be accepted as a viable option, the impact of the composted pesticide waste on terrestrial plants and animals as well as the potential for leaching needs to be evaluated. The Toxic Compounds Leaching Procedure (TCLP), recommended by the United States Environmental Protection Agency (USEPA), provides an evaluation of leaching potential. This method is designed to simulate the physical conditions at the bottom of landfills and may overestimate leaching potential, but is the most accepted procedure to date.

Chemical analysis of the composted material may be used to provide information on the extractable concentration of pesticide and common metabolites. There are some difficulties with using this information as a sole criterion in evaluating the hazard that these composted pesticide wastes present to terrestrial ecosystems. First, even if the pesticide degradation products can be predicted and isolated, their toxicity to beneficial soil organisms is frequently unknown. Second, environmental fate studies using radiolabelled material have demonstrated that a substantial fraction of parent compound or degradation product may become unextractably bound to organic matter and, therefore, is unidentifiable by routine analytical chemistry. Although these residues are unable to be extracted by rigorous methods, in some cases they may still be bioavailable to beneficial soil invertebrates, such as earthworms (Fuhremann and Lichtenstein, 1988; Haque et al., 1982; Ebert, 1992) but not in all cases (Riley, 1986). When considering land applying the composted pesticide material the federal Resource Conservation Recovery Act (RCRA) regulations need to be consider. The application of RCRA to the land application of pesticide contaminated soil may provide some guidance for our disposal interests.

The earthworm species *Eisenia foetida* (Savigny) was selected as a representative beneficial soil macroinvertebrate. The European Economic Community (EEC), the Organization for Economic Co-operation and Development (OECD), the U.S. EPA, and the American Society for Testing and Materials (ASTM) recommend the use of *E. foetid* for standardized laboratory soil toxicity studies (EEC, 1985; OECD, 1984; Greene et al., 1989; ASTM, 1995). *E. foetida* is susceptible to the toxic action of many xenobiotics, however, there is good evidence that it is among the more hardy of earthworms. (Roberts and Dorough, 1985).

Diazinon was selected for use in these studies for several reasons. The work of Petruska et al. (1985) generated a considerable amount of information on the fate of ^{14}C-diazinon in the compost environment. In previous field studies using diazinon, it was found to have a moderate half-life in the composting environment. However, radiolabelled fate studies indicated that a substantial amount of the radioactivity was unextractably bound to the compost material (Petruska et al., 1985; Frederick et al., 1996). Evaluating the bioavailability of diazinon in compost is, therefore, relevant even if it is found in low concentrations in the extractable fraction. The primary hydrolysis product, 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) is known to be practically non-toxic. The low toxicity of IMHP makes the interpretation of toxicity results from this study less complicated.

This research consisted of two studies. The first study was a field study designed to evaluate the toxicity of composted diazinon to earthworms, *E. foetida*. The second study was a laboratory-scale radiolabel study designed to examined the bioavailability of diazinon and its degradation products in soil amended with composted Δ-2-^{14}C diazinon.

2. Literature Review

2.1 Review of Groundwater Contamination by Pesticides

2.1.1 Pesticides in U. S. Ground Water

Barbash and Resek (1996) provided an excellent review of pesticides in U. S. groundwater. Groundwater is used by approximately 50% of the nation's population. In agricultural areas over 95% of the population rely on groundwater for drinking water (Barbash and Resek, 1996; review Moody et al., 1988). There are two principal concerns regarding contamination of groundwater by pesticides. First, elevated concentrations of pesticides in groundwater may make it unsuitable as drinking water. Second, contaminated groundwater may spread to surface waters where it may have an impact on aquatic ecosystems and drinking water.

Pesticides from every major class have been detected in meteoric, surface, and groundwater, but have been detected in ground water more than any other part of the earth's hydrosphere (Barbash and Resek, 1996). Pesticide concentrations in groundwater resulting from non-point source pollution are generally very low. "The concentrations of most agricultural pesticides have been found to be below existing criteria established for protecting human health in over 99 percent of the wells sampled in agricultural areas (Barbash and Resek, 1996)". Pesticide concentrations from point sources, however can be much higher, and in intensive agriculture areas there may be considerable overlap with those contaminated by point sources (Barbash and Resek, 1996; review Baker et al., 1989).

Pesticides in ground and surface water that result from point source pollution may be at much higher concentrations. In addition, contaminated ground and surface water from point sources in heavy agricultural areas may be so common that Hallberg (1986c) coined the term quasi-point source to describe the widespread effect. For example, "in intensive agricultural areas, such as corn-belt states like Illinois or Iowa, there are over 1500 potential point-source type sites because of the dominance of agriculture throughout these states (Hallberg, 1989; reviews Hallberg, 1986c, 1988a; Long, 1988)." Point source pollution by pesticides arises from the routine handling of pesticides including manufacturing, transport, mixing, and loading. Essentially at any stage that concentrated pesticide is being handled there is a risk of accidental release or improper disposal.

In the immediate vicinity of local farm chemical supply dealerships pesticide concentrations in wells have been high enough to warrant closing the wells in several states (Hallberg, 1986a; reviews Hallberg, 1985; 1986c; Holden, 1986). These situations were generally not cases where anyone was clearly suspected of negligence (Hallberg, 1986a). In these cases, pesticide concentrations in local groundwater increased by 100 times or more (Hallberg, 1986a). Beneath agrochemical facilities maximal concentrations of pesticides in surficial soils typically exceed 1,000 μg/kg for most of the compounds detected. "Hallberg (1985) noted that pesticide concentrations in soils and ponded surface waters near handling, loading, and equipment rinsing areas at agrochemical handling facilities in Iowa are often as high as those in the original formulations (Barbash and Resek, 1996; review Hallberg, 1986a)." The high variability of pesticide concentrations in soils and groundwater beneath mixing-and-loading facilities indicates that management practices greatly effect levels of contamination (Barbash and Resek, 1996). The study by Habecker (1989) concluded that "spillage is an ongoing problem especially in the mixing/loading and pesticide equipment parking areas" (Barbash and Resek, 1996; review Habecker, 1989).

In addition to detecting pesticides, adjuvants used in pesticide formulations and transformation products have been detected in groundwater (Barbash and Resek, 1996; review Garrett et al., 1976; Kelley, 1985; Kelley and Wnuk, 1986; Hallberg, 1988; Steichen et al., 1988; Cherryholmes et al., 1989; Domagalski and Dubrovsky, 1991; and Kolpin and Thurman, 1995) as well as beneath agrochemical mixing-and-loading facilities (Barbash and Resek, 1996; review Long, 1989). The relatively slow rates of transformation of surfactants, coupled with their comparatively low affinities for soil organic carbon makes them likely contaminants of ground water. Transformation products may, at times, be in higher concentrations than the parent pesticide. Analysis for pesticide transformation products has historically been neglected; it is, however, becoming more common. In many areas, transformation products are detected more frequently than parent compounds in groundwater. "It is now widely recognized that a decrease in the measured concentration of a pesticide in the subsurface over time--i.e., its "dissipation"-is rarely caused by its complete mineralization to simple substances such as water, carbon dioxide, inorganic ions and nitrogen gas. Instead, the dissipation of a pesticide *in situ* arises from the simultaneous action of a variety of processes that usually lead to relatively minor modifications in its structure, or its transport away from the point of measurement. (Barbash and Resek, 1996)"

2.1.2 Factors Affecting Groundwater Contamination

The greatest factor impacting pesticide detection in groundwater is its use patterns. "In general, the compounds for which detections were reported by both the NPS (National Pesticide Survey) and the PGWDB (Pesticides in Ground Water Database) are among those that have been used most extensively (Barbash and Resek, 1996)". However, the factors affecting pesticide mobility that are most relevant to our disposal system are those related to organic matter/pesticide interactions.

Creeger (1986) considers a pesticide a potential threat to groundwater if the chemical possesses one or more of the following criteria: a water solubility >30 mg/L; a partition coefficient < 5; a hydrolysis half-life > 25 weeks; a soil half-life > 2 to 3 weeks (Kuhn and Suflita, 1989; reviews Creeger, 1986). The tendency of a pesticide to interact with subsurface solids is described by partitioning coefficients keyed to a specific soil (K_p) or normalized to soil organic carbon (K_{oc}) (Barbash and Resek, 1996). The tendency for a solute to interact with the non-aqueous phase strongly influences the rate at which the solute moves through the soil, the concentration in the aqueous phase, and the degree to which the non-aqueous phase serves as a repository for the solute (Barbash and Resek, 1996). This interaction may be predicted by soil organic-carbon partition coefficient, or K_{oc}, where $K_{oc} = K_p / f_{oc}$. K_p is the equilibrium partitioning coefficient between the aqueous phase and subsurface solids and f_{oc} is the mass fraction of organic carbon in the soil. For soil with f_{oc} values of > 0.001 (i.e., when the mass fraction of organic matter in soil exceeds 0.1 percent), retardation of organic solutes in soil is dominated by natural organic carbon (Barbash and Resek, 1996; review McCarty et al., 1981; Schwarzenbach and Westall, 1981). Large quantities of organic matter in soil reduce the leaching of pesticides through the combined effects of enhanced microbial activity (Barbash and Resek, 1996; review Hurle and Walker, 1980; Felsot et al., 1982; Nash, 1988; Goetz et al., 1990; Harper et al., 1990; and Norris et al., 1991) and hydrophobic sorption.

Pesticides that would otherwise not be expected to be found in groundwater may be carried by dissolved organic matter to groundwater, a phenomenon known as colloid transport (Barbash and Resek, 1996). This is relevant to disposal of the treated pesticide rinsewater as well as composted pesticide material. Treated pesticide rinsewater from our system certainly contains high concentrations of water soluble organic matter. Composted pesticide waste would also be expected to contain large amounts of water soluble organic matter that may leach if this material were land applied. Hydrophobic compounds may also reach ground water by nonequilibrium transport through porous medium under saturated conditions, a phenomenon known as preferential transport (Barbash and Resek, 1996).

Other factors will influence the potential for groundwater contamination. The formulation is one factor. For example "controlled-release" formulations may retard the release of pesticides into soil water (Barbash and Resek, 1996; review Bayer, 1966). The geology of the aquifer is another factor. Shallow, unconfined, and unconsolidated aquifers and mature karst areas are the hydrogeologic environments most susceptible to pesticide contamination. The design and age of the well is also an important factor. Springs and dug, bored or driven wells show consistently higher frequencies of pesticide detection than do drilled wells (Barbash and Resek, 1996).

Developing more efficient means of delivering pesticides to target pests may dramatically reduce groundwater contamination in the future. Although only a fraction of the pesticide applied through routine agricultural activities reaches ground and surface water, this fraction is comparable to the amounts that typically reach target pests (Hallberg, 1986). This underscores the relative inefficiency with which pesticides are currently delivered to target pests and demonstrates the need for more efficient methods.

2. 1.3. Diazinon in Ground and Surface Water

The ranges of observed concentrations for diazinon in groundwater, as reported by the Pesticide Ground Water Data Base (PGWDB), were 0.01-3.2 μg/L and the frequency that the concentration in sampled wells exceeded the health advisory limit of 0.6 μg/L was 0.051% (no MCL has been set for diazinon) (Barbash and Resek, 1996). According to the PGWDB, 42 of the 3,884 wells tested contained detectable levels of diazinon (Barbash and Resek, 1996). The low persistence and low application rates for organophosphates may explain why this class of compounds is not frequently detected in ground water (Barbash and Resek, 1996).

Although diazinon is infrequently detected in ground water, in surface water and municipal water, particularly in urban areas, it may present a considerable problem. The Trinity River Basin NAWQA study-unit team collected 80 samples from urban streams in the Dallas-Fort Worth metropolitan area and 80 samples from agricultural streams (Land, 1996). Diazinon was detected in all the urban samples and about 60% of the agricultural samples. It was also the only insecticide to exceed HA levels. It exceeded this level in

15% of the samples. In most of the agricultural streams diazinon concentrations were at or near the method of detection limit.

Municipal waste water treatment plants, have been having difficulty with diazinon contaminated effluents. Over the whole U. S., less than 1% of Publicly Owned Treatment Works (POTWs) have experienced failures due to diazinon. Nonetheless, such failures can prove to be extremely expensive (Merchant and Gugliuzza, 1987). Failure of Fort Worth to meet CWA standards could result in fines of up to $25,000 per day. Conventional waste water treatment plants are designed to remove sewage, not chemical contaminants and diazinon at low levels may pass through these plants unaltered (Tierney, 1993). Since the passage of the Clean Water Act in 1988, wastewater treatment plants with capacities greater than one million gallons per day must monitor wastewater effluent using biomonitoring techniques as part of TREs (toxicity reduction evaluation) (Merchant and Gugliuzza, 1997). These bioassays are very sensitive to low levels of diazinon. Concentrations as low as 0.35-0.6 ng/ml can cause acute toxicity to one of the test species, *Ceriodaphnia dubia.* The most likely source for contamination of municipal waters is related to consumer or commercial dumping of excess formulated material into sewers. Stormwater contamination could also be occurring from misdirected applications to storm water gutters, consumer dumping, and landscape runoff, among other sources (Texas A&M, 1993). The city has been working with the EPA to meet CWA through a city-wide education plan designed to prevent pesticide pollution of wastewater (Merchant and Gugliuzza, 1987).

2.2 Options for Disposal of Pesticide Waste Water

Noyes (1992) states "the best way to minimize pesticide waste is to not create it”. In addition to careful calibration, there are several technologies under development that hope to minimize the amount of pesticide applied and apply it in such a way to generate no rinsate. Direct injection at the nozzle is a technology that would eliminate tank mixing and reduce rinsate through mixing of the formulation and diluent at the nozzle. Other technologies hope to reduce the amount of non-target contamination (e.g., reduced-volume application coupled with auxiliary electrical or aerodynamic forces; target-sensing sprayer controllers; and electrostatic charged droplets).

New technology that reduces the amount of pesticide waste generated, however, is still far from eliminating waste. There continues to be a need for environmentally safe, efficient methods for disposing of pesticide rinsewater (Seiber, 1992). Current options for disposing of pesticide rinsates include respraying, disposal as hazardous waste, and treatment with carbon sorption, or evaporation / degradation tanks. Several states require bulk storage facilities that include washing and mixing/loading bulk storage facilities within containment to capture wastewaters (Loundsbury, 1992). The most commonly used option for this captured wastewater is to respray the material as dilute solution or use as a diluent for subsequent pesticide batches. Legally, this must be done at label rates on label approved crops (Dwinell, 1992). Disposing of pesticides as hazardous waste, treatment with carbon sorption, or evaporation/degradation tanks are options that are less widely used because of the expense and difficulties in complying with regulations (Dwinell, 1992).

Each of these options comes with important legal considerations as well. The first legal issues that must be considered when managing pesticide rinsates, involve the storage of the rinsate. "Pesticides that are individually separated and identified, or that are in an identified, target compatible reusable mix are considered rinsates, not hazardous waste, by EPA. There is no limit on storage time of rinsates. However, it is best to store only what you can use immediately. Rinsates can be used as make-up water for upcoming loads of identical field mixes to be sprayed on label registered target crops. EPA considers mixed or combined rinsates that are not identifiable, as hazardous waste that must be legally disposed of within 90 days by a licensed hauler and a toxic waste facility" (Noyes, 1992).

There have been technological advances that make the respraying of rinsates far less labor intensive. For example, aerial applicators now have the option of rinsing and spraying their equipment in air, while they are still at the site. "Currently, at least two companies market 20-23 gallon in-hopper rinsing systems for in-flight hopper and plumbing 'triple-rinsing' that greatly improves pesticide application efficiency by spraying the rinsate on the target during the final trip. This minimizes or eliminates rinsate handling on mixing loading sites. (Noyes, 1992)"

The two options that are acceptable by federal regulations for treating pesticide rinsates are carbon filtration and evaporation/degradation pits. The cost of carbon filtration systems is $20 to $50 thousand dollars, with an operating cost of approximately $500 to $1,000 per disposal of filters and sludges (Dwinell, 1992). For some large pesticide application operations this is economically feasible. Industry sources estimate about forty GAC filtration systems are in use for pesticide rinsewater treatment (Dwinell, 1992). The capital cost of evaporation/degradation tanks is in the range of $20,000 to $50,000, and minimal operational cost during its lifetime (Dwinell, 1992). The lifetime of the system, depending on its durability, should be 15 to 20 years (Dwinell, 1992). When the system is dismantled the soil must be tested for hazardous residues. If it contains compounds listed as hazardous waste the soil must be disposed of as hazardous waste. If it does not exhibit hazardous characteristics the soil may be disposed of by land application. The greatest difficulty with this system, therefore, is that these systems may have a day of reckoning when the soil must be disposed of and tested for hazardous waste. Another problem encountered with these systems is the rate of evaporation may not be fast enough, particularly at northern latitudes (Dwinell, 1992).

These treatment systems must comply with two federal regulations, the Clean Water Act (CWA), and the Resource Conservation and Recovery Act (RCRA) (Dwinell, 1992). Before any treated waste water may be released from a treatment system, a National Pollution Discharge Elimination System (NPDES) permit is required. This permit specifies the amount of water, the location of the discharge and the quality of the water (Dwinell, 1992). To obtain a National Pollution Discharge Elimination System (NPDES) permit, however, these pesticide residues may need to be below one part per billion. As a result, pesticide rinsate treatment systems are designed to eliminate the need to discharge treated rinsewater. Carbon filtration systems are designed to be closed loop systems where the treated rinsate is used in subsequent tank rinses. Evaporation / degradation tanks eliminate wastewater discharges by evaporation. Pesticides that are designated hazardous waste by RCRA, and pesticide rinsates that are combined and not identifiable, are considered hazardous waste by the EPA and are regulated under RCRA. In addition, any of the equipment used to treat the hazardous waste is also considered hazardous waste. The disposal of this waste is extremely expensive (Dwinell, 1992). In developing our pesticide rinsate disposal system we will also need to consider these regulations.

2.2.1. Alternatives for Disposal of Rinsates

There has been extensive research on other alternatives for treating pesticide wastes. To date none of these technologies are available to the agrochemical community, largely because of difficulties associated with field scale operation and federal regulations. It has proven difficult for many of these technologies to be scaled-up from laboratory conditions due to cost considerations or technical problems. Hapeman-Somich (1992) reviews these developing technologies, which include; chemical degradation, high energy lamps, infrared incineration, electrolysis, ozonation, and biological degradation and sorption.

In developing new technologies for field pesticide waste disposal technologies, Seiber (1992) states that the following items would be desirable in the research, development, and demonstration phases: 1) simple chemical and bioassay tests to evaluate effluents; 2) side-by-side comparison tests of two or more competing technologies; 3) clear-cut regulatory targets, so that we will know for what we are aiming.

2.3. Contaminated Soil

Past uncontrolled disposal practices at agrochemical facilities, which have caused high levels of soil and groundwater contamination, continue to present a serious problem (Fritz, 1992). Many agrochemical dealers have, in most cases inadvertently, contaminated soil and water at their dealerships with pesticides (Myrick, 1992). Options for disposal of these contaminated soils are very expensive, particularly in soils containing pesticides listed as hazardous waste under RCRA. Cleanup of soil contaminated with hazardous waste from a single facility could cost from one to five million dollars. Those pesticides listed as hazardous waste under RCRA do not have "de-minimus" values assigned to them, which means that they are considered hazards at any concentration. In addition, if drinking water has been contaminated, the responsible party is liable under state and federal law. Providing alternative sources of drinking water during the clean-up process can be extremely expensive. (Buzicky et al., 1992)

Options for treating soil contaminated with hazardous materials include: excavation and burial in a chemically secure landfill, vapor extraction, chemical precipitation, vitrification, thermal desorption, and incineration (Skladany and Metting, 1993; review U.S. EPA, 1988). Many of these treatments do not destroy the contaminants. They are rather bound in a modified matrix or transferred from one phase or location to another. One alternative option being considered for disposal of pesticide contaminated soil is the land application of the pesticide-contaminated waste at FIFRA-approved label rates. Landfarming composted pesticide waste generated by our disposal process may have similar considerations to that of contaminated soil. Under the land application approach the soil is viewed as a "new inert diluent" of the pesticide serving as a carrier for the active ingredient (s) within the pesticide formulation. Landfarming may be legal under RCRA because it represents reuse of the product and the EPA believes it should be legal under FIFRA because the material is applied at label rates on label-approved crops. This approach is very dependent on the location and pesticide mixture of the soil. Landfarming was made legal in Illinois (Felsot et al., 1992). ..."Although many states are considering regulations allowing landfarming for disposal of pesticide-contaminated soils, the effectiveness of the technology, methods for stimulation of degradation rates, and possible off-site movement of contaminants has hardly been studied" (Felsot, 1992).

Felsot (1992) designed a study to compare herbicide behavior following landfarming of different rates of contaminated soil with herbicide behavior following conventional application by spraying at the same rates. The following four criteria were used to determine if remediation of pesticide-contaminated soil using land application was successful; 1) pesticide residues in positive controls (conventionally treated plots) and contaminated soil-treated plots (landfarmed plots) after two growing seasons do not differ significantly; 2) contaminant concentrations and toxicity in leachates and runoff water are at levels not significantly different from or are even lower than concentrations and toxicity in positive controls; 3) crop phytotoxicity is not greater than expected from conventional sprays; 4) residues of pesticides in crops should not violate established U.S. EPA tolerances. Land application of composted pesticide material from our rinsate disposal system may use the same justification. With proper management of this material, including separate storage and proper labeling of the compost material, the material would be better characterized than contaminated soil from a waste site or from an evaporation/degradation pit.

2.4. Bioremediation: General Overview

The Superfund Amendments and Reauthorization Act (SARA) 1993 encourages the selection of alternative remedies for the clean-up of contaminated sites (Ritter and Scarborough, 1995). Bioremediation is one alternative strategy. There are two fundamental approaches to bioremediation; the microbial approach, where a site or contaminated material is augmented with one or more species of contaminant specific microorganisms; and the microbial ecology approach, where physical and chemical factors are adjusted to enhance degradation by the natural consortium of microorganism (Ritter and Scarborough, 1995). A third approach allows the intrinsic properties of the soil to degrade the toxicant without addition of nutrients or microorganisms.

Bioremediation may further be classified as '*in situ*' or '*ex situ*'. *In situ* bioremediation involves adding a biological agent, such as enzymes or microorganisms (bioaugmentation) or adding nutrients to stimulate biological degradation, to the contaminated soil without the use of dig-and-haul technology. *Ex situ* bioremediation involves the removal of the contaminated soil before treatment (Skladany and Metting, 1993).

Contaminants may be either metabolized by biological agents or co-metabolized (Felsot, 1989). When a contaminant is metabolized, the contaminant provides metabolic energy for the biological agent. This process is typically growth linked, unlike co-metabolism (Alexander, 1981). The phenomenon known as enhanced pesticide degradation is the result of metabolism (Felsot, 1989). When a contaminant is co-metabolized, the contaminant does not provede metabolic energy for the biological agent. Because co-metabolism is not a growth linked process degradation of the contaminant is typically slow. Plants and animals are able to metabolize contaminants but these transformations are typically modest compared to the effects of heterotrophic bacteria and fungi (Alexander, 1981).

The degradation of a toxic compound by microorganisms typically will detoxify the compound, however, some compounds may also be activated to more toxic compounds by microorganisms (Alexander, 1981). The dominant paradigm in pollution control and site remediation industries equates bioremediation with mineralization (Skladany and Metting, 1993). Most currently used pesticides are able to be biologically transformed and, in some cases, completely mineralized (Karns, 1992). Enzymes have been shown specifically degrade pesticides and lignin-peroxidase enzymes have been shown to degrade pesticides by non-specific mechanisms (Karns, 1992).

It is impractical to discuss all the factors that will influence biotransformation rates of pesticides in soil and organic matter. A few of the major factors that will influence biotransformation rates include 1) presence of an appropriate microorganism or microbial consortium, 2) availability of the contaminant of interest to the microorganisms or consortium, and 3) provision of suitable environmental conditions (Skladany and Metting, 1993; review Anderson, 1989). Although absolute recalcitrance is likely only a property of synthetic polymers such as polyethylene, PVC, etc. pesticides may be very recalcitrant in specific environments or highly degradable in others (Alexander, 1981). Environmental factors on which biotreatability studies most often focus are; 1) proportions and availability of contaminants, supplementary substrates, and nutrients; 2) moisture requirements; 3) O_2 requirements; 4) temperature; and 5) the presence of inhibitory compounds or other limiting factors (Skladany and Metting, 1993). There are several explanations as to why a chemical may be refractory to microbial destruction: first is that "biological evolution has pursued a narrow range of chemical pathways", therefore, "if no enzyme has evolved to convert a molecule to an intermediate in an existing biochemical pathway, it is unlikely that the compound will be acted on biologically"; second is if a compound is unable to penetrate a microbial cell and extracellular enzymes cannot act on the compound; third, if the concentration of a compound or energy value is too low for a microbial species, otherwise able to degrade the compound, will not be able to sustain itself; forth, "a number of compounds that are normally readily degradable are rendered resistant when they are complexed with resistant organic materials”. (Alexander, 1981)

Biological treatment of contaminated soils faces four major challenges (Skladany and Metting, 1993): 1) waste heterogeneity; 2) concentration effects: too low of a concentration may not adequately support microbial growth or stimulate the induction of suitable enzymatic systems, high concentrations may inhibit microorganisms; 3) compound persistence or toxicity; 4) maintaining conditions suitable for microbial growth, this is particularly true for *in-situ* applications.

2.5. Review of Composting

Haug (1980) defined composting as, “the biological decomposition and stabilization of organic substrates under conditions which allow development of thermophilic temperatures as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land without adverse environmental effects" (Poprasert, 1989; reviews Haug, 1980). "The ecological response to various physical factors in composting systems can be compared with what would be observed in soil ecosystems, except for the greatly compressed time context."..."In soils, physical factors, such as temperature, moisture content, or bulk density, are largely imposed externally, whereas, in composting systems, these physical qualities are a consequence of internal events (Miller, 1993)".

The main objectives of composting are to stabilize organic waste, destroy plant and animal pathogens, reclaim nutrients and soil, and dry sludges (Poprasert, 1989). Composted material may be used in agriculture and horticulture, the production of selective substrates for mushroom cultivation; alternatively the composting process may be used in the treatment of organic wastes including, leaves, sludges, refuse, hazardous wastes, etc., which may or may not be carried out with the production of a compost product (Miller, 1993).

Aerobic compost typically goes through the following stages (Poprasert, 1989). At first there is a latent phase, during which microorganisms need to acclimatize to and colonize the fresh compost. Microbial metabolism then warms the compost to the mesophilic level. At this stage bacteria are predominant. Common genera of bacteria found in compost include *Bacillus, Clostridium,* and *Pseudomonas* (Miller, 1991). Additional metabolic heat warms the compost to the thermophilic phase. During the thermophilic phase temperatures are at their highest, and pathogen destruction and waste stabilization are most effective. Waste stabilization is mainly the result of bacteria, which are the main decomposers of proteins and carbohydrates (Polprasert, 1989; reviews Strom, 1985a). As readily available nutrients become less available, metabolic heat will decrease and the compost will cool. Fungi and actinomycetes will develop in the compost following the decline in readily available nutrients, to degrade more resistant organic materials such as cellulose and lignins. During this maturation phase, second level consumers will move in to feed on the microbial populations. These include mites, beetles, nematodes, protozoa, rotifers, diptera, isopods, snails, slugs, collembola and earthworms. The various feeding guilds of mites effect the decomposition of organic matter (Siepel and Maaskamp, 1994). Third level consumers will move in to feed on the secondary consumers. These include; centipedes, rove beetles, carabid beetles, pseudoscorpions, flatworms and ants. Actinomyces becomes the dominant group of microorganisms. This phase favors humification; the transformation of complex organics to humic colloids closely associated with minerals (iron, calcium, nitrogen, etc.) and finally to humus. Ammonia is biologically oxidized to nitrite (NO_2^-) and then nitrate (NO_3^-).

The most important nutrient parameter in compost is the C/N ratio, which ideally should be about 25:1. Phosphorus (P) is next in importance; sulfur (S), calcium (Ca), and other trace nutrients are also important (Poprasert, 1989). Between 20 and 40% of C substrate in organic waste is assimilated into new microbial cells in composting (Poprasert, 1989; reviews Alexander, 1961).

Composting may be conducted under aerobic or anaerobic conditions. Aerobic composting is more typically used because the organic waste becomes stabilized more rapidly and more completely. “Under suitable conditions, aerobic composting normally takes about 10 to 30 days, while anaerobic composting can last from 45 to 100 days” (Poprasert, 1989). Aerobic composting results in the production of CO_2, NH_3, water and heat. Anaerobic composting results in methane, CO_2, NH_3, trace amounts of other gases, and other low-molecular-weight organic acids. NH_3 is further oxidized to become nitrate (NO_3^-) during the maturation or curing phase. Oxygen serves two purposes in aerobic composting: 1) as a terminal electron acceptor in respiration, 2) as a substrate required for oxygenase enzymes to degrade saturated alkanes, aromatic hydrocarbons and halogenated hydrocarbons (Polprasert, 1989; reviews Finstein et al., 1980). Biddlestone et al., (1988) recommended that the oxygen concentration should remain between 10 and 18% for maximum thermophilic activity, which is achievable in forced air composting. Mathur (1991) reported that the long standing "rule-of-thumb" for minimum oxygen concentration in compost is 5% (Judge, 1996; reviews Mathur, 1991). Due to the heterogeneity and bulky character of compost there always exists anaerobic sites in aerobic composting (Poprasert, 1989). The particle size of the compost materials and management practices will effect aeration of the compost (Poprasert, 1989). Particle sizes should be small enough to allow for sufficient aeration and availability of nutrients to microorganisms, but fine particle sizes may require bulking agents. Aeration may be achieved by non-mechanical means such as turning of the compost, insertion of perforated bamboo poles or dropping of the compost from floor to floor. Forced air aeration is a more effective mechanical method. In compost systems that lack forced air aeration, anaerobic and facultative conditions exist in the interior of the compost piles.

A moisture content between 50 and 80% should be adequate for most composting (Poprasert, 1989). Peat moss based composts are generally hydrated at 80 to 90% water/wet matrix (w/w) because of its water holding capacity (Judge, 1996; reviews Mathur, 1991). Apparently, the additional water does not decrease air flow in the peat moss matrix. Peat moss can hold up to 20 times its dry weight in water (Judge, 1996; reviews Bulganina et al., 1983). Low moisture can result in inhibition of biological processes. Optimum matrix potentials for bacterial colonization are above approximately -20 kPa (Miller, 1993). High moisture can result in anaerobic conditions and leaching of nutrients and pathogens (Poprasert, 1989).

To optimize both breakdown of organic matter and pathogen destruction, 55 °C is the optimum temperature (Poprasert, 1989). High temperatures are beneficial for killing pathogens but if they increase above 65-80 °C, fungi, actinomycetes and most bacteria will become inactive. The design and size of the compost are important to facilitate one temperature range or the other (Judge, 1996). The maximum desirable composting temperature based on species diversity is 60 °C, which is the same temperature recommended based on decomposition rate (Strom, 1985a). Water is the greatest thermal regulator in compost. Potential mechanisms for heat loss include radiation, conduction, evaporative cooling and sensible heating of air. Approximately 90% of the heat is removed through evaporative cooling, with only 10% through sensible heating (Miller, 1993;

reviews MacGregor et a., 1981). Radiation may be ignored and conduction is only significant in small composting masses.

Aerobic composting usually occurs at circumneutral pH. The pH in compost may drop in the first few days due to the production of volatile fatty acids, after which the pH will become neutral again (Poprasert, 1989). Under anaerobic conditions the pH may drop as well, due to the production of fatty acids (Miller, 1993). The pH of compost may rise to approximately 8.5 because of ammonification. As ammonia is oxidized, the pH will drop to 8.5 to 8.0 (Miller, 1993). The pH of compost is generally self correcting and, therefore, the initial pH does not need to be corrected (Miller, 1993)".

2.5.1. Fate of Pesticides in Compost

The fate of pesticides in compost has been studied for two purposes. The first has been to evaluate the fate of pesticides incidentally found in yard waste and sewage. The second has been to evaluate the potential for using composting as a means to detoxify pesticides. The latter scenario has generally involved much higher pesticide concentrations.

Diazinon is the most widely used lawn care pesticide, with approximately 5,000 tons used per year. A statewide study in Illinois found diazinon up to 5 (μg/g) in the majority of incoming yard trimming samples at compost sites (Frederick et al., 1996). Lemmon and Pylypiw (1992) studied the degradation of diazinon, chlorpyrifos, isofenphos and pendimethalin associated with grass in a homeowner composting environment. In all cases less than 1 μg/g was found 14 days post application and no detectable residues were found after three weeks of composting. Fredrick et al. (1996) studied the fate of diazinon, 2,4-D, and pendimethalin, each at concentrations of 10 μg/g, in a composting system designed to simulate the temperature and aeration found in windrows. 2,4-D was found to mineralize to CO_2 fairly rapidly (50% conversion in 50 days), which approximately paralleled the rate of mineralization of total carbon in the yard trimmings. Mineralization rates for diazinon and pendimethalin were much slower, 10 and 13% in 50 days, respectively. Only minor fractions of 2,4-D, diazinon and pendimethalin were found to be released as volatiles although the temperature was maintained at 55 to 60 °C through much of the 50 day composting period. In addition, leachability of all three pesticides was low. Less than 0.01 μg/g 2,4-D, 0.01 μg/g diazinon and 0.1 μg/g pendimethalin of the original 10 μg/g was found in the final compost. Diazinon was rapidly hydrolyzed to IMHP, and this accounted for 30% of the initial 10 μg/g. Very little of the 2,4-D and pendimethalin were recovered as degradation product products. Fifty percent of the 2,4-D, 50% of the diazinon and 83% of the pendimethalin were found to be associated with humic materials or unextractable from the final compost. (Fredrick, 1996)

A chemical survey of U.S. sewage sludges reported the routine occurrence of many of the priority pollutants (Racke and Frink, 1989; reviews USEPA, 1982). Over 100 municipalities are currently composting their sewage sludge and interest is steadily increasing (Racke and Frink, 1989; reviews Goldstein, 1987). The resultant compost products are used as soil conditioners for horticultural and agricultural purposes. Racke and Frink (1989) investigated the fate of carbaryl (naphthyl-1-^{14}C-carbaryl) during the composting of sewage sludge. The concentration was 1.8-2.2 μg/g on sludge - wood chip mixtures. Only 3-4% of the initial ^{14}C could be extracted from compost at the end of the composting cycle. In all cases, less than 0.1% of the applied carbaryl was recovered in this extract. Between 1.6 and 4.9% of the carbaryl was recovered as $^{14}CO_2$. The great majority was recovered as unextractable residues remaining in the compost.

Other studies have evaluated the potential for using composting as a method for disposing of pesticide waste. Berry et al. (1993) evaluated the suitability of solid-state fermentation (SSF) techniques to dispose of rinsates containing atrazine and carbofuran. High concentrations of atrazine (1400 to 8000 μg/g) and carbofuran (800 to 5000 μg/g) were added to bioreactors containing either steam-exploded wood or sphagnum peat moss. In peat moss bioreactors solvent extractable atrazine and carbofuran represented less than 0.2% and less than 0.05% of the initial concentration within 480 days of composting, respectively. In steam exploded wood bioreactors, solvent extractable atrazine represented 20% of the initial concentration following 320 days, whereas, solvent extractable carbofuran represented less than 0.05% of the initial concentration within 480 days. High concentrations of carbofuran may have been toxic to microorganisms as indicated by the low bioreactor temperatures in the first 40 days. A gram-negative bacterium (rod shaped) was isolated that used carbofuran as a sole C and energy source (Taraban and Berry, 1992). The amount of either atrazine or carbofuran in leachate was inversely related to incubation time. In addition, the percentage of solvent extractable that was leachable decreased with time. This may have been the result of increased association with intraparticle micropores. After 50 days of incubation, no carbofuran was detected in leachates. After 480 days of incubation, enough atrazine persisted in the composted peat material that initially contained 8230 μg/g atrazine to elicit a toxic response in an oat (*Avena* *sativa* L.) that was exposed to a 20% dilution of this material in soil. Exposure of western corn root borer larvae (*Diabrotica virgifera virgifer* LeConte) to soil containing 20% composted peat from bioreactors did not result in increased mortality.

Berry et al. (1993) also evaluated the suitability of solid state fermentation (SSF) techniques to dispose of chlorpyrifos and metolachlor. Pesticides were added to bioreactors containing either sphagnum peat moss, steam-exploded wood, or a wheat straw-horse manure mixture. At the end of the composting period (290 days), 0.5, 24, and 0.6% of the initial 1000 μg/g chlorpyrifos was extractable in each of the bioreactor types, respectively. Degradation of chlorpyrifos, however, was extremely slow and was observed only after cornmeal was added to the bioreactor initially and following 250 days of operation. Production of the primary hydrolysis product, TCP, in bioreactors containing horse manure or peat remained low. Following the addition of corn meal, there was a persistent decrease in concentrations of extractable TCP. Of an initial 2000 μg/g concentration of metolachlor 23, 49 and 0.3% was extractable in each of the bioreactors, respectively. The rapid rate of metolachlor degradation in bioreactors containing wheat straw - horse manure was surprising considering its slow degradation in the peat moss and steam-exploded wood bioreactors. It is possible that conjugation played an important role in the disappearance of metolachlor from wheat straw - horse manure bioreactors. Leaching of chlorpyrifos from either sorbent was minimal. Leaching of metolachlor from wheat straw - horse manure was much less than from peat. Additional work has been conducted by the Pesticide Waste Disposal Group at VPI and SU. Section 2.5 focuses on this group's work with diazinon.

A report by the Snell Environmental Group classified the following pesticides based on their susceptibility to degradation in compost after 30 days: trifluralin (high, 76-95%); atrazine, lindane, endrin, and methoxychlor (moderately high, 51-75% reduction); chlorpyrifos, chlordane, and silvex (2-(2,4,5-trichorophenoxy) propionic acid (moderate, 31-50% reductions); 2,4-D (moderately low, 16-30% reduction); and were DDT, dieldrin, and toxaphene (low susceptibility = 0-15% reduction) (Snell Environmental Group, 1982). Compost consisted of a mixture of shredded newspaper, manure, wastewater treatment plant sludge, sawdust, peat moss, soil, powdered milk, and fertilizer. The mechanism of dissipation was difficult to discern from this study. The methods for trapping volatile compounds were not adequate and the compost was not analyzed for the presence of metabolites that would unequivocally prove that degradation had occurred.

2.6. Pesticide Waste Disposal Group's Field Work with Diazinon

Early experiments by the Pesticide Waste Disposal Group focused on applying rinsate materials directly to biodegradation pits that contained peat moss or steam exploded wood. Hetzel et al. (1989) determined that peat moss is a very effective sorbent of diazinon, corresponding to loading rates of up to 0.4 g diazinon/g of peat moss. Degradation experiments demonstrated that high cumulative concentrations of diazinon (total 66,000 μg/g) were degraded to undetectable levels after one year, following the final application of diazinon. Mullins et al. (1989) found that bioreactors containing initial diazinon concentrations of 4000 to 32,000 mg/kg contained less than 1 to 8 mg/kg following 18 weeks of composting, respectively. In degradation pits containing peat moss / dairy cattle manure, diazinon initially degraded to IMHP, followed by disappearance of this hydrolysis product over the following nine weeks. Studies on the effects of moisture and nutrients on diazinon degradation rates indicated that as much as 99% of the diazinon degradation occurred in the four weeks before the addition of water and nutrients. This was likely due to the low pH (2.2 to 3.3). Air samples taken three days before and three days after the addition of nutrients contained 14 and 131 ng/L diazinon, respectively. Judge et al. (1990) determined that the sorption of 5000 μg/g chlorpyrifos (Dursban 4E and diazinon (Diazinon 2E) could be increased from 40% to above 90% by the addition of $Ca(OH)_2$ to steam exploded wood. This was likely the result of demulsification of the pesticide formulation. A one step process was developed that provided for the demulsification and sorption of formulated pesticides. Later, a second sorption step, using a column, was added to the process, to remove additional pesticide from the rinsewater (Hetzel et al., 1992). Mullins et al. (1992a) demonstrated that 98 (± 1.4) % of diazinon from a 5000 μg/ml solution could be removed by demulsification sorption and filtration. For comparison, 99.9 (± 0.1) % removal was obtained using activated carbon. Further unpublished data from the Pesticide Waste Disposal Group has demonstrated that a simulated rinsate contaminated with diazinon at 1500 μg/ml contained levels as low as 0.02 μg/ml following treatment. The diazinon removed from this waste water by peat moss has been shown to degrade to non-detectable levels after 60 days of composting. Results of radiolabelled fate studies conducted by our group (Petruska et al., 1985) are covered in Section 3.6 Degradation of Diazinon and Environmental Fate.

2.7. Physical and Toxicological Properties of Diazinon

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4yl phosphorothioate) is a broad spectrum, non-systemic organophosphorous insecticide and nematicide. It acts as an acetylcholinesterase inhibitor resulting in classic cholinergic signs (Sumner et al., 1985).

The following are some physical properties of diazinon relevant to its environmental fate. Diazinon has a boiling point of 83 to 84 °C at 268 mPa, a vapor pressure of 18.8 mPa at 20 °C, an octanol-water partition coefficient of 4.0, water solubility of 40 μg/ml at 25 °C and is miscible with most organic solvents (Merck Index, 1983). The volatility of diazinon is 2.4 mg / m^3 at 20 °C and 18.6 mg / m^3 at 40 °C. Diazinon has a half-life of 30 days and a soil sorption K_{oc} = 1,000 E (Wauchope et al., 1992). Combined data on diazinon's water solubility, affinity for soil and stability suggests little tendency to leach (Sumner et al., 1988). Arienzo et al. (1994) determined diazinon to be slightly mobile in soils with a low or medium OM content and immobile in those with a high OM. The octanol-water partitioning coefficient indicates a potential for diazinon to accumulate in biological organisms. It has been found to concentrate in fish at a maximum level of 300 to 360 times the water concentration (Sumner et al., 1988).

Diazinon is moderately toxic to mammals with an acute oral LD_{50} of 250 mg/kg and 285 mg/kg in male and female rats, respectively and an acute dermal LD_{50} of 900 mg/kg and 455 mg/kg in male and female rats, respectively (Gains, 1969). The no observable effects limit in the most sensitive species of monkeys was determined to be 0.025 mg/kg/day over a long term study (Sumner et al., 1985 cites CIBA-GEIGY, unpublished report). Diazinon is more toxic to birds and fish. The LD_{50} for several bird species ranges from 3 to 40 mg/kg (Sumner et al., 1985 cites Honeycutt, Sumner and Beavers, unpublished report). The LC_{50} for several species of fish ranges from 0.4 to 8 μg/ml (Sumner et al., 1985 cites CIBA-GEIGY, unpublished report). Diazinon also was shown to have sublethal effects on the chironomid *Chironomus tentans* at 3 ng/ml (Morgan, 1986). Egg hatch was delayed, the duration of the larval stage was increased, percentage pupation decreased and adult emergence was decreased slightly.

2.8. Degradation of Diazinon and Environmental Fate

Diazinon may degrade by biotic or abiotic processes. It appears that the hydrolysis of diazinon to IMPH is governed primarily by abiotic processes. Enhanced degradation of diazinon by soil microorganisms, however, can become a predominant factor in soils repetitively exposed to diazinon. The mineralization of IMHP appears to be almost an exclusively biotic process and several studies have demonstrated microbial consortia capable of IMHP mineralization.

2.8.1 Oxidation and Hydrolysis

Diazinon and its potential degradation products are provided in Figure 1. Oxidation of diazinon to the more toxic form, diazoxon, occurs in both plant and animal tissue. (McEwen and Stevenson, 1989). In vertebrates, oxidation takes place in the liver microsomes, in the presence of $NADPH_2$ and oxygen. In insects, oxidation takes place in the fat body and the metabolite is excreted (Buchel, 1986). Gomaa et al. (1969) reported that the rate of oxidation to diazoxon approximately doubles for every 10 °C rise in temperature from 10 °C to 60 °C. Diazoxon has not been isolated from soils (Sumner et al., 1985). Degradation of diazoxon applied to a silt loam soil at pH 8.1 and 25 °C was found to follow a definite linear curve and the half-life in soil was found to be 18 hours (Getzin, 1968).

Diazinon and diazoxon hydrolyze to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP). This product has very low toxicity and exists in two isometric forms; a ketone and an enol (Gomma et al. 1969). Ruzicka et al. (1967) demonstrated that the diazinon hydrolysis to IMHP was pseudo 1^{st} order (Ruzicka et al., 1967). The hydrolysis of diazinon and diazoxon is pH catalyzed in the pH range of 3.1 to 10.4 and at an ionic strength of 0.02 M (Gomma et al., 1969). At a pH of 8.4 the rate of hydrolysis of diazoxon is about ten times that of diazinon (Gomma et al., 1969). The hydrolysis of diazinon and diazoxon is catalyzed both by acid and alkaline conditions. The pH of most natural waters ranges from 5.5 to 8.5 and the temperature is less than 25 °C. Under these conditions, diazinon will have a relatively long residual life (Gomma et al., 1969). Hydrolysis of diazinon in soils appears to be adsorption rather than acid catalyzed (Konrad et al., 1967). The reaction in soil appears to be a rapid adsorption, followed by

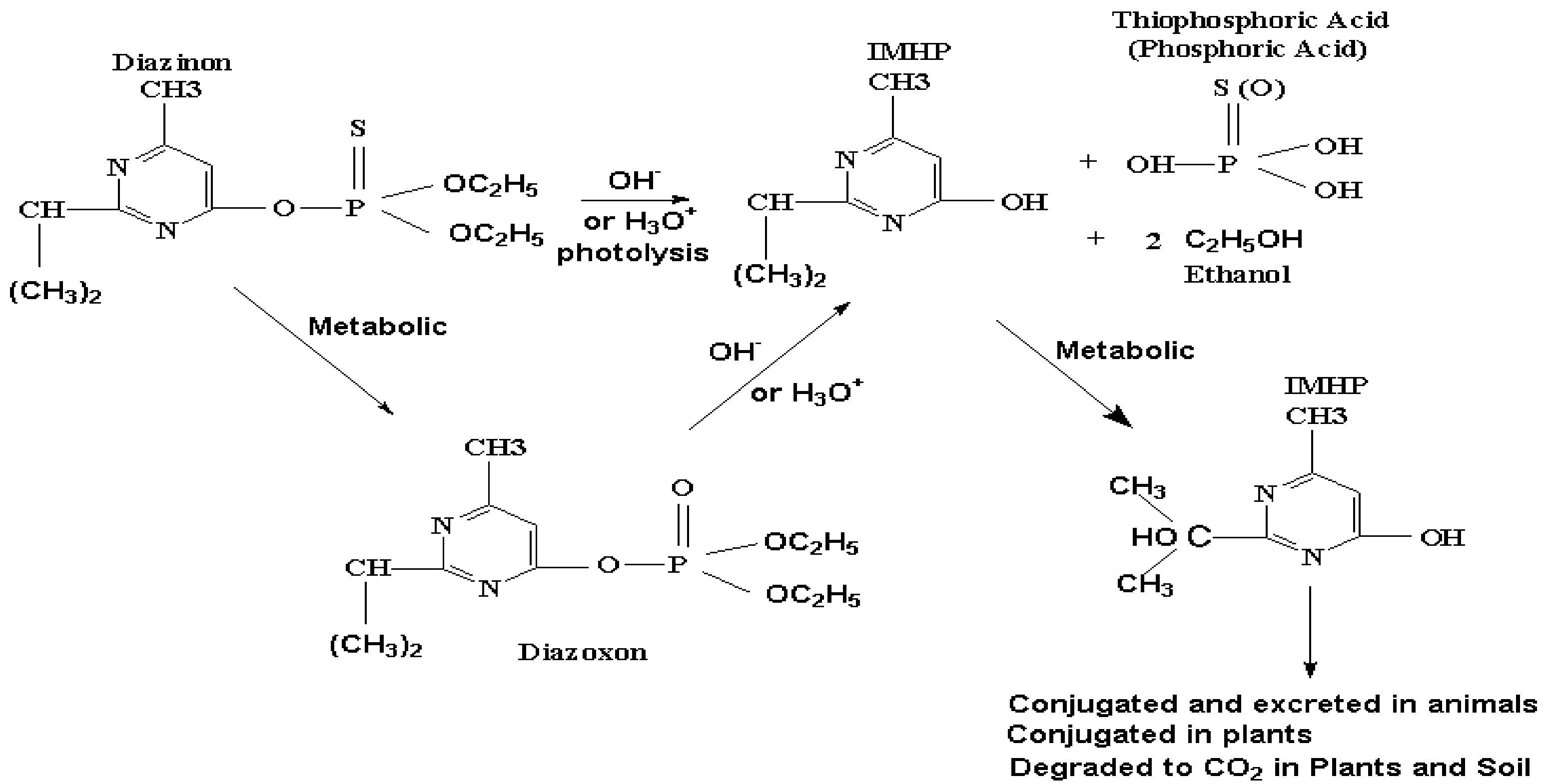


Figure 1. The chemical and biological degradation of diazinon.

degradation at the adsorption sites and then release of the degradation products into the aqueous phase. Degradation rates are closely related to the extent of initial soil adsorption (Konrad et al., 1967). In soil-free aqueous systems, however, diazinon hydrolysis can be acid or alkaline catalyzed (Konrad et al., 1967).

In almost all of the soil fate studies reviewed there were large accumulations of IMHP (Petruska, 1985; Frederick et al., 1996; Ahmad and Forgash, 1975; Konrad et al., 1967; Sethunathan and MacRae, 1969; Sumner et al., 1988; Barik and Munnecke, 1982; Caldwell and Raushel, 1991). This is particularly true under anaerobic conditions, such as in rice paddy soils. In some studies, where significant quantities of unidentified bound residues were formed or relatively rapid mineralization occurred, IMHP did not accumulate in the soils (Sethunathan and Pathak, 1971; Sethunathan, 1972; Getzin, 1968).

2.8.2 Microbial Mineralization of Diazinon

Microbial isolates have been shown to enhance hydrolysis and mineralization of diazinon. Gunner and Zuckerman (1968) isolated an *Arthrobacter sp.* and a *Streptomyces sp*. that were capable of degrading diazinon by a synergistic relationship. Sethunathan (1972) isolated a *Flavobacterium sp*. from the water in a diazinon treated rice field that had an exceptional capability to metabolize diazinon as a sole carbon source. Diazinon that was incubated in rice field water was converted to metabolites within the first 3 days and then mineralized to CO_2 within the next 2 days. Sethunathan and Yoshida (1973) reported that the *Flavobacterium* sp. was able to hydrolyze diazinon under aerobic and anaerobic conditions but it occurred more rapidly under aerobic conditions. This microorganism required aerobic conditions to mineralize diazinon. Matsumura and Boush (1968) investigated the ability of the soil fungus *Trichoderma viride* to degrade various radiolabelled insecticides, including diazinon. ^{14}C-diazinon was incubated for 24 hours in a liquid medium with a culture of *T. viride.* After the 24 hour incubation, water soluble metabolites represented 24% of total activity and solvent soluble radiolabel represented only 0.1%.

Getzin (1968) found 35% mineralization of ^{14}C-diazinon after 20 weeks of incubation in soil. Little CO_2 was released in fumigated soils. Unpublished data by CIBA-GEIGY found approximately 50% mineralization of ^{14}C-diazinon after 180 days of incubation in soil (Sumner et al., 1988). In contrast, Sethunathan and MacRae (1969) found only 0.4 to 0.8% mineralization of diazinon after 50 days of incubation in submerged soils. Hsu and Bartha (1979) studied mineralization rates of diazinon in both rhizosphere and root free soil. Following one month of incubation 13% of diazinon was mineralized in soil containing rhizosphere, whereas root-free soil samples were able to mineralize only 5%.

Composting experiments with diazinon have only been modestly successful at mineralizing diazinon. Peat moss composting of ^{14}C-ring labeled diazinon, resulted in 0.2% mineralization after 50 days (Petruska et al., 1985). A study on the degradation of pesticides in composted yard waste yielded approximately 11% mineralization of radioactive ring-labeled diazinon after 50 days of composting (Frederick et al., 1996).

2.8.3 Volatility of Diazinon in Laboratory and Field Studies

Diazinon demonstrates relatively low volatility in laboratory and field studies. A radiolabelled fate study in soil recovered less than 1% of radioactivity as volatiles following 2 weeks of incubation at 25 °C. (Getzin and Rosefield, 1966). Frederick et al. (1996) only recovered 0.2 % of radiolabelled diazinon as volatiles following 50 days of composting at temperatures of 55 to 60 °C. However, in benchtop composting experiments that simulated forced air composting, 22% of ^{14}C-diazinon was accounted for as ^{14}C-volatiles (Petruska et. al., 1985).

2.8.4 Formation of Bound Residues

Studies with ^{14}C-ring labeled diazinon in soil indicate that a large fraction of the radiolabelled material becomes unextractably bound to the soil. In organic soil, sandy loam, silty loam and clay loam, unextractable radioactivity represented 20 to 30% of the original application after 20 weeks (Getzin and Rosefield, 1966). After incubating a silt loam soil for 20 weeks, the unextractably bound radioactivity represented 35 % of the original application (Getzin, 1968). Unpublished data by CIBA-GEIGY indicated that approximately 20% of the radioactivity from ^{14}C-diazinon can be accounted for as unextractably bound following 180 days of incubation in soil (Sumner et al., 1988).

A large fraction of the radiolabelled material becomes unextractably bound in compost, as well. In a laboratory composting experiment with peat moss, Petruska et al. (1985) found 15 % of the applied radioactivity to be associated with NaOH-soluble humic and fulvic acids after three weeks of composting. Combustion of the compost, following extraction with organic solvents and NaOH, accounted for 25 % of the initial radioactivity. The association of radioactivity with NaOH-extractable material suggests binding, because this material does contain significant micropores. Radioactivity associated with the combustion fraction may be bound, associated with micropores, or both. Frederick et al., (1996) found 20% of the radioactivity applied as diazinon was associated with humic material and approximately 32 % was found to be unextractable after 50 days of composting.

2.8.5 Abiotic Factors in the Degradation of Diazinon

As indicated at the beginning of this discussion, diazinon degradation results from a combination of biotic and abiotic factors. The greatest effect of abiotic factors is on the hydrolysis of diazinon (Getzin, 1968). There have been some reports that indicate that hydrolysis of diazinon is almost solely the result of abiotic factors. Getzin (1968) found degradation rates for 20 μg/g diazinon to be similar in autoclaved and non autoclaved soil. Getzin and Rosefield (1968) found rates of diazinon degradation (15 to 25 μg per cubic centimeter of soil) to be similar in autoclaved, gamma-radiation sterilized and non-sterilized soils.

Getzin (1968) found higher temperatures, moisture and soil acidity to accelerate the decomposition of diazinon (20 μg/g). There is conflicting evidence on the effect of type, with respect to organic matter and silt, loam, clay content, on the degradation rate of diazinon. Only studies that compared soil types, while keeping other factors constant, are useful for evaluating the effect of soil type on diazinon degradation. Studies by Suett (1971) and Rosefield (1966) did this, but provided conflicting results. In addition, unless soils were sterilized, the effect of soil type may be microbial rather than physical-chemical. Suett (1971) found that, seven months after the application of diazinon, 1% of the applied diazinon remained in a sandy loam (1.9% O.M.); whereas a peaty loam (18.1% O.M.) contained 10% of the applied diazinon. Alternatively, Getzin and Rosefield (1966) found the half-lives for diazinon in organic soil, sandy loam, silt loam and clay loam to be similar in all four soils. The half-lives were found to be 2 to 3 weeks, and less than 8% remained after 20 weeks. After 20 weeks at 25 °C, Getzin (1968) found 10 to 15% of the applied diazinon unaltered in a silty loam. In contrast, Sethunathan and MacRae (1969) found that only 2 to 6% of the applied diazinon activity remained in soils at 50 and 80 days after application, respectively, to submerged tropical soils.

2.8.6 Biotic Factors in the Degradation Rate of Diazinon

Other reports have indicated a role of microorganisms in the hydrolysis of diazinon. Sethunathan and MacRae (1969) found losses of diazinon from sterilized soil to be much slower than from unsterilized soil samples, indicating a role or microorganisms in diazinon degradation. The half-lives for diazinon in sterilized soil and unsterilized soil were 8.8 and 33.8 days, respectively. Bro-Rasmussen et al. (1968) determined the half-life (RL_{50}) of diazinon in two different soils, at two levels of water content, with two different diazinon concentrations (equivalent to 1 and 10 kg/ha), and in steam treated and untreated soil. All four of the factors influenced diazinon degradation with steam treatment having the greatest effect on the rate of diazinon degradation and diazinon concentration having the least effect. The half-life of diazinon in this experiment varied considerably ranging from 22 to 80 days. Additional evidence for the influence of microorganisms on diazinon degradation rates is provided by "enhanced microbial degradation" studies, discussed below.

2.8.7 Selection of Soil Microorganisms by Diazinon and Microbial Toxicity

There is some evidence that the presence of diazinon in soil may select for microorganisms that are capable of using it as a carbon and energy source. The majority of the studies that have investigated the microbial degradation of diazinon have been focused on decreased efficacy of diazinon in controlling a target pest. The case of diazinon failing to control brown plant hoppers is one example of enhanced degradation (Felsot, 1989). Diazinon was the first organophosphate that failed to control a pest due to enhanced microbial degradation. Reports on the enhanced degradation of diazinon following repeat applications with diazinon include Sethunathan (1981; 1982) and Forrest et al., (1981).

Robson and Gunner (1970) demonstrated diazinon's selective effects on soil microorganisms. Exposure to diazinon diminished O_2 uptake and growth in some, but not all, bacterial cultures. Diazoxon inhibited bacterial O_2 uptake, but only retarded their growth rather than completely inhibiting it. The effect of diazoxon was likely reduced by its relatively rapid decomposition. IMHP had no effect on growth or oxygen uptake and the ammonium salt of diethylphosphorothioate increased oxygen uptake. In a mixed culture exposed to diazinon, where two organisms were sensitive to diazinon but a third was not, the resistant form was subsequently shown to become the dominant form.

Somasundaram et al. (1990) studied the toxicity of several pesticides and their hydrolysis products to *Photobacterium phosphoreum*. The EC_{50} for diazinon was found to be 10 μg/g whereas the EC_{50} for IMHP was found to be 886 μg/g. Diazinon and IMHP do not appear to inhibit the N fixing capabilities of *Acetobacteriacae*, an important group of free living nitrogen-fixing bacteria, at concentrations up to 500 μg/g (Wood and MacRae, 1984).

2.8.8 The Use of Enzyme Preparations in Diazinon Degradation

Munnecke and Hsieh (1974) adapted a mixed microbial culture for growth on parathion to detoxify concentrated parathion in agricultural waste. An enzyme preparation from this mixed culture was shown to hydrolyze seven technical grade organophosphate insecticides, including diazinon, at rates 40 to 1005 times faster than the recommended chemical treatment with 1.0 N sodium hydroxide (pH 10) (Munnecke, 1976). Munnecke (1980) tested the ability of a bacterial enzyme preparation to detoxify waste from diazinon EC 25%. The ability to degrade formulated pesticides at concentrations similar to spray solutions (0.02-0.04%) and rinsed containers (1%) was investigated. The formulated pesticides did not appear to inhibit enzymatic hydrolysis and enzymatic hydrolysis was significantly faster than chemical hydrolysis procedures that were recommended at the time. Barik and Munneck (1982) used purified enzyme isolated from a *Pseudomonas* sp. to hydrolyze high concentrations of diazinon using a fixed bed reactor and in soil. Diazinon concentration in soil ranged from 125 to 10,000 μg/g. The 1,250 μg/g concentration degraded to 2 μg/g in 24 hours and the 10,000 μg/g concentration degraded 98% in 24 hours. To optimize conditions for the enzyme, the temperature of the soil needed to be increased to 38 °C and the pH to 9. They concluded that the cost of enzyme treatment seems realistic for treating emergency situations, particularly in the case of high concentrations in soils. Caldwell and Raushel (1991) immobilized a phosphotriesterase from *Pseudomonas diminuta* onto a trityl agarose matrix and placed it in a glass column reactor. Several organophosphates including diazinon were shown to be hydrolyzed by the immobilized enzyme. Diazinon, 0.08 mM at pH 9.0 in 10% methanol, was hydrolyzed to greater than 99% completion at a flow rate of 55.5 mL/hr.

2.8.9 Stability of Diazinon in Water

Diazinon was found to be more stable in natural and experimental aqueous solutions than lindane, endosulfan and methylparathion (Ferrando et al., 1992). After 96 hours 44 and 48 % of 1 μg/mL unformulated diazinon remained in natural and experimental water, respectively. The water was well aerated (40-100% of the saturation limit) and exposed to a 12 hour photoperiod. The half-life was estimated to be 81 and 89 hours in natural and experimental waters, respectively.

2.8.10 Adsorption of Diazinon to Soil, Clay and Humic Acids

Diazinon adsorption in soils with high organic matter content depends almost entirely upon the organic matter content (Arienzo et al., 1994). When organic matter is low, the significance of other soil properties on adsorption becomes relevant (e.g., silt + clay content) (Arienzo et al., 1994). In soils with widely variable organic matter, silt and clay contents, the clay content was the most significant parameter in the adsorption of diazinon (Arienzo et al., 1994). Arienzo et al. (1994) found adsorption isotherms for ^{14}C-diazinon to be S-type in soils with an organic matter content less than 0.88%, otherwise they were L-type. Sorption isotherms for diazinon sorbed by humic acids of molecular weights greater than 300 kdaltons are L-type and adsorption was complete within 3 minutes (Saint-Fort and Visser, 1988). In humic acids with molecular weights greater than 300 kdaltons, interparticle diffusion of diazinon into the sorbent was the rate limiting process. The maximum adsorption capacity of the 300 kdalton fraction increased with increasing acidity but the bonding energy constant remained the same. This suggests that the mechanism of adsorption was the same under both pH conditions. No interaction of diazinon occurred with the humic acid fraction that had molecular weight less than 10 kdaltons, or with humic acid fraction that had molecular weight from 10 to 300 kdaltons (Saint-Fort and Visser, 1988).

2.9. Biology of Earthworms

Earthworms belong to the class Oligochaeta and inhabit soil or fresh water. The two most important families of terrestrial Oligochaeta in Europe, North America, Australia and Asia are the Megascolecidae and the Lumbricidae. The megascolecids and their close relatives comprise more than half of the known species and are widely distributed outside the paleartic zone; whereas Lumbricidae is the most important family for human welfare and is the dominant family in the paleartic zone (Edwards and Bohlen, 1996). The distribution of earthworm in North America is closely related to the expansion of the Quaternary ice caps (Edwards and Bohlen, 1996). These ice caps physically exterminated earthworm populations and earthworms were slow in recolonizing these northern latitudes. In the U.S. today there are 33 species of lumbricids (Edwards and Bohlen, 1996). Relatively few of the terrestrial Oligocheata families are endemic to America and only two genera of lumbricids, *Bimastos* and *Eisenoides* are endemic (Edwards and Bohlen, 1996) “Five species are commonly found in North American soils: *Allolobophora caliginosa*, the common field worm, *Allolobophora chlorotica*, a rather inactive, green colored worm; *Eisenia foetida*, the yellow and maroon-banded manure worm; *Lumbricus rubellus*, the red worm; and *Lumbricus terrestris*, the night crawler or dew worm (Roberts and Dorough, 1985).”

Earthworms activity increases the porosity and aeration of soils and improves their hydraulic properties and structural stability. Earthworms are important in promoting short, rapid cycles of nutrients. Their activity mixes the soil horizons and helps to bury above ground litter. Earthworms move from 2 to 250 tons per ha per annum of soil from lower strata to the soil surface (Edwards and Bohlen, 1992).

2.9.1 Overview of Earthworm Biology and Ecology

The following basic earthworm physiology is obtained from Edwards and Bohlen (1996). The principle systematic features of earthworms include: bilateral symmetry; external segmentation with corresponding internal segmentation; no skeleton and only a thinly pigmented cuticle; all segments contain setae except the first two; an outer layer of circular muscles and an inner layer of longitudinal muscles; and they are hermaphroditic. Earthworms have a large coelomic cavity and a closed vascular system. Transverse septa divide the cavity into segmental portions. These septa are generally perforated by pores, which allow coelomic fluid to pass freely though them.

The earthworm digestive system consists of a buccal chamber, pharynx, esophagus, crop, gizzard and intestine. The intestine is a simple cylindrical tube where most of the absorption occurs. The intestine has a region of longitudinal folds in the medial dorsal line that increase its surface area, known as the typhlosole. Movements of the body wall during locomotion help with the passage of gut contents, which may be as much as 20% of the total live weight.

Nephridia are the main organ for nitrogen excretion in earthworms and are paired in each of the segments except the first three and the last (Edwards and Bohlen, 1996). The urine of earthworms contains ammonia, urea and to a limited extent uric acid. Like freshwater animals, earthworms maintain a constant concentration of body fluids and excrete hypotonic urine. Approximately half of the nitrogenous material excreted by earthworms is excreted as mucus and greater than 50% of the total loss of assimilated energy for earthworms goes into the production of mucus. Mucus acts as a lubricant during movement through the soil, binds soil in burrows, serves a protective barrier against noxious materials and may be used to stimulate the proliferation of gut microorganisms to aid in the digestion of food (See Section 3.8.2). Lavelle et al. (1983) reported that a population of the tropical earthworm species *Pontoscolex corethrurus* could excrete mucus to the equivalent of 20% of the total organic matter of the soil in which they lived. Scheu (1991) estimated the carbon loss due to mucus excretion to burrow walls and by the intestinal tract in *O. lacteum* to represent 63% of the total carbon loss by earthworms (including respiration and mucus excretion). Little of the intestinal mucus produced during gut passage is voided in casts because of reabsorption in the posterior intestine (Scheu, 1991).

The earthworm nervous system is very sensitive to chemicals, touch and light (Edwards and Bohlen, 1996). The main chemical transmitters in the central nervous system of earthworms are acetylcholine, probably adrenaline, noradrenaline and possibly 5-hydroxy-tryptamine (5HT) (Edwards and Bohlen, 1996; review Mill, 1982). The ganglia of *E. foetida* contain two cholinesterases, which may be similar to mammalian acetylcholinesterase and butyrylcholinesterase (Vigh-Teichmann and Goslar, 1968). *L. terrestris* also contains two main cholinesterases. The ventral nerve cord only contains the acetylcholinesterase type and the only butyrylcholinesterase type activity was found in blood vessel walls (Edwards and Bohlen, 1992; reviews Silver, 1984; Teräväinen, 1969). The central nerve cord of *L. terrestris* exhibits intense cholinesterase activity. Both acetylcholinesterase and other cholinesterases are present and have identical distribution, being located primarily in the supportive glial cells surrounding the neurones (Teräväinen, 1969).

The following information on earthworm mating strategies is from Edwards and Bohlen (1996). Earthworms are semi-continuous or continuous breeders producing offspring at most times of the year; however, peak cocoon production occurs in spring and early summer in the northern hemisphere. Earthworms are hermaphroditic, but contain more complicated genital systems than most unisexual animals. Most earthworm species reproduce by cross-fertilization, although some can produce cocoons parthenogenically. Asexual reproduction by fragmentation and regeneration occurs in some species of aquatic *Microdrili* but does not seem to occur in terrestrial earthworms. *L. terrestris* mates on the surface, but many species mate below ground. After copulation, which takes approximately 1 hour, the earthworms separate. The clitellum produces a secretion that hardens over its surface. Then the worm moves backwards drawing the tube over its head, when the worm is completely free, the ends of the tube close. The cocoon contains nutritive albuminous fluid, ova and spermatozoa. The number of ova in each cocoon ranges from 1 to 20 for lumbricid species, but usually only one or two survive and hatch. The time for a cocoon to hatch ranges from 3 weeks to 3 months, depending on the species, population density, age structure, available food, moisture and temperature. The life span of Lumbricids in the field is likely a few months although they may potentially live 4 to 8 years.

Water constitutes up to 85% of the fresh weight of earthworms (Edwards and Bohlen, 1996). Water conservation is very important for earthworms because their thin cuticle and mucus coating provide little protection. Earthworms apparently lack a mechanism to maintain a constant internal water content (Edwards and Bohlen, 1996; review Kretzschmar and Bruchou, 1991). Earthworms may lose up to 80-85% of their moisture without mortality. Excess water does not appear to significantly effect earthworms. The earthworm species *A. chlorotica*, *A. long*, *D. subrubicunda*, *L. rubellus* and *L. terrestris* were shown to be able to survive from 31 to 50 weeks in soil totally submerged beneath aerated water. The pH optimum for earthworms is species dependent, but they are generally more tolerant of acidic than basic conditions.

2.9.2 Earthworm Feeding Strategies

Earthworms are the most important invertebrates in the first stage of decomposition in many ecosystems. The fragmentation of organic matter by earthworm digestion exposes a greater surface area to microorganisms (Edwards and Bohlen, 1996). The main food sources of earthworms are leaf litter, living and dead roots, humic reserves, occasionally root exudates, leachates and percolates from the canopy and leaf litter (Lavelle, 1988). Addition of bacteria to leaves can increase their palatability (Edwards and Bohlen, 1996; reviews Wright, 1972). Many pesticides can make food substrates unpalatable to earthworms (Edwards and Bohlen, 1996).

The earthworm's strategy for nutrient acquisition may be divided into three classes, endogeic, epigeic, and anecic, based on the soil profile in which the species lives and feeds. The feeding strategies of different earthworm species allow these species to coexist by exploiting different food resources and habitat space (Edwards and Bohlen, 1996). Most earthworm communities contain 3-6 species. From northern latitudes to the equator the successively dominant groups are epigeic, anecic and meso- and oligohumic endogeic (Edwards and Bohlen, 1996; review Lavelle, 1983).

Epigeic earthworms live in the litter or the immediate topsoil and feed on high quality resources, such as leaf litter (Lavelle, 1988). They generally do not, however, incorporate the ingested organic material into soil (Edwards and Bohlen, 1996). The surface litter environment is not as protective as deeper soil profiles; therefore high mortality rates must be balanced with high reproductive rates (Lavelle, 1988). They use high quality resources and are small to allow for high metabolic activity when conditions are favorable. Selection for smaller and fast moving traits is also the result of high predatory pressure (Edwards and Bohlen, 1996 cites Bouché, 1977).

In experiments using ^{13}C-surface litter and ^{14}C-root derived carbon in soil, the epigeic species (*L. rubellus*) primarily assimilated ^{13}C from surface litter; although they did assimilate some ^{14}C-soil carbon (Zhang and Hendrix, 1995). In analyzing the gut content of Lumbricidae worms, Judas (1992) found that the gut contents of the epigeic species, *L. castaneus,* was high in organic matter (61% of the dry wt.) and consisted mainly of non-particulate detritus. Epigeics and anecics have a more simple intestine than endogeics with a small typhlosole that lacks secondary folds (Edwards and Bohlen, 1996).

Endogeic species live within the soil profile and feed on lower quality resources concentrated around plant roots and near the surface (Lavelle, 1988). They generally mix the ingested organic material with the mineral fraction (Edwards and Bohlen, 1996). They are generally sluggish and experience low predation (Edwards and Bohlen, 1996 cites Bouché 1977). Lavelle (1988) further categorized endogeic earthworms as "polyhumic", "mesohumic", and "oligohumic". The "polyhumic" species selectively ingest soils with high organic matter. They search out the upper organic soil layers or concentrations in the soil profile. Small species may selectively ingest the fine organic-rich fraction of the soil. The "mesohumic" species ingest the upper 10-15 cm of the soil indiscriminately taking in both organic and mineral fractions. The "oligohumic" species feed on the poor organic matter of deep horizons (30-40 cm) in tropical soils. Although endogeics consume large quantities of soil humus, they preferentially assimilate easily decomposable organic compounds with a mean turnover time of a few years (Martin et al., 1992). Neuhauser et al. (1978) found that the epigeic species, *Eisenia foetida*, was unable to degrade ring [^{14}C]- methoxy [^{14}C]- and side chain [^{14}C]- lignin presented in food but was able to degrade cinnamic 2-[^{14}C] acid offered as food and vanillin 5-[^{14}C] injected. Cinnamic acid is a precursor of lignin and vanillin is a product of the oxidative decomposition of lignin. Scheu (1993a) found that the mineralization of ^{14}C-labeled lignin was increased in the presence of *Octolasion lacteum* (Oerley) and *Lumbricus castaneus* (Savigny) when incubated with brown rotted beech and white rotted beech than when incubated with brown rotted beech and white rotted beech alone. Scheu (1993b) found that processing of soil by the earthworm, *Octolasion lacteum*, tended to reduce overall lignin mineralization in limestone soils that did not contain beechwood. Scharpenseel et al. (1989) found that earthworms from temperate regions were unable to assimilate resistant organic matter (Martin et al., 1992; review Scharpenseel et al., 1989). Contrary evidence has been observed for geophagous tropical earthworm species, which appear to be able to assimilate "resistant" soil organic matter. Martin et al. (1992) demonstrated that young *M. anomala* are able to assimilate young organic matter, such as fresh plant debris and coarse soil organic matter, as well as fine clay-associated soil organic matter.

In experiments using ^{13}C-surface litter and ^{14}C-root derived carbon the endogeic species, *Aporrectodea caliginosa*, assimilated more root derived soil carbon but also assimilated ^{13}C-derived from surface litter (Zhang and Hendrix, 1995). The activity of endogeic earthworms leads to homogenization, mineralization and humification of organic matter (Shaw and Pawluk, 1986a; review Bal, 1982). In analyzing the gut content of Lumbricidae worms, Judas (1992) found that in the gut contents of the endogeic species, *A. caliginosa*, *A. rosea*, *O. lacteum*, and *O. cyaneum*, were higher in organic matter than the soil horizon, but lower than the epigeics and anecic species. The endogeics' gut contents contained no particulate organic matter and higher mineral fractions than that of the epigeics and anecics (Judas, 1992). Endogeics tend to have a more complex intestine with a large typhlosole that often has secondary folds (Edwards and Bohlen, 1996).

Anecics live in the soil and produce vertical burrows. They feed on partially decomposed organic matter, are capable of incorporating organic matter into soil and striping small pieces from larger food sources using their mouth parts (Edwards and Bohlen, 1996). Anecics draw litter down into their burrows, consuming the litter once it has undergone sufficient degradation (Wallwork 1983). These food stores are often enough to survive inclement conditions on the soil surface. Anecics experience high predation when they are at the surface but are able to withdraw rapidly into their burrows (Edwards and Bohlen, 1996 cites Bouché 1977). In analyzing the gut content of Lumbricid worms, Judas (1992) found that the anecic species, *Lumbricus terrestris*, had a higher median organic gut content than any of the endogeic or epigeic species found in this study. In addition it was the only species with high amounts of particulate plant remains in the gut.

The time it takes for food to pass through the gastrointestinal tract of earthworms appears to be related to feeding strategy. Epigeics consume food sources less frequently and process it more slowly than endogeics (Piearce, 1978). The time for soil to pass through the gastrointestinal tract of an anecic species, *L. terrestris*, was found to be 12 to 20 hours (Edwards and Lofty, 1977); an epigeic species, *L. rubellus*, was found to be 6 to 8 hours (Daniel and Anderson, 1992); and an endogeic species, *E. foetida*, was found to be 3 to 4 hours (Edwards and Lofty, 1977). The residence time also appears to be dependent on the activity in which the earthworm is engaged. It takes about 20 hours for food to pass through the intestine of *L. terrestris* during feeding but only 12 hours when they are forming burrows (Parle, 1963a). They also take in soil non-selectively when forming burrows (Parle, 1963a).

Although earthworm strategies for nutrient acquisition are generally divided into endogeic, epigeic and anecic, these strategies represent the possible extremes. The feeding strategies of most earthworms fall somewhere in among these three categories (Edwards and Bohlen, 1996). In addition the feeding strategies of some earthworm species are very unique. For example *Eisenia lucens*, feeds specifically on rotten wood (Jolly et al., 1993).

In earthworm castings, mineral granules adhere to soil organic matter leading to water stable aggregates. Shipitalo and Protz (1989) proposed the following model for aggregate formation in earthworm casts. Litter and soil are ingested and intimately mixed. Litter is then fragmented, a liquified soil -debris mixture forms in the anterior digestive tract. Organic debris is further fragmented by the gizzard, which grinds food with the aid of mineral particles. Lignified components resist fragmentation and remain as relatively large, intact pieces, whereas less lignified and softer tissues are lightly fragmented and become part of the soil plasma. Physical breakdown of tissue, combined with microbial activity, digestive enzymes and other secretions, result in complete decomposition of some organic matter and release and formation of bonding agents. Humified portions of the soil organic matter serve as the foci for aggregate formation. Spatial redistribution of compacted soil appears to be possible down to size orders of silt grains through earthworm activity (Altemüller and Joschko, 1992). To a greater extent, it appears that existing bonds are destroyed by earthworm ingestion (Zhang and Shrader, 1993). Nevertheless, earthworms are advantageous for the stabilization of reformed aggregates. Aging and drying of casts facilitates close approach and bonding of plant and microbial polysaccharides and other organic compounds to clay, thereby stabilizing the new microaggregates (Shipitalo and Protz, 1989).

2.9.3 Interactions of Earthworms with Soil Microorganisms

Lavelle (1988) described three possible systems that may be used by earthworms to assimilate nutrients. It may be that earthworms switch between these systems as quantity and quality of available nutrients change. The first possible system is where earthworms use their own digestive enzymes to extract nutrients from soil organic matter, fungi, bacteria or protozoa. Protease, lipase, amylase, lichenase, cellulase and chitinase activities have been described in the intestines of earthworms (Laverack, 1963; reviews Van Gansen, 1958). It is, however, difficult to determine if the cellulase, chitinase, lipase and protease enzymes are produced by the earthworm itself or allochthonous microorganisms (Laverack, 1963; Lee, 1985).

The second possible system is known as a "mutualistic digestive system" (Barois and Lavelle, 1986; Martin et al., 1987; Barois, 1992; Trigo and Lavelle, 1993). The “hypothesis of mutualism” was proposed by Barois and Lavelle (1986) as an explanation for earthworms obtaining their required nutrients from poor organic matter. Mucus production and increased moisture in the earthworm gut stimulate microbial metabolism. This increased microbial metabolism degrades soil organic matter to simpler compounds that may be assimilated by the earthworm. The mucus and moisture are then reabsorbed in the posterior part of the gut. Whether microbes remain or are themselves digested further in the gut has not yet been answered (Trigo and Lavelle, 1993).

The third possibility is an intermediate system: the “external rumen” (Swift et al., 1979). In this system, earthworms use the organic compounds that are made available by litter and soil microorganisms. Giving microorganisms a chance to grow on leaf litter collected in earthworm galleries or earthworms eating their own castings may be related to this. Parallels between the fecal microbiology of ruminants and earthworms' castings may be evidence for the external rumen system (Shaw and Pawluk, 1986a).

There is evidence that earthworms cannot exist on pure microbial cultures; they need mixed cultures of microbial species (Edwards and Bohlen, 1996). As food, bacteria are of minor importance, algae are of moderate importance, fungi and to a lesser extent protozoa, are of major importance (Edwards and Fletcher, 1988). It is generally accepted that bacteria and actinomycetes are not commonly consumed by earthworms and may proliferate in the process of gut passage (Kristufek et al., 1992). Miles (1963) demonstrated that *E. foetida* cocoons were unable to develop to maturity when only provided with fungi and bacteria but required protozoa to develop to sexual maturity.

The microbial composition changes qualitatively and quantitatively during passage through the earthworm intestine (Pedersen and Hendriksen, 1993). *Eisenia lucens* is an extreme example of this. This earthworm feeds almost exclusively in rotten wood. “Of the microbes isolated from the gut of *Eisenia lucens*, *Vibrio* spp. accounted for 83% of the total bacteria *Streptomyces limpanii* accounted for 90% of the actinomycetes, and these species were of relatively low abundance in the wood substrate where the earthworms were living (Edwards and Bohlen, 1996; review Contreras, 1980)”. Earthworms tend to shift the balance from fungi to bacteria by increasing soluble organic matter and preferentially feeding on fungi (Edwards and Bohlen, 1996). The numbers of culturable aerobes and facultative anaerobes have been shown to increase logarithmically from the foregut to the hindgut in the epigeic species (*L. rubellus*) (Kristufek et al., 1992). A similar increase was seen for actinomycetes and micromycetes. However, in the endogeic species, *A. caliginosa*, numbers of bacteria and actinomycetes decreased from the foregut to the midgut, and then remained unchanged from the midgut to the hindgut. It was hypothesized that the diet of epigeic species, *L. rubellus*, which is rich in readily available nutrients, enabled the digestive canal to operate as a "fermenter". The endogeic species, *A. caliginosa*, however, consumes food high in more recalcitrant organic matter and not in readily available nutrients, therefore, consumed the bacteria as a nutrient source (Kristufek et al., 1992). The influence of earthworms on microorganisms may be related to bactericidal activity (Lassegues et al., 1981; Valembois et al., 1982). However, earthworm antibacterial factors have not yet been reported from the earthworm intestinal tract (Pedersen and Hendriksen, 1993). Many soil microorganisms are in a dormant state until conditions become suitable. Thick and thin walled spores tend to lose little viability through the earthworm intestine (Edwards and Bohlen, 1996; review Huffman and Purdy, 1964).

The short term effect of earthworms is to increase microbial decomposition of organic matter. The long term effect of earthworms may be to decrease microbial decomposition of organic matter by physically protection it in the castings (Edwards and Bohlen, 1996; reviews Martin, 1991; Lavelle and Martin, 1992). In aging casts, slower growing soil fungi begin to dominate and microorganisms become dormant (Edwards and Bohlen, 1996). Bohlen and Edwards (1995) speculated that earthworms decrease microbial biomass by releasing the nutrients fixed in microorganisms, but this activity is overshadowed in an environment where there are organic inputs into the soil by the stimulatory effect of these inputs.

The development of fungal hyphae as well as aging and drying can increase the stability of earthworm casts (Edwards and Bohlen, 1996; reviews Swaby, 1949; Parle, 1963b; Marinissen and Dexter, 1990; Shipitalo and Protz, 1988, 1989; Barois et al., 1993). Some bacteria secrete polysaccharide gums that can contribute to aggregate stabilization in earthworm castings (Edwards and Bohlen, 1996; reviews Geoghagan and Brain, 1948). Microscopic examination shows that bacteria and bacterial produced polysaccharides are abundant in casts and may serve to bond mineral particles (Shaw and Pawluk, 1986b; Altemüller and Joshko, 1992). Altemüller and Joschko (1992) used the combined techniques of fluorescent staining and thin section microscopy to observe the distribution of microorganisms in the casts. Relatively large bacteria in the shape of short rods were primarily found in dense colonies, forming streaks between soil material and not in any accordance with plant tissues. These bacterial streaks mark areas of high slime content in which bacterial activity was at its maximum in fresh castings.

Karsten and Drake (1995) found aerobic and anaerobic microorganisms to be more numerous in the guts of both the epigeic earthworm species, *L. rubellus*, and the endogeic earthworm species, *Octolasion lacteum*, than in the beech forest soils from which they were obtained. The overall enumeration results indicated that microorganisms capable of growth under anaerobic conditions were two to three orders of magnitude more numerous in earthworm intestines than the soil from which they were obtained. In earthworms, the anaerobic microbial growth potentials were approximately equal to aerobic microbial growth potentials; whereas, in soil samples, the aerobic microbial growth potentials were much greater than anaerobic microbial growth potentials. These results suggested that "the earthworm gut might constitute a micro-habitat enriched in microbes capable of anaerobic growth and activity" in otherwise well aerated soils. The only evidence of indigenous microorganisms associated with earthworms comes from Jolly et al. (1993). Using scanning electron microscopy, these researchers observed segmented, filamentous organisms attached to the hindgut epithelium of *L. terrestris* by "socket-like" attachments. The physical linkages observed by Jolly et al. (1993) in the hindgut of *L. terrestris* "do not necessarily prove the existence of truly indigenous microbial strains, but they do indicate that some microbial strains are highly adapted to living in the alimentary tract of earthworms" (Edwards and Bohlen, 1996; review Jolly et al., 1993). Using light microscopy, Karsten and Drake (1995) observed mixtures of rods, cocci, and spore forming microbes in earthworm gut homogenates with no single dominant morphological type in gut homogenates.

There is strong evidence that microorganisms are able to enter earthworm cocoons of *Eisenia fetida* from the soil in which they were produced (Zachmann and Molina, 1993). I do not believe that from this study it is known whether microorganisms are capable of entering the cocoon once it is formed or during its formation. It is worth noting that earthworms pass their cocoon over their mouth as it is being formed. It would be interesting to see if the parent inoculates the cocoon at this point in cocoon production.

Earthworms not only disperse microorganisms important in food consumption but also plant associated mycorrhizae and other root symbionts, biocontrol agents, and microbial antagonists of plant pathogens as well as microorganisms that act as pests (Edwards and Bohlen, 1996). Several researchers have demonstrated the ability of earthworms to promote the dispersal of beneficial soil microorganisms through soils; including pseudomonads, rhizobia and mycorrhizal fungi (Edwards and Bohlen, 1996; reviews Buckalew et al., 1982; Doube et al., 1994a; Doube et al., 1994b; Madsen and Alexander, 1982; Reddell and Spain, 1991; Rouelle, 1983; Stephens et al., 1994a).

2.9.4. Effects of Earthworms on Available Nutrients

Scheu (1990) stated that "nutrient immobilization and mineralization is mainly caused by soil microflora; however, the microbial nutrient turnover is considered to be driven by the activity of soil fauna". The effects of earthworms on soil microflora depend on the nutrient status of the ecosystem. Earthworms appear to have a greater impact on soil microflora in later successional stages of terrestrial ecosystems by making nutrients available (Scheu, 1990). Binet and Trehen (1992) found that the total ^{15}N release from rye-grass litter into soil was three times larger in microcosms with *L. terrestris* than without. Estimates of earthworms' contribution to nitrogen turnover in cultivated soils range from 3 to 63 kg N per ha per yr (Binet and Trehen, 1992). Kale et al. (1992) studied the potential of using vermicompost to reduce the need for chemical fertilizers. Plants in plots where vermicompost was used had considerably greater *Mycorrhizae* association with their roots; 10% being infected in vermicompost plots versus only 3% in controls. There was more P and considerably more N (almost 4 times) taken up by plants in vermicompost plots than those that received the recommended NPK application.

2.9.5. Detoxication of Xenobiotics by Earthworms

Much of the work on earthworm detoxication mechanisms has been done by Jørgen Stenersen and his colleges. Stenersen (1984) provides an excellent review on this subject. Much of the following information is obtained from that review.

A major function of detoxication enzymes is to increase the polarity of xenobiotics, thereby, making them more water soluble and excretable (Nakatsugawa and Nelson, 1982). Microsomal monooxygenases or mixed function oxygenases (MFOs) are present in many terrestrial phyla and mediate the oxidation of a variety of non-polar xenobiotics (Stenersen, 1984). Oxidative metabolism in earthworms has been shown to oxidize aldrin to dieldrin (Nelson et al., 1976); parathion to paraoxon (Nakatsugawa and Nelson, 1982; Stenersen, 1979b); and carbofuran to 3-hydroxy carbofuran (Stenersen et al., 1973; Gilman and Vardanis, 1974). Subcellular distribution, requirement of NADPH and oxygen, and sensitivity to CO indicated that aldrin epoxidase in earthworms is a typical mixed function oxidase involving cytochrome P-450 (Nelson et al., 1976). The highest activity was found in the gut wall and the typhlosole (Nelson et al., 1976).

Glutathione S-transferases make hydrophobic xenobiotics more water soluble and, therefore, excretable by conjugating them with the tripeptide, glutathione. Yang (1986) defines conjugations as “biosyntheses in which natural or foreign compounds or their metabolites combine with readily available, endogenous conjugating agents (e.g., glucuronic acid, sulfate, acetyl, methyl, glycine) to form conjugates”. In addition to their function in detoxication, almost all conjugation mechanisms can act on natural substrates, serving a role in intermediary metabolism. There are two types of conjugation reactions; those where an activated conjugating agent is formed and those where an activated substrate is formed (Yang, 1986; reviews Williams 1968). Conjugations are generally considered “secondary detoxication” mechanisms, because they are typically preceded by other reactions, particularly oxidations (Yang, 1986). GSH-dependent transferases, however may be considered a “primary detoxication mechanism”, because they can catalyze the primary attack on organophosphates, organothiocyanates, and organochlorine compounds. The diversity of GSH-S-transferases allows conjugation with a diversity of substrates (Yang, 1986). The substrate must, however, contain an electrophilic center. The glutathione may conjugate with the xenobiotic by adding to a double bond, cleaving an epoxide bridge, or by substitution of a halogen or other leaving group (Stenersen, 1984).

Worms seemed to be devoid of transmethylase activity and “ligandin” properties (Stenersen and Øien, 1981). The inability to conjugate γ-HCH (Stenersen and Øien, 1981) may in part explain the long lasting effect that a single dose of γ-HCH has on earthworms (Stenersen, 1979a). Similarly *L. rubellus* was shown to lack the ability to selectively eliminate certain PCB congeners (Larsen et al., 1992), which is an ability higher animals posses.

Stenersen et al. (1979) demonstrated glutathione S-transferase activity in six Lumbricid species. Qualitative comparison of the enzymes in different species using isoelectric focusing appeared to follow a taxonomic pattern. *E. foetida* contained on the average about twice as much glutathione as the other earthworm species. No correlation between glutathione concentration and GSH S-transferase activity could be made, however. Stenersen and Øien (1981) studied the properties of GSH S-transferases in nine species of worms. The distribution of in the tissues of *A. longa* and *L. terrestris* was studied. Enzymes were present in all the tissues that were investigated. The nephridia had the highest activity; therefore a role in excretion is likely. The crop and ventral nerve cord were also high in activity, but surprisingly low activity was found in chloragogenous tissue. GSH S-transferase activity in *E. foetida* is very complex (Stenersen and Øien, 1981). Anion exchange and hydroxyl apatite adsorption chromatography demonstrated that GSH S-transferase activity in *E. foetida* was the result of several enzymes. Different peaks from ion exchange chromatography showed different activity toward various substrates. The narrow substrate specificity of these glutathione s-transferases in earthworms indicates that they may function more in the excretion of endogenous catabolic products than in the detoxication of xenobiotics.

2.9.6. *Eisenia foetida*

The earthworm species that I chose to use in my studies was *Eisenia foetida*. *E. foetida* is divided into two species (Øien and Stenersen, 1984). Two color variants formerly describes as the subspecies *E. foetida typica* and *E. foetida unicolor* were found to have differing esterase patterns and produced sterile hybrids. These subspecies have been named *Eisenia fetida fetida* and *Eisenia fetida andrei*. Bacteriostatic lipoproteins in the two subspecies exhibit the same bacteriostatic activities, but different biochemical characteristics. *E. f. andrei* appears to be very different biochemically from *E. f. fetida* and perhaps derived from *E. f. fetida* by the eradication of certain genes (Lassegues et al., 1981; Roch et al., 1984). Shepard (1988) also showed differences between *E. fetida* and *E. andrei* in cocoon production and hatching (ASTM, 1995; reviews Shepard, 1988). Øien and Stenersen (1984) suggest that the two subspecies *E. foetida typica* and *E. foetida unicolor* be named *Eisenia fetida* (Savigny) and *Eisenia unicolor* (André), respectively. Because these subspecies have only recently been designated species, many of the references to *E. foetida* in this review do not distinguish between *Eisenia fetida* (Savigny) and *Eisenia unicolor* (André). Where the original author made this distinction, I refer to them by there new species name, not as *E. foetida*.

E. foetida is a ubiquitous endogeic species that is commonly found associated with manure and has, therefore, earned the common name, manure worm (Roberts and Dorough, 1985). It is also known as a red wiggler. *E. foetida* requires copious amounts of decaying organic matter and can be found in decaying logs, compost and manure piles (Roberts and Dorough, 1985). Soil consumption for *E. foetida* is estimated to be 16 mg soil / individual / day (ASTM 1995; reviews Stafford and Edwards 1985). The nitrogen excretion rate of *E. foetida* has been estimated at 0.4 mg/g/day, which is very high relative to other earthworm species (ASTM, 1995).

The live cycle of *E. foetida* from cocoon to first reproduction may vary from 51 days at 25 °C, to greater than 166 days at 13 °C (ASTM 1995; reviews Tomlin and Miller, 1980). Once sexually mature, *E. foetida* is a prolific breeder, producing a cocoon every 7 to 10 days. Cocoons hatch in 14 to 21 days and can produce 1-11 hatchlings; however, generally contain two to seven young (Roberts and Dorough, 1985; ASTM, 1995). *E. foetida* is the only Lumbricid species found to average greater than one worm/cocoon, averaging 3.3 (Edwards and Bohlen, 1996; reviews Evans and Guild, 1948a; Edwards, 1988). *E. foetida*'s maximum life expectancy is 4 to 5 years; however, 1-2 years is more common (ASTM, 1995; reviews Reynolds, 1977)

E. fetida prefers soils with a pH of 8.0 to 8.5 (Edwards and Bohlen, 1996; review Rivero-Hernandez, 1991) but can tolerate a pH range from 4.0 to 8.0 (Edwards and

Bohlen, 1996; review Edwards, 1988). Kaplan et al, (1980), however, found that *E. foetida* , exposed to pH values less than 5 or greater than 9, died within 1 week; optimum pH for weight gain centered around 8.0. Van Gestel et al. (1992b) studied the influence of temperature, moisture and soil pH on growth and reproduction of *Eisenia andrei.* The objective of the study was to explain variability between ecotoxicity-reproduction studies. This study was carried out in artificial soil as described by the EEC (1985) and finely ground cow dung was added as a food source. Cocoon production was optimal at 20 °C, 85% moisture and was significantly reduced at pH ≥ 8. Considering results from this study Van Gestel et al. recommend that the pH limits recommended by the EEC (6.0 ± 0.5) should be followed. However, since lower pH values do not appear to inhibit reproduction, pH values should range between 5 and 6 for reproduction tests. The optimal moisture content for cocoon production of 85% (m /m) exceeds field capacity (55%), which is recommended by the EEC (1985). The moisture content of soils should, therefore, be higher for reproduction tests than for adult mortality tests. Tsukamoto and Watanabe (1977) demonstrated that increasing the incubation temperature for *E. foetida* cocoons from 10 °C to 25 °C in 5° intervals decreased cocoon hatchibility, number of young worms emerging from each cocoon and development time of cocoons. The growth of young *E. foetida* over a 200 day period, however, increased with increasing temperature. The optimal temperature for survival was 15 °C, however temperatures between 10 °C and 25 °C did not have a dramatic effect of survival. Grant (1955) showed that the temperature optimum for *E. foetida* was 15.8 to 23.2 °C. In addition, the upper lethal limit for *E. foetida* was 25 °C following 48 hours of exposure. *E. foetida* was not able to survive exposure to 0 °C for 48 hours.

2.10. Single Species Toxicity Testing

Biological monitoring in some cases may eliminate the need for chemical measurement (Miller et al., 1985; review Dowd, 1984) or support and / or direct comprehensive chemical characterization of environmental samples (Miller et al., 1985). “The major advantage of this approach is that the biological response to a complex mixture of chemicals integrates the effects of environmental variables such as solubility, pH, antagonism, synergism and time of exposure, all of which affect toxicity to the test organisms (Miller et al., 1985)."

Single species toxicity tests are commonly used to estimate the hazard that a particular xenobiotic may pose to a group of organisms using a representative species. There are three assumptions that must be accepted if one is to accept single species toxicity testing as estimate of hazard to an ecosystem (Cairns, 1992).

1. If one finds and uses "the most sensitive species" in toxicity testing and finds a concentration and time of exposure causing no observable deleterious effects, all other species and the ecosystems in which they occur will automatically be protected. By extension, if fish and aquatic invertebrates are used as test organisms, testing microbial response is unnecessary.
2. It is possible to extrapolate from the results of single-species laboratory tests in containers low in environmental realism to natural complex ecosystems.
3. Natural ecosystems are uniformly sensitive to different forms of stress.

Although single species toxicity tests are a valuable tool for estimating hazard, their shortcomings in representing a complex ecosystem must be recognized. Moreover, it is generally accepted that mortality is a relatively insensitive parameter of questionable ecological relevance (Van Gestel et al., 1988).

E. foetida has gained favor as a "representative" species for practical reasons. It can be readily reared in laboratory conditions, thereby allowing for year round toxicity testing and has a relatively short life cycle. The European Economic Community (EEC), the Organization for Economic Co-operation and Development (OECD), the USEPA, and the American Society for Testing and Materials (ASTM) have all used *E. foetida* for standardized laboratory toxicity studies (EEC, 1985; OECD, 1984, Greene et al., 1989; ASTM, 1995).

If *E. foetida* is to be used as a representative test organism, the relative toxicity of various xenobiotics to *E. foetida* should be compared to other species that it is intended to represent. Miller et al. (1985) compared toxicological profiles for algae (*Selenastrum capricornutum*), daphnia (*Daphnia magna*), earthworms (*E. foetida*), microbes (*Photobacterium fisherii*), mixed sewage microorganisms and plants; wheat "Stephens," (*Trifolium pratense* L.) and cucumber, "Spartan Valor," (*Cucumis sativa*) exposed to selected heavy metals, herbicides and insecticides. Algae and daphnia were the most sensitive test organisms to heavy metals and insecticide followed in order of decreasing sensitivity by Microtox (*Photobacerium fisherii*), DO depletion rate, seed germination and earthworms. Higher plants were most sensitive to 2,4,-D, followed by algae, Microtox, daphnia and earthworms. The herbicides 2,4-D and 2,4,5-T are very toxic to mammals but are only moderately toxic to earthworms and fish (Roberts and Dorough, 1984a). 2,4-dichlorophenol and 2,4,5-trichlorophenol, however, are extremely toxic to earthworms and fish, whereas they are only moderately toxic to mammals (Roberts and Dorough, 1984a).

Edwards and Bohlen (1992) concluded that the susceptibility of different species of earthworms does not appear to differ greatly. What may be more important is the species' behavioral patterns as it relates to exposure (See Section 2.13). Callahan et al. (1994) found good correlation in toxicity for 68 chemicals among the earthworm species, *E. fetida*, *Allolobophora tuberculata*, *Eudrilus eugeniae*, and *Perionyx excavatus*, using the 2-day standard filter paper test (CT) and the 14-day standard artificial soil test (ST). Haque and Ebing (1983) studied the effects of formulated pesticides, including eight fungicides, five herbicides, a plant regulator and ten insecticides, on *L. terrestris* and *E. foetida* using a 14-day soil test. The toxicity of these pesticides differed for the two species; however, the two species were exposed to different soil types, thereby, making results difficult to interpret. Heimback (1985) compared the results of toxicity studies using *E. foetida andrei* in standard artificial soil and *L. terrestris* in a sandy loam amended with 10% ground peat moss. There was a high correlation (0.81) between the two species for the 23 pesticides studied. Seven of the 23 pesticides tested fell outside the 95% confidence limits for the correlation curve; of these, four were highly toxic. Roberts and Dorough (1984a) found good correlation in toxicity between *E. foetida* and *L. rubellus* for three to four of the ten pesticides tested using the filter paper test. Pesticides that did not show good correlation were slightly more toxic to *L. rubellus*. Neuhauser et al. (1986) used the EEC filter paper test and the artificial soil test to compare the toxicity of ten chemicals to four earthworm species, *Allolobophora tuberculata*, *Eisenia fetida*, *Eudrilus eugeniae* and *Perionyx excavatus*. The correlation among species within tests was very high. The largest difference between species resulted from the carbaryl-filter paper test, with *E. fetida* being the least sensitive. Van Gestel and Ma (1988) found that *Eisenia andrei* demonstrated lower LC_{50} values in response to chlorophenols than *L. rubellus*, even though *L. rubellus* accumulated higher concentrations in their tissue. Callahan et al. (1994) found similar toxicity values for *E. fetida*, *Allolobophora tuberculata*, *Eudrilus eugeniae*, and *Perionyx excavatus* exposed to 62 chemicals, suggesting that *E. fetida* may be a representative for these species. Stafford et al. (1986) found *E. foetida* to be the most sensitive species for the availability of heavy metals and dredged sediments (ASTM, 1995; reviews Stafford et al., 1986).

There has been considerable evidence that earthworm sensitivity towards cholinesterase inhibiting insecticides is species specific. Stenersen (1979a) found *E. foetida* to be the most tolerant species to a number of cholinesterase-inhibiting insecticides when compared to *A. caliginosa*, *A. chlorotica* and *L. rubellus*.

The carbamates appear to have differing modes of action in *E. foetida* and *L. terrestris*. *L. terrestris* develops severe tonic tremors, sores and ulcers before dying; whereas, *E. foetida* only develops long lasting tonic tremors (Stenersen, 1979a). Carbofuran is 1/6 as toxic to *E. foetida* as it is to *L. terrestris* when it is injected, and it is 1/2 as toxic in soil (Gilman and Vardanis, 1974). Stenersen et al. (1973), also found carbofuran to be much more toxic to *L. terrestris*, whereas aldicarb was much more toxic to *E. foetida andrei*. Both species took up carbofuran proportional to their size, but *E. foetida* excreted 95% of it in 48 hours; whereas, *L. terrestris* only excreted 10% of it in 48 hours. *E. foetida* did, however, demonstrate characteristics of carbofuran poisoning including coiling and random muscle contractions. Roberts and Dorough (1984a) found a similar narrow range of toxicity for N-methylcarbamate to *E. foetida* and *L. rubellus* , and carbofuran showed equal toxicity to both species. Other carbamates, however, were an order of magnitude more toxic to *L. rubellus* than to *E. foetida* and aldicarb was 160 times more toxic to *L. rubellus* than to *E. foetida*.

Organophosphates, except for acephate, were found to be 10 to 50 times more toxic to *L. rubellus* than to *E. foetida* (Roberts and Dorough, 1984a). The organophosphate parathion and its oxygen analog paraoxon appear to have the same mode of action in both *E. foetida* and *L. terrestris*. However, detoxication mechanisms in these two species for parathion are quite different. The low toxicity of parathion to *E. foetida* may be explained by its low rate of activation to paraoxon followed by a high detoxication rate of paraoxon (Stenersen, 1979b). *L. terrestris* ‘ cholinesterase, however, is severely inhibited by parathion. Ma and Bodt (1993) studied the effects of chlorpyrifos on six species of earthworms in standardized artificial soil. The species studied in increasing order of sensitivity to chlorpyrifos were *E. veneta*, *E. fetida*, *A. longa*, *A. caliginosa*, *L. terrestris* and *L. rubellus*. The most sensitive species, *L. rubellus*, was an order of magnitude more sensitive to chlorpyrifos than *E. fetida*. Cocoon production was also measured for the most sensitive species, *L. rubellus*, and the least sensitive species, *E. veneta*. The 14-day

EC_{50} for cocoon production in *E. veneta* was 121 µg/g and the 14-day LC_{50} for this species was 1184 µg/g. The 14-day EC_{50} for cocoon production in *L. rubellus* was only 9.5 µg/g and the 14-day LC_{50} for this species was 129 µg/g. This study demonstrates that there is not only a large difference between species in adult toxicity, but this toxicity is also reflected in cocoon production. This study also demonstrates that cocoon production is a far more sensitive measure of ecotoxicity than adult mortality. Kula and Kokta (1992) found *E. fetida* to be more sensitive than *A. caliginosa* and *A. longa* to parathion and propoxur exposed in OECD artificial soil. *A. chlorotica* and *E. fetida* had a similar sensitivity to parathion. Stenersen et al. (1992) evaluated the toxicity of a carbamate (carbaryl) and an organophosphate (paraoxon) to three species of earthworm (*E. andrei, E. fetida, and E. veneta*). They used the standard OECD filter paper test and characterized the inhibition patterns of cholinesterases in each of the species. It appears from this work and that of Ma and Bodt (1993) that *E. andrei* and *E. fetida* are more sensitive to organophosphorous inhibiting insecticides than *E. veneta*. This is not, however, as dramatic as the higher sensitivity of *E. veneta* and other earthworm species to carbamates compared to *E. andrei* and *E. fetida*. Because *E. veneta* is more similar to other earthworm species, particularly in sensitivity to carbamates, Stenersen et al. (1992) recommend that *E. veneta* be used for the test organism for cholinesterase-inhibiting pesticides rather than *E. fetida*.

2.11. Toxicity Testing Using Earthworms

Earthworms have several characteristics that make them suitable as key bio-indicator organisms. They are ubiquitous; are large and easy to handle; can be readily collected and identified; are known to be affected by and take up a number of organic and inorganic xenobiotics; are easily bred in the laboratory; and their longevity makes it likely that few controls will die during the test period (Goats and Edwards, 1988). It has been proposed to use the earthworm as a marker species for soil invertebrates, as the rat and fathead minnow serve as marker species for mammals and fish, respectively (Roberts, 1984). The earthworm has been selected as a key indicator organism for ecotoxicity testing of industrial pollutants by: the European Economic Community (EEC); the Organization for Economic Co-operation and Development (OECD); the Food and Agriculture Organization of the United Nations (FAO); the American Society for Testing Materials (ASTM, 1995); by many national pesticide registration authorities including the US EPA (Greene et al., 1989); and environmental pollution committees (Goats and Edwards, 1988). Earthworms are becoming widely used in testing the registration of new chemicals, particularly pesticides (Gibbs et al., 1996).

Lofs-Holmin and Bostrom (1988) reviewed literature regarding the effects of pesticides on soil fauna. After reviewing 60 laboratory and 63 field experiments with earthworms, they found considerable variation between tests in the methodology used, and a lack of information provided. These shortcomings made it difficult to repeat tests and compare results and highlighted the need for standardized techniques.

2.11.1 Earthworm Toxicity Testing Under Field Conditions

Field tests have historically been inadequate in use of controls, replication, adequately defining the dose used, information on the soil type, and variability in assessing effects (Lofs-Holmin and Bostrom, 1988). The major advantage of these tests is that they are entirely environmentally relevant. Suggestions were made by Edwards and Bohlen (1992) for standardizing field experiments.

Methods for sampling earthworm populations are categorized as being passive or active (Edwards and Bohlen, 1996). The sorting of earthworms from soil is a passive method, whereas any method that stimulates the earthworm to leave the soil is an active method. Washing and sieving methods may be used to aid in the recovery of small earthworms and cocoons. These methods, however, are time consuming and tend to damage worms. Electrical methods are variable depending on moisture content and pH; in addition it is difficult to determine the exact volume of soil from which earthworms are obtained. A 0.55% formalin solution is an effective chemical method for bringing earthworms to the surface. Mustard, however, has been shown to be more effective with less environmental impact (Edwards and Bohlen, 1996; review Gunn, 1992). Flooding soil with formalin or mustard tends to be biased toward species with wide and deep burrows. A method similar to a Burlese funnel may be used to collect earthworms from matted surfaces. Edwards and Bohlen (1992) recommend hand sorting the first 5-10 cm of soil and then pouring dilute formalin on to it to recover the endogeic and anecic species, respectively. Earthworms may be marked and recaptured using a water-soluble non-toxic dye that persists on the earthworms for several months (Edwards and Bohlen, 1996; review Meinhardt, 1986). Others have used radioisotopes such as ^{198}Au or small pieces of

^{182}Ta wire inserted into the coelom for mark and recapture studies (Edwards and Bohlen, 1996).

2.11.2 Earthworm Toxicity Testing Under Laboratory Conditions

There have been several laboratory methods used for testing the toxicity of xenobiotics to earthworms (Heimback, 1988). Earthworm related species, such as *Tubifex*, have been used. Xenobiotics have been injected into earthworms. The effects of a toxicant may be tested directly on the earthworm nervous system by using such methods as the Giant Axon Conduction Velocity Test (GACVT). Earthworms have been fed by forced feeding, offering contaminated food pellets or placing contaminated food at the soil surface. Earthworms have been exposed to xenobiotics in a number of different matrices, for example immersion in solutions, filter paper tests, natural soil, artificial soil, and silica matrices. Edwards and Bohlen (1992) reviewed the effectiveness and relevance of these various laboratory tests. The immersion test can provide very reproducible results but it is difficult to relate this to the dose in soil, due to sorption. In tests using topical application the mucus impairs the diffusion of the toxicant into the earthworm. Moreover, the toxicant is sloughed off with the mucus. Results from injection tests are often inconclusive because the earthworms are damaged and effects of the toxicant are often difficult to distinguish from the effects of the solvent carrier. Forced feeding a contaminated gel by injection into the esophagus is an awkward process; earthworms had to first be anesthetized and it was only practical for large species. Laboratory tests using natural soil are very dependent on the physiochemical characteristics of the soil. The filter paper test does not take into account adsorption onto organic matter or clay. The silica matrix, ‘artisol’, test provides a sorption medium of fine silica but it may not accurately reflect natural soil. The artificial soil test has the advantage of being standardized and relevant to soil conditions (Edwards and Bohlen, 1992). The filter paper test, artificial soil test and artisol test are considered possible standard methods by the European Economic Community (Heimback, 1988). The filter paper test provides skin contact; the artificial soil and artisol test provide gut uptake as well as skin contact. (Heimback, 1984). The contact filter paper tests that I have conducted, indicated that there may be some potential for gut uptake during these tests as well. I observed castings of filter paper in the vial following these tests.

In the OECD artificial soil test; the LC_{50}, the no observable effects concentration (NOEC), the lowest observed effects concentration (LOEC), and weight change, are determined (OECD, 1984). The LC_{50} can be determined with a high degree of accuracy, whereas the NOEC-LOEC lacks precision from a statistical point of view (Heimbach, 1992). Chloroacetamide may be used as a positive control for toxicity. Chloroacetamide is very toxic to earthworms and is only poorly adsorbed onto soil. There is contradictory evidence whether its toxicity is affected by soil pH (Heimbach and Edwards, 1983; review Van Gestel and Van Dis, 1988). Other studies have benomyl as a positive control toxicant. Benomyl, however, is not a good positive control because its toxicity is primarily derived from its metabolite (Haque and Ebing, 1983)

2.11.3 Comparison Between Toxicity Tests

Callahan et al. (1985) compared the value of information obtained from the 48-hour filter paper test, the 14-day artificial soil test and the Giant Axon Conduction Velocity Test (GACVT). The 48-hour filter paper test was a short test and highly reproducible. It is however, limited to the detection of water-soluble toxicants. Negative results should not be considered conclusive evidence that the contaminated soil is non-toxic. The 14-day soil test is best suited to assess chemical impacts of known or unknown chemicals in soil. The Giant Axon Conduction Velocity protocol (GACVT) can detect neurotoxins in complex mixtures and as single compounds. This test can detect sublethal neurotoxic effects in shorter periods of time and at lower concentrations than mortality tests.

Several studies have shown poor correlation between the filter paper test and both the artificial soil test and artisol test (Callahan, 1994; Heimback, 1984; Neuhauser, 1986; Van Gestel and van Dis, 1988). Callahan et al. (1994) tested the toxicity of 68 chemicals to four earthworm species using the 48-hour filter paper test (CT) and the 14-day artificial soil test (ST). In 24 of 34 tests, chemicals were more toxic in the (ST) exposure than the (CT) exposure, regardless of species. Also, earthworms were more sensitive to chemicals at low concentrations in the artificial soil test than in the contact filter paper test. Exposure time and intestinal uptake in the soil test may have been the factors affecting toxicity. Heimback (1984) found a high correlation between the artificial soil test and artisol test. Heimback suggested using the artisol test as a method because of its ease in handling and standardizing. Van Gestel and van Dis (1988) determined the effects of pH and organic matter content in two natural sandy soils on the toxicity of four chemicals known to show different adsorption behavior in different soils. Their results support the use of artificial soil as a representative natural soil.

A field test was developed by Heimback (1992) to evaluate ecological significance of laboratory data obtained using the standard artificial soil toxicity test. Twenty-one field tests on 12 different products were conducted at the highest recommended application rate and at four times this rate. There was a good correlation between laboratory results and effects on earthworm populations in the field**.**

2.11.4 Measuring Sublethal Effects

Edwards and Bohlen (1992) described many of the sublethal symptoms that have been observed in earthworms. These include: malformations; effects on activity, such as coiling and mucus extrusion; effects on nervous and physiological functions, such as impairment of locomotion, particularly those related to AchE inhibition; and effects on growth and reproduction.

Haque and Ebing (1983) studied the effects of formulated pesticides, including eight fungicides, five herbicides, a plant regulator and ten insecticides, on *L. terrestris* and *E. foetida* using a 14-day soil test. The sublethal effect of weight loss was independent of the lethality of the pesticide. The behavioral effects caused by insecticides were more severe than those caused by herbicides or fungicides. The most common reaction of *L. terrestris* after 8 days of exposure was coiling of the body and longitudinal muscle contraction, after which the body became rigid and sometimes swellings appeared on the body surface. Worms also appeared to stop feeding, dehydrate, and loose weight.
Gibbs et al. (1996) developed an accurate technique for measuring adult growth and reproduction. There was an obvious need for food substrate, such as fermented alfalfa or peat moss with fermented alfalfa, to stimulate earthworm reproduction. Van Gestel et al. (1988) compared two methods for determining the viability of cocoons produced in toxicity experiments and described a new method for incubating cocoons on artificial substrate and extracting juvenile earthworms from the substrate.

Van Gestel et al. (1992a) compared the sensitivity of survival, growth, cocoon production and cocoon viability as endpoints for evaluating the toxicity of nine chemicals to *Eisenia andrei*. Cocoon production was the most sensitive endpoint for cadmium, chromium, paraquat, fentin, benomyl, and phenmedipham. Cocoon hatchability was the most sensitive for pentachlorophenol and carbendazim. Growth was the most sensitive parameter followed by cocoon hatchability and cocoon production for parathion.

Earthworms are able to detect and avoid chemicals (Edwards and Lofty, 1977; Slimack, 1996). This ability is likely related to food selection and it has been suggested that earthworms can leave trails that contain pheromones (Edwards and Bohlen, 1996; reviews Rosenkoeffer and Boice, 1983). Gilman and Vardanis (1974) determined the potential for *E. foetida* and *L. terrestris* to avoid carbofuran by placing contaminated soil in the center of a bucket and non-contaminated soil around this. Carbofuran appeared to repel *E. foetida* but not *L. terrestris*. Yeardley et al. (1996) developed a test that used earthworm avoidance as a sensitive indicator of soil contamination at hazardous waste sites.

Earthworms are able to concentrate heavy metals in their tissues and therefore may be useful as a biomonitoring tool. Krivoltsky et al. (1982) suggested that earthworms were one of the best indicators of radioactive soil pollution (Edwards and Bohlen, 1996; review Krivoltsky et al., 1982).

2.12. Toxicity of Diazinon to Earthworms

Diazinon is moderately toxic to earthworms (Edwards and Bohlen, 1992). Potter et. al. (1990) studied the effect of pesticides on earthworms and thatch degradation in turfgrass research plots. Diazinon 14 G applied at the highest label rate (4.48 kg a.i. / ha) caused 40 to 60% reductions in earthworm numbers in three of four tests. Earthworm populations recovered to only a 22% reduction in 29 days following treatment with diazinon. Ruppel and Laughlin (1977) exposed *L. terrestris* to soil contaminated with diazinon, to simulate a broadcast dosage. The LC_{50} for Diazinon 14G was determined to be greater than the equivalent of 32 lb. a.i. / A following 3 days of exposure. Edwards et al. (1971) found diazinon to have no effect on earthworm populations when applied at the recommended rate for killing pests. Kring (1969) did not find any dead earthworms on the surface of soil treated with 4.48, 2.24, or 0.56 kg / ha diazinon.

Ebere and Akintonwa (1995) determined the 24 hour LC_{50} of diazinon to *L. terrestris* to be 31.9 μl/L in aqueous solution. Lord et al. (1980) found a diazinon concentration of 10 μg/ml in water to be toxic to *L. terrestris* and *A. longa*.

There have been several studies regarding the effects of acetylcholinesterase inhibiting insecticides on earthworms. Because there have not been as in depth of studies on diazinon, the following addresses information on other acetylcholinesterase inhibiting insecticides. Stenersen (1979a) studied the toxicity of the carbamate insecticides; aldicarb, carbofuran and carbaryl to *E. foetida*, *A. chlorotica*, *L. rubellus* and *A. caliginosa*. This work indicated four sublethal effects related to cholinesterase activity: 1) a tendency to curl up rather than escape into soil when poked or expose to bright light; 2) earthworms taking a longer time to bury themselves when placed on the soil surface; 3) tonic tremors, which is a stiffening of the organism; and 4) dehydration. Dehydration may be a defense mechanism that the earthworm used to rid its body of the toxin. Roberts (1984) defined symptoms leading to death for *E. foetida* exposed to N-methylcarbamate insecticides and organophosphorus insecticides. Symptoms appear to be very similar to those described in Stenersen (1979a). N-methyl carbamate poisoning occurs more rapidly and includes characteristic formation of swellings that eventually burst and become lesions. Similar lesions have not been observed for organophosphorus insecticides.

The carbamates are very toxic to earthworms despite their moderate in-vivo anticholinesterase activity. The organophosphate, paraoxon, however, is much less toxic to earthworms despite its severe cholinesterase depression and relatively slow recovery (Stenersen et al., 1973). The high toxicity of carbamates relative to organophosphates in earthworms, as well as relative species sensitivities, may be explained by a description of their esterase inhibition patterns. Stenersen, (1980a) verified the existence of two cholinesterases in *E. foetida* by selective inhibitors and heat denaturation. These enzymes were separated by ion exchange chromatography (Stenersen, 1980b): E1 consisted of several isoenzymes of equal molecular weight. Stenersen (1979c) studied the inhibition of and reactivation of cholinesterases E1 and E2 in *E. foetida* following in-vivo exposure to carbamates and organophosphates. The objective of this study was to explain the relative toxicity of organophosphates and carbamates in terms of cholinesterase inhibiting activity. E1 was the most sensitive to all the cholinesterase inhibitors tested. E1 was inhibited faster by paraoxon than E2, but it was also reactivated more readily. When E2 was inhibited, but E1 was less inhibited, earthworms showed no symptoms of cholinesterase inhibition. The cholinesterase E2 appeared to be carbaryl resistant whereas E1 was carbaryl-sensitive. Carbaryl caused prolonged depression of the E1 cholinesterase. Earthworms could survive strong and long-lasting inhibition of E1 when E2 was not inhibited, but this caused tonic tremors. The E1 cholinesterase appeared to be the most important in normal performance. The E2, however, can prevent death when E1 is inhibited. *E. foetida* appears to be able to survive severe cholinesterase inhibition provided it is transient.

2.13. Bioavailability of Xenobiotics to Earthworms

Belfroid et al., (1994b) describe the two ways that earthworms may acquire xenobiotics, passive absorption through the body wall and intestinal uptake. Edwards and

Bohlen (1992) stated that earthworms may accumulate xenobiotics either through capillary water or by contact with chemicals on the surface of soil particles.

Edwards and Bohlen, (1992) described the factors affecting exposure to include the chemical's properties, soil type, environmental conditions, and behavior of the earthworms. Chemical characteristics affecting exposure include water solubility, volatility, lipid / water partitioning coefficient, adsorptive capability and persistence in soils. Soil characteristics also will affect exposure. Low soil moisture may increase sorption. Clay and organic matter in soil will also increase sorption. Sorption of nonionic organic compounds by soil in aqueous systems is controlled mainly by the organic matter content of the soil; by contrast sorption by dry and subsaturated soils from nonpolar organic solvents and from vapor phase is determined mainly by the mineral type and content (Chiou, 1989). The cation exchange capacity, which is greatly affected by clay and organic matter, will affect adsorption. The pH of the soil will affect the ionic state of the soil contaminant as well as the adsorptive capacity of the soil. Temperature will affect the contaminant's water solubility and vapor pressure. Increased temperature may increase the binding of strong bonds (e.g., covalent and perhaps hydrophobic and hydrogen bonding) but may decrease the binding if it is as weak adsorption process such as Van der Waals forces (Bollag and Loll, 1983). Cation exchange mechanisms are unaffected by temperature. The sorption of nonpolar organics is independent of temperature (Hasset and Banwart, 1989; review Chiou et al., 1979; Hasset et al., 1980). Other than in low organic matter soils, properties such as clay content, soil pH, hydrous oxide content, etc. have little effect on sorption of nonpolar organic compounds (Hasset and Banwart, 1989).

Pesticides and their degradation products may bind to soil organic matter by chemical mechanisms ranging from sorptive forces to covalent bonding or by physical entrapment in micropores. Bonding mechanisms postulated for pesticides on humic acids include; H-bonding, ion exchange, Van der Waals forces, protonation, charge transfer, ligand exchange, hydrophobic interaction and coordination via a metal ion (Saint-Fort and Visser, 1988).

2.13.1 Effects of Soil Properties on Bioavailability

2.13.1.1 Work with Soil

Van Gestel and van Dis (1988) determined the effects of pH and organic matter content in two natural sandy soils on the toxicity of four chemicals known to show different adsorption behavior in different soils. Toxicity was affected by soil pH and organic matter. The differences in LD_{50}'s between earthworms exposed to the high organic matter artificial soil and the low-organic-matter sandy soil were not greater than a factor of 3-4, however.

The presence of earthworm may also affect the soil physical-chemical properties that influence pesticide bioavailability. Meharg (1996) studied the bioavailability of atrazine to soil microbes in the presence of *L. terrestris*. The presence of *L. terrestris* did not affect the concentration of atrazine in soil solution; their presence did, however initially increase KCl^{-} extractable label. The rate of mineralization in the presence of earthworms was double that of controls. Meharg hypothesized that mucilage secretions in the present study may be the obvious reason for increased binding of atrazine and its metabolites to soil exchange sites, though change in soil structure and microbial community may be partially responsible. He cited evidence by Edwards et al. (1992) and Stehouwer et al. (1994) showing greater binding of atrazine on burrow walls than the bulk soil. Increased bioavailability and increased microbial metabolic activity will act synergistically to increase the rate of mineralization.

Lord et al. (1980) studied the uptake of pesticides from soil and water by earthworm whole bodies and earthworm solids. They described the uptake of chemicals by earthworms as being an equilibrium between chemical in the worm, chemical in the soil water and chemical adsorbed onto soil organic matter. Calculations were based on the assumptions that the earthworm uptake from the soil aqueous phase would be similar to uptake from aqueous solutions and the concentration in the soil aqueous phase would be determined mainly by organic matter content; provided that the chemical was non-polar and stable. Results indicated that these concentrations in the soil aqueous phase were unlikely to be reached because of metabolism of the xenobiotic and slow diffusion of the chemicals in soils. Whole earthworms (*L. terrestris* and *A. Longa*) absorbed over 90% of diazinon from aqueous solutions. Uptake of diazinon was rapid for the first six hours and half of the equilibrium concentration was accumulated in the first two hours. The concentration in whole earthworms reached an equilibrium in 24 hours. The uptake of diazinon by macerated earthworm tissue was much more rapid reaching equilibrium in a few minutes. Earthworms were also exposed to soil contaminated with 1 µg/g diazinon, based on dry weight of soil. The soil concentration decreased to 0.5 µg/g after 32 days of incubation. The concentration in earthworm initially increased to a maximum of approximately 0.4 µg/g, based on fresh weight, following 18 days of incubation. The concentration in soil at this time was approximately 0.8 μg/g. The concentration in earthworms then decreased to approximately 0.2 μg/g following an additional 24 days of exposure.

2.13.1.2 Work with Soil Models

Additional information on the bioavailability of sorbed soil contaminants has been gathered for microorganisms. The primary objective of these studies was to determined degradation rates of sorbed soil contaminants. These models may also be relevant to earthworms, however, provided that earthworms are only able to obtain contaminants from soil solution. Most sorption rate models developed and applied to date can be divided into several categories: first-order rate models and two-site rate models; pore diffusion models; surface-diffusion models; or combined pore and surface-diffusion models (Miller and Pedit, 1992).

Miller and Alexander (1991) developed a model for the biodegradation by non-growing microbial populations of sorbed organic chemicals that are readily desorbed. This model combines information obtained from sorption isotherms under aseptic conditions and the rate of biodegradation in the absence of sorbent. Miller and Pedit (1992) demonstrated that, even with relatively uniform subsurface materials, long term sorption-desorption phenomena may be complicated by the slow nature of sorption and desorption processes and by the degradation of the contaminant from solution and solid phases at different rates.

Scow and Hutson (1992) developed a mathematical diffusion-sorption-biodegradation (DSB) model to describe the biodegradation of an organic chemical in the presence of spherical microporous aggregates, under conditions of substrate limitation. The DSB model was tested experimentally by Scow and Alexander (1992). The DSB model was found to simulate the biodegradation of phenol and glutamate originating inside aggregates of kaolinite clay as well as the biodegradation of glutamate in an experimental system containing gel-exclusion chromatography beads.

Studies conducted by Scow and Hutson (1992) and Scow and Alexander (1992) used a non-adsorbing solute and mainly nonattached bacteria, conditions that are rarely found in nature (Harms and Zehnder, 1995; review Gray and Parkinson, 1968). Harms and Zehnder (1995) used a defined model system, in which 3-chlorodibenzofuran (3CDF) was the organic contaminant, porous Teflon granules were the sorbent, and *Sphingomonas* sp. strain HH19K was the test organism, to examine the bioavailability of a sorbed compound for a bacterium attached to a sorbent. Adsorption between the 3CDF and the Teflon, mainly took place inside the pores of the granules. The kinetics of desorption could be ascribed to sorption-retarded radial diffusion. 3CDF consumption by the *Sphingomonas* sp., which were irreversibly adhered to the exterior of the Teflon granule, drove desorption rates considerably. They proposed that the rate at which sorbed substrate becomes available to organisms is influenced by 1) the specific affinity of the degrading organisms (i.e., their ability to reduce the aqueous substrate concentration) and 2) the tendency of the organisms to adhere to the sorbent.

2.13.2 Effects of Earthworm Behavior on Bioavailability

The behavior of the earthworm will also affect exposure (Edwards and Bohlen, 1992). When contaminants are associated with a particular soil horizon, the species' habitat space is an important consideration. Epigeic and anecic earthworms would be more affected by xenobiotics associated with surface litter. "*Aporrectodea longa* seems to be less susceptible to pesticides than many other species of earthworm, because it can burrow deeply into the soil and enters an obligatory diapause during the summer (Edwards and Bohlen, 1996)." Juvenile earthworms may be unable to burrow deeply into soil and can therefore be affected more severely (Edwards and Bohlen, 1996).

L. terrestris moves through well-defined burrows and therefore only comes into contact with a small part of the soil volume (Lord et al., 1980). Pesticides have been shown to diffuse slowly into the permanent burrows of earthworms (Edwards and Bohlen, 1992; reviews Edwards, 1983). Theoretically water cannot move from soil micropores into macropores unless the macropores are already saturated (Edwards et al., 1989; reviews Gardner, 1979). It may the case, particularly in no-till fields, that the burrows of *L. terrestris* serve as conduits for a large fraction of the pesticide laden infiltrating water after rain storms (Edwards et al., 1992, 1989, 1988).

If earthworms are able to acquire xenobiotics at a greater rate through feeding than through dermal exposure, then their feeding behavior and avoidance behavior may effect bioavailability. Results of Belfroid et al. (1994a) suggest that significant intestinal uptake of hydrophobic contaminants does take place and it increases with increasing hydophobicity of the chemical. Elimination rate constants also decrease with increasing hydrophobicity (Belfroid et al., 1995). Belfroid et al. (1994a) found that the bioaccumulation factor of hydrophobic compounds in earthworms, *Eisenia andrei*, was greater in soil than in aqueous system. If passive adsorption of interstitial water was the only mode of uptake, higher bioaccumulation factors would have been expected in the aqueous environment that in soil. These results are contradictory to the conclusions of Van

Gestel and Ma (1988) and Larsen et al., (1992) that suggested that passive adsorption was of primary importance.

2.13.3 Bioavailability of Bound Residues

2.13.3.1 General Overview of Bound Residues

The formation of unextractably- bound or incorporated residues plays a significant role in the fate of many pesticides in different environments. These bound residues will likely escape detection by conventional analytical methods (Khan, 1991). The term bound residue may be determined by the method of extraction. A residue that may be unextractably bound using one extraction method may be extractable with other solvents or reaction conditions (Bollag and Loll, 1983). The incorporation of toxic xenobiotics into soil humus generally reduces leaching, bioavailability and toxicity (Bollag, 1991).

It is important to understand some of the fundamental properties of humus and the humification process to understand the nature of bound residues. Brady (1974) defines humus as “a complex and rather resistant mixture of brown or dark brown amorphous colloidal substances synthesized from the tissues of various organisms” (Bollag and Loll, 1983; review Brady, 1974). “Humic material is usually divided into 3 fractions: 1. humic acid, which is soluble in dilute alkali and precipitates upon acidification of the alkaline extract; 2. fulvic acid, which is soluble in acid and base; and 3. humin, which is insoluble in both acid and dilute alkali (Bollag and Loll, 1983)”. The fundamental structures of these fractions are aromatic polymers made up of nitrogen heterocycles, quinones, phenols and benzoic acids. The aromatic polymers have carboxyl, hydroxyl, carbonyl and thiol groups; which may act as binding sites and aliphatic moieties (Bollag and Loll, 1983; review Stevenson, 1982). Fulvic acid contains more oxygen but less carbon than humic acid. Most of this oxygen is as hydroxyls, carbonyls, etc.; whereas, in humic acid oxygen is in the core structure as ether and ester linkages. Fulvic acid has a molecular weight ranging from 280 to 2100 daltons (Bollag and Loll, 1983; review Schnitzer, 1978). The molecular weights of humic acids range from 1400 to 1,000,000 daltons (Bollag and Loll, 1983; review Stevenson, 1982). Humin is similar to humic acid in its analysis and properties but it is insoluble in dilute alkali. This insolubility may be the result of complexes with inorganic soil materials (Bollag and Loll, 1983; review Schnitzer, 1978). Four hypotheses have been proposed to explain the humification process (Bollag and Loll, 1983). These include: 1) the plant alteration hypothesis, where lignin and other resistant plant materials in soil undergo only slight changes to form high molecular weight humic acids, which gradually decompose to fulvic acids, water and CO_2; 2) the microbial synthesis hypothesis, microorganisms produce intracellular humic polymers by normal metabolism of plant remains and when they die they release these polymers into the soil; 3) the chemical polymerization hypothesis, in which soil microbes produce amino compounds and phenols and release this into the environment where they polymerize; and, 4) the cell autolysis hypothesis, in which both plant and microbes die, autolyze and their residues polymerize. All four these processes likely occur but their relative predominance is unknown. One of the most important polymerizing reactions is oxidative coupling, which is a free radical reaction that links phenols, anilines, amino acids or other compounds into polymers. This reaction is caused by plants, microorganisms, metals and clays.

There may be a relationship between the amount of binding of a pesticide or degradation product and the nature of substitution on the amino nitrogen; degradation products containing secondary or primary amino functional groups may become part of the bound residue in soil (Khan, 1991). Certain classes of pesticides and pesticide degradation products strongly adsorb to soil organic matter but do not form covalent bonds (Bollag and Loll, 1983). The nature of binding of phenols and anilines to soil organic matter can range from sorptive forces such as charge-transfer, hydrogen bonding, and hydrophobic interactions to irreversible covalent bonds (Bollag, 1991). Two mechanisms by which xenobiotics can become incorporated into soil organic matter have been proposed: 1) surface bound where the xenobiotic attaches directly to the reactive sites on colloidal organic surfaces; and 2) incorporation, where the xenobiotic is incorporated into the structure of newly formed fulvic and humic acids during the process of humification (Bollag, 1991; reviews Stevenson, 1976; Bartha, 1980; Bollag, 1983).

Physical entrapment may play a role in the formation of bound residues. Humic materials may consist of phenolic and benzenecarboxylic acids joined by hydrogen bonds to form a molecular sieve-type polymeric structure of considerable stability (Khan, 1991; reviews Schnitzer and Hoffman, 1965). One of the characteristics of this proposed structure is that it would contain voids or holes of different molecular dimensions that could trap organic molecules such as pesticides and/or their degradation products. (Khan, 1991)" (See Figure 2).

Khan (1991) used a thermoanalytical method described by Schnitzer and Hoffman (1965) to obtain information on the nature of bound residues of pesticides and their degradation products. The technique involved isothermal High Temperature Distillation (Khan, 1991; reviews Khan and Hamilton, 1980). In the humic fraction COOH and OH groups are eliminated between 200 and 400 °C, decomposition of the humic nuclei occurs at 450 to 550 °C. The pesticide residues that linked to with phenolic-OH and -COOH groups of the SOM may be released during their degradation. Alternatively, the thermal decomposition of the organic matter's structure may also result in the release of these bound residues.

2.13.3.2 Bioavailability of Bound Residues to Microorganisms

Bollag et al., (1988) incubated phenolic pesticide metabolites with laccase and a naturally occurring phenol in the presence of *R. praticola*. The experiment demonstrated that the toxicity of the phenolic pesticide decreased in the presence of laccase due to transforming and/ or polymerizing reactions (Bollag, 1991; reviews Bollag et al., 1988). Dec and Bollag (1990) studied the release of bound ^{14}C-chlorophenols from humic complexes. 4-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and pentachlorophenol were covalently bound to a synthetic humic acid polymer and incubated with soil microbial cultures. Over a 10 week period, relatively small fractions of the chlorophenolic compounds were released. This release was accompanied by simultaneous mineralization of 1.2 to 6% of applied chlorophenolic compound. Most of the chlorophenol remained bound to the humic material. In a similar study, Dec et al. (1990) compared the release of ^{14}C-labeled 2,4-dichlorophenol (DCP) that was incorporated versus DCP that was surface bound. They also attempted to clarify whether the release of chlorophenol from synthetic humic material is similar to that from natural humus. Residues of DCP incorporated in the DCP polymer appeared to be bound more tightly than DCP bound to humic materials. Synthetic humic acids also appeared to bind DCP more strongly than natural humic acids. Khan and Behki (1990) demonstrated that two *Pseudomonas* species, known to be capable of atrazine transformation, were able to release and transform bound atrazine residues.

Hsu and Bartha (1974b) found that soil-bound radiolabelled 3,4-dichloroaniline (DCA)was mineralized to $^{14}CO_2$, at a rate of 1% per week. Steinberg et al. (1988) demonstrated that indigenous soil bacteria were unable to degrade residues of EDB in fumigated soils, while freshly added ^{14}C-EDB was mineralized within days in the same soil, at comparable concentrations. (Pignatello, 1989; reviews Steinberg et al., 1987)

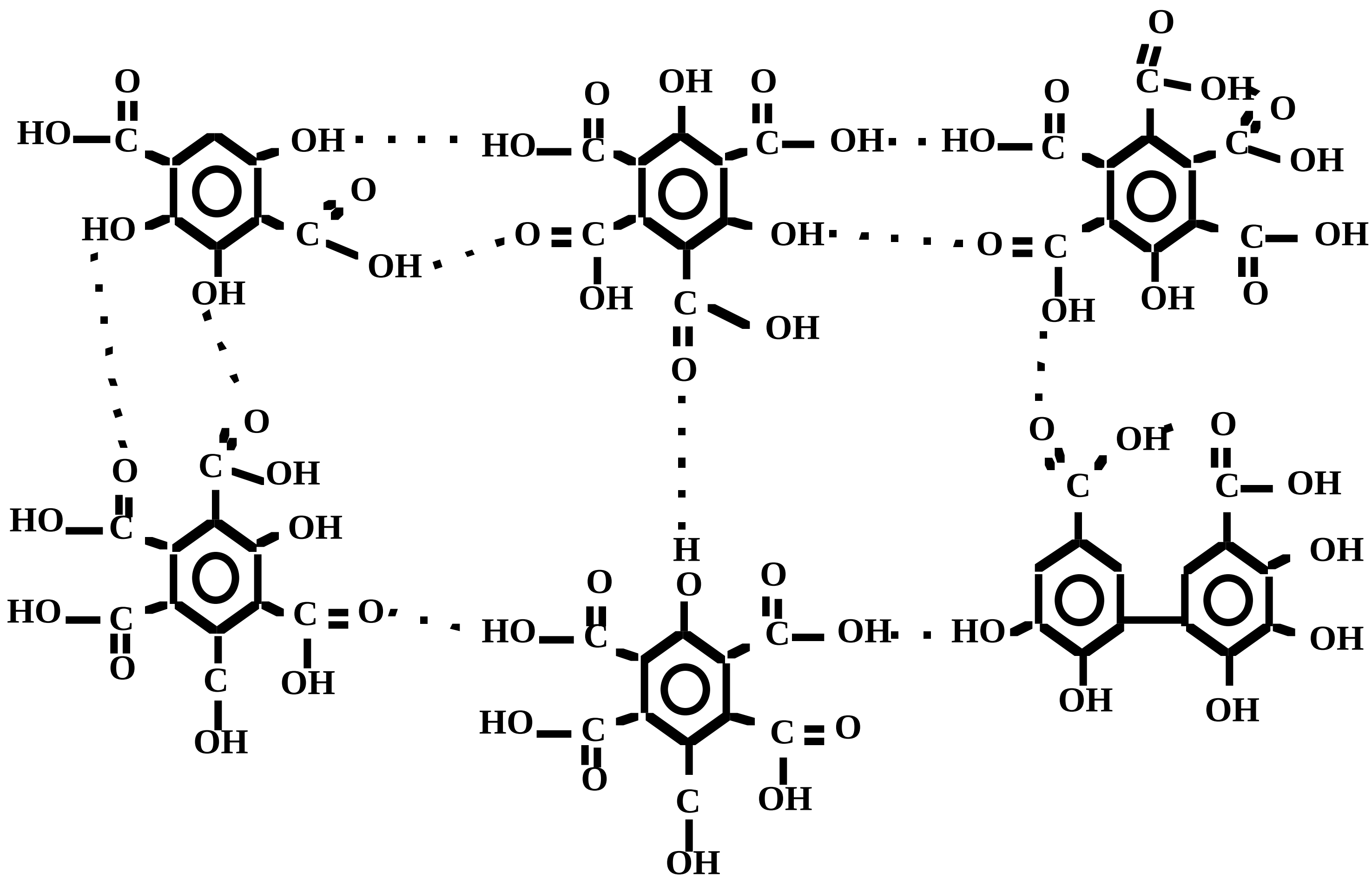


Figure 2. Partial structure of humic materials showing how these can act as “molecular sieves”. (Adapted from Schnitzer and Khan, 1972).

2.13.3.4 Bioavailability of Bound Residues to Earthworms

Although bound residues may be less bioavailable to earthworms, there is evidence that bound residues may be released by earthworm activity and incorporated into earthworm tissue. Studies on the bioavailability of bound residues are scarce. This, however, is an issue that may need further elucidation considering the fact that the fate of so many pesticides includes the formation of bound residues.

Fuhremann and Lichtenstein (1988) found that unextractable bound residues of soil-bound methyl [^{14}C] parathion residues were able to be taken up by earthworms (*L. terrestris*) following 2, 4 and 6 weeks of exposure. The extraction procedure to determine unextractable bound residues involved nine, 6 hour soxhlet extractions with various solvents over a 12 day period. After this, soil was incubated for 19 days and extracted a tenth time for 6 hours. Following this extraction regime, 33 % of the ^{14}C-residues remained. Earthworms were exposed to this unextractable fraction. Following 6 weeks of incubations it was calculated 30 earthworms would absorb approximately 3% of the bound residues of in 5000 g of soil. In earthworms that had accumulated radioactivity from bound residues, 58-66 % of these residues became again bound in the earthworm. In earthworms exposed to fresh residues of methyl [^{14}C] parathion only 18-33% of the residues became bound in the earthworm tissue. However, earthworms incubated in freshly deposited methyl-parathion soil had 13 times more radioactivity than those incubated in bound methyl-parathion soil. In soil containing freshly deposited methyl [^{14}C] parathion, ^{14}C-residues concentrated in the anterior end. In soil containing bound deposited methyl [^{14}C] parathion, ^{14}C-residues concentrated in the middle section. The presence of earthworms also increased the relative concentration of extractable residues in the soil. Following an incubation of 42 days with worms, 3.8% of the methyl [^{14}C] parathion was extractable from the soil. In soils incubated without earthworms only 2 % of the methyl [^{14}C] parathion was extractable. It is worth noting that only 51% of the total radioactivity in this study was accounted for in the mass balance.

Haque et al. (1982) found that soil-bound [^{14}C] 'Hydroxymonolinuron'-β–Δ-glucoside residues in earthworms (*L. terrestris*) reached a concentration equal to that of the surrounding soil (based on dry weight) following 42 days of exposure. Freshly deposited (not bound) [^{14}C] 'Hydroxymonolinuron'--β–Δ -glucoside accumulated, in worm whole bodies to a concentration greater than the surrounding soil following 21 days of incubation. Residues remaining after one, 18 hr extraction with chloroform : methanol : water (1 : 1 : 1 by volume) and one 18 hr soxhlet extraction with methanol : chloroform (1 : 1 by volume) were considered to be unextractable. Using a procedure by Roberts and Stranden (1988), the fulvic acids, humic acids and humin were extracted from the soils; 29% of the radiocarbon was associated with fulvic acid, 20% with humic acid, and 9% with the humin fraction. Solubilization by means of 0.5 M acid and alkali appeared to be a criterion for predicting the bioavailability of bound phenylurea residues. The half life of the soil bound residues was estimated to be about 4.6 years based on mineralization of hydroxymonolinuron-B-D-glucoside. They also stated the mineralization on phenylureas may be dependent on the turnover rate of humic substances.

Riley et al. (1986) found that paraquat soil residues are for the most part unavailable to earthworms. After applying paraquat to a field site at the recommended rate of 2 kg/ha and at a rate of 112 kg/ha, earthworm whole bodies, including gut contents, contained 0.6-0.8 μg/g and 30-90 μg/g paraquat, respectively. However, after dissecting *L. terrestris*, the gut contents were found to contain 111 μg/g paraquat whereas the whole bodies only contained 0.3 μg/g fresh weight. Earthworms were also shown to be able to eliminate paraquat rapidly. After treating soil at the recommended rate of 2 kg/ha, earthworms were found to contain 1.8 μg/g of paraquat. However, after re-incubating these earthworms for 36 hours on uncontaminated soil, residues in earthworms were undetectable (< 0.1 μg/g). It is not stated whether earthworms were extracted after the removal of gut contents or not. If the gut content was not removed, this elimination may have been due to evacuation of the earthworm's gut contents in the 36 hour period, rather than a detoxication mechanism.

Ebert (1992) cultured *E. foetida* and *L. rubellus* in two soil types (sandy loam and loamy sand) containing extractable and bound residues of ^{14}C-labeled bentazon. Bentazon is known to be quickly transformed in the soil, resulting in the incorporation into humic substances. Before earthworms were exposed to the soils, approximately 10% of the total radioactive residues were extractable from sandy loam and approximately 4 % total radioactive residues were extractable from loamy sand. In loamy sand the primary extractable ^{14}C compound was N-methylbentazon. In sandy loam the primary extractable ^{14}C compound was bentazon. Following 14 days of exposure, earthworms were prepared by killing them in 4% formalin. They were then either dissecting longitudinally and their gut contents were flushed or were dissecting transversely without removing their gut contents. Others were frozen in liquid N and dissected transversely without removing the gut contents. The various fractions of the earthworm were all combusted. Apparently no effort was made to identify the ^{14}C compounds in the earthworm. In both soil types the concentration of radioactive residues was higher in worm tissues than in initial extracts of the soil. Earthworms of both species held in sandy loam took up less radioactivity than those held in loamy sand. The fact that concentrations of radiolabelled material were higher in earthworms than in initial extracts of soil is not in itself evidence for release of bound residues. Earthworm tissues may simply have concentrated the extractable residues. In order to prove that earthworms were able to release unextractably- bound residues of bentazon or degradation products, it would be necessary to expose earthworms to soil that had already been exhaustively extracted. Tissues of the alimentary canal generally contained more activity than those of the remaining body. In loamy sand, *L. rubellus* contained significantly higher amounts of radioactive residues in its posterior gut than *E. foetida*. In *L. rubellus*, the posterior parts of the body contained significantly higher amounts of radioactive residues than the anterior segments, irrespective of the mode of tissue preparation. In *E. foetida*, the posterior parts of the body contained similar amounts of radioactive residues than the anterior segments, irrespective of the mode of tissue preparation. Differences between the amounts of ^{14}C-labeled material in earthworm preparations with or without gut contents were greater for *E. foetida* than for *L. rubellus.*

3 . Chapter 3 Project I: The Toxicity of Composted Diazinon Waste to Earthworms (*Eisenia foetida*)

3.1 Abstract

Studies were conducted to evaluate the toxicity of composted diazinon sorbent to the earthworm *Eisenia foetida*. A system that is being developed by the Pesticide Waste Disposal Group was used to treat a simulated diazinon rinsate (1500 μg/mL). Following a two-step sorption process, the sorbent material was open-air dried in a greenhouse, amended with nutrients and composted. After 30 and 60 days of composting, the material was incorporated into soil. Earthworms (*E. foetida*) were exposed to this amended soil. Mortality and sublethal effects (ability to burrow, curling, and weight change) were determined at 48 hour intervals for 18 days, at which time mortality in controls exceeded 10%. Earthworms that were exposed to soil amended with uncomposted diazinon sorbent and soil amended with 30-day composted diazinon sorbent demonstrated sublethal effects and mortality. Earthworms that were exposed to soil amended with 60-day composted diazinon sorbent did not demonstrate sublethal effects or mortality.

3.2 Introduction

A process for cleaning up pesticide rinsewater using sorption onto an organic matrix followed by biodegradation is being developed (Hetzel et al., 1989; Mullins et al., 1989; Judge et al., 1990; Hetzel et al., 1992). This process has been demonstrated to be very effective at removing diazinon from waste water and then degrading it in a composting environment (Mullins et al., 1992). We are now at the point where we must consider options for disposing of composted pesticide wastes. The most practical option would be on site land application of this material. In the case of composted diazinon sorbent, the hazard that this material would present cannot be determined solely by chemical analysis. The fate of diazinon in the composting environment includes the formation of significant quantities of unidentifiable bound residues (40%) (Petruska et al., 1985). It has been demonstrated that earthworms are able to acquire radioactivity when exposed to soil containing bound radioactive pesticide residues (Fuhremann and Lichtenstein, 1988; Haque et al., 1982; Ebert, 1992). It is, therefore, necessary to supplement chemical analysis of the composted diazinon sorbent with bioassays to evaluate the potential hazard of land applying this material. Bioassays will also need to be conducted with other pesticides that are known to form bound residues (see Section 2.11.3 Bioavailability of Bound Residues) or that are degraded to products of unknown toxicity.

3.3 Materials and Methods

3.3.1 Demulsification, Sorption and Composting of Diazinon and Controls

3.3.1.1. The Sorption Demulsification Process.

A detailed description of the equipment used in the sorption/demulsification process is available (Phillips, 1994). The sorption/demulsification process was used to treat four replications of diazinon contaminated waste water and three replications of uncontaminated tap water (controls). For each of the four diazinon replications, 133 L (35 gal.) of tap water was contaminated with diazinon 4E (48.5% A.I.) at a calculated concentration of 1500 mg/L (pH = 7.4 ± 0.07; conductivity 156 ± 16). A sample of this initial simulated waste water was taken for analysis of diazinon concentration. Samples of the untreated diazinon rinsate were either frozen in plastic containers at -20 °C for or refrigerated in glass containers at 4 °C until they could be extracted. The control replications treated uncontaminated tap water using the sorption/demulsification process. Step one was a mixing process that involved destabilization of the diazinon emulsion and sorption onto peat moss. A 19 L (5 gal.) bucket (2.6 ± 0.5 kg. dry wt.) of sphagnum peat moss was then added to the 133 L (35 gal.) of either diazinon contaminated water or tap water. Hydrated lime [$Ca(OH)_2$; 0.9 ± 0.1 kg wet wt.] was then added. The peat moss, $Ca(OH)_2$ and water were then mixed for 4 hours. During this mixing process, rinsate from the outer liner was recirculated back into the inner liner. After 4 hours of mixing, the inner liner was then raised and the aqueous phase was filtered from the sorbent material. Sharkskin filter (50 cm diameter; Schleicher & Schuell, general purpose, medium to fine particle retention) paper overlaid with a fine mesh screen trapped fine particulates during this filtering process. Step two involved the sorption of the diazinon that was not captured in step one by a peat moss packed column. A column, constructed from 8 inch (20.3 cm) PVC pipe, was packed with a 19 L (5 gal.) bucket (2.6 ± 0.6 kg dry wt.) of peat moss. This column was pre-equilibrated with water. The filtered rinse water from the first sorption step was then pumped onto the top of this column. The column flow rate was slow (approximately 40 mL/min) to allow for efficient sorption of diazinon. The final treated rinsate was then sampled and contained in a 209 L (55 gal.) plastic barrel. Sorbent material from steps one and two were dried on black plastic in a greenhouse until they could be composted. Sorbents from the four diazinon rinsate treatments dried for 4, 5, 8, and 10 weeks, respectively. Sorbents from the first two control rinsate treatments were dried for 13 weeks, and the third control rinsate treatment was dried for 14 weeks.

3.3.1.2. Composting of Sorbent Material.

The step one and two sorbents from each rinsate treatment were combined. The combined sorbent material from control runs and diazinon runs were each amended with the following nutrients based on total wet weight; 1% corn meal, 0.3% ammonium nitrate, 0.02% N/P/K (15/30/15) fertilizer, and 4% vegetable oil. Ammonium nitrate and N/P/K fertilizer were added as a solution. An inoculum solution was made from a previous diazinon bioreactor and added to the sorbent. In addition, approximately 12 % peanut hulls (weight/weight) and 3% grass clippings (weight/weight) were added to the sorbent material. Water was added qualitatively and the moisture content was later determined quantitatively. The nutrients were mixed with the sorbent material on a wooden platform using a rototiller and shovel to ensure homogeneity. The amended control sorbent was then divided into three equal portions and the diazinon sorbent was divided into four equal portions. These portions were placed into 38 L mesh bags (1.5 mm pore size). A sample of the material in individual bags was taken to analyze for diazinon concentration and pH and for earthworm toxicity testing. The mesh bags were made of a cotton/polyester material and the seams hand sewn with nylon rope. The bags were prewashed with hot water and a mild bleach to remove dyes that might have interfered with analytical analysis or bioassays.

The three bags that contained control sorbent were placed into a single bioreactor and the four bags that contained diazinon sorbent were placed into an adjacent bioreactor. The bioreactors were 1 m^3, constructed with wood and lined with aluminum sheeting to insulate the compost and protect the wood. The floor of the bioreactor was elevated on 2” x 4” (5.1 x 10.2 cm) studs, each containing 3/4 inch (1.9 cm) holes and fixed with a 1/4 inch (0.64 cm) mesh galvanized steel screen to increase ventilation. The top of the bioreactor was equipped with two vents, as well. A more detailed description of these bioreactors is provided by Judge (1996) (Section 4.3.2.2.4.).

Amended sorbent from diazinon and control rinsate treatments did not provide an adequate volume for optimum composting conditions. Previous studies have demonstrated that the volume of sorbent material generated from sixteen rinsate treatments was adequate for optimum composting conditions. An additional 494 L of compost was, therefore, used as filler in the control bioreactor and 456 L was used in the diazinon bioreactor. The same proportions of nutrients as well as grass clipping and peanut hulls, which were added to the control and diazinon runs, were added to the filler material. The nutrients were mixed as described previously. This material was then used to surround the bags that contained either diazinon or control sorbent.

The amended sorbent material from control and diazinon runs, as well as the amended filler material for both bioreactors, were weighed following the addition of nutrients and water. This was done to determine the change in biomass during composting cycles. The total wet weight was determined to be 88 kg for amended control sorbent, 405 kg for filler compost in the control bioreactor, 95 kg for amended diazinon sorbent, and 386 kg for filler compost in the diazinon bioreactor. The average percentage moisture for the diazinon and control bioreactors were 67 % and 69 %, respectively.

Following 30 days of composting, additional amendments were added to the control and diazinon sorbents and to the filler material. The proportion of each of these amendments was the same as was used initially. Grass clippings and peanut hulls, however, were not added. Sorbent from individual bags were mixed in large plastic tubs using an electric drill equipped with a 2 inch drill auger. A sample of the material in individual bags was taken to analyze for diazinon concentration and for earthworm toxicity testing. The mass of the amended sorbent materials from control and diazinon rinsate treatments, as well as the amended filler material for both bioreactors, were determined following the addition of nutrients and water. The total wet weight, was determined to be 84 kg for amended control sorbent, 362 kg for compost filler in the control bioreactor 95 kg for amended diazinon sorbent, and 385 kg for compost filler in the diazinon bioreactor The average percentage moisture for the diazinon and control bioreactors were 69 ± 3 % and 68 ± 1 % (std. dev.), respectively. The average percentage moisture for the diazinon and control bioreactors following 60 days of composting were 68 ± 3 % and 81 ± 1 % (std. dev.), respectively. A sample of the material in individual bags was taken to analyze for diazinon concentration and for earthworm toxicity testing.

3.3.1.3. Determining Temperature Profile.

The temperatures in the control and diazinon bioreactors were monitored at 1 min intervals using a data logger (Model 21X, Campbell Scientific Inc., Logan, UT). Five probes were placed in both the diazinon and control bioreactors and a single probe was used to measure ambient temperature. Temperatures were monitored in each of the individual sorbent material bags, in the filler material near the edge of the compost, and ambient temperatures at the surface of the earthworm pit. Temperatures were averaged and recorded at 60 min intervals.

3.3.1.4. Extraction and Analysis.

3.3.1.4.1 Diazinon Extraction - Water

Untreated rinsate (1 mL) was diluted with 400 mL of tap water in a 500 mL separatory funnel. Sodium sulfate (5 g) was added and shaken until it dissolved. The water was then extracted with two consecutive 50 mL aliquots of methylene chloride by shaking vigorously for 2 min. The methylene chloride extract containing diazinon was dried by passing through a sodium sulfate column and then concentrated on a rotary evaporator with Keeper's solution to approximately 2 mL. These samples were then quantitatively transferred to centrifuge tubes with hexane and concentrated as needed for analysis using a nitrogen evaporator (Organomation, Model 111). Samples were analyzed on a Tracor 540 equipped with a FPD phosphorus detector. The column packing was 10% OV 101 and the dimensions of the column were 1.95 m x 0.635 cm (I.D.). The oven, detector and inlet temperatures were 195, 240 and 225 °C, respectively. Spikes and blanks were run with each extraction series. The concentration of the spikes was 1000 μg/mL and the recovery was 88.5%.

3.3.1.4.2 Diazinon Extract - Compost

The following extraction procedure was used for each series compost samples. Compost material (20 g) was weighed and placed into a 500 mL French square bottle. The samples were then sonicated with 250 mL of acetone at medium speed setting (4-5) for 2 min using a Polytron Ultrasonic generator Model PCU-1. This material was then filtered using a Buchner funnel with 7 mm Whatman #1 filter paper. The French square bottle was rinsed with two, 15-20 mL portions of acetone, each time adding it to the sample. The filtrate was then transferred to a 1000 mL separatory funnel. The filtering flask was rinsed with acetone and the rinsate added to the sample. The filtrate was shaken vigorously for 1 min with 100 mL of hexane. Saturated sodium sulfate (10 mL) and 190 mL of distilled water were added to the separatory funnel. The aqueous phase was extracted three times with 50 mL of hexane by shaking vigorously for 1 min. After each extraction, the aqueous phase was given 10 min to separate from the organic phase. The three, 50 mL portions of hexane were combined in a separatory funnel and the aqueous phase discarded. Saturated sodium sulfate solution (20 mL) and 180 mL of distilled water were added to the combined hexane extracts. The separatory funnel was gently shaken for 30 sec and the layers given time to separate. The aqueous layer was discarded and the hexane layer was drained through a sodium sulfate column into a 500 mL Erlenmeyer flask. The separatory funnel was rinsed with two, 15-20 mL portions of hexane; this rinsate was added to the sodium sulfate column. The combined hexane extract was then concentrated using a rotary evaporator and quantitatively transferred to a centrifuge tube. Additional concentration was done using a nitrogen evaporator for analysis, as needed. Samples were analyzed using a Tracor 540 gas chromatograph equipped with a FPD phosphorous detector. The column packing was 10% SP21000; 100/120 Supelcoport and the dimensions of the column were 2 m x 4 mm ID. The oven, inlet and detector temperatures were, 195, 225 and 240 °C, respectively. Spikes and blanks were run with each extraction series. Recovery for 10 μg/mL and 2000 μg/mL spiked samples were 92.8 % and 98.6 %, respectively.

3.3.2 Toxicity Testing of Composted Sorbent to Earthworms (*Eisenia foetida*)

3.3.2.1. Rearing and Care of Earthworm Culture.

Earthworms (*Eisenia foetida*) cocoons were purchased from Carolina Biological Supply Company in two lots of 100. *E. foetida* were cultured in a 1.2 m x 1.2 m x 0.6 m high underground bin. The bin was constructed of treated lumber and lined with 26 gauge aluminum sheeting in order to prevent leaching of the toxicants from the treated lumber in to the bin. Sixteen, 0.6 cm holes were drilled into the bottom of the bin to allow for water drainage. These holes were covered with a 1.5 mm mesh screen to prevent the escape of earthworms and immigration of other earthworm species into the bin. The bin was buried in shady woods. Bedding consisted of peat moss, top soil and cow manure at a 1:1:1 ratio, based on volume. The soil was Groseclose topsoil obtained from a farm in Montgomery Co., Va. and prepared by passing it through a 0.3 cm mesh screen. For specifications on this soil (see Section 3.2.2.2.). The cow manure had passed the heating stage but was still moist. Moisture levels in the bin were maintained qualitatively. Initially, corn meal was added two times per week, however, fungus began to grow on the excess corn meal;

therefore, the feeding schedule was reduced to once per week. The feeding regime was further reduced when the anticipated need for use in toxicity testing was reduced. A backup earthworm culture was also maintained in the controlled temperature room in the greenhouse. Temperatures in this greenhouse room were maintained at approximately 20 °C.

3.3.2.2. Preliminary Toxicity Studies.

Prior to exposing *Eisenia foetida* to composted diazinon waste, several experiments were conducted with other pesticidal materials. This was done for two reasons. The first was to develop a reliable method for evaluating toxicity of composted material using *E. foetida*, and the second was to evaluate the toxicity of these other composted pesticidal materials to *E. foetida.*

Three experiments were used to examine the toxicity of the composted pesticidal materials to *E. foetida*. In the first study, a 1:1:1 mixture of soil, cow manure and compost was used as an earthworm bedding. In the second study earthworms were exposed to Groseclose soil amended with 10% compost, based on dry weight. In the third study earthworms were exposed to Groseclose soil amended with 10% compost, based on volume. Sphagnum peat moss was used in place of compost for controls in each of the studies. Materials from three bioreactors, which had been used to dispose of mixtures of pesticides (diazinon / chlorpyrifos, atrazine / metolachlor, and diazinon / lindane / malathion / captan), were incorporated into Groseclose topsoil. The Groseclose topsoil was screened using a 1/8 inch (0.32 cm) screen. This soil was a silty loam soil (30% sand, 61%silt, 8.4% clay) with 1.4% organic matter and a pH of 5.8. The cow manure used in the first experiment had passed through the heating stage. Soil amendments from the three bioreactors and peat moss controls were each replicated three times. The bedding material for each test group was made in a single batch. The bedding was mixed for 15 min using a 1/3 H.P., 8 inch (20.3 cm) drill press equipped with a paint mixing blade and with a spade. Moisture levels were established at the water holding capacity of the soil. The bedding was incubated for 12 hours in the controlled environmental chamber before the worms were introduced.

Eisenia foetida used in this study were all sexually mature adults and were harvested from the field culture the same day of the test. Ten *E. foetida* were placed into each of the control and experimental replicates and incubated for 3 weeks in a walk-in controlled environmental chamber. Temperatures in the chamber were maintained between 18 and 20 °C by using an air conditioner and an electric space heater; each having a thermostat. The room was thoroughly insulated to help maintain a constant temperature. Constant lighting was provided by an overhead fluorescent bulb. Burying response time was measured weekly. Mortality was monitored at 3 1/2 day intervals. Mortality for earthworms was determined by observing no response following probing of the anterior end of the earthworm.

A behavioral response was selected to measure the earthworms physical condition. The earthworm behavior of burrowing into soil when exposed to a bright light was measured. This behavioral response has been observed in earthworms to be inhibited by acetylcholinesterase inhibiting insecticides (Stenersen,1989a; Stenersen et al., 1992). The same control amended soil was used for each of the test groups in order to provide a consistent bedding surface between test groups. This soil was placed in a 1 quart potting container and very loosely packed by hand. A 40 W floodlight was placed 0.4 m above the surface of the container. Ten earthworms from an individual container were placed onto the surface of the soil. The number remaining on the surface was counted at 1 min intervals until all ten worms had burrowed into the soil.

3.3.2.3. Exposure of Earthworms to Composted Diazinon and Controls.

A laboratory study was conducted to evaluate the toxicity of composted diazinon sorbent to *E. foetida*. Screened (0.3 cm) Groseclose topsoil was amended with either uncomposted, 30- or 60-day composted diazinon sorbent. The Groseclose topsoil from the same batch used in preliminary studies (Section 3.2.2.2). Corresponding controls were made by amending screened Groseclose topsoil with uncomposted, 30- or 60-day composted control sorbent. The compost was 10 % of the total soil mass, based on dry weight. Each of these compost samples were replicated 16 times.

Composted sorbent material was first ground using an electric coffee grinder in order to make the particle sizes small enough for earthworm ingestion. This grinding process produced the following gradient of particle sizes; 49 % < 0.15 mm, 38 % from 0.15 mm to 0.3 mm, and 14 % > 0.3 mm. Composted sorbent material (11 g, dry wt.) was added to 100 g (dry wt.) of screened (0.3 cm) Groseclose soil. The specifications for Groseclose soil are provided in Section 3.2.2.2. The soil and compost were contained in a pint size mason jar (0.48 L) with several holes for aeration. Water was added to the compost and soil prior to mixing to bring the total moisture up to 30% of the wet weight. Amended soil was first mixed by hand and then mechanically with a 1/2 inch (1.3 cm) drill press equipped with a paint mixing blade. The container was then emptied onto a tray where it was mixed by hand. This step was important because it ensured homogeneity in the corners of the container and reduced soil compaction. At this time, I noticed that soils amended with composted organic matter appeared to form cohesive spherical structures; whereas, soil amended with uncomposted material appeared to be more fragmented. I suggest that this may be due to an increasing lignin to cellulose ratio during the composting process. These soil samples were then placed in a controlled environment room where temperatures were maintained at approximately 20 °C. Continuous light was provided in this room by two, 4-foot fluorescent 40 W cool white bulbs.

In order to provide an adequate number of sexually mature *E. foetida* for this experiment, earthworms were harvested from the outdoor culture (Turf Grass Center culture) and the culture in the controlled environment room (greenhouse culture). The greenhouse culture had been established from the Turf Grass Center culture. In addition the burying response times and mean weight from the representative organisms in the two cultures were not determined to be significantly different. Earthworms from these two cultures were combined before adding them to the amended soil. Soil samples received either four or five earthworms. Care was taken, however, to have the same number of earthworms in the soil amended with diazinon compost and the corresponding controls. Each of the soil containers received one earthworm before any received two, until all of the earthworms were delivered. This was done to reduced bias associated with picking more or less healthy earthworms first. Each individual earthworm was rinsed with distilled water, blotted to dryness and weighed in a Petri dish containing moist filter paper before being delivered to the soil. Individual earthworms were again weighed following 14 days of incubation. Earthworms were incubated in 0.48 L glass jars. The lids of these jars contained several holes for ventilation. These jars were kept in the controlled environment room described in Section 4.2.2.

3.3.2.4. Mortality

Mortality was determined for each soil sample at 24 or 48 hour intervals over 3 weeks. Mortality was corrected for Abbott's formula (Finney, 1971). After 18 days of incubation, the average mortality in the controls had exceeded 10%. This visually appeared to be due to dehydration. At each time interval, earthworms were sorted from the soil. Earthworms were considered to be dead when there was no response when probing them at the anterior end. The 95% confidence intervals for the LT_{50}s of the means of each soil type was determined using a probit computer program.

3.3.2.5. Burying Response Time

Earthworm's ability to burrow into soil, when exposed to a bright light, is inhibited by acetylcholinesterase inhibiting insecticides (Stenersen,1989a; Stenersen et al., 1992). Burrowing response time was used as a measurement of sublethal toxic effects. Burrowing response times were measured for earthworms from all soil samples, following 12 hours of exposure. The burrowing response, for earthworms from all soil samples, was also measured following 7 and 13 days of exposure. Measuring burying response for all of the earthworms at 24 and 48 hours would have been very labor intensive. The burying response times were, therefore, only measured for earthworms exposed to soil amended with uncomposted diazinon sorbent and corresponding controls each time mortality was determined. It was assumed that symptomology would develop first in earthworms exposed to soil amended with uncomposted diazinon sorbent.

To measure burying response times, first the soil samples were spread out onto a tray. Earthworms were removed from the soil and the soil was poured back into the glass jar and tapped on the table to help ensure consistent compaction. The surviving earthworms from each soil sample were placed on the surface of the soil to which they had been exposed. Earthworms were then exposed to a 40 W floodlight mounted 0.4 meters over the surface of the soil. The number of earthworms remaining on the surface was counted at 30 sec intervals for up to 7 min. Earthworms were considered buried when half of their body was below the soil surface.

3.3.2.6. Curling Effect

Earthworms, intoxicated by acetylcholinesterase inhibiting insecticides, are known to demonstrate a curling effect (Stenersen et al., 1992; Stenersen, 1989a; Stenersen et al., 1983). Earthworms exposed to uncomposted diazinon sorbent and diazinon sorbent composted for 30 days demonstrated this effect following 7 days of exposure. These earthworms curled up and became very turgid. The number of earthworms demonstrating this effect was counted each time that mortality was determined. This value was corrected by using Abbott's formula. This effect may have occurred in earthworms before 7 days of exposure but the effect was not measured.

3.4 Results

3.4.1 Results from Demulsification, Sorption, Degradation Field Study

The concentration of the untreated diazinon rinsates were 1,150 ± 71 μg/mL (mean ± std. dev.) The concentration of step two filtrates was not determined during this study. However, similar runs, using the same process and rinsate concentration, resulted in concentrations of diazinon in the treated rinsewater to be 0.3 μg/mL (Hetzel et al., unpublished data). The pH of the uncomposted, 30 day composted and 60 day composted diazinon sorbents were 8.6 ± 0.04, 8.2 ± 0.2, 7.7 ± 0.3 respectively (mean ± std. dev.) The concentrations of diazinon in the uncomposted, 30 day composted and 60 day composted diazinon sorbents were 58 ± 32, 7 ± 5, 5 ± 4 μg/g (dry wt.), respectively (mean ± std. dev.) The uncomposted concentration of diazinon, refers to the concentration measured in the sorbent immediately before composting. The sorbent was allowed to sit open on plastic sheets in a greenhouse for 4 to 10 weeks before mixing and composting. This was done to represent procedures that would be encountered in field practice. Assuming that the rinsate treatment system was capable of removing 100% of the diazinon from the rinsate, and that there was no diazinon degradation, the concentration of diazinon on the combined sorbents would be approximately 29,400 μg/g of sorbent (dry wt.).

The temperature profile of the bioreactor is provided in Figure 3. The temperatures in the bioreactors followed the expected curve after the first nutrient amendments. However, temperatures increased little above ambient temperatures following the second nutrient amendments. Traditionally, temperatures have not increased as much following the second series of amendments to the bioreactors as temperatures following the first series of amendments. It may be that the addition of grass clippings and peanut hulls was needed in the second series of amendments to stimulate microbial metabolic activity. In addition, the second series of amendements were added on November 2nd when the ambient temperatures were below 10 °C. The compost was removed from

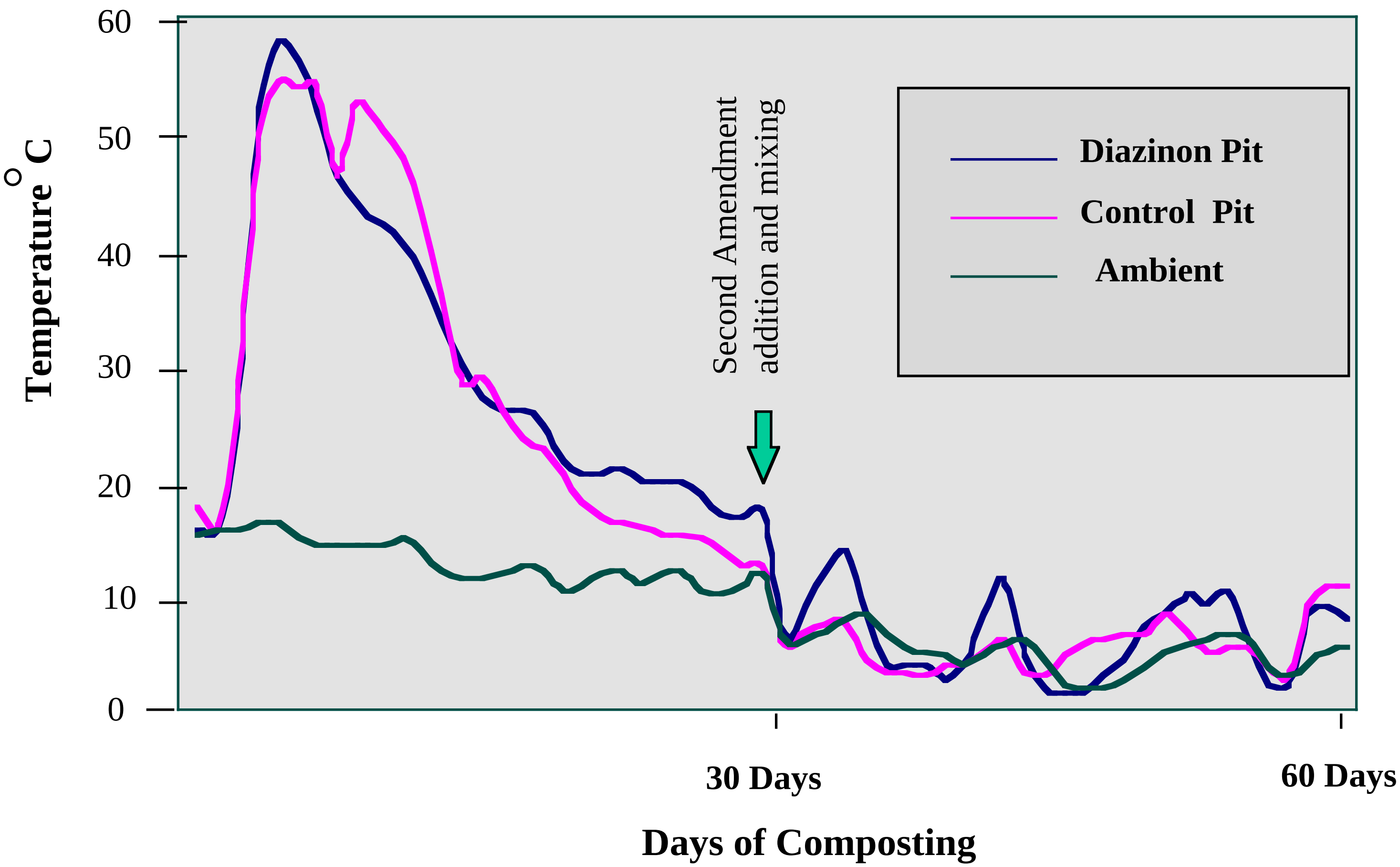


Figure 3. Temperature profile for field bioreactors.

the bioreactor for a 8 to 10 hours during this process, and cold water was added to the compost. It may be that the microbial consortium entered a dormant state because of the low ambient temperatures. They were then unable to generate enough metabolic heat to overcome the low ambient temperatures and become active. In addition the biomass in the bioreactor may not have been large enough to overcome heat loss, due to the low ambient temperatures. The total biomass loss in the first 30 days of composting based on was 0 and 0.5 kg (dry wt.) or 0 and 0.6% of initial compost mass, in the diazinon sorbent and control sorbent, respectively.

3.4.2 Toxicity Testing with *Eisenia foetida*

3.4.2.1 Results from Preliminary Toxicity Studies.

The results from the earthworm toxicity studies on composted diazinon/lindane/malathion/captan, diazinon/chlorpyrifos, and atrazine/metolachlor are presented in Table 1. Diazinon / chlorpyrifos compost was extremely toxic to earthworms. The lowest exposure that these earthworms experienced was 10% compost based on dry weight. These earthworms experienced 100% mortality within one week. It may be inferred that the toxicity was due to chlorpyrifos or its degradation products because the diazinon/lindane/malathion/captan compost was non-toxic to *E. foetida*. The results for composted atrazine/metolachlor varied among toxicity tests. Earthworms experienced mortality in the first two toxicity tests, which had the higher compost concentrations, but did not demonstrate mortality in the third toxicity test, which had the lowest.

3.4.2.2 Toxicity of Composted Diazinon Waste

3.4.2.2.1 Mortality Data.

Mortality of *Eisenia foetida* exposed to soil amended with uncomposted , 30-day composted and 60-day composted diazinon sorbent is presented in Figure 4. The LT_{50}s and corresponding 95% confidence intervals were 14.97 d (14.12 to 15.82), 14.6 d (14.06 to 15.14), and indeterminate for soil amended with uncomposted, 30-day composted, and 60-day composted diazinon sorbent, respectively. The 95% confidence interval for the LT_{50} of earthworms exposed to soil amended with 60-day composted diazinon sorbent was

Table 1. Preliminary Study: Exposure of *E. foetida* to a Series of Composted-Mixed Pesticides

Test 1. Groseclose topsoil amended with 10% composted material (by dry weight)

Compost material	% Mortality after 4 weeks of exposure (Mean ± S. E. M.[1])
Control	0 ± 0 a[2]
Diazinon / Lindane / Malathion / Captan	0 ± 0 a
Diazinon / Chlorpyrifos[3]	100 ± 0 b
Atrazine / Metolachlor	43 ± 25 a,b

Test 2. Groseclose topsoil, composted material and cow manure (1:1:1 by volume)

Compost material	% Mortality after 8 days of exposure (Mean ± S. E. M.)
Control[1]	0 ± 0 a
Diazinon / Lindane / Malathion / Captan	0 ± 0 a
Diazinon / Chlorpyrifos[4]	100 ± 0 b
Atrazine / Metolachlor	48 ± 18 a,b

Test 3. Groseclose topsoil amended with 10% composted material (by volume)

Compost material	% Mortality after 8 days of exposure (Mean ± S. E. M.)
Control[1]	0 ± 0 a
Diazinon / Lindane / Malathion / Captan	0 ± 0 a
Diazinon / Chlorpyrifos[5]	100 ± 0 b
Atrazine / Metolachlor	0 ± 0 a

[1] Means ± standard error of the mean; based on 3 replicates each containing 10 earthworms

[2] Means followed by different letters are significantly different at the $\alpha = 0.01$ level (Tukey).

[3] There was 100% mortality in this group within 1 week of exposure.

[4] There was 100% mortality in this group within 3.5 days of exposure.

[5] There was 100% mortality in this group within 2 days of exposure.

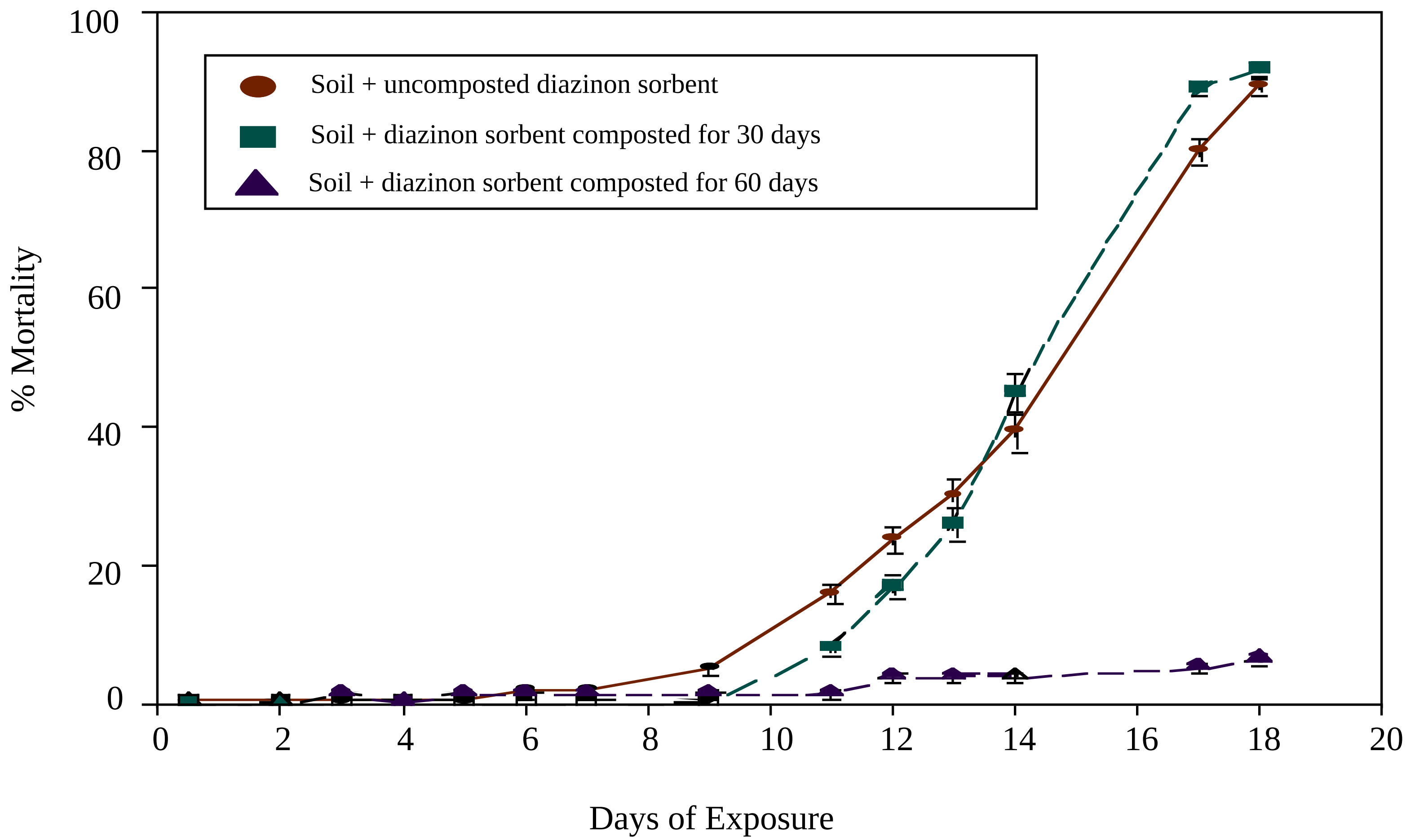


Figure 4. Percent Mortality (corrected for controls using Abbott's formula) of *Eisenia foetida* exposed to uncomposted, 30-, and 60-day composted diazinon sorbent. Means ± SEM are plotted. Each mean is based on 16 soil replicates.

indeterminate because the mortality did not exceed control mortality. On the basis of these confidence intervals, earthworms exposed to 60-day composted diazinon did not show mortality significantly different from zero. The LT_{50} values obtained from earthworms exposed to 60-day composted diazinon were, however, significantly different from earthworms exposed to uncomposted diazinon and 30-day composted diazinon. The LT_{50} value obtained for earthworms exposed to uncomposted diazinon was not significantly different than the LT_{50} for earthworms exposed to 30-day composted diazinon.

3.4.2.2.2 Sublethal Effect

The times required for surviving earthworms to burrow into soil while being exposed to a bright light was measured following 7 and 14 days of incubation in amended soils and are presented in Figure 5. Following 7 days of exposure, there was no discernible difference between the burrowing response times of earthworms in each experimental group and its respective control group. However, after 14 days of exposure, earthworms exposed to uncomposted and 30-day composted diazinon required significantly longer time to burrow into the soil than their respective controls indicating sublethal impairment ($\alpha = 0.05$). Earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbent for 14 days required approximately 250 to 300 sec to burrow into the soils; whereas, their corresponding controls only required approximately 1/2 the time (100 to 125 sec) to burrow into the soil. Earthworms exposed to soil amended with 60-day composted diazinon sorbent and its corresponding control also only required approximately 100 to 125 sec to burrow into the soil. This indicates significant impairment in earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbent and no impairment in earthworms exposed to soil amended with 60-day composted diazinon sorbent.

On the seventh day of the incubation, it became apparent that earthworms exposed to uncomposted diazinon sorbent or 30-day composted diazinon sorbent were showing characteristic curling effects associated with acetylcholinesterase inhibition. This effect was measured by counting the number of surviving earthworms demonstrating this curling behavior. Results of these observations, presented as the mean percentage of live earthworms demonstrating this effect for the 16 replicates of each experimental group, are presented in Figure 6. Because these observations are presented as the average

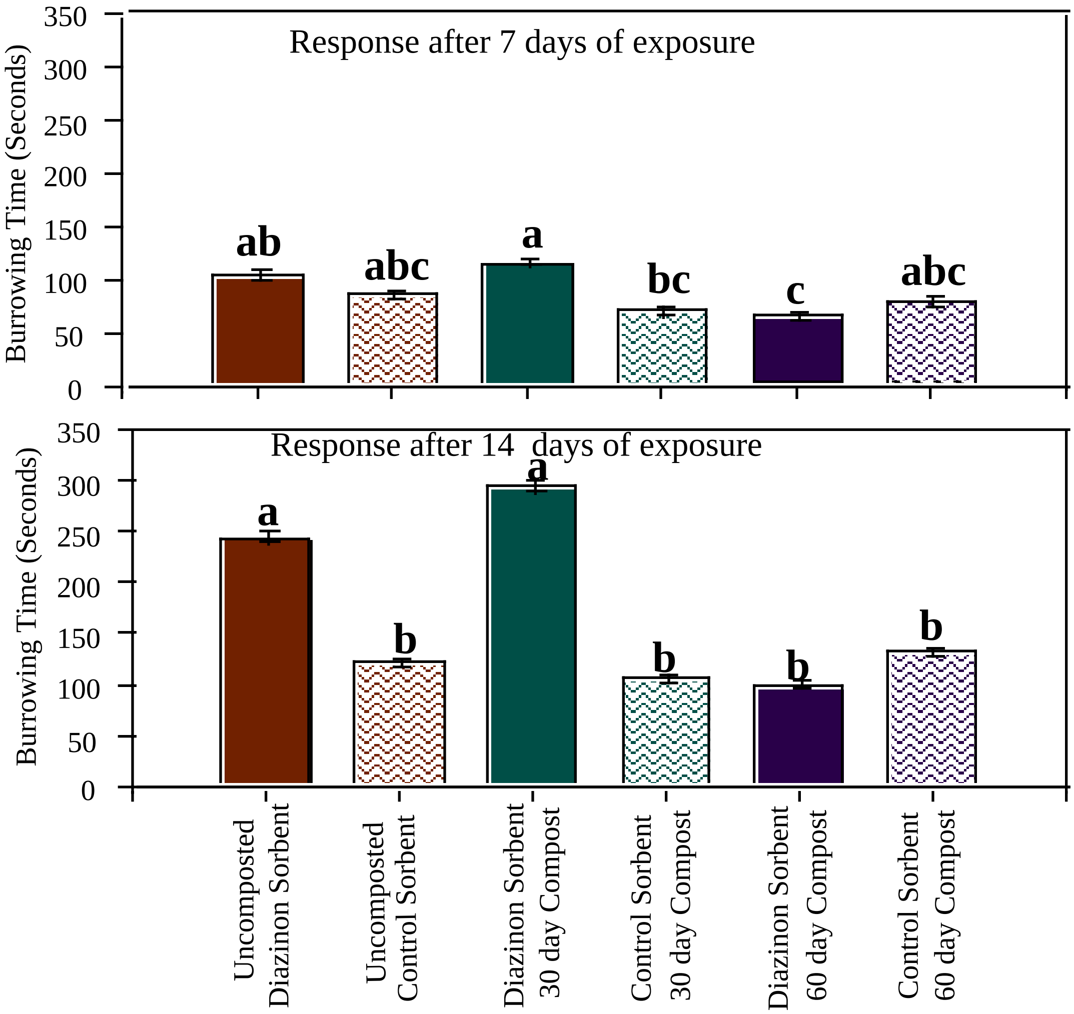


Figure 5. Burrowing response times for *E. foetida* exposed to amended soils for 7 days and 14 days. Means ± SEM are plotted for 16 replicates. Each mean is an average of the total number of live earthworms from the 18 day mortality study. Burrowing response times corresponding to different letters are significantly different at the $\alpha = 0.05$ level (Tukey-Kramer Multiple Comparison Test).

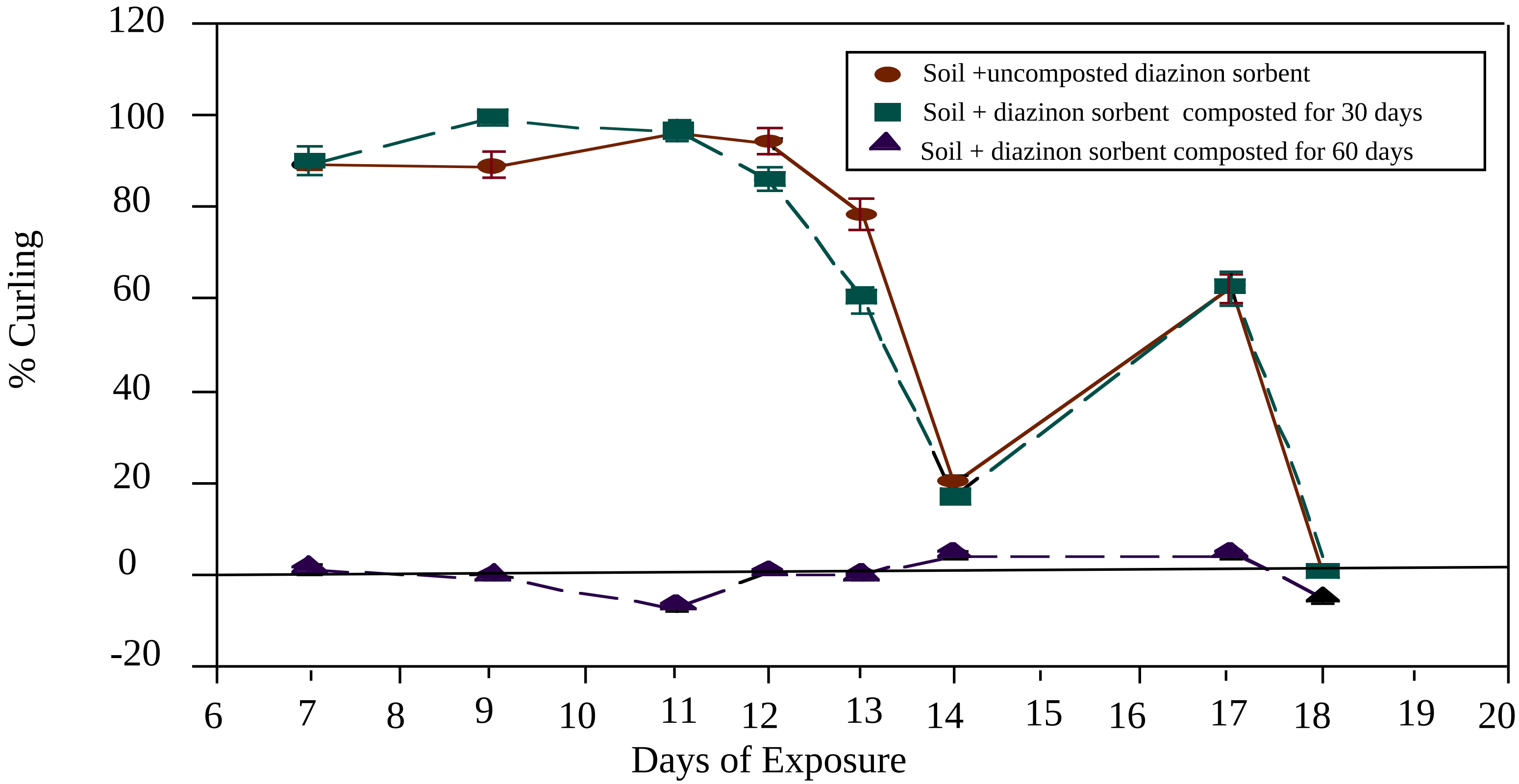


Figure 6. Percentage of live *E. foetida* showing curling effects following exposure to amended soil, corrected for Abbott's formula. Means ± SEM are plotted (Error bars are absent when the error was less than the mean symbol). Each mean is an average of the total number of live earthworms in each of 16 replicates, which varied with time due to mortality.

percentage of live earthworms, interpretation of results in the latter part of the incubation period becomes difficult. Before dying, earthworms would become turgid and stop demonstrating this curling effect. By comparing results presented in Figure 6 with mortality data in Figure 4, it appears that a few of the earthworms continued to show curling effects before becoming turgid and dying. After 14 days of exposure, there was a peak in increasing percentage curling effect (Figure 6). This percentage curling effect, however, is a percentage of the number of living earthworms. It can be seen in Figure 4 that mortality was increasing rapidly from 50% at this time. This peak in increased percentage curling effect, therefore, represented only a fraction of the total number of test organisms. This effect was also captured photographically and with a video camera. Figure 7 is a series of representative video clips that may be viewed by clicking on the following: uncomposted control sorbent; uncomposted diazinon sorbent; 30-d composted control sorbent; 30-day composted diazinon sorbent; 60-d composted control sorbent; and 60-d composted diazinon sorbent. It should be noted that these video clips are looped and the jerking motion in the film is an artifact of the video loop, not the earthworm behavior.

The change in weight of earthworms during the 14-d incubation period is presented in Table 2. Although all of the control earthworms experienced approximately 11% weight loss in the first 13 days of incubation, earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbents experienced 33 and 38% weight loss, respectively. Dehydration was likely the greatest factor in weight change during the study. The percentage moisture was not determined for these earthworms, however, so the relative contribution of dehydration to weight change could not be definitively determined.

Table 2. Live Weight of Earthworms Before and After 13 Days of Exposure to Soil Amended with Uncomposted, 30-day Composted and 60-day Composted Diazinon sorbent.

Soil amendment	Worm wt. before exposure (mg)[1]	N[3]	Worm wt. after 13 days (mg)[1]	N[3]	% Weight change[2]
Control sorbent uncomposted	321 ± 88	48	286 ± 82	48	11% (0.012 / NS)
Diazinon sorbent uncomposted	320 ± 84	64	216 ± 53	53	33% (< 0.001 / S)
Control sorbent composted for 30 Days	285 ± 98	60	256 ± 88	59	10% (0.098 / NS)
Diazinon sorbent composted for 30 Days	296 ± 88	82	185 ± 50	60	38% (< 0.001 / S)
Control sorbent composted for 60 Days	291 ± 99	60	256 ± 82	58	12% (0.00018 / S)
Diazinon sorbent composted for 60 Days	285 ± 88	80	230 ± 84	78	19% (< 0.001 / S)

[1] Mean ± Standard Deviation

[2] T-test (two tailed) comparing worm wt. after 13 days of exposure to before exposure (P-value) (S = significant / NS = not significant; $\alpha = 0.01$)

[3] N = number of earthworms weighed

3.5 Discussion

3.5.1 Preliminary Toxicity Studies

The difference among the three preliminary toxicity studies may, at least in part, be explained by different compost concentrations in each toxicity study. Composted chlorpyrifos appears to be extremely toxic to *E. foetida*. Even at the lowest concentrations of compost (10% V/V), 100% of the earthworms died within 2 days of exposure. Further study has determined that this mortality is likely the result of a toxic metabolite (unpublished data). The atrazine/metolachlor compost is was moderately toxic to *E. foetida*, resulting in 50% mortality in the two tests that had the higher concentrations of compost. These preliminary studies were conducted in part to develop a reliable method for testing the toxicity of composted pesticide material to *E. foetida*. Although each of these methods produced reliable results and little mortality in the control, laboratory tests using natural soil are very dependent on the physiochemical characteristics of the soil (Edwards and Bohlen, 1992). The European Economic Community (EEC) method discussed in the Chapter 2 should, therefore, be used in future toxicity studies. This method would allow for comparison between results performed in by different laboratories. The artificial soil test has the advantage of being standardized and relevant to soil conditions (Edwards and Bohlen, 1992).

3.5.2 Toxicity of Composted Diazinon Waste

3.5.2.1 Correlating Chemical Analysis and Earthworm Toxicity Testing

In previous studies, the diazinon concentration in the combined step 1 and 2 sorbents after treatment of the diazinon rinsewater was approximately 14,000 µg/g of sorbent (dry wt.), dropping off rapidly due to the high pH environment from the $CaOH_2$ (Mullins et al., unpublished data). Diazinon concentrations were generally found to be high in the step one sorbent, and much lower in the step two sorbent (Mullins et al., unpublished data). The diazinon concentration in the uncomposted diazinon sorbent (58 ± 32 µg/g) was almost an order of magnitude greater than the diazinon concentrations in the 30- and 60-day composted diazinon sorbent (7 ± 5 and 5 ± 4 µg/g, respectively). Based on these differenced in diazinon concentration, earthworm response to soil amended with uncomposted diazinon sorbent would be expected to be much greater than to soil amended with 30- and 60-day composted diazinon sorbent. Similar earthworm responses would also be expected following exposure to soil amended with 30- or 60-day composted diazinon sorbent. However, this was not the case. Earthworm response to soil amended with uncomposted diazinon sorbent was statistically indistinguishable from earthworm response to soil amended with 30-day composted diazinon sorbent. Earthworm response to soil amended with 60-day composted diazinon sorbent, however, was different from both the uncomposted and 30-day composted diazinon sorbent, and was indistinguishable from controls. This was the case when comparisons of mortality, burrowing response time, curling effect and weight change were made. It may be that the methods used to extract diazinon from the compost did not reflect the diazinon concentrations that were bioavailable to *E. foetida*. The chemical extraction methods may have been able to extract diazinon that was not bioavailable to *E. foetida*. This diazinon may have become increasingly associated with the compost matrix during the second 30-days of composting, and, therefore, less available or unavailable to the earthworms. Alternatively, it may be the earthworms were able to remove unextractably bound residues from the 30-day composted diazinon sorbent, which they were not able to remove from the 60-day composted diazinon sorbent. Earthworms have been shown to be capable of accumulating unextractably bound residues of pesticides from soil (Fuhremann and Lichtenstein, 1988; Haque et al., 1982; Ebert, 1992). It is less likely that the difference in toxicity between earthworms exposed to soil amended with 30- and 60-day composted diazinon sorbents was due to a toxic metabolite. The dominant metabolite would be expected to be IMHP and, although its earthworm toxicity is not specifically known, it has very low toxicity to many other organisms (Sumner et al., 1985). In any case, this shows why biological assays in addition to chemical analysis are important in determining the hazard that a compound may present.

3.5.2.2. Symptomology of *Eisenia foetida*

A timeline for mortality and each of these sublethal effects is presented in Figure 8. This figure brings together information presented in Figures 4, 5 and 6. The timeline shows that, within the first 7 days, curling effect was present in 100% of the earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbent. The burrowing response time of these earthworms, however, was not impaired at this time. Earthworms began to die following 10 days of exposure to soil amended with

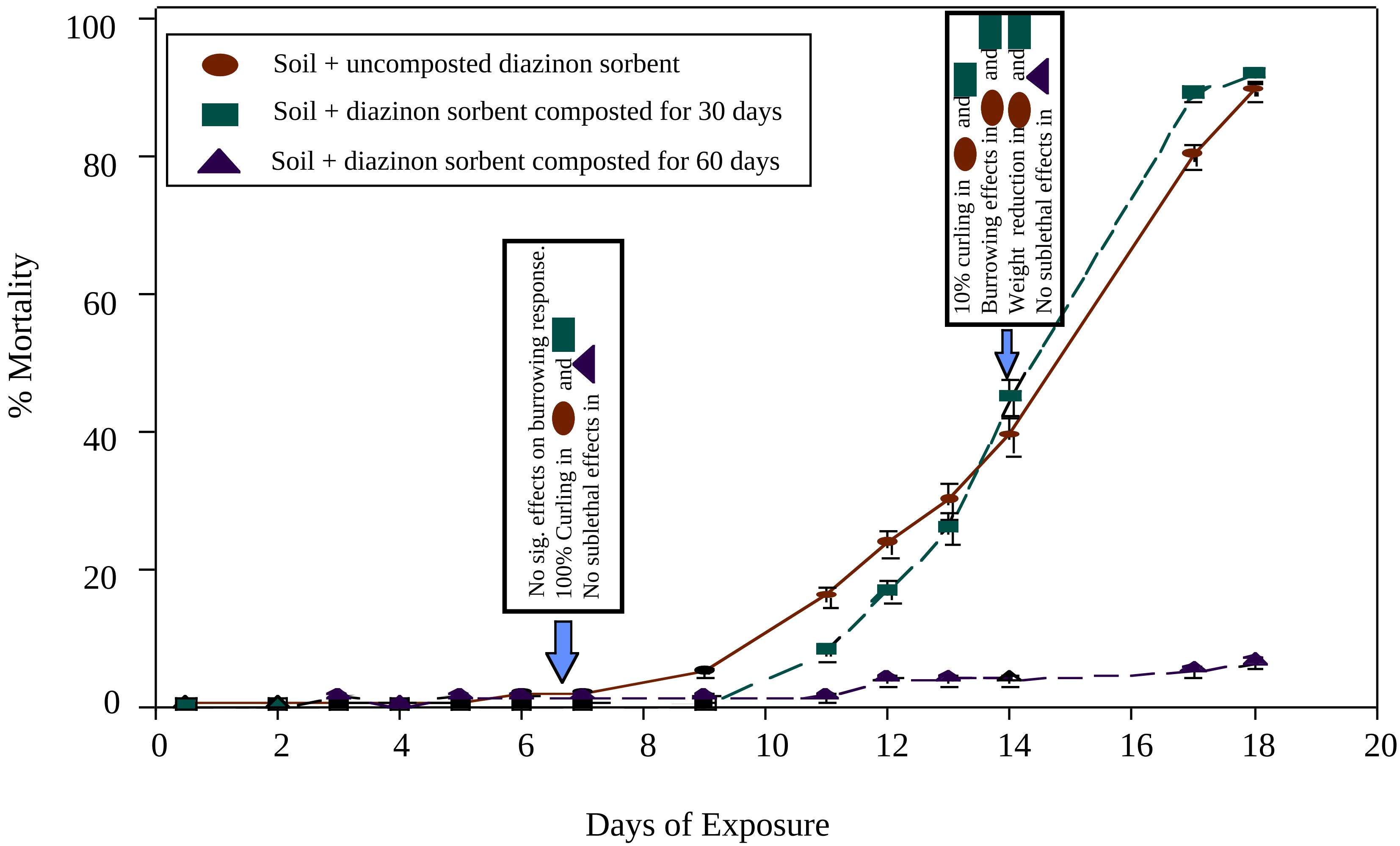


Figure 8. Timeline of mortality and sublethal effects from exposure of *E. foetida* to soil amended with uncomposted, 30-day composted and 60-day composted diazinon sorbent.

uncomposted or 30-day composted diazinon sorbent. After 14 days of exposure to soil amended with uncomposted or 30-day composted diazinon sorbent, approximately 40% of the earthworms had died. At this time only 10% of the remaining live earthworms continued to show the curling effect. Their ability to burrow into the soil, however, was significantly impaired and they had experience 33 and 38% weight loss, respectively.

Earthworms, exposed to soil amended with uncomposted and 30-day composted diazinon sorbent, demonstrated sublethal affects characteristic of acetylcholinesterase inhibition and in the chronological order that has been reported in the literature (Roberts, 1984; Stenersen, 1979a). Earthworms from these two test groups first began to show curling effects within the first 7 days of exposure. Both Roberts (1984) and Stenersen (1979a) reported this curling effect as the first symptom leading to death for earthworms exposed to organophosphate and carbamate insecticides. This symptom began to disappear and earthworms became turgid prior to death at 12 to 14 days of exposure. This also correlates well with the symptomology reported by Roberts (1984) and Stenersen (1979a). Stenersen (1979a) observed that *E. foetida* were unable to bury themselves following a single exposure to 12.5 to 50 μg/mL carbaryl or carbofuran. Two days after the single exposure the earthworms required 15 min to bury themselves. Thirty-five days after the single exposure to 50 μg/mL carbaryl or carbofuran the earthworms still curled up rather than trying to escape when touched or stimulated by heat and light from a photolamp (Stenersen, 1979a). In Stenersen (1979a) the curling effect was, therefore, the more sensitive and persistent sublethal indicator of toxicity. This was also the case in this study. The curling effect was very dramatic within the first 7 days of exposure; whereas, earthworms' ability to bury themselves was not impaired after 7 days of exposure to soil amended with uncomposted and 30-day composted diazinon sorbent. After 14 days of exposure to uncomposted or 30-day composted diazinon sorbent, the earthworms required twice as much time to bury themselves as their corresponding controls, but the sublethal effect of curling had disappeared.

This study was originally designed to have a duration of less than 14 days; therefore, provisions were not made to monitor or maintain soil moisture during the test period. Mortality in controls, however, did not exceed 10% within the first 14 days of the test; therefore, earthworms continued to be observed for 18 days. Although control mortality did not exceed 10%, all the earthworms in this study (experimental and controls) visually appeared to experience some dehydration late in the study. Therefore, some of the mortality in earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbent may have resulted from a synergistic dehydration effect. Earthworms exposed to acetylcholinesterase inhibiting insecticides are known to dehydrate (Roberts, 1984; Stenersen, 1979a). The dehydration initially results in the earthworms becoming turgid followed by turgid paralysis. This may a mechanism used by *E. foetida* to rid its body of toxins. The decreasing soil moisture after 14 days may have contributed to the mortality in these organisms that were also dehydrating because of exposure to diazinon. Quantitative evidence of increased dehydration in earthworms exposed to soil amended with uncomposted and 30-day composted diazinon sorbent is also presented in Table 2.

Each of these three sublethal effects should be looked for in earthworms when conducting a toxicity study on acetylcholinesterase inhibiting insecticides. The curling effect appears to be the most sensitive, but it is transient and it may be missed if the insecticide concentration is too high or observation interval is too long. The burrowing response time is not as sensitive as curling effect but may be more sensitive than mortality data. The dehydration effect is cumulative and does not appear to be as transient. Dehydration may not, however, be as specific to the mode of action of acetylcholinesterase inhibiting insecticides; rather it may result from an earthworm detoxication mechanism.

4. Chapter 4 Project II: Studies on the Fate of ^{14}C-diazinon in Compost, Amended Soil and Earthworms

4.1 Abstract

A laboratory-scale radiolabeled experiment was conducted to determine the bioavailability of Δ-2-^{14}C-diazinon and its degradation products to *Eisenia foetida* , after composting and amendment to soil. The composting conditions in this study were designed to simulate the field study with respect to diazinon concentration, nutrients, temperature and composting time. The fate of Δ-2-^{14}C-diazinon during this composting period was determined. The composted diazinon sorbent was then amended to Groseclose topsoil and earthworms were incubated in this amended soil for up to 14 days. The fate of composted Δ-2-^{14}C-diazinon during this incubation period was determined.

Very little of the Δ-2-^{14}C-diazinon was mineralized to $^{14}CO_2$ (0.08%), during the 60-day composting period. However, approximately 50% of the radioactivity moved from the acetone extractable fraction into the NaOH extractable and unextractable fractions. Diazinon accounted for 85% of the radioactivity in the acetone extractable fraction of uncomposted Δ-2-^{14}C-diazinon; whereas it only accounted for 1 and 0.5% of the radioactivity in the acetone extractable fraction of 30- and 60-day composted Δ-2-^{14}C-diazinon, respectively. The majority of the remaining activity in the acetone extractable fractions of these two treatments were as ^{14}C-IMHP (81 and 87%, respectively).

Radioactivity became increasingly incorporated into the soil-bound fractions during the 14 d earthworm incubation. The majority of acetone extractable radioactivity from soil amended with 60-day composted ^{14}C-diazinon remained at the origin when analyzed using HPTLC/autoradiography. The majority of the remaining radioactivity was determined to be 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP). Spots corresponding to diazinon and diazoxon were barely distinguishable from background radioactivity. Earthworms accumulated radioactivity when exposed to soil amended with 60 day composted ^{14}C-diazinon. The majority of this activity became unextractably bound within the earthworm tissue and the majority of the radioactivity that was acetone extractable did not migrate from the origin when analyzed using HPTLC/autoradiography.

4.2 Introduction

A laboratory scale study with Δ-2-^{14}C-diazinon was conducted. The objectives of this study were to evaluate the fate of composted diazinon in amended soil and earthworms (*E. foetida*). This laboratory-scale study was designed to simulate conditions that might occur in the field. In a previous study, which evaluated the fate of ^{14}C-diazinon (100 μg/g) in a composting environment, no diazinon was found in the organic solvent extractable fraction of the compost after 30 days of composting (Petruska et al., 1985). A significant amount of radioactivity, however, was unextractably associated with the humic acid and fulvic acids (15%) and with the humin (25%). It is not known whether these unextractably bound residues may be released following soil incorporation or by the actions of earthworms. These bound residues are more likely in the form of less toxic metabolites but may also be incorporated diazinon. Radiolabeled materials were employed to evaluate the bioavailability and fate of composted diazinon following soil incorporation.

4..3 Materials and Methods

4.3.1 Composting of ^{14}C-Diazinon

Peat moss samples were contaminated with Δ-2-^{14}C-diazinon, amended with nutrients, and placed in the controlled environmental chamber, described in Section 4.3.3, to simulate field composting conditions. Three, 36 g (dry wt.) samples of peat moss were finely ground using an automatic coffee grinder. This grinding process produced the following gradient of particle sizes; 49 % < 0.15 mm, 38 % from 0.15 mm to 0.3 mm, and 14 % > 0.3 mm. Calcium hydroxide [($Ca(OH)_2$)], 5.66 g, was mixed with each of the 36 g peat moss samples. The pH of this mixture was 8.8. Each of the peat moss / $Ca(OH)_2$ mixtures were transferred to a 350 mL glass composting vessel. This composting vessel was a larger version of the 74 mL composting vessel used by Judge (1996).

A radiolabeled sample of Δ-2-^{14}C-diazinon was obtained as a gift from Ciba-Geigy Corporation (now Novartis). In addition Ciba-Geigy provided the following non-radioactive standards used for chromatography; diazinon (96.8%), diazoxon (0.89 mg/mL), pyrimidine thiol (98%), and IMHP (99%). The specific activity of the Δ-2-^{14}C-diazinon was 35.8 μCi/mg, the chemical purity was determined to be 99.8% by gas chromatography and the radiochemical purity was determined to be 98.5 % by thin layer chromatography. The entire 200 μCi sample was dissolved in 500 mL of acetone. Two, 0.5 μL samples of this solution were counted for radioactivity. In addition, a 19.5 μL aliquot of this sample was saved for thin layer chromatography / autoradiography. The remaining Δ-2-^{14}C-diazinon standard (480 μL) was mixed with 3.498 mL of diazinon 4E. The Δ-2-^{14}C-diazinon-spiked emulsion was then quantitatively transferred with 180 mL of tap water to a larger beaker. The total volume of this emulsion was 189 mL. This emulsion was stirred continuously using a magnetic stirrer. Each of the three peat moss / $Ca(OH)_2$ mixtures was contaminated with 55 mL of the emulsion. The emulsion was delivered in 5 mL aliquots using a 22 gauge glass syringe. After adding each aliquot, the peat moss / $Ca(OH)_2$ mixture was mixed with a stainless steel spatula. The 5 mL aliquots were delivered sequentially to the three composting vessels, until the entire 55 mL of emulsion was delivered to each of the vessels. Subsamples of the emulsion (2 μL) were taken before, midway, and after the stock solution was added to the peat moss. The total concentration of diazinon in each of the three peat moss / $Ca(OH)_2$ mixtures was calculated to be 12 mg/g (dry wt.). Four, 2 μL, samples of the water emulsion were analyzed using fluorescent HPTLC and autoradiography.

Amendments were added to each of the three peat moss / $Ca(OH)_2$ mixtures, in proportions similar to field studies. After adding each of the amendments described below, the contaminated peat moss / $Ca(OH)_2$ mixtures were thoroughly mixed, using a stainless steel spatula. Liquids were added using a glass syringe, equipped with a 22 gauge needle. Inoculum was added using an 1 mL Eppendorf pipette that had an enlarged tip. Each of the contaminated peat moss / $Ca(OH)_2$ mixtures received the following amendments: ammonium nitrate (0.492 g); N/P/K fertilizer (15-30-15; 31 mg in 5 mL of water); corn meal (1.94 g); vegetable oil (0.889 g); and inoculum (3 g of compost from the same diazinon bioreactor used for inoculum in the field study in 6 mL of tap water). Water was added to bring the total calculated moisture up to 62 % of the total wet weight. A sample (15.9 g, wet wt.; 6 g dry weight) was taken from each of the composting vessels and frozen. The three composting vessels were then placed under a constant air stream, in a hood for 48 h, to allow for evaporation of surfactants and for amendement equilibration within the matrix. The pH after this 48 h incubation period was 6.7. Air exiting the composting vessels passed through two polyurethane foam plugs, to trap (radioactive) volatiles. It is worth noting that this equilibration period was much shorter than the storage time of field sorbent material, before composting; which was 48 to 80 days and in field experiments, there was significant disappearance of extractable diazinon during this drying time. The first of the three composting vessels was frozen after the 48-hr equilibration period. The remaining two composting vessels were placed in the controlled environmental chamber for 30 and 60 days, respectively (see Figure 9).

4.3.2. The Compost Cycle: Equipment design, Temperature Recording, and Trapping of CO_2 and Volatiles.

The controlled environment chamber was designed to maintain up to 60 °C, to simulate composting temperatures or 15 °C for earthworm incubation. Volatile compounds and CO_2 were removed from air leaving individual composting vessels. A diagram of the controlled environmental chamber is provided in Figure 10. A heat exchanger was placed along the walls of a 38 L cooler. The heat exchanger consisted of 10 coils of 1/2 inch (1.3 cm) PVC tubing around the inside of the cooler. A water bath, equipped with a recirculating pump (Brinkman Lauda K-2/R), circulated water through the PVC tubing and regulated the water temperature. A profile of the temperatures in the environmental chamber during the composting cycle is presented in Figure 11.

Air was sampled by the drawing of air through the system using a Gast® pressure vacuum pump. By drawing air through the system, rather than pushing it through, the potential hazard associated with leaks in the system was reduced; since air would leak into the system rather than out from the system. Air circulated through the system for 2 min at approximately 4.8 L/min, at 30 min intervals. Air entering the compost samples, first passed through two, 500 mL Erlenmeyer flasks containing water, to saturate the air with water vapor. Air entered each composting vessel at the top of the compost, such that only the air space above the compost was sampled. Air exited the vessel from the center of the lid. Air leaving individual composting vessels passed through two polyurethane foam plugs to trap organic volatiles and then through a buret, containing 3 mL of 5 N KOH, to remove CO_2. The air from all the composting vessels was then combined in a glass manifold

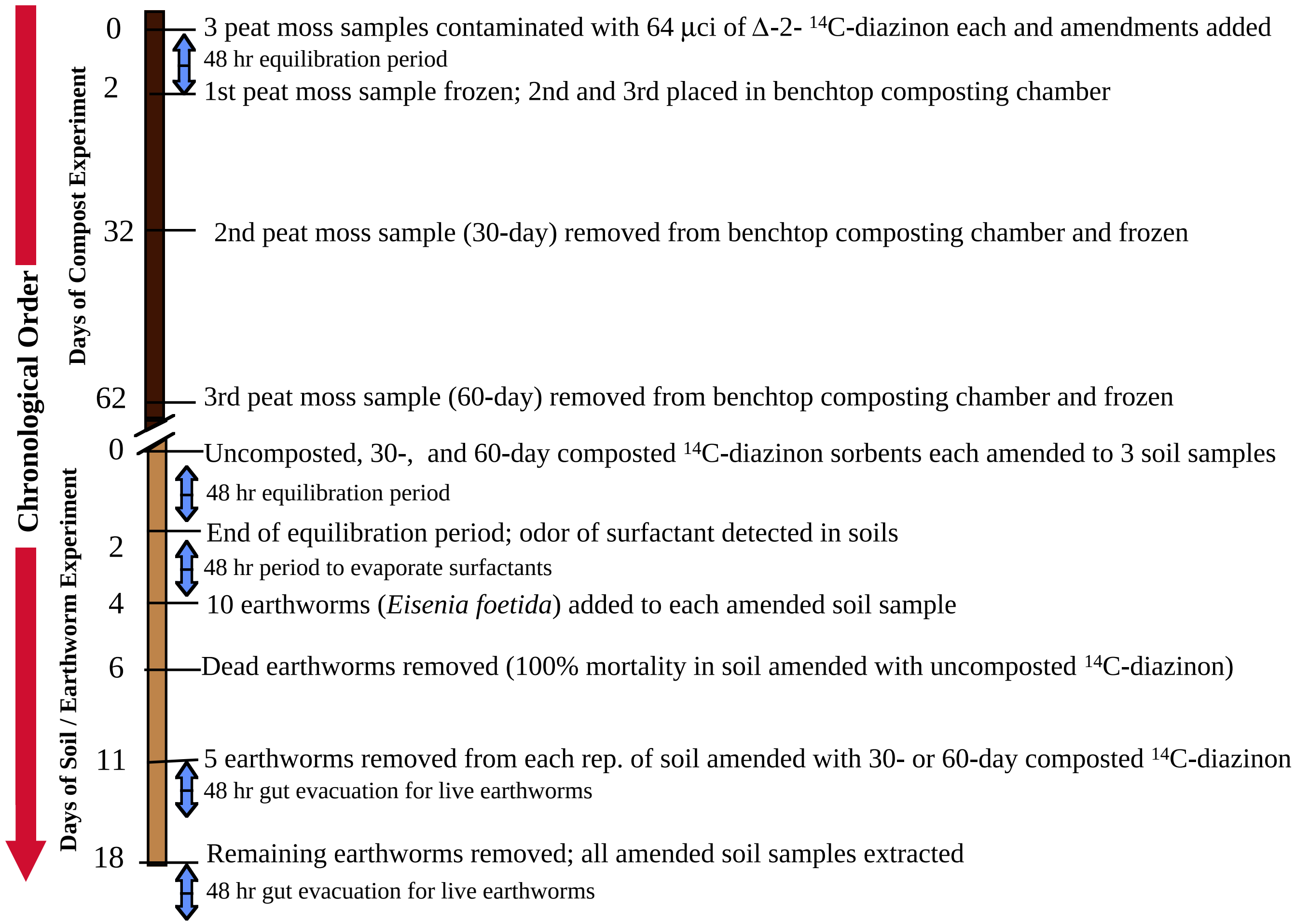


Figure 9. Timetable for ^{14}C-diazinon radiolabeled experiment: Contamination of peat moss with ^{14}C-diazinon and composting, followed by amendment of composted material to Groseclose topsoil and earthworm exposure

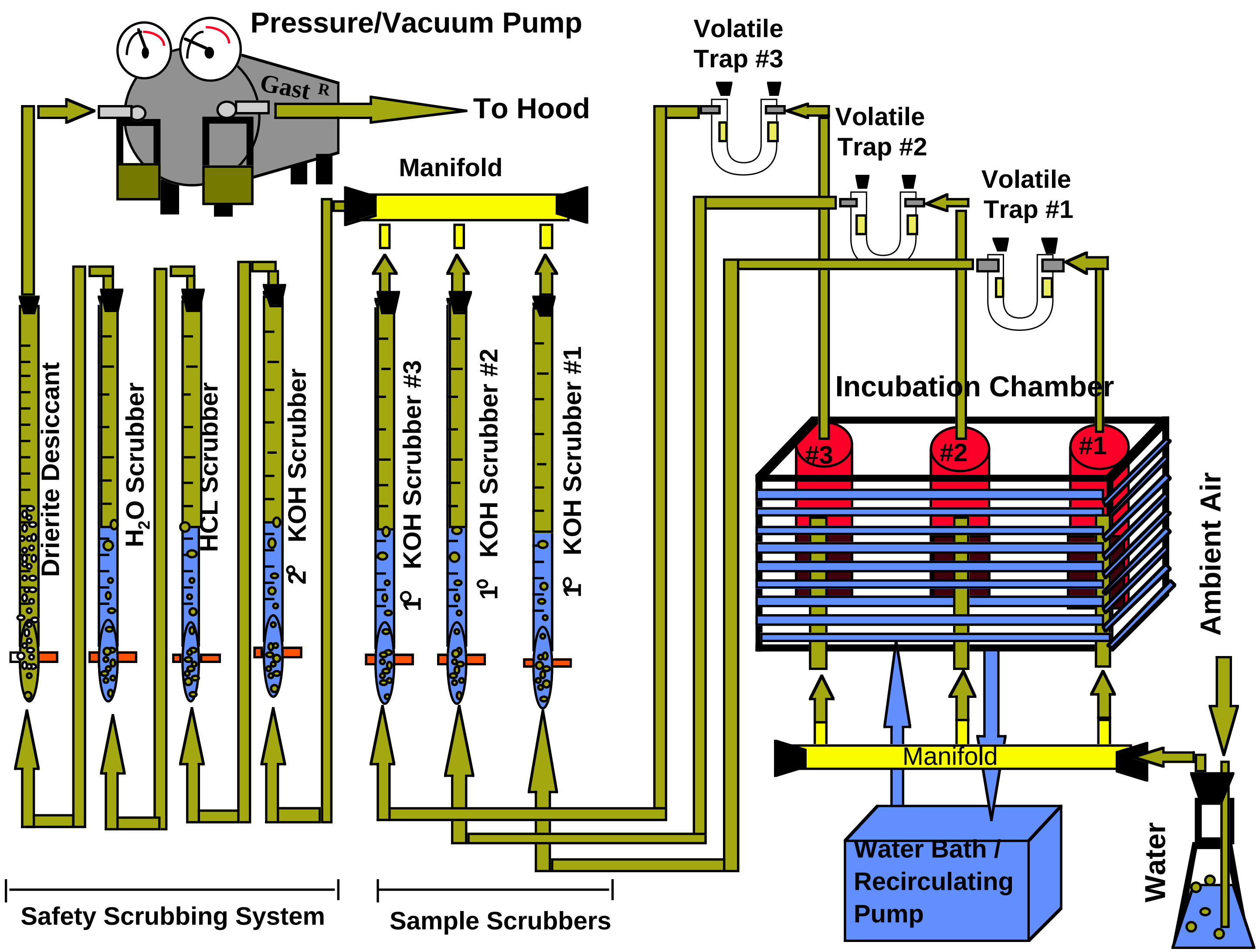


Figure 10. Schematic of the controlled environmental chamber used in the laboratory radiolabeled study.

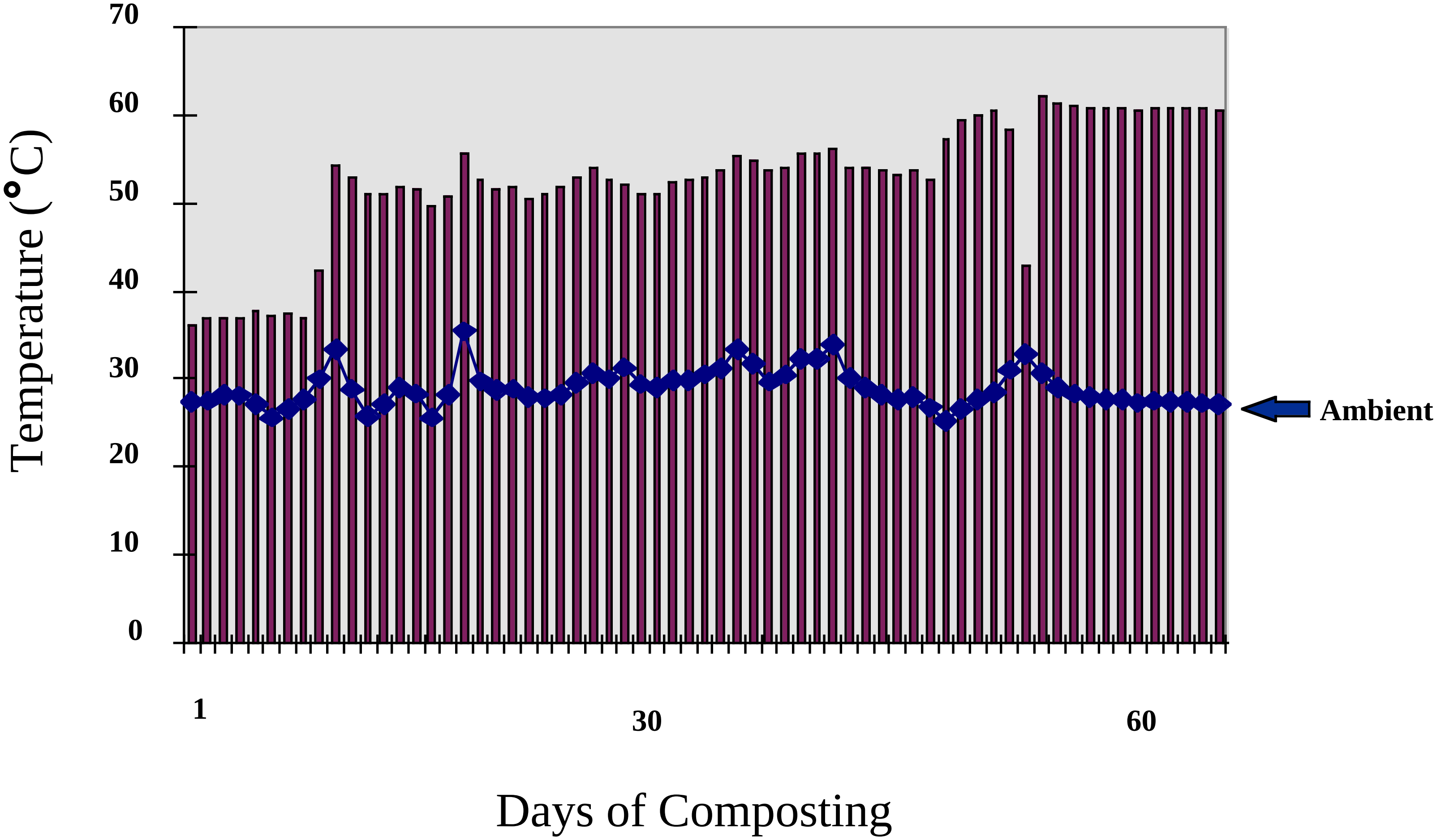


Figure 11. Temperature profile of laboratory radiolabeled study, during compost incubation.

before passing through a single buret containing 3 mL of 5 N KOH, which served as a $^{14}CO_2$ safety scrubber. Following KOH scrubbers, it passed through 5 mL of 0.02 N HCL to neutralize vapors, 5 mL of water to remove any remaining pH effects, and finally, drierite to remove water vapor. Air leaving the air sampling system, was transferred to a hood, as an additional safety precaution. Air tubing was primarily Teflon tubing (0.063 x 0.125 cm), however, 1/4“ ID Tygon tubing was used sparingly for connections. Connections between these tubing sizes were made with modified 1 cc tuberculin syringes; the rubber seals were backed up with silicon sealant. Fittings for the top of the burettes (air scrubbers) were made using 18 gauge needles inserted through 00 rubber stoppers.

4.3.3 Exposure of Earthworms (*Eisenia foetida*) to Composted and Uncomposted ^{14}C-Diazinon

4.3.3.1. Soil Incorporation of Composted and Uncomposted ^{14}C-Diazinon

The soil incorporation of composted and uncomposted ^{14}C-diazinon was designed to simulate the land application of compost waste and provide a medium for earthworm bioavailability studies. ^{14}C-Diazinon sorbent (10 g, dry wt.), which had been composted for 0, 30 or 60 days, was amended to Groseclose topsoil (90 g, dry wt.). For characteristics of Groseclose topsoil see Section 3.2.2.2. These three experimental groups were each replicated three times. The sorbent was added to each of the soil replicates in small aliquots until all the replicates had received 10 g (dry wt.) of sorbent material. ^{14}C-diazinon sorbent material was thoroughly mixed with the soil using a stainless steel spatula. Water was mixed into the amended soil to a calculated soil moisture of 30% of the wet weight. The amended soil samples were placed in the incubation chamber for 24 h to allow for equilibration. Before the earthworms were placed into the soil, I detected an odor of surfactants in soil amended with uncomposted ^{14}C-diazinon sorbent and a slight odor in soil amended with 30-day composted ^{14}C-diazinon sorbent. This surfactant odor had not been detectable in uncomposted, 30- or 60-day compost from the field study. Therefore, the soil samples were left open under a hood in a heated sand bath at about 60 °C for 48 h, to allow time for the surfactant time to evaporate from the soil.

4.3.3.2. Exposing earthworms to amended soil.

Ninety sexually mature *E. foetida* were harvested from the greenhouse culture. These earthworms were individually rinsed with distilled water and weighed in a Petri dish on moist filter paper. Ten sexually mature *E. foetida* were delivered to each soil sample. *E. foetida* were delivered such that each of the soil samples received one worm before any received two, until all soil samples received ten. This was done to avoid bias in earthworm selection. These earthworms were observed to see if they burrowed into the amended soil. After 10 min, only six of the thirty earthworms had burrowed into the soil amended with uncomposted (0 day) ^{14}C-diazinon sorbent; whereas, all the earthworms had burrowed into the soil amended with ^{14}C-diazinon sorbent composted for 30 and 60 days.

The amended soil samples were placed into a controlled environmental chamber. This was the same chamber that was used for the composting part of the experiment with a few modifications (see Section 4.4.3). The optimal temperature for incubating *E. foetida* is 15 to 20 °C, so the heat exchanger was used to cool the environmental chamber. A temperature profile of the environmental chamber during the incubation period is provided in Figure 12. Amended soils were contained in 1 L glass jars fitted with a Teflon lined lid. Air entered and exited through the 18 gauge needles, which were inserted through 00 stoppers, in the lid of the jar. Air from the three replicate soil samples of each test group was combined before passing through the air scrubbing system. The air scrubbing system was the same as that described for the composting of ^{14}C-diazinon sorbent (Section 4.3.2).

4.3.3.3. Removal and Preparation of Earthworms for Storage.

Earthworm mortality was first determined after 2 days of exposure. Earthworms that had died were removed, rinsed with distilled water, and stored at 3 °C. After 7 days of exposure, five earthworms were removed from each of the three replicates of 30 day and 60 day composted ^{14}C-diazinon amended soil. Since all of the earthworms that were exposed to soil amended with uncomposted ^{14}C-diazinon sorbent died following 2 days of exposure, no earthworms were removed from this test group. Copious amounts of fecal castings were present on the surface of soil amended with 30- and 60-day composted ^{14}C-diazinon sorbent indicating feeding activity. Dead earthworms were rinsed with distilled water, blotted to dryness, weighed and then stored separately from living earthworms at

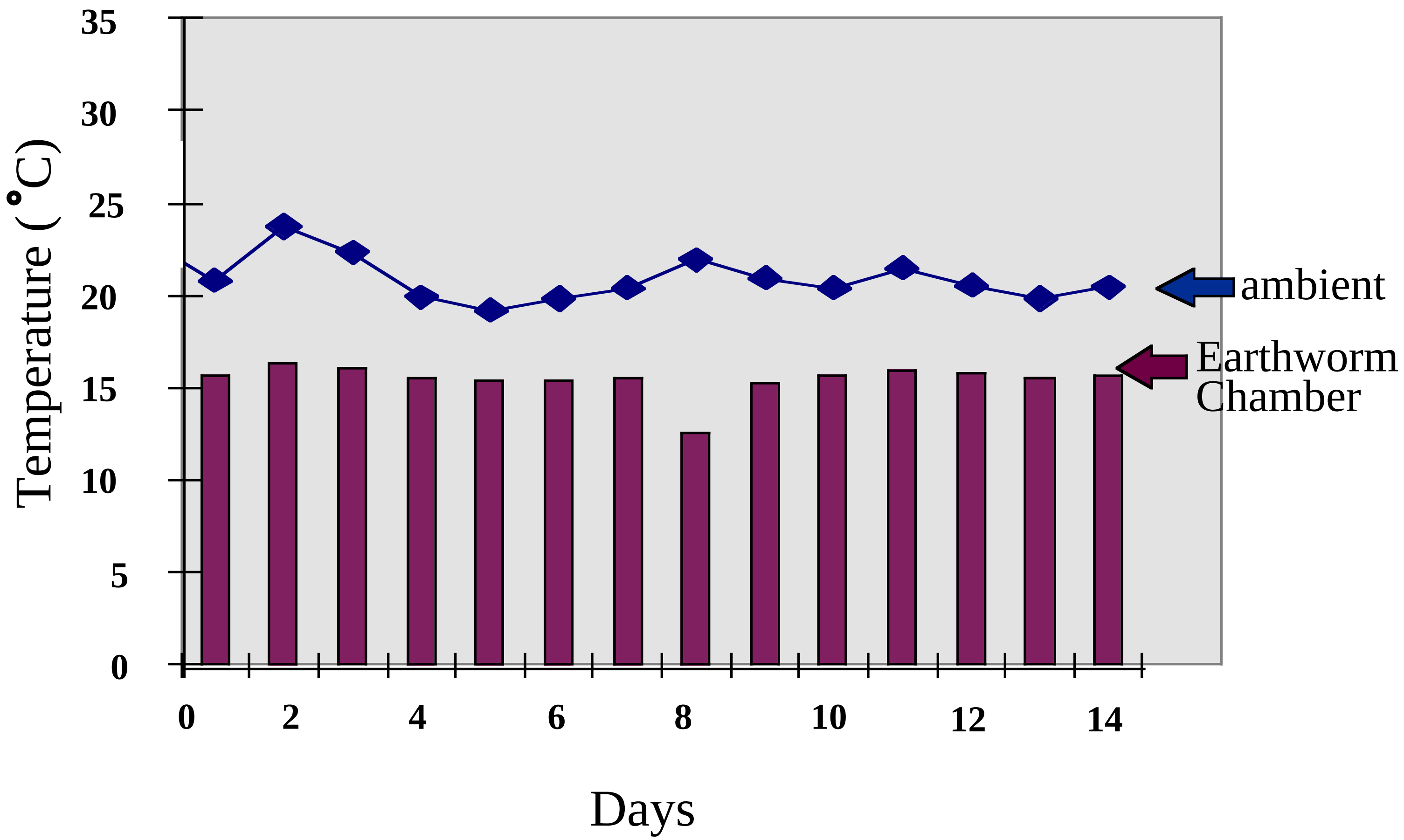


Figure 12. Temperature profile of laboratory radiolabeled study, during earthworm incubation in soils amended with uncomposted, 30-day composted and 60-day composted ^{14}C-diazinon sorbent.

3 °C. Living earthworms were individually rinsed with distilled water, blotted to dryness and weighed on an analytical balance.

A method for gut evacuation was used, which was modeled after Lord et al., (1980) and Belfroid et al. (1994b). Each group of living earthworms was placed into a Petri dish containing Whatman #1 filter paper, moistened with 2 mL of water. Three Petri dishes, also containing Whatman #1 filter paper, moistened with 2 mL of water, served as evaporation controls. The evaporation controls were used when estimating the mass of fecal material excreted by each group of worms. Earthworms were incubated in the gut evacuation chambers for 24 h in the dark. Feces clinging to the exterior surface of the earthworms were removed with a spatula and returned to the gut evacuation chamber. Earthworms were then rinsed with distilled water, blotted to dryness and weighed on an analytical balance. Earthworms that did not survive the gut evacuation process were stored separately from those that did survive. Copious amounts of feces were generated by earthworms from all six soil samples (soil amended with 30- and 60-day composted ^{14}C-diazinon sorbent).

After 14 days of exposure, the remaining earthworms were removed. Copious amounts of fecal casts were present in both the 30- and 60-day compost amended soils, indicating feeding activity. The castings in soil amended with 30-day composted ^{14}C-diazinon were wet trailings on the walls of the glass containers; whereas, castings from soil that was amended with 60-day composted ^{14}C-diazinon were as pellets. I qualitatively estimated that castings constituted approximately 50% of the soil amended with 60 day composted ^{14}C-diazinon. Earthworms that had been exposed to soil amended with 60-day composted ^{14}C-diazinon sorbent were transferred to a second gut evacuation chamber for 24 h and then to a third gut evacuation chamber for 36 h. This was to determine if a 24 h gut evacuation was sufficient for the worms to evacuate all of their gut contents. These chambers were also weighed before and after gut evacuation to estimate the mass of the feces collected in each gut evacuation chamber. However, extraction of the gut evacuation chambers (see Section 4.6.2.4) was a more accurate way of determining the fraction of gut material that was evacuated with each sequential gut evacuation than determining fecal mass.

Earthworms were stored in groups based on the following criteria: soil experimental group and replicate number; exposure time; alive or dead when removed from the soil;

survival during the gut evacuation process; and, finally, the duration of the gut evacuation process. Worms, grouped on these criteria, were stored in glass test tubes at 2 to 3 °C for 26 to 50 days before extraction.

4.3.4 Extraction and Analysis

4.3.4.1 Characterizing the Fate of Diazinon in Compost.

4.3.4.1.1. Exhaustive Extraction of Compost; Acetone, 1N NaOH, and Combustion.

Compost samples (1.5 g, dry wt.) were placed in a 100 mL Sorval (Model 18105) Omni Mixer vessel with 15 mL of acetone. After 12 h, they were mixed at full speed using a Sorval Model 18105 Omni mixer for 5 min, and sonicated for 30 min in a Fisher Scientific Ultrasonic Generator (Model FS 28 11). Solids were precipitated from the acetone fraction by centrifugation at 3900-4000 RPM (2800 x g) for 20 min, using a Fisher Scientific Marathon 6K Centrifuge. The supernatant was decanted from the pellet. The total radioactivity in the supernatant was based on gravimetric measurements. This was achieved by using the total supernatant weights and the weight of a 250 μL aliquot of the supernatant on a Mettler AE 163 analytical balance. Scintiverse Bio-HP scintillation fluid (4 mL) was added to the 250 μL sample aliquots contained in 5 mL minivials and the radioactivity determined using a Beckman Model LS 6500 Multi-Purpose Scintillation Counter. A smaller volume of sample was counted if the ESCR was below 0.25. Counting efficiencies were typically greater than 90%. An additional 15 mL of acetone was then added to the pellet and was re-extracted using the same method, minus the 12 h soaking step. Uncomposted diazinon sorbent required four extractions for exhaustive extraction, whereas 30- and 60 day composted diazinon sorbents required seven. This extraction procedure was repeated until the radioactivity in the extracts was indistinguishable from background counts.

After the final acetone extraction, 15 mL of 1 N NaOH was added to each sample. Samples were allowed to stand for 12 h in 1 N NaOH, and the samples were then extracted for 5 min using an Omni Mixer at maximum speed, and sonicated for 30 min using an ultrasonic generator. Solids were precipitated from the acetone fraction by centrifugation at 3900-4000 rpm (2800 x g) for 20 min. The total radioactivity in the supernatant was determined using the "gravimetric" method described for acetone extracts. For some extracts, a 50 µL aliquot of the NaOH fraction was counted rather than 250 µL to reduce quenching effects. An additional 15 mL of 1 N NaOH was then added to the precipitated fraction and was re-extracted using the same method, minus the 12 h soaking step. Extractions were repeated until the radioactivity in the extracts were indistinguishable from background counts. After the first two extractions, however, samples were extracted with 25 mL of 1 N NaOH rather than 15 mL. This was done because the 15 mL extracts appeared to be saturated and using a greater NaOH volume reduced the number of extractions required for exhaustive extraction.

A 2 to 3 g (wet wt.) aliquot of the precipitated compost material was weighed into a 1.5 mL Eppendorf microcentrifuge tube, using an analytical balance. A similar size aliquot was weighed into a combustion crucible, at the same time. The aliquot in the microcentrifuge tube was placed in a sand bath at 60 to 80 °C for a 48 h period to determine the moisture content. The aliquot in the combustion crucible was combusted using a Harvey Biological Oxidizer (R. J. Harvey Instruments Corp., USA). The catalyst zone was 800 °C and the combustion zone was 900 °C, with a 4-min burn time. The target flow for O_2 and N_2 were both 350 cc/min. (320-380 cc/min.). $^{14}CO_2$ generated from the combustion process was trapped in 8 mL of $^{14}CO_2$ scintillation cocktail. The recoveries for blank peat moss samples, spiked with 24,000 dpm of ^{14}C-diazinon, were 89 ± 10%. These recoveries were used to correct for the combustion efficiency of the compost samples.

4.3.4.1.2. Determination of Radioactivity in CO_2 and Volatiles.

A method modified from Kuyper and Aghdashi (1972) was used to distill $^{14}CO_2$ from the KOH solution, in preparation for liquid scintillation counting (LSC) (Mullins and Eaton, 1978). A 500 µL aliquot of 80% ethanolamine was placed on the bottom of a 20 mL scintillation vial. The vial was tilted and rotated by hand to coat the inner surface of the scintillation vial. Scintillation vials were then placed into a vial holder that was tilted at 45° and rotating at 6 rpm, to keep the walls of the scintillation vial coated. A 500 µL aliquot of the KOH solution from the $^{14}CO_2$ trap was placed in a specialized glass thimble (Kuyper and Aghdashi, 1972). The thimble was then placed into the ethanolamine-coated scintillation vial and a cap, which had an 18 gauge needle inserted through it, was loosely placed on the vial. A 1 mL syringe was then used to deliver 500 µL of 25 N, H_2SO_4 to the thimble well via the 18 gauge needle and the cap was immediately tightened. The cap of the ethanolamine-coated scintillation vial was tightened and the vial was placed into the tilted, rotating (6 rpm) vial holder for 2 h. The cap assembly and thimble were then removed, 8 mL of Scintiverse Bio-HP scintillation fluid was added to each sample and samples were counted using LSC.

The glass U-shaped tubes, used in the trapping of volatiles, were rinsed with 15 mL of acetone to remove ^{14}C-volatiles that may have condensed in the tubes. This acetone was then combined with the polyurethane foam plugs in a beaker for 30 min. After soaking, foam plugs were sonicated for 30 min in a sonicator. The foam plugs were then squeezed to dryness and re-extracted using a 30 min sonication with 8.5 mL of acetone. A 250 µL aliquot was counted from each of the acetone extractions. Foam plugs were re-extracted until the radioactivity in the extracts were indistinguishable from background counts.

4.3.4.1.3. Chromatography and Autoradiography of Compost Acetone Extracts.

A series of extractions was required to exhaustively extract acetone soluble radioactivity from the compost. These sequential extractions were combined and concentrated to provide an appropriate specific activity for High Performance Thin Layer Chromatography (HPTLC) and Thin Layer Chromatography (TLC). Both HPTLC and TLC were used, in combination with autoradiographic techniques, to identify radiolabeled metabolites of ^{14}C-diazinon. However, only TLC was used to quantify these metabolites because a larger volume of sample could be used.

The acetone-extraction series from individual compost samples was qualitatively transferred to a single glass container. To evaluate the transfer efficiency, containers were rinsed with 5 mL of acetone after the qualitative transfer, and 250 µL of this was counted using LSC. The lid of containers for combined extracts were equipped with two, 18 gauge syringe needles inserted through 00 rubber stoppers, to provide and entrance and exit for gas. Pre-purified N gas was used to replace the headspace over combined acetone extracts.

This was done to reduce the risk of oxidation, during the concentration process. Combined acetone extracts were kept at 45 to 55 °C using a heated sand bath. Combined acetone extracts were concentrated to 1 mL and were then quantitatively transferred to graduated centrifuge tube. The glass containers, in which combined acetone extracts had been concentrated, were rinsed and counted following the transfer to estimate transfer efficiency. Gases leaving the combined acetone extract samples, during the concentration processes, passed through two polyurethane foam plugs to trap any volatiles. This was also conducted in a hood as an added measure of safety. A 100 µl aliquot of the combined-concentrated acetone extracts was counted using LSC. It was important not to have the samples consist of two phases (acetone and aqueous) prior to chromatography analysis. Combined acetone extracts were, therefore, concentrated to near dryness to remove most of the water in the concentrated extracts. The acetone extracts were warmed in a warm water bath and evaporated under pre-purified N gas. This concentration process was conducted in a closed air system and volatiles were trapped in polyurethane foam plugs to estimate loss. It was determined that water could be evaporated from the samples without losing radiolabeled compounds.

Fluorescent HPTLC plates (Merck, Silica Gel 60 F_{254}) were used to analyze combined-concentrated acetone extracts of compost samples. Two solvent systems were used. Solvent system I was a chloroform : acetone (8:1) solution. Solvent system II was an ethyl acetate : benzene : chloroform : propanol (2:2:1:1) solution (J. A. Petruska et al., 1985; Nicholson et al., 1981). In a previous study by Petruska et al. (1985), the Rf-values for diazinon, pyrimidine thiol, diazoxon and IMHP were 0.88, 0.58, 0.38 and 0.08, respectively, using solvent system I and 0.86. 0.81, 0.65, and 0.28, respectively, using solvent system II. HPTLC plates were prerinsed with their respective solvent systems to remove any contaminants. The plates were then activated at 110 °C for a minimum of 2 h and stored in a desiccator until use. Each of the combined concentrated acetone extracts was spotted-over with 2 µg each of the following standards; diazinon, diazoxon, pyrimidine thiol, and IMHP. In addition, each HPTLC plate was spotted with approximately 2,400 dpm of ^{14}C-diazinon standard. At the same time that HPTLC plates were spotted with 2 µL of combined-concentrated acetone extracts, 2 µL was also placed directly into scintillation fluid and counted to determine radioactivity. Autoradiography was used to correlate radioactive metabolites of ^{14}C-diazinon with standards of diazinon, diazoxon, pyrimidine thiol and IMHP. Each of the HPTLC plates was replicated twice. The first in the pair was exposed to x-ray film (Kodak XRP-1) for 2 weeks and the second for 4 weeks before film development (Kodak GBX developing solution for 10 min.; stop bath for 1 min. and fixing solution for 10 min.). Contact prints of the developed x-ray film were then made using Kodak F-3 bromide print paper and a Simon Omega Enlarger (F-22, 45 sec). Photographs of the HPTLC plates were taken under ultraviolet light (254 nm) using Kodak T-Max 100 film and printed in identical dimensions as the contact prints of the x-ray film. The contact prints of x-ray film and printed photographs of HPTLC plates were then compared to identify radioactive compounds.

Acetone extracts were also analyzed by TLC to identify and quantify the radioactive compounds. The 20 x 20 cm plates were prepared by mixing 85 g of silica gel type GF/Florescent, 10-40 mm, (Sigma Chemical Co.) with 160-165 mL of distilled water in a Waring blender. This silica suspension was then immediately poured into a Desaga-Brinkmann TLC plate spreader and spread at 1.3 mm thickness. The TLC plates air dried for 4-5 h before activating at 120 °C, for a minimum of 2 h. The TLC plates were stored in a desiccator until use. The TLC plates were equilibrated with the sovent vapors for 30 min in the chromatography chambers before chromatography was initiated. Solvent systems I and II, described for HPTLC plates, were also used with TLC plates. Each of the combined concentrated acetone extracts was spotted-over with 25 µg each of the following standards; diazinon, diazoxon, pyrimidine thiol, and IMHP. In addition, each TLC plate was spotted with approximately 4,800 dpm of ^{14}C-diazinon standard. At the same time that TLC plates were spotted with 25 µL of combined-concentrated acetone extracts, 25 µL was also placed directly into scintillation fluid and counted to measure the total activity in the sample. Autoradiography was used to correlate radioactive metabolites of ^{14}C-diazinon with standards of diazinon, diazoxon, pyrimidine thiol and IMHP. X-ray film (Kodak^{R}) was exposed to TLC plates for a minimum of 30 days prior to detection (visualization of spots). The development of x-ray film, production of contact prints, and UV photography of the TLC plates was identical to the materials and methods used for HPTLC plates. The contact prints of x-ray film and printed photographs of TLC plates were compared to identify radioactive metabolites. Spots that were either visible on the autoradiogram or under ultraviolet light were scraped from the surface of the TLC plates and quantitatively placed into scintillation vials along with 4 mL of liquid scintillation fluid. Extreme care was taken at this point, due to the hazardous nature of the radioactive dust. Plates were scraped under a hood without the fan running. The scraping was done on aluminum foil as well as disposable sorbent pads. A dust mask was worn and the area was thoroughly swiped before and after final cleanup. The threshold for observing radioactivity using autoradiography was approximately 100 dpm. The threshold, however, was dependent on the precision of the radioactive spot. Some spots, containing radioactivity in the 100 to 200 dpm range, were not detected by autoradiography. In addition the threshold was related to the amount of time that the x-ray film was exposed to TLC plates.

4.3.4.2 Characterizing the Fate of Diazinon in Amended Soil and Earthworms.

4.3.4.2.1 Exhaustive Extraction of Amended Soil with Acetone and NaOH and Sample Combustion.

The protocol for extracting soil samples was the same as compost samples, with two exceptions. The mass of each soil sample extracted was 5 g (dry wt.) and the Omni mixer was run at approximately 1/3 its maximal speed. The protocol for soil combustion following exhaustive extraction was also the same. The recovery for blank soil spiked with 24,000 dpm of ^{14}C-diazinon was 56 ± 5% %. These recoveries were used to correct for the combustion efficiency of the compost samples. See Section 4.3.4.1.1 for the extraction protocol for compost samples.

4.3.4.2.2. Determination of Radioactivity in CO_2 and Volatiles.

The protocol for trapping of CO_2 and volatiles and their subsequent extraction was the same as for compost samples (see Section 4.3.4.1.2).

4.3.4.2.3. Extraction of Earthworm Tissues.

As described in Section 4.3.3.3, earthworms were stored in groups based on the following criteria: soil experimental group and replicate number; exposure time; alive or dead when removed from the soil; survival during the gut evacuation process; and, finally, the duration of the gut evacuation process. All of the earthworm tissue from each group was extracted in 100 to 300 mg aliquots. The earthworm groups were not homogenized before removing subsamples to better represent the heterogeneity among earthworms within each experimental replicate group. An attempt was made to represent every group each time extractions were made to reduce the effect of storage time when comparing groups. Aliquots of earthworm tissue, approximately 100 to 300 mg, were weighed into a glass homogenizing tube using a Mettler AE 163 analytical balance. Acetone (3 mL) was added to the homogenizing tube using an Eppendorf micropipet. The worm tissue was then homogenized for 5 min by hand, using a glass pestle. The homogenate was transferred to a thick-walled centrifuge tube, using three, 1 mL aliquots of acetone to rinse the glass homogenizer. Solids were precipitated from the acetone extract by centrifuging for 5 min on a Dynac centrifuge at 1060 x g. The supernatant was decanted into a glass test tube. The total activity in the supernatant was determined gravimetrically by counting 250 µL subsample using LSC and comparing the subsample's mass to the mass of the total supernatant. The pellet was resuspended in 3 mL of acetone and rehomogenized. The total activity of this homogenate was determined gravimetrically by counting a 250 µL subsample using LSC. The homogenate was then centrifuged for 5 min at 1060 x g. The total activity of the supernatant was determined gravimetrically by counting a 250 µL subsample. The pellet was resuspended in 1.5 mL of acetone and stored at -20 °C. To quantitatively determine the amount of radioactivity remaining in the earthworm tissue, the pellet was combusted. The method for trapping and counting $^{14}CO_2$ from the combustion process was the same for soil and compost samples (see Section 4.3.4.1.1).

4.3.4.2.4. Extraction of Gut Evacuation Chambers.

The filter paper from individual gut evacuation chambers was cut into small squares and placed with all the fecal castings into a 50 mL Nalgene centrifuge bottle. In addition, the individual gut evacuation chambers were triple rinsed with acetone and the acetone added to the Nalgene centrifuge bottle. The total mass of the acetone, filter paper, and castings was determined on a Mettler AE 163 analytical balance. The castings and filter paper were first mixed using a stainless steel spatula. The centrifuge bottles were then vortexed at maximum speed for 30 sec and sonicated for 30 min in a Fisher Scientific ultrasonic generator (Model FS 28 11). The samples were given 14 h to soak in the centrifuge bottles and then were again sonicated for 30 min. The castings and filter paper were precipitated from the acetone extract by centrifuging at 2800 x g (3900-4000 RPM's) for 15 min, using a Fisher Scientific Marathon 6K Centrifuge. The total activity in the supernatant was determined gravimetrically by counting a 250 μL subsample using LSC. This extraction procedure was repeated until the dpm in the subsample of supernatant was indistinguishable from background radioactivity.

After the last acetone extraction, 15 mL of 1 N NaOH was added to the centrifuge bottle. Samples were allowed to stand for 24 h and then vortexed at full speed for 30 sec and sonicated for 30 min. The earthworm casting and filter paper were precipitated from the NaOH extract by centrifuging at 2800 x g (3900-4000 RPM's) for 15 min. The total radioactivity in the supernatant was determined gravimetrically by counting a 250 μL subsample using LSC. The radioactivity in the first extracts was indistinguishable from background; therefore, additional extractions were not necessary.

4.3.4.2.5. Chromatography and Autoradiography of Soil and Earthworm Acetone Extracts

The protocol for TLC, HPTLC and autoradiography of soil and earthworm acetone extractions was the same for compost acetone extractions (see Section 4.3.4.1.3).

4.4 Results

4.4.1 Composting of ^{14}C-Diazinon

A profile of the radioactivity recovered by sequential extractions of compost with acetone and NaOH is provided in Figure 13. In each of the cases, most of extractable radioactivity was recovered in the first extraction. Only a small amount of extractable radioactivity remained after the first two extractions. The 30- and 60-day composted ^{14}C-diazinon sorbents consistently required more extractions than the uncomposted ^{14}C-diazinon sorbents for exhaustive extraction. After 15 extractions with NaOH, radioactivity was still being extracted from the 30- and 60-day composted ^{14}C-diazinon sorbents. This may represent a degradation of the humic structures by NaOH and subsequent release of incorporated radioactivity.

The mass balance of radioactivity from the composting of ^{14}C-diazinon is provided in Table 3. Following 30 and 60 days of composting, only 0.17 and 0.13% of the ^{14}C-diazinon had mineralized, respectively, and only 0.01 and 0.03% was released as

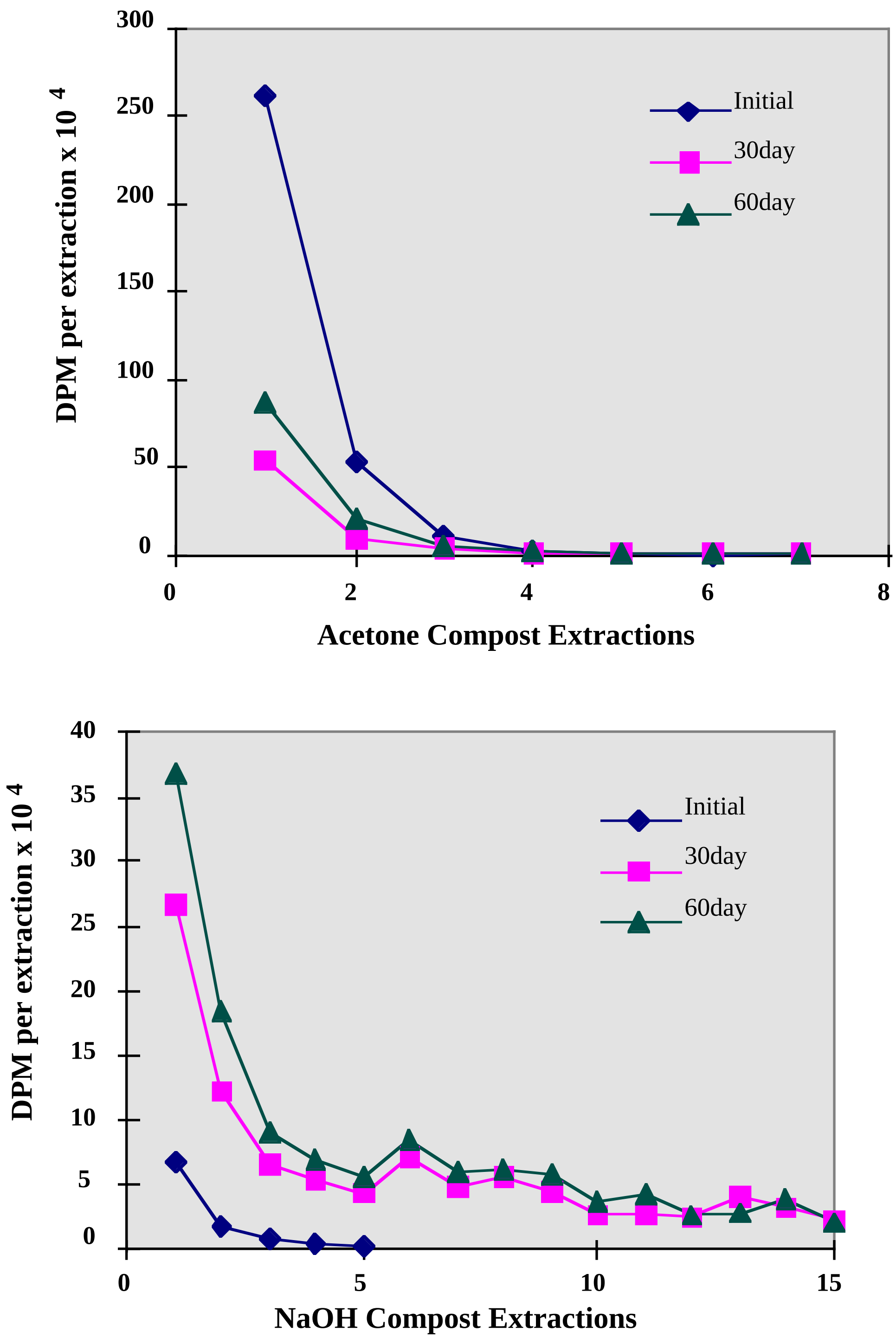


Figure 13. Radioactivity removed by sequential compost extractions.

Table 3. Mass Balance of Radioactivity After Composting of Δ-2-^{14}C-Diazinon in a Peat Moss Matrix for 30 and 60 Days.

Fraction analyzed	0 Day μCi[1] $\pm$ std. dev. (% of activity added[2])		30 Day μCi $\pm$ std. dev. (% of activity added)		60 Day μCi $\pm$ std. dev. (% of activity added)	
CO_2	0.03	(0.05 %)	0.1	(0.16 %)	0.08	(0.13 %)
Volatile organics	0	0 %	0.01	(0.02 %)	0.02	(0.03 %)
Acetone extraction	45 $\pm$ 0.5	(70 %) a	14 $\pm$ 3	(22%) b	16 $\pm$ 0.1	(25 %) b
Alkaline extraction	1 $\pm$ 0.04	(2 %) a	13 $\pm$ 2	(20%) b	17 $\pm$ 3	(27 %) b
Residue combustion	0.6 $\pm$ 0.3	(1 %) a	6 $\pm$ 2	(9 %) b	8 $\pm$ 2	(13 %) b
Total Radioactivity	47	(73 %)	33	(51 %)	41	(65 %)

[1] Percentage of radioactivity relates to the total radioactivity added to each compost sample as (64 μCi or 1.4 x 10^8 dpm).

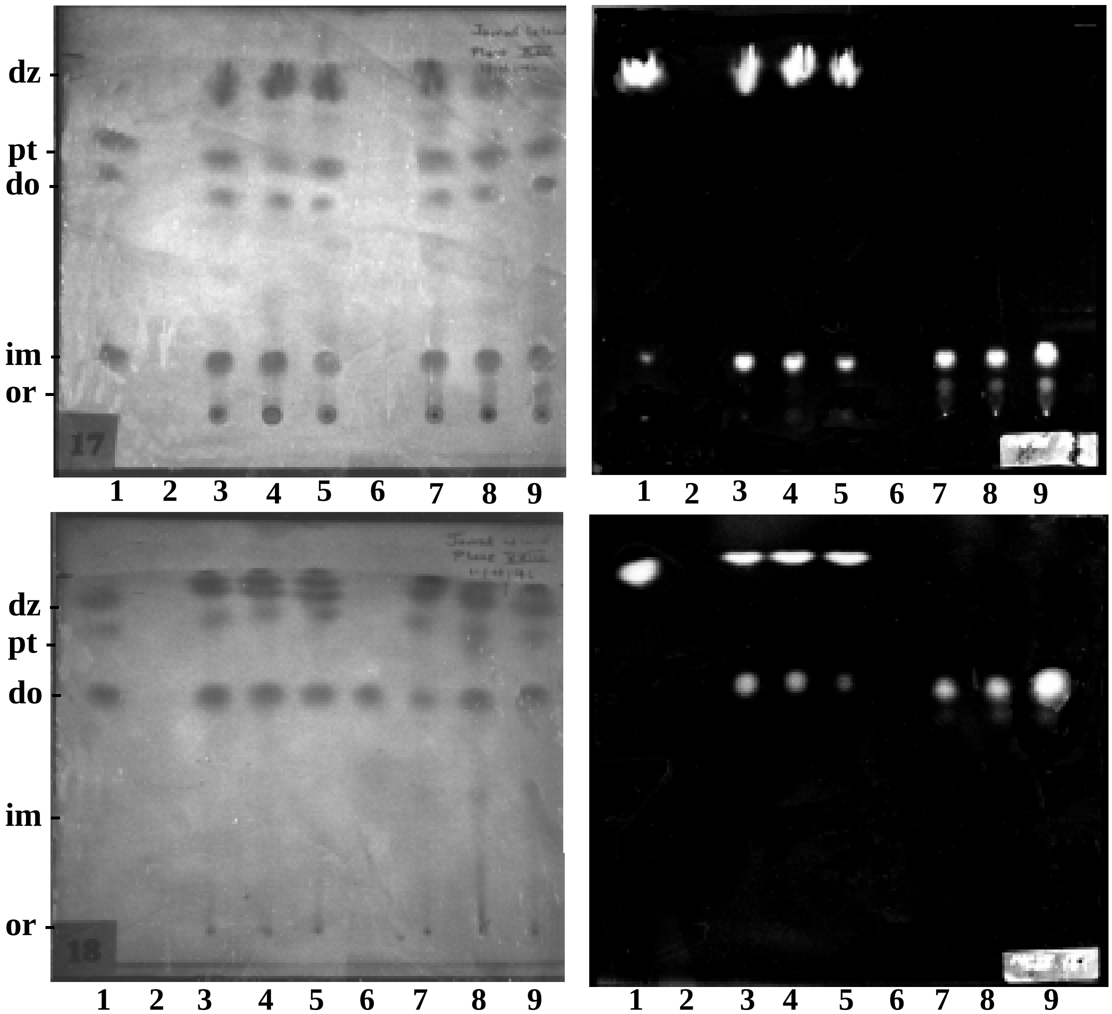
[2] Values within the same row that are followed by different letters are significantly different at $\alpha = 0.05$ (Tukey-Kramer Multiple Comparison).

volatiles. The small amount of $^{14}CO_2$ that was captured from the uncomposted ^{14}C-diazinon sorbent was captured during the 48 h equilibration period.

The majority of the radioactivity in the uncomposted ^{14}C-diazinon sorbent was extractable with acetone (70 %); whereas, only 2% of the radioactivity remained in the NaOH soluble fraction following exhaustive acetone extraction. These results were significantly different from the 30- and 60-day composted ^{14}C-diazinon sorbents (α = 0.05). There was a significant decrease in acetone extractable radioactivity and in the 30- and 60-day composted ^{14}C-diazinon samples the acetone extractable decreased from 70% in the uncomposted ^{14}C-diazinon sample to 22 and 25%, respectively (α = 0.05). There was a corresponding increase in the NaOH extractable radioactivity of the 30- and 60-day composted ^{14}C-diazinon samples from 2% in the uncomposted ^{14}C-diazinon sample to 20 and 27%, respectively (α = 0.05). There was also a corresponding increase in the in the unextractable radioactivity (combustion fraction) of the 30- and 60-day composted ^{14}C-diazinon samples from 1% in the uncomposted ^{14}C- diazinon sample to 9 and 12%, respectively (α = 0.05). There was not, however, any significant difference in the acetone extractable, NaOH extractable, or unextractable radioactivity between the 30- and the 60-day composted ^{14}C-diazinon sorbent (α = 0.05).

Autoradiograms from HPTLC and TLC chromatography are presented in Figures 14 through 19. These plates provide a visual presentation of the data provided in Table 4. HPTLC plates were used to identify radioactive metabolites, but not to quantify radioactivity. Extracts from uncomposted and 60-day composted ^{14}C-diazinon sorbents were chromatographed on the same HPTLC plates. The results from this plate and its replicate plate are presented in Figures 14 and 15, respectively. Extracts from 30-day composted ^{14}C-diazinon sorbents were chromatographed on a single HPTLC plate. The results from this plate and a replicate plate are presented in Figures 16 and 17, respectively. The autoradiogram corresponding to each HPTLC plate is presented adjacent to it in congruent orientation.

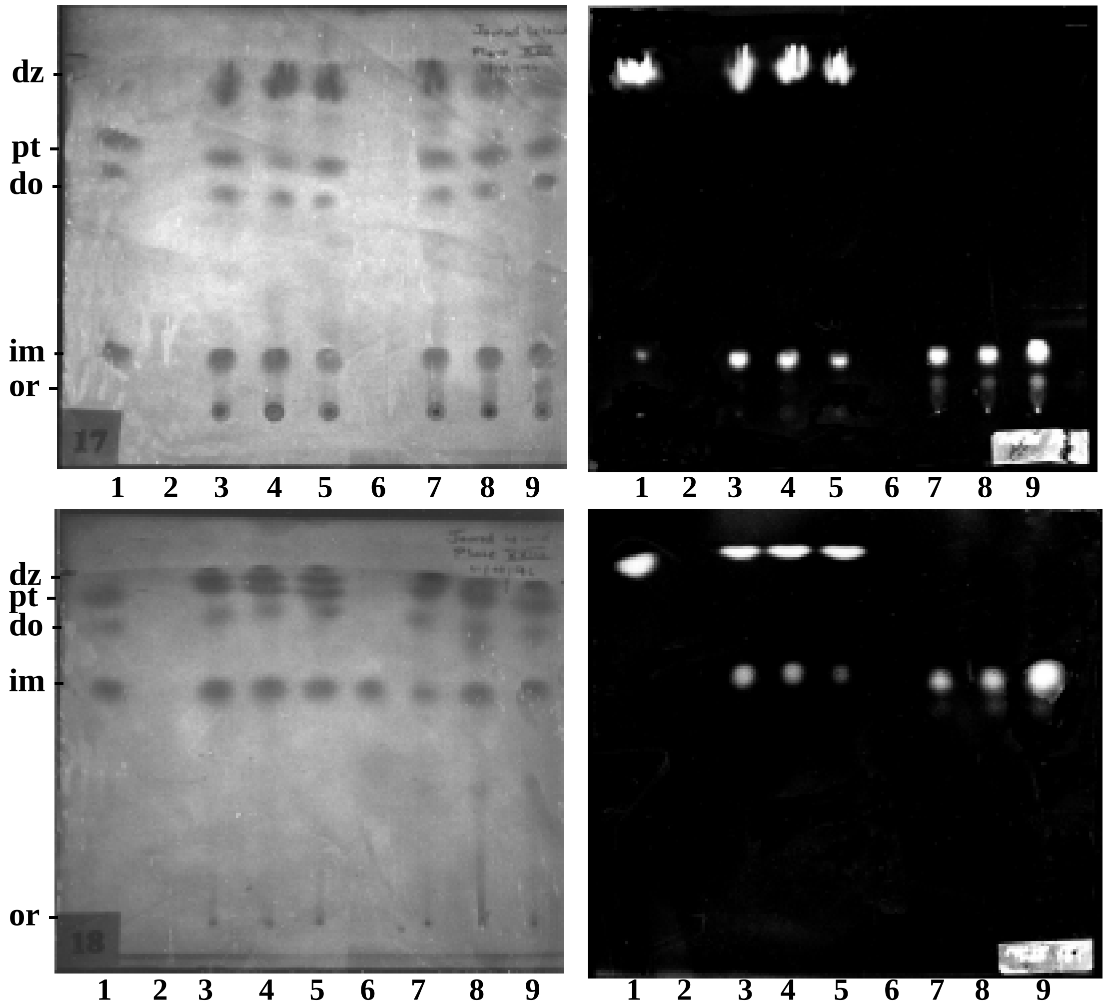
TLC plates were used to both identify radioactive metabolites, and to quantify radioactivity. Extracts from uncomposted and 60-day composted ^{14}C-diazinon sorbents were chromatographed on the same TLC plates (Figure 18). Extracts from 30-day composted ^{14}C-diazinon sorbents were chromatographed on a single TLC plate (Figure



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately] (Top plate lacked diazinon in the mixed standard); origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #4 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #5 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
Lane #6 Blank (Bottom Plate Spotted with 2µl of Oxypyrimidine Std.)
Lane #7 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl) (Because of lost activity from R.1, R.2 was spotted twice)
Lane #8 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #9 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)

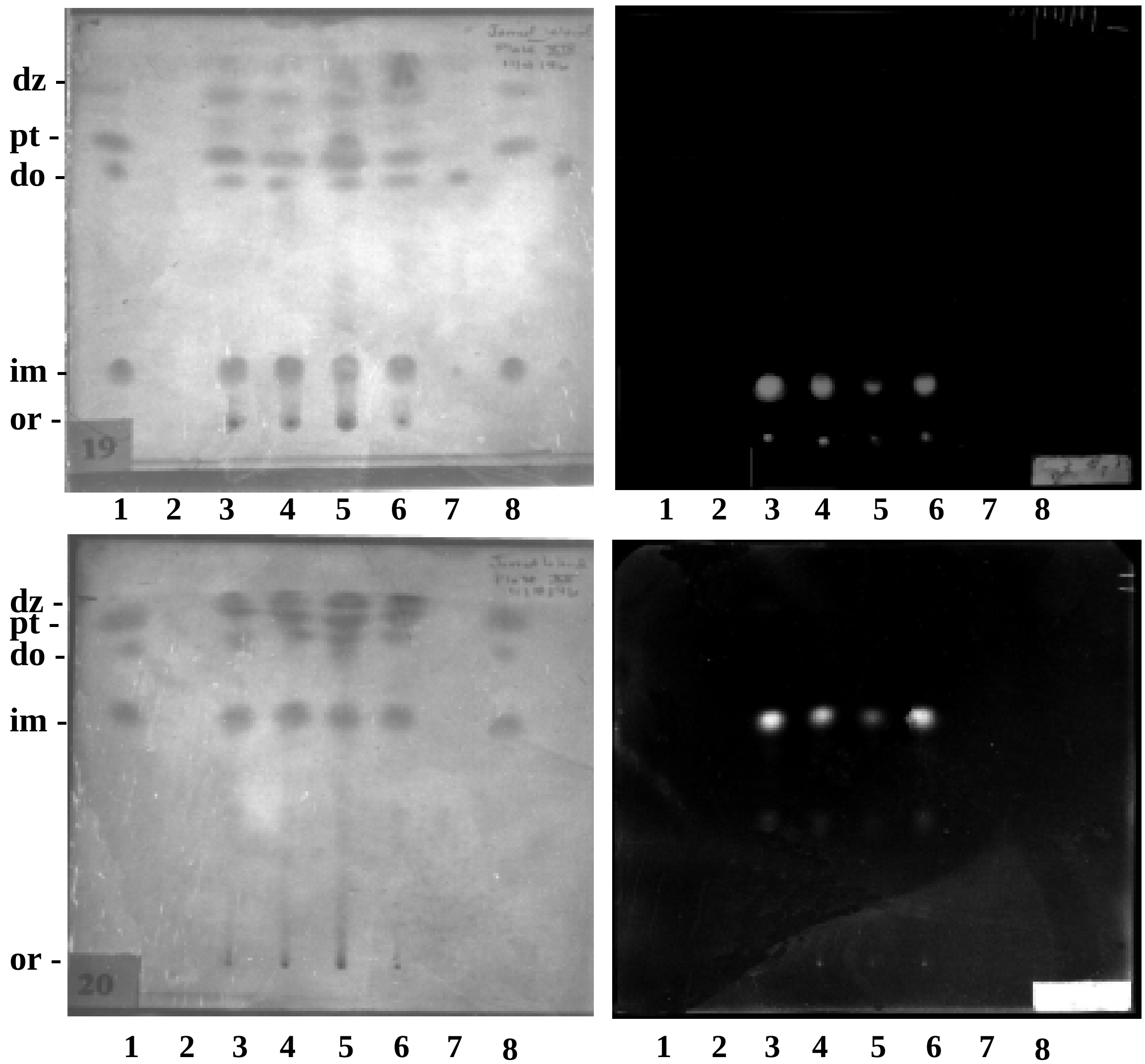
Figure 14. HPTLC of uncomposted ^{14}C-diazinon sorbent and 60-day composted ^{14}C-diazinon sorbent.



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I   Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately] (Top plate lacked diazinon in the mixed standard); origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #4 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #5 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
Lane #6 Blank (Bottom Plate Spotted with 2µl of Oxypyrimidine Std.)
Lane #7 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
(Because of lost activity from R.1, R.2 was spotted twice)
Lane #8 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #9 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)

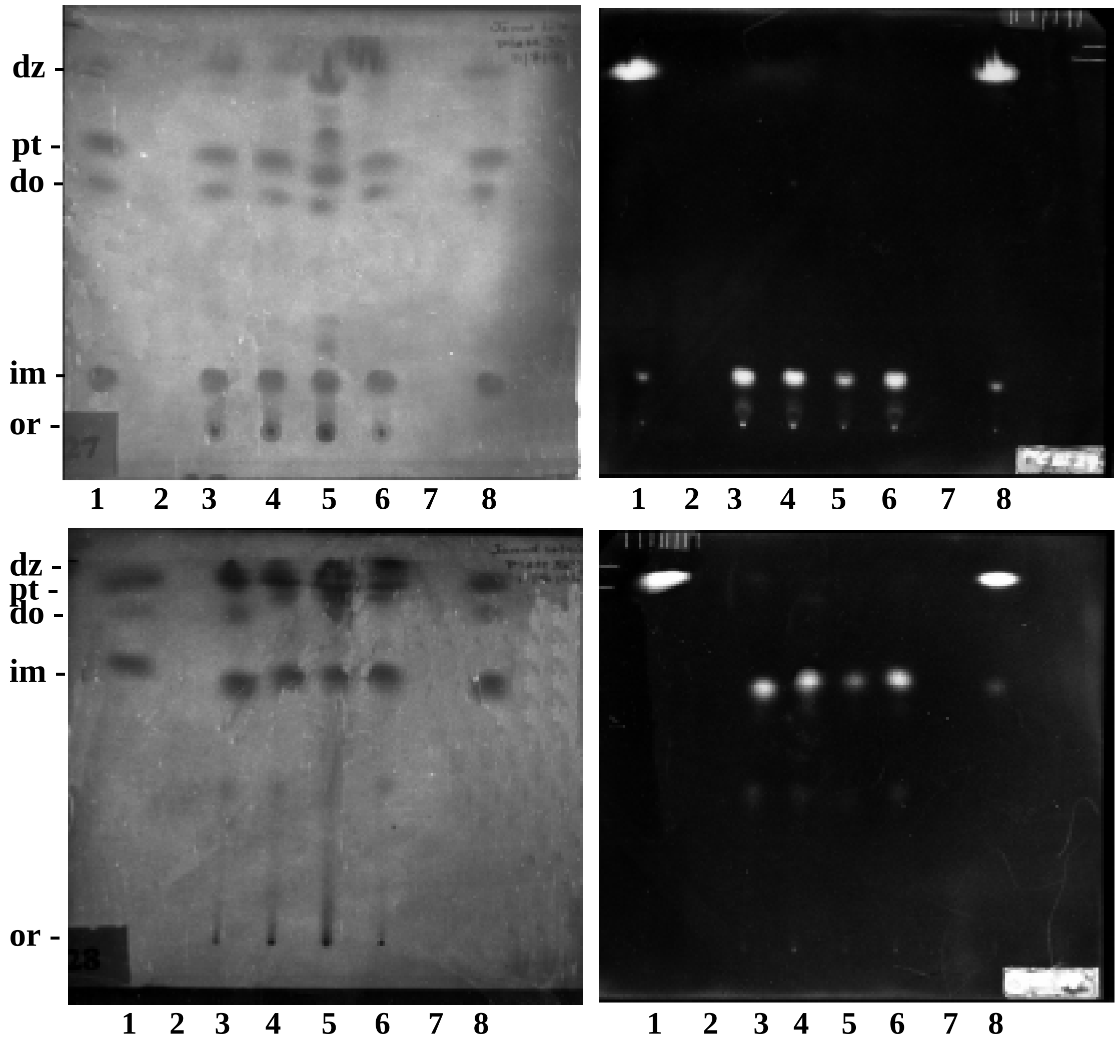
Figure 15. HPTLC of uncomposted ^{14}C-diazinon sorbent and 60-day composted ^{14}C-diazinon sorbent (replicate plate).



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I   Bottom: Solvent System II

Lane #1 Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #4 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #5 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
(Some activity was lost from R.3, therefore, R.3 was re-extracted and.spotted as R.4)
Lane #6 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 4 (2 µl)
Lane #7 Blank
Lane #8 Mixed Standard, no spike over with ^{14}C-Diazinon

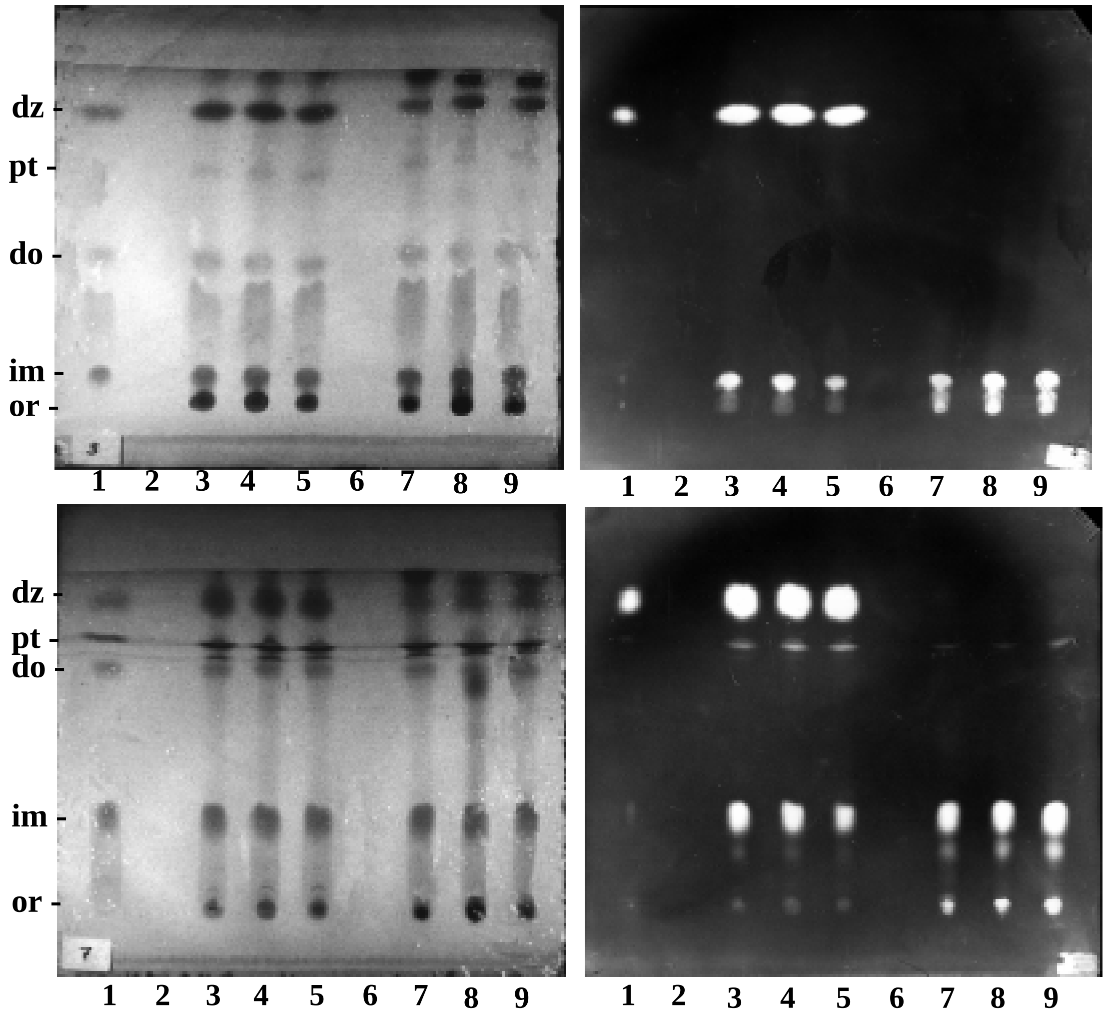
Figure 16. HPTLC of 30-day composted ^{14}C-diazinon sorbent.



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #4 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #5 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
(Some activity was lost from R.3, therefore, R.3 was re-extracted and .spotted as R.4)
Lane #6 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 4 (2 µl)
Lane #7 Blank
Lane #8 Mixed Standard plus 1 µl of ^{14}C-Diazinon Standard (2,400 dpm)

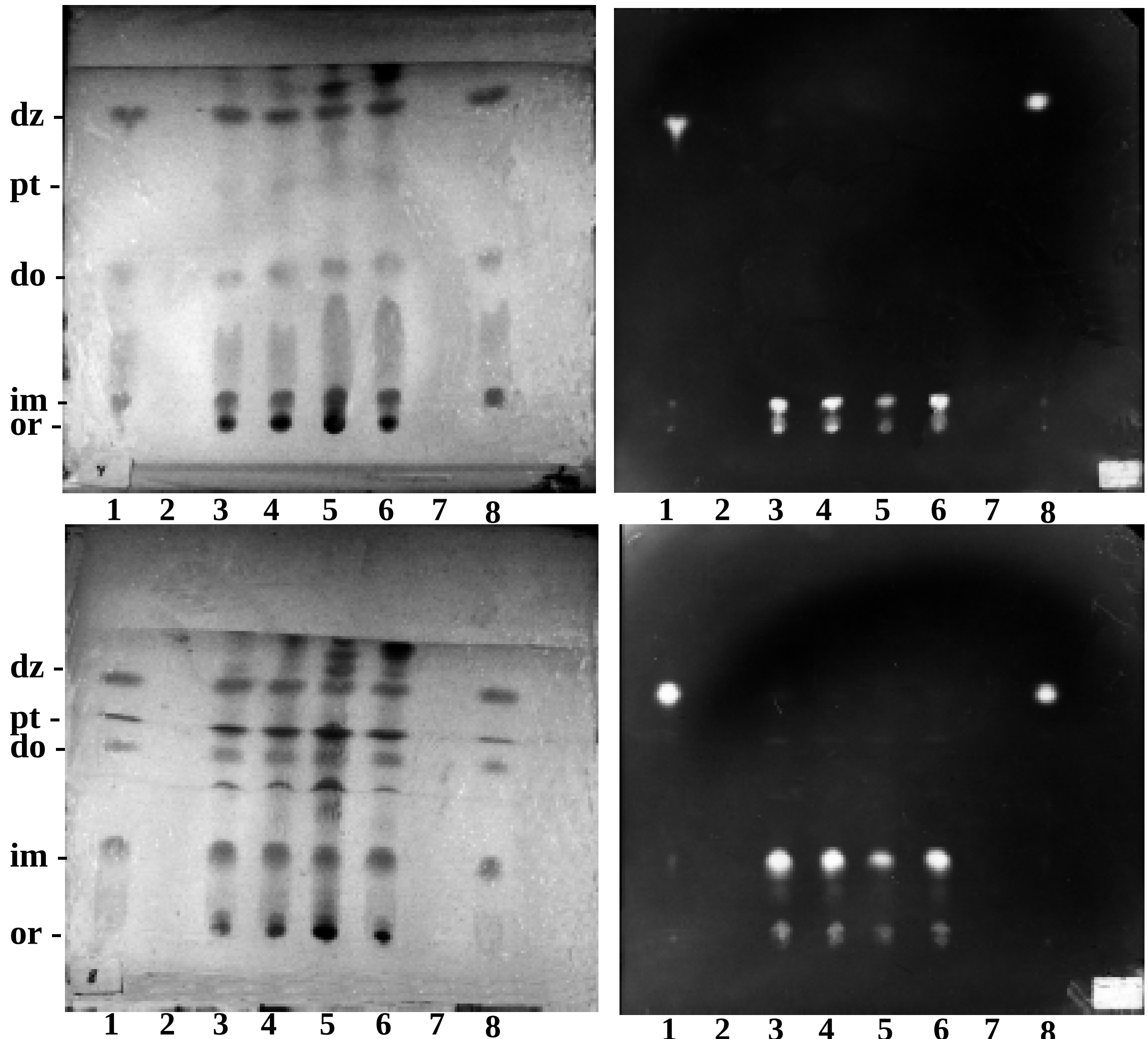
Figure 17. HPTLC of 30-day composted ^{14}C-diazinon sorbent (replicate plate).



Thin Layer Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I   Bottom: Solvent System II

Lane #1 2 µl of ^{14}C-Diazinon Standard (4,800 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Uncomposted ^{14}C-Diazinon Sorbent R. 1 (25 µl)
Lane #4 Mixed Standard plus Uncomposted ^{14}C- Diazinon Sorbent R. 2 (25 µl)
Lane #5 Mixed Standard plus Uncomposted ^{14}C- Diazinon Sorbent R. 3 (25 µl)
Lane #6 Blank
Lane #7 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
(Because of lost activity from R.1, R.2 was spotted twice)
Lane #8 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
Lane #9 Mixed Standard plus 60-Day Composted ^{14}C-Diazinon Sorbent R. 3 (25 µl)
[1] Bottom plate's mixed standard contains a new oxypyrimidine standard

Figure 18. TLC of uncomposted ^{14}C-diazinon sorbent and 60-day composted ^{14}C-diazinon sorbent.



Thin Layer Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 2 µl of ^{14}C-Diazinon Standard (4,800 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately] origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 1 (25 µl)
Lane #4 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
Lane #5 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (25 µl)
(Some activity was lost from R.3, therefore, R.3 was re-extracted and .spotted as R.4)
Lane #6 Mixed Standard plus 30-Day Composted ^{14}C-Diazinon Sorbent R. 4 (25 µl)
Lane #7 Blank
Lane #8 Mixed Standard plus 2 µl of ^{14}C-diazinon standard (4,800 dpm) [1]
[1] mixed standard in Lane # 8 of bottom plate contained a new pyrimidine thiol standard.

Figure 19. TLC of 30-day composted ^{14}C-diazinon sorbent.

Table 4. Tabulation of Thin Layer Chromatographic Analysis of ^{14}C-Diazinon Metabolites in the Acetone Extractable Fractions of Compost After Composting in a Peat Moss Matrix for 30 and 60 Days. [1]

Comparison of Compost Time	Ave. dpm Spotted[1]	Percentage of radioactivity spotted at the origin: Origin[2]	IMHP	Pyr. thiol	Diazoxon	Diazinon	Total % of dpm Spotted
Solvent system I							
Uncomposted ^{14}C-diazinon	41,206	2 ± 0.6 c	20 ± 6.8 c	0.1 ± 0.03 a	0.1 ± 0.02	85 ± 8.2 a	107
30-day composted ^{14}C-diazinon	9,988	16 ± 0.8 b	81 ± 1 .8 a	0.5 ± 0.2 a	0.2 ± 0.1	1 ± 0.5 c	99
60-day composted ^{14}C-diazinon	18,111	18 ± 2.8 b	89 ± 3.9 a	0.1 ± 0.01 a	0.1 ± 0.04	0.2 ± 0.1 c	107
Solvent system II							
Uncomposted ^{14}C-diazinon	41,206	0.8 ± 0.13 c	20 ± 8.4 d	2 ± 0.06 bc	0.2 ± 0.06	85 ± 8.2 a	108
30-day composted ^{14}C-diazinon	9,988	11 ± 1.0 b	80 ± 2.2 a	0.6 ± 0.26 c	0.3 ± 0.03	0.9 ± 0.6 c	93
60-day composted ^{14}C-diazinon	18,111	10 ± 0.9 b	85 ± 1.4 b	1 ± 0.4 bc	0.3 ± 0.01	0.6 ± 0.5 c	97

1 As percentage of total radioactivity based on the average dpm in a 25 µL subsample of the three concentrated acetone extracts from each treatment (25 µL was also spotted at the origin for TLC). The percentage is based on a ratio of the dpm per spot divided by the total dpm in a 25 µL subsample x 100%.

2 Values within the same column that are followed by different letters are significantly different at the $\alpha = 0.05$ level (Tukey-Kramer multiple comparison). For those values that are not followed by a letter, the compound was detected in less than three replicates (i.e. diazoxon).

19). The autoradiogram corresponding to each TLC plate is presented adjacent to it in congruent orientation. HPTLC and TLC plates were wrapped in Saran Wrap. The Saran Wrap was not removed when these plates were being photographed, to reduce the hazard associated with dust. These photographs were re-touched to reduce the glare from the Saran Wrap. The original scanned images as well as the hard copies are archived. Care was taken to not alter spots associated with compounds.

Quantitative results from TLC of the solvent extractable fraction using solvent systems I and II are presented in Table 4. The majority of the radioactivity in the uncomposted ^{14}C-diazinon was as diazinon (85%) and approximately 20% was IMHP. Following 30 days and 60 days of composting, most of the ^{14}C-diazinon had degraded to ^{14}C-IMHP, which accounted for 80 to 90% of the radioactivity. There was not, however, a significant difference in radioactivity as ^{14}C-IMHP between the 30- and 60-day compost extracts. TLC of extracts from 30- and 60-day composted ^{14}C-diazinon revealed 10 to 20% of the radioactivity remaining at the origin; whereas, less than or equal to 2% of the radioactivity remained at the origin from extracts of the uncomposted ^{14}C-diazinon. Most importantly, less than or equal to 1% of the spotted radioactivity could be accounted for as ^{14}C-diazinon following 30 and 60 days of composting. The difference in ^{14}C-diazinon concentrations in acetone extracts of 30- and 60-day composted ^{14}C-diazinon was not significant ($\alpha = 0.05$).

4.4.2. Characterizing the Fate of Diazinon in Amended Soil and Earthworms.

4.4.2.1 Determination of Radioactivity in CO_2 and Volatiles

The mass balance from incorporation of composted ^{14}C-diazinon into Groseclose topsoil is provided in Table 5. Following soil incubation, only 1.3, 0.8 and 0.7% of the ^{14}C-diazinon had mineralized to $^{14}CO_2$ in soil amended with uncomposted, 30-day composted, and 60-day composted ^{14}C-diazinon, respectively. It is worth noting that a higher percentage of the radioactivity was appeared to be mineralized from the soil amended with uncomposted ^{14}C-diazinon than from soils amended with 30- and 60-day composted ^{14}C-diazinon. Only 0, 0.002 and 0.005% of the radioactivity was released as volatiles from

Table 5. **Mass Balance of Radioactivity After Groseclose Soil was Amended with Δ-2-^{14}C-Diazinon Composted in a Peat Moss Matrix for 30 and 60 Days and Earthworms were in the Amended Soils for 14 Days.**

Fraction Analyzed	0 Day µCi ± std. dev.[1]	(% of activity added)[2]	30 Day µCi ± std. dev.	(% of activity added)	60 Day µCi ± std. dev.	(% of activity added)
CO_2	0.2	(1.3 %)	0.13	(0.8 %)	0.11	(0.7 %)
Volatile organics	0		0.002	(0.01 %)	0.005	(0.03 %)
Soil acetone extraction	0.9 ±0.02	(6 %) a	1.3 ± 0.16	(8 %) a	1.0 ± 0.03	(6 %) a
Soil alkaline extraction	1.1 ± 0.1	(7 %) a	3.9 ± 0.25	(24 %) b	4.4 ± 0.06	(28 %) c
Soil residue combustion	1.1 ± 0.3	(7 %) a	4.4 ± 1.7	(28 %) b	4.4 ± 0.7	(28 %) b
Worm acetone extraction	0.09 ± 0.01	(0.6 %) a	0.005 ± 0.005	(0.03 %) b	0.003 ± 0.000	(0.02 %) b
Worm residue combustion	0.008 ± 0.002	(0.05 %) a	0.008 ± 0.002	(0.05 %) a	0.011 ± 0.000	(0.07 %) b
Gut evacuation chamber	N/A	-------	0.003 ± 0.000	(0.02 %) a	0.008 ± 0.000	(0.05 %) b
Total	3.4	(21 %)	9.7	(61 %)	10	(64 %)

[1] Percentage of radioactivity relates to the total radioactivity added to each soil sample as compost-sorbed radioactivity (16 µCi or 3.5×10^7 dpm, based on the radioactivity added to peat moss before composting, minus losses due to CO_2 and volatility during composting and the mass of compost amended to soil).

[2] Values within the same row that are followed by different letters are significantly different at $\alpha = 0.05$ (Tukey-Kramer Multiple Comparison).

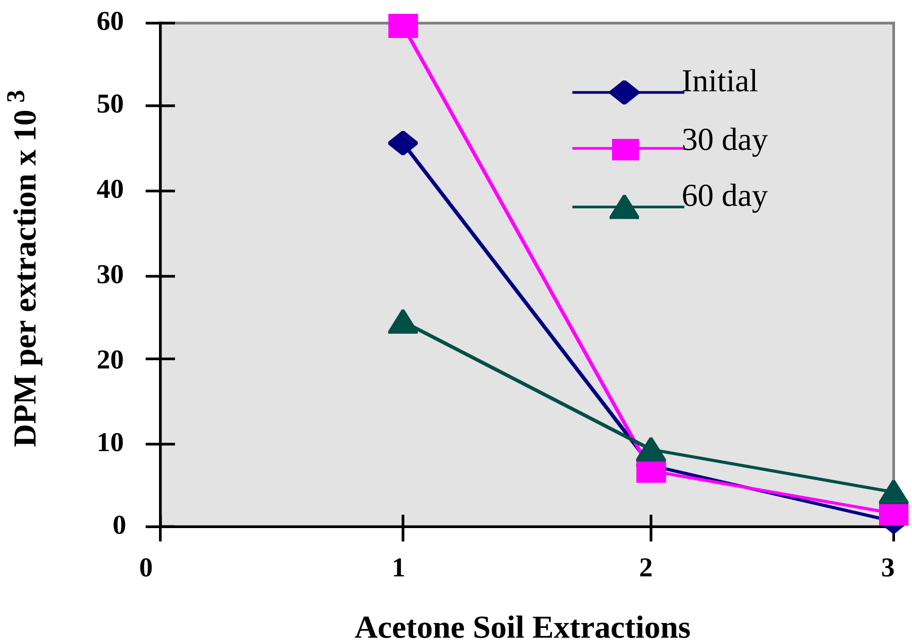
the soil amended with uncomposted, 30-day composted, and 60-day composted, respectivly. This low volatility is not surprising considering the low temperatures of the earthworm incubation chambers 15 to 20 °C.

4.4.2.2. Exhaustive Extraction of Amended Soil with Acetone and NaOH, and Combustion.

A profile of the radioactivity recovered by sequential extractions of soil with acetone and NaOH is provided in Figure 20. In each of the cases the majority of radioactivity was recovered in the first extract, and almost all the radioactivity in the first two extracts. Exhaustive NaOH extraction of radioactivity from soil amended with 30- and 60-day composted diazinon sorbent, however, required several extractions. The recovered radioactivity in soil amended with uncomposted ^{14}C-diazinon sorbent was distributed between the acetone extractable (6%), the alkaline extractable (7%), and the combustible (7%) fractions. The total recovery for the soil amended with uncomposted ^{14}C-diazinon sorbent was only 22%. There was significant effort to account for this radioactivity but it remains unclear if this radioactivity was lost as uncaptured volatiles or as incomplete combustion. This is further addressed in the discussion. In soils amended with 30- or 60-day composted ^{14}C-diazinon sorbents, the majority of the radioactivity was found in the NaOH extractable and combustion fractions, with approximately equal radioactivity in both fractions (25 to 30%). There was not any significant difference in either NaOH extractable radioactivity or unextractable radioactivity between the soil amended with 30- and 60-day composted ^{14}C-diazinon sorbent ($\alpha = 0.05$). Only 8 and 6% of the radioactivity was recovered in the acetone extractable fraction from soil amended with 30-and 60-day composted ^{14}C-diazinon sorbent, respectively. The radioactivity in the acetone extractable fraction of these two soils was not also significantly different ($\alpha = 0.05$).

4.4.2.3 Chromatography and Autoradiography of Soil Acetone Extracts

Autoradiograms from HPTLC and TLC chromatography are presented in Figures 21 through 26. HPTLC plates were used to identify radioactive metabolites, but not to quantify radioactivity. Extracts from soil amended with uncomposted or 60-day composted ^{14}C-diazinon sorbents were chromatographed on the same HPTLC plates. This plate and a



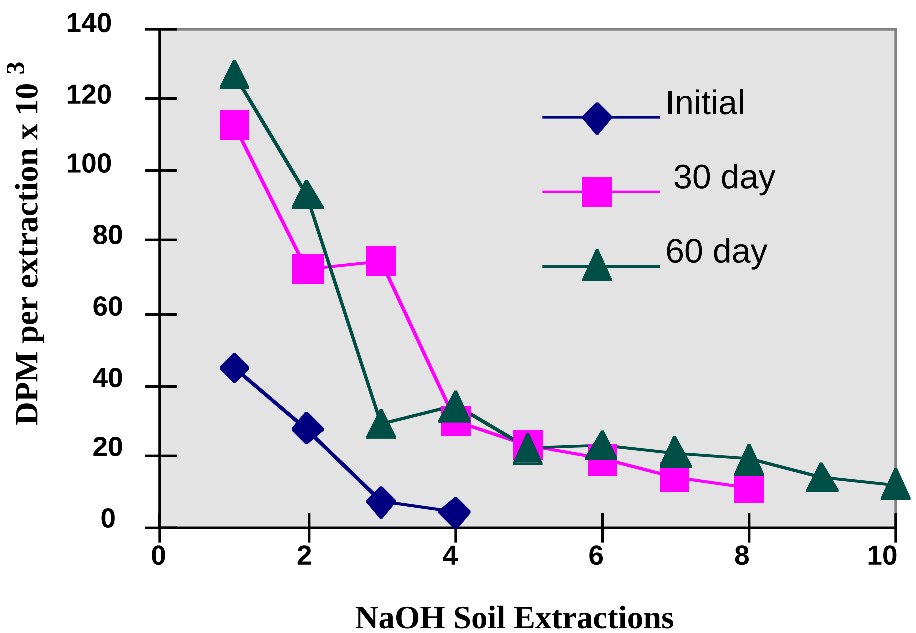
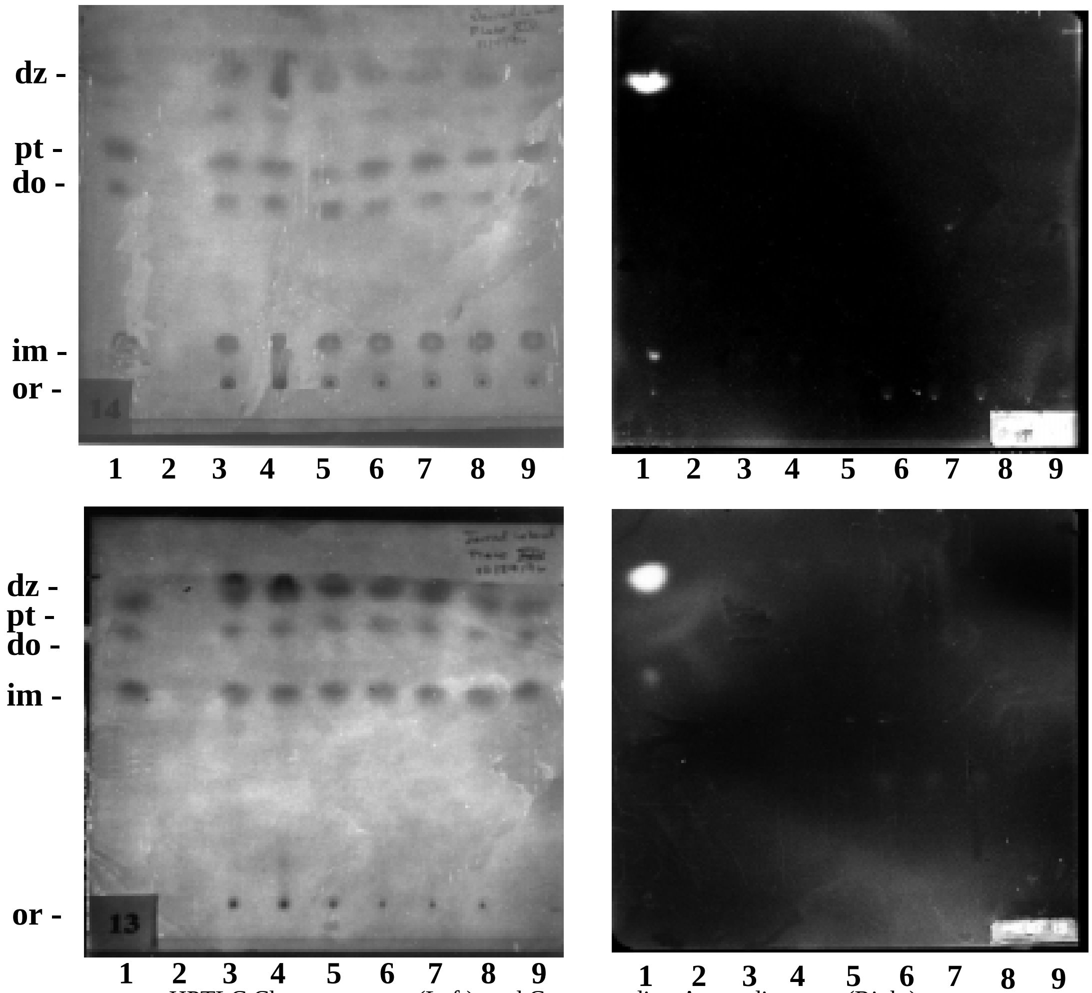


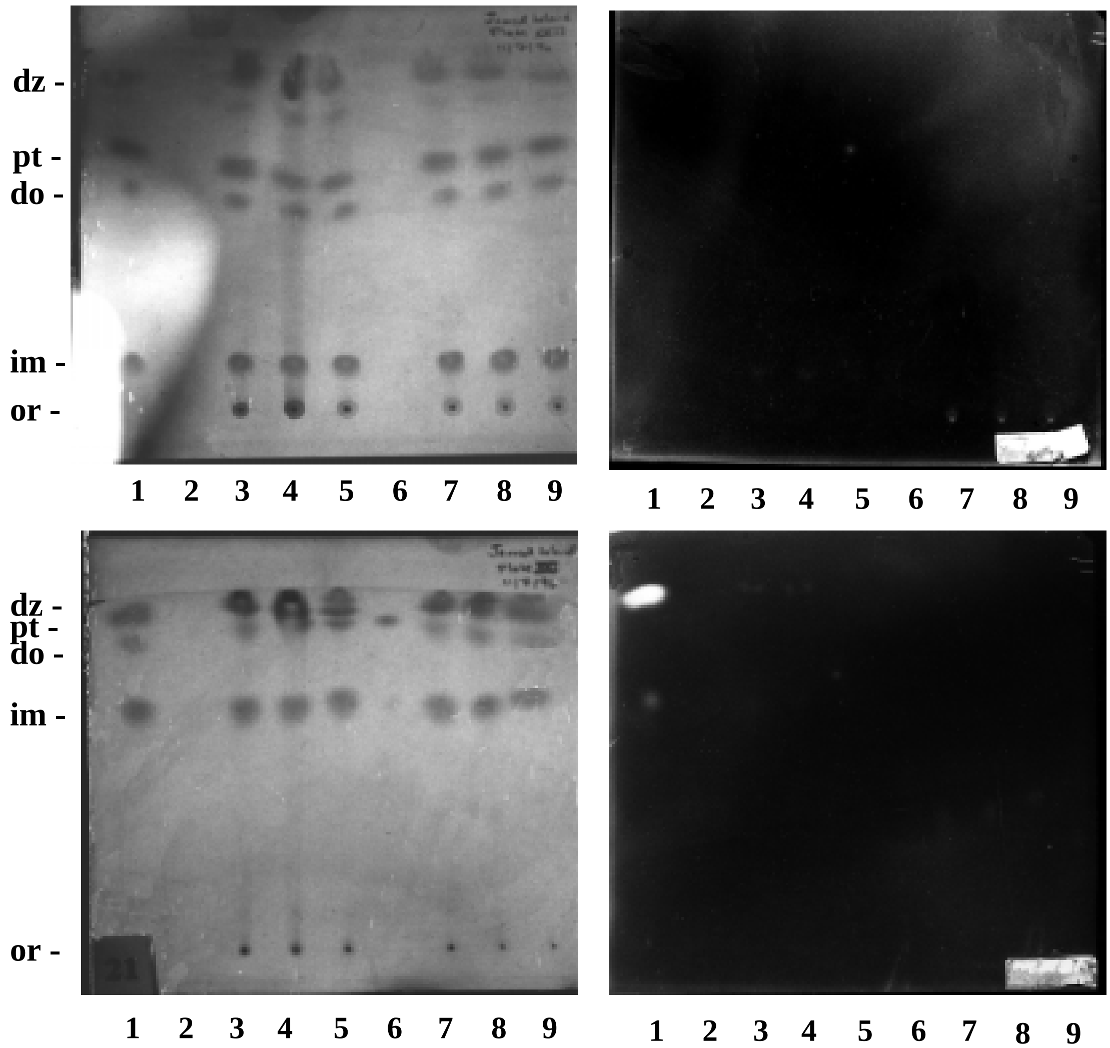
Figure 20. Radioactivity removed by sequential soil extractions.



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 1 µl of ^{14}C- Diazinon Standard (2,400 dpm); Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon R. 1 (2 µl)
Lane #4 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon R. 2 (2 µl)
Lane #5 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon R. 3 (2 µl)
Lane #6 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon R. 1 (2 µl)
Lane #7 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon R. 2 (2 µl)
Lane #8 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon R. 2 (2 µl) (Top Plate)
Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon R. 3 (2 µl) (Bottom)
Lane #9 Mixed Standard plus Soil Amended w/ 60- Day Composted ^{14}C-Diazinon R. 3 (2 µl) (Top Plate)
Blank (Bottom Plate)

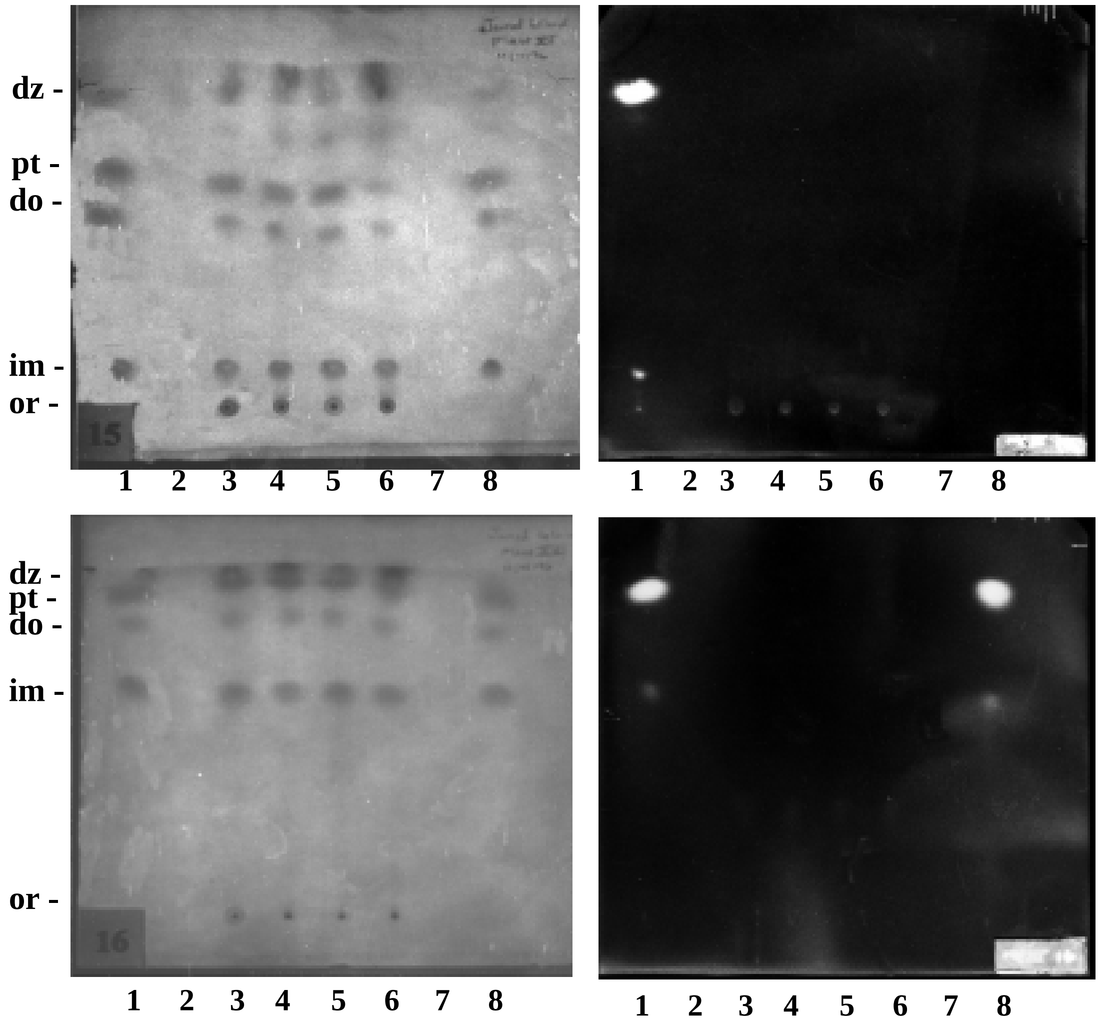
Figure 21. HPTLC of soil amended with uncomposted ^{14}C-diazinon sorbent and soil amended with 60-day composted ^{14}C-diazinon sorbent.



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm; only in bottom plate) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #4 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #5 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
Lane #6 Blank (2 µl of Diazoxon on Bottom Plate)
Lane #7 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon Sorbent R. 1 (2 µl)
Lane #8 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #9 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)

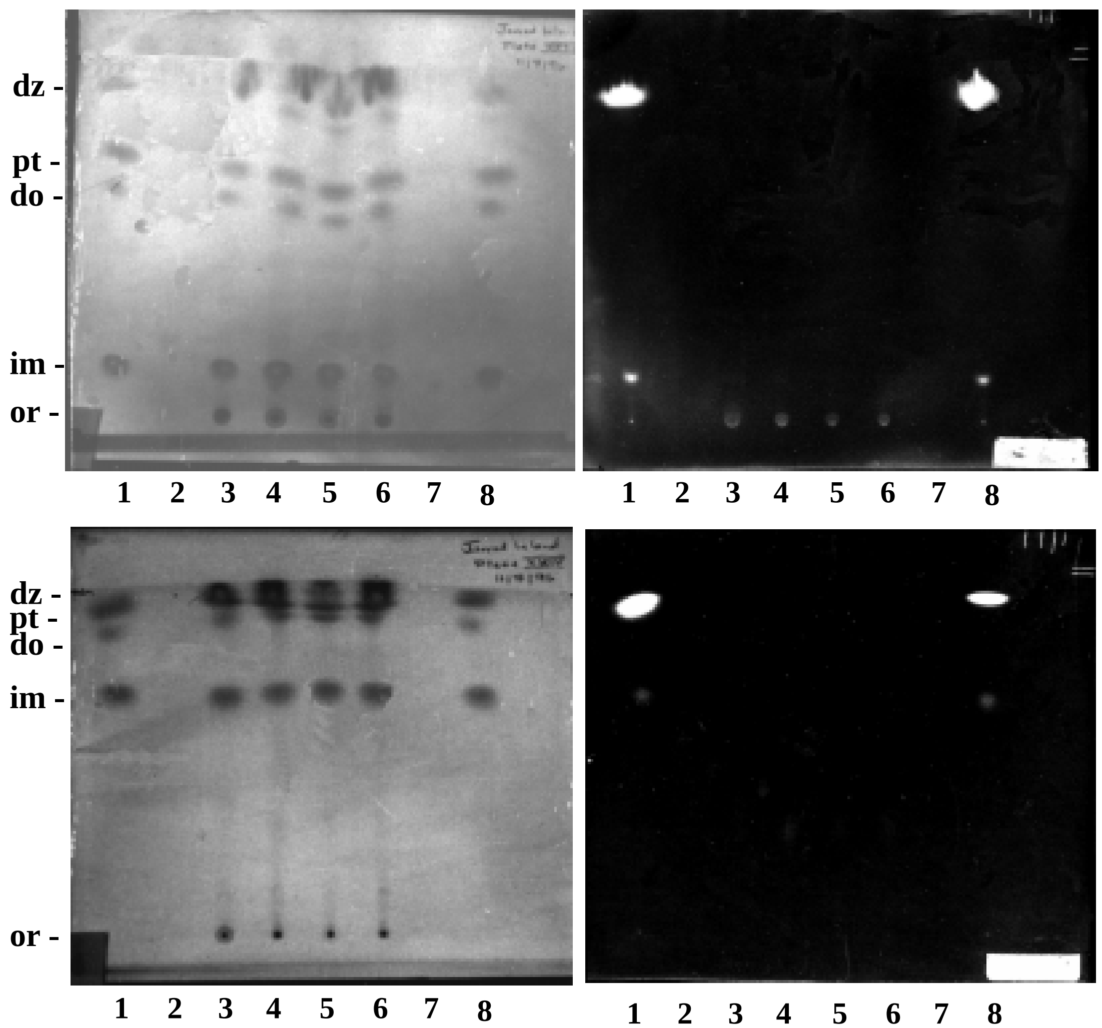
Figure 22. HPTLC of soil amended with uncomposted ^{14}C-diazinon sorbent and soil amended with 60-day composted ^{14}C-diazinon sorbent (replicate plate).



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ 30- Day Composted ^{14}C-Diazinon Sorbent R. 1a (2 µl)
Lane #4 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 1b (2 µl) (R.1 was extracted twice, this is the second extract of R.1)
Lane #5 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #6 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
Lane #7 Blank
Lane #8 Mixed Standard plus 1 µl of ^{14}C-Diazinon Standard (2,400 dpm; bottom plate only)

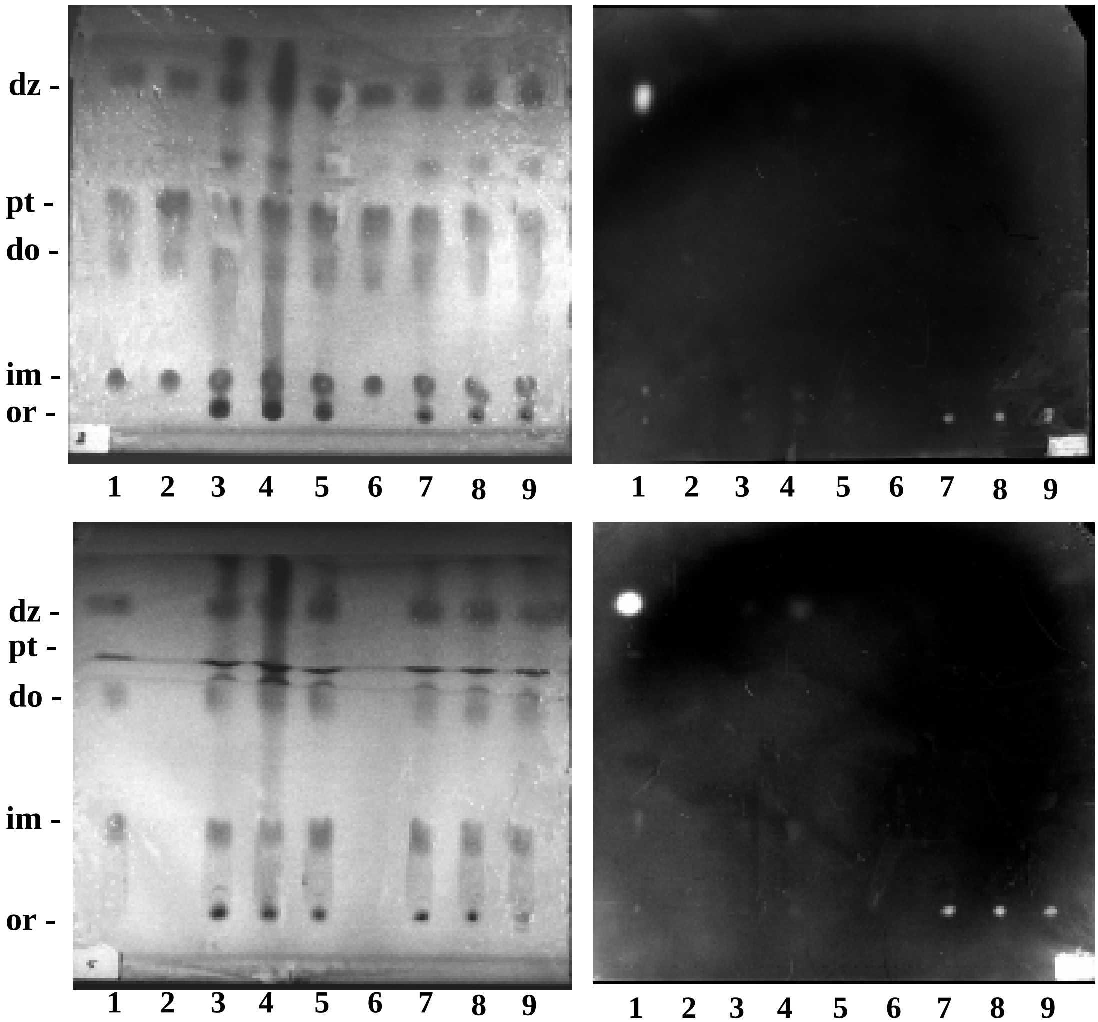
Figure 23. HPTLC of soil amended with 30-day composted ^{14}C-diazinon sorbent.



HPTLC Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I   Bottom: Solvent System II

Lane #1 1 µl of ^{14}C-Diazinon Standard (2,400 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 1a (2 µl)
Lane #4 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 1b (2 µl) (R.1 was extracted twice, this is the second extract of R.1)
Lane #5 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (2 µl)
Lane #6 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (2 µl)
Lane #7 Blank
Lane #8 Mixed Standard plus 1 µl of ^{14}C-Diazinon Standard (2,400 dpm)

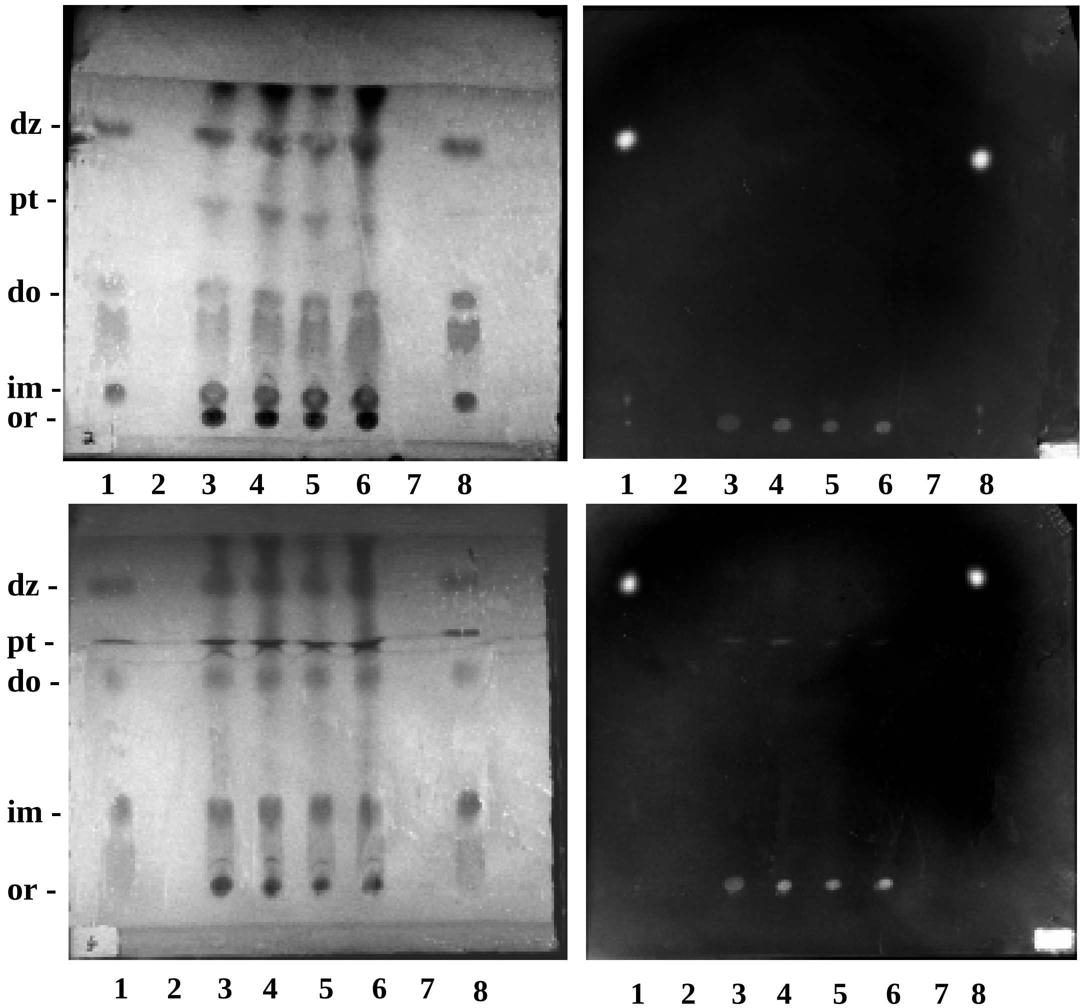
Figure 24. HPTLC of soil amended with 30-day composted ^{14}C-diazinon sorbent (replicate plate).



Thin Layer Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I Bottom: Solvent System II

Lane #1 2 µl of ^{14}C-Diazinon Standard (4,800 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]; origin (or)
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 1 (25 µl)
Lane #4 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
Lane #5 Mixed Standard plus Soil Amended w/ Uncomposted ^{14}C-Diazinon Sorbent R. 3 (25 µl)
Lane #6 Mixed Standard (Top); Blank (Bottom)
Lane #7 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon Sorbent R. 1 (25 µl)
Lane #8 Mixed Standard plus Soil Amended w/ 60-Day Composted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
Lane #9 Mixed Standard plus Soil Amended w/ 60- Day Composted ^{14}C-Diazinon Sorbent R. 3 (25 µl)

Figure 25. TLC of soil amended with uncomposted ^{14}C-diazinon sorbent and soil amended with 60-day composted diazinon sorbent.



Thin Layer Chromatograms (Left) and Corresponding Autoradiograms (Right)
Top: Solvent System I   Bottom: Solvent System II

Lane #1 2 µl of ^{14}C-Diazinon Standard (4,800 dpm) plus Mixed Standard [diazinon (dz), pyrimidine thiol (pt), diazoxon (do), and IMHP (im) spotted separately]
Lane #2 Blank
Lane #3 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 1 (25 µl)
Lane #4 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 1 (25 µl) (R.1 was extracted twice, this is the second extract of R.1)
Lane #5 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 2 (25 µl)
Lane #6 Mixed Standard plus Soil Amended w/ 30-Day Composted ^{14}C-Diazinon Sorbent R. 3 (25 µl)
Lane #7 Blank
Lane #8 Mixed Standard plus 2 µl of ^{14}C-Diazinon Standard (4,800 dpm)

Figure 26. TLC of soil amended with 30-day composted ^{14}C-diazinon sorbent.

similar plate are presented in Figures 21 and 22, respectively. Extracts from soil amended with 30-day composted ^{14}C-diazinon sorbents were chromatographed on a single HPTLC plate. The results from this plate and a replicate plate are presented in Figures 23 and 24, respectively. The autoradiogram corresponding to each HPTLC plate is presented adjacent to it in congruent orientation. TLC plates were used to both identify radioactive metabolites, and to quantify radioactivity. Extracts from soil amended with uncomposted or 60-day composted ^{14}C-diazinon sorbents were chromatographed on the same TLC plate (Figure 25). Extracts from soil amended with 30-day composted ^{14}C-diazinon sorbents were chromatographed on a single TLC plate (Figure 26). The autoradiogram corresponding to each TLC plate is presented adjacent to it in congruent orientation. The images of these chromatograms were also re-touched to reduce the glare from Saran Wrap.

Quantitative results TLC of the solvent extractable fraction using solvent systems I and II are presented in Table 6. The majority of the radioactivity in the soil amended with uncomposted ^{14}C-diazinon was distributed between diazinon (38 and 41% using solvent systems I and II, respectively) and IMPH (36 and 32% using solvent systems I and II, respectively). In soil amended with 30- and 60-day composted ^{14}C-diazinon, most of the radioactivity did not move from the origin during chromatography (65 to 80%). Whereas, only 16 and 11% of the radioactivity remained at the origin from extracts of the soil amended with uncomposted ^{14}C-diazinon using solvent systems I and II, respectively. The majority of the remaining activity in soil amended with 30- and 60-day composted ^{14}C-diazinon was as ^{14}C-IMHP. In soil amended with 30-day composted ^{14}C-diazinon, 11 and 12% of the activity could be accounted for as IMHP using solvent systems I and II, respectively. In soil amended with 60-day composted ^{14}C-diazinon, 15 and 10% of the activity could be accounted for as IMHP using solvent systems I and II, respectively. There was no significant difference between 30- or 60-day composted ^{14}C-diazinon for any of compounds detected using TLC ($\alpha = 0.05$).

4.4.2.4. Extraction of Earthworm Tissues.

The percentage of total radioactivity accumulated in earthworm tissues is presented in Table 5, along with the mass balance for radioactivity from soil amended with ^{14}C-diazinon compost. The percentage of total soil radioactivity that could be accounted for in acetone extracts of earthworm tissue was an order of magnitude greater (0.09%) in the soil

Table 6. Tabulation of Thin Layer Chromatographic Analysis of ^{14}C-Diazinon Metabolites in the Acetone Extractable Fractions of Soils.[1]

Soil amendment	Ave. dpm Spotted[1]	Percentage of radioactivity spotted at the origin					Total % of dpm spotted
		Origin	IMHP	Pyr. thiol	Diazoxon	Diazinon	
Solvent system I							
Soil + uncomposted ^{14}C-diazinon	525	16 ± 4.6 a	36 ± 2.1 a	0.4 ± 0.4 a	2 ± 0.5	38 ± 9.8 a	92
Soil + 30-day composted ^{14}C-diazinon	1,120	68 ± 3.4 b	12 ± 0.4 b	3 ± 5.6 a	1 ± 0.3	8 ± 2.5 b	92
Soil + 60-day composted ^{14}C-diazinon	714	80 ± 2.8 b	15 ± 0.6 b	1 ± 0.8	2 ± 0.4	5 ± 1.6 b	103
Solvent system II							
Soil + uncomposted ^{14}C-diazinon	525	11 ± 2.8 a	32 ± 2.2 b	8 ± 3.3 a	2 ± 1.4	41 ± 8.5 a	94
Soil + 30-day composted ^{14}C-diazinon	1,120	68 ± 5.3 b	11 ± 0.8 c	8 ± 1.0 a	0.8 ± 1.2	6 ± 2.5 b	94
Soil + 60-day composted ^{14}C-diazinon	714	65 ± 3.4 b	10 ± 0.1	5 ± 0.8 a	4 ± 0.8	5 ± 0.3 b	89

1 Values expressed as percentage of average dpm in a 25 µL subsample of the three concentrated acetone extracts from each treatment (25 µL was also spotted at the origin for TLC). The percentage is based on a ratio of the dpm per spot divided by the total dpm in a 25 µL subsample x 100%.

2 Values within the same column that are followed by different letters are significantly different at the $\alpha = 0.05$ level (Tukey-Kramer multiple comparison). For those values that are not followed by a letter, the compound was detected in less than three replicates (i.e. diazoxon).

amended with uncomposted ^{14}C-diazinon sorbent than in soil amended with 30- or 60-day composted ^{14}C-diazinon (0.005% and 0.003%, respectively). The difference in acetone soluble radioactivity from earthworms exposed to soil amended with 30- or 60-day composted ^{14}C-diazinon was not significant ($\alpha = 0.05$). The amount of unextractable radioactivity was similar for earthworms exposed to soil amended with uncomposted, 30-day, or 60 day composted ^{14}C-diazinon (0.008, 0.008, and 0.011%, respectively). Earthworms exposed to soil amended with uncomposted ^{14}C-diazinon accumulated approximately seven times the amount of radioactivity as compared to earthworms exposed to soil amended with 30- and 60-day composted diazinon sorbent. The amount of radioactivity that became unextractably bound within the earthworms (combustion fraction) increased with increasing composting time. This information may be obtained by dividing the amount of radioactivity in the worm residue combustion (Table 5) by the total radioactivity in the earthworm (worm acetone extraction plus worm residue combustion, Table 5) a value for the percentage of total associated radioactivity that was unextractably bound can be calculated. The percentage of total earthworm associated radioactivity that was unextractably bound to earthworm tissue was 8% for soil amended with uncomposted ^{14}C-diazinon, 61% for soil amended with 30-day composted ^{14}C-diazinon , and 79% for soil amended with 60-day composted ^{14}C-diazinon.

Quantitative results from TLC of the solvent extractable fraction of earthworms are presented in Table 7. The distribution of radioactive metabolites in earthworm exposed to soil amended with uncomposted ^{14}C-diazinon sorbent corresponds to the distribution of radioactive metabolites in uncomposted ^{14}C-diazinon sorbents (Tables 4 and 7). In uncomposted ^{14}C-diazinon sorbent, diazinon and IMHP accounted for 85 and 20% of the radioactivity, respectively (see Table 4, solvent systems I and II). In earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent, diazinon accounted for 82 and 81% of the spotted radioactivity using solvent systems I and II respectively and IMHP accounted for 23% of the radioactivity in both solvent systems I and II (see Table 7, solvent systems I and II).

In earthworms exposed to soil amended with 30- and 60-day ^{14}C-diazinon sorbent, the majority of the radioactivity remained at the origin of the TLC plates. This may represent radioactive compounds that have been conjugated by any number of earthworm metabolic pathways or conjugating detoxication mechanisms such as glutathion S-

Table 7. Thin Layer Chromatographic Analysis of ^{14}C-Diazinon Metabolites in the Acetone Extractable Fraction of Earthworms After 14 Days of Incubation in Groseclose Soil Amended with 30- and 60-Day Composted ^{14}C-Diazinon Sorbent.[1]

Soil ammendment	Exposure (days)	Alive or Dead	Ave dpm spotted	Percentage of radioactivity spotted at the origin: Origin[2]	IMHP	Diazoxon	Pyr. thiol	Diazinon	Total % of spotted dpm
Solvent System I									
Uncomposted diazinon	2	D	2,883	16 ± 6	23 ± 2	2 ± 2	0.6 ± 0.4	82 ± 6	124 ± 9
30-day composted diazinon	7	D	246	48 ± 4	11 ± 2	1 ± 0.2	0 ± 0	3 ± 0.03	63 ± 5
30-day composted diazinon	7	A	396	38 ± 7	6 ± 0.8	2 ± 1.5	0.2 ± 0.4	3 ± 0.8	49 ± 7
60-day composted diazinon	7	A	674	40 ± 1	9 ± 4	1 ± 0.2	0.6 ± 1	0.8 ± 0.5	51 ± 7
30-day composted diazinon	14	A	290	32 ± 3	9 ± 0.6	4 ± 1	0 ± 0	0.2 ± 0.2	45 ± 3
60-day composted diazinon	14	A	420	48 ± 5	6 ± 2	0.4 ± 0.6	0.3 ± 0.1	4 ± 5	59 ± 0.5
Solvent System II									
Uncomposted diazinon	2	D	2,883	16 ± 7	23 ± 2	2 ± 2	1 ± 0.7	81 ± 6	123 ± 10
30-day composted diazinon	7	D	246	40 ± 0.6	6 ± 3	17 ± 8	10 ± 4	2 ± 0.7	75 ± 13
30-day composted diazinon	7	A	396	33 ± 4	4 ± 0.9	8 ± 0.8	11 ± 8	3 ± 0.2	59 ± 4
60-day composted diazinon	7	A	674	36 ± 3	3 ± 1	8 ± 1	4 ± 1	0.9 ± 0.4	52 ± 4
30-day composted diazinon	14	A	290	31 ± 0.9	2 ± 2	10 ± 4	13 ± 1	0.2 ± 0.2	56 ± 5
60-day composted diazinon	14	A	420	45 ± 0.8	3 ± 1	18 ± 8	6 ± 1	2 ± 0.01	74 ± 7

[1] Values expressed as percentage of radioactivity relates to the total radioactivity spotted onto the TLC plate as a subsample of the total radioactivity in each group of earthworms.

transferases (Stenersen, 1984). As mention earlier, the amount of radioactivity that became unextractably bound within the earthworms increased with increasing composting time (Table 5). This supports the idea that radioactivity remaining at the origin of TLC plates may be in some way incorporated into earthworm tissue. The total percentage of spotted radioactivity recovered after scraping was relatively low for samples were low radioactivity was chromatographed. This was likely an artifact of trying to recover low radioactivity.

4.4.2.5. Extraction of Gut Evacuation Chambers.

The percentage of total radioactivity excreted in earthworm casts is provided in Table 5, along with the mass balance for soil amended with ^{14}C-diazinon sorbents. Although there were significant differences among the three test groups regarding total activity, this is not an experimentally relevant comparison. This is because gut evacuations were only collected from those earthworms that survived. To test the effectiveness of the gut evacuation procedure, some earthworms were incubated for a second and third gut evacuation. The radioactivity in the second and third gut evacuation chambers was indistinguishable from background, indicating that the majority of the activity was excreted in the first gut evacuation. In these replicates, however, the concentration of radioactivity in the first gut evacuation chamber was very low as well. The efficacy of this gut evacuation procedure may, therefore, need to be re-evaluated using earthworm with higher initial concentrations of radioactivity in their intestines.

4.4.3. Mortality During the Exposure Period.

The mortality that occurred during exposure of earthworms to soil amended with ^{14}C-diazinon compost, is presented in Figure 27. The mortality from the laboratory radiolabeled study was much greater than the field study. The lower mortality observed in the field study was likely due to diazinon degradation (97%; see Section 3.4.1) prior to composting.

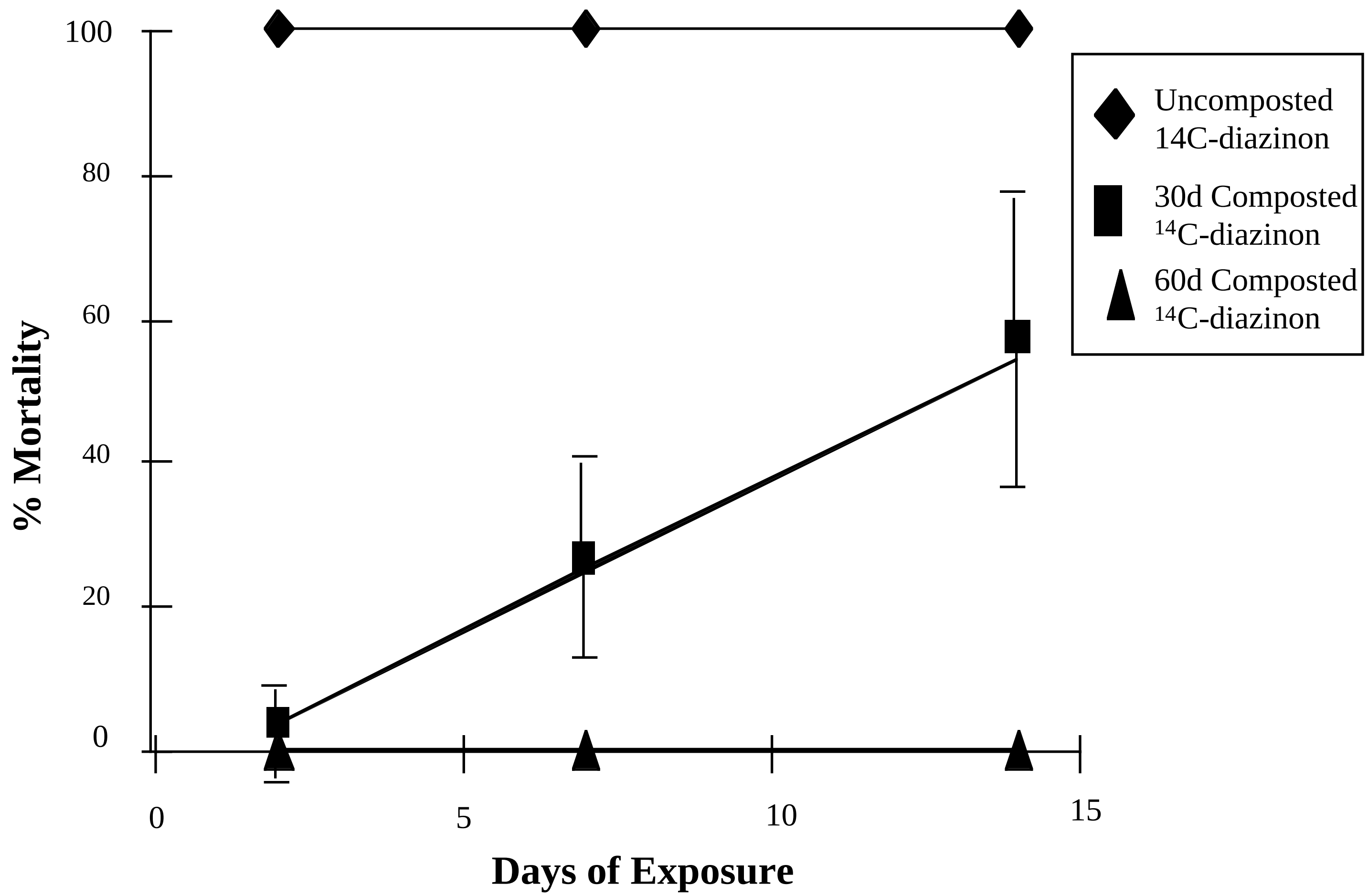


Figure 27. Mortality of *Eisenia foetida* following exposure to Groseclose soil amended with uncomposted, 30- , and 60-day composted ^{14}C-diazinon sorbent. Means ± standard errors are plotted (N=3 soil replicates; there were 10 earthworms in each soil replicate).

4.5 Discussion

This study evaluated the fate of Δ-2-^{14}C-diazinon in compost, compost amended soil and earthworms exposed to the amended soil. Δ-2-^{14}C-diazinon was incubated in a nutrient-amended peat moss matrix under simulated composting conditions for 30 and 60 days. This composted material was then amended to Groseclose topsoil, and earthworms (*E. foetida*) were incubated in this amended soil for up to 14 days. The fate of Δ-2-^{14}C-diazinon in compost has been reported on previously (Petruska et al., 1985) and will be compared with the results from this study. The composting process significantly reduced acetone extractable diazinon concentrations but little of the diazinon was mineralized. This is the first study to evaluate the fate of composted diazinon in amended soil. The effects of the soil environment on the fate of diazinon in amended soil are not distinguishable from earthworm effects in this study. However, it appears that diazinon continues to be degraded and incorporated following soil incorporation. After 14 days of incubation in amended soil, 30- and 60-day composted Δ-2-^{14}C-diazinon appeared to become further associated with humic and fulvic acids and the unextractable soil fractions (alkaline extractable and residue combustion fractions, see Tables 3 and 5). Little mineralization of Δ-2-^{14}C-diazinon occurred during the soil incubation period, but mineralization rates in amended soil were greater than that which occurred in the composting environment. This may have been related to: increased bioavailability in the soil matrix; a greater potential to mineralize compounds after they had degraded in the composting environment; greater microbial activity in the amended soil either due to environmental conditions or the effects of earthworms on the soils physical matrix or microbial metabolism. The uptake of diazinon and its degradation products by earthworms complements toxicology information presented in Chapter 3. Earthworms exposed to soil amended with uncomposted Δ-2-^{14}C-diazinon sorbent contained large quantities of diazinon; whereas, those that were exposed to 30- and 60-day composted Δ-2-^{14}C-diazinon contained approximately seven times less radioactivity and no compounds of known toxicological significance. The results of this experiment will be discussed in three parts: 1) the fate of Δ-2-^{14}C-diazinon in compost (Figure 28) ; 2) the fate of composted Δ-2-^{14}C-diazinon in amended soil (Figure 29); and 3) the fate of Δ-2-^{14}C-diazinon in earthworms exposed soil amended with composted Δ-2-^{14}C-diazinon (Figure 29).

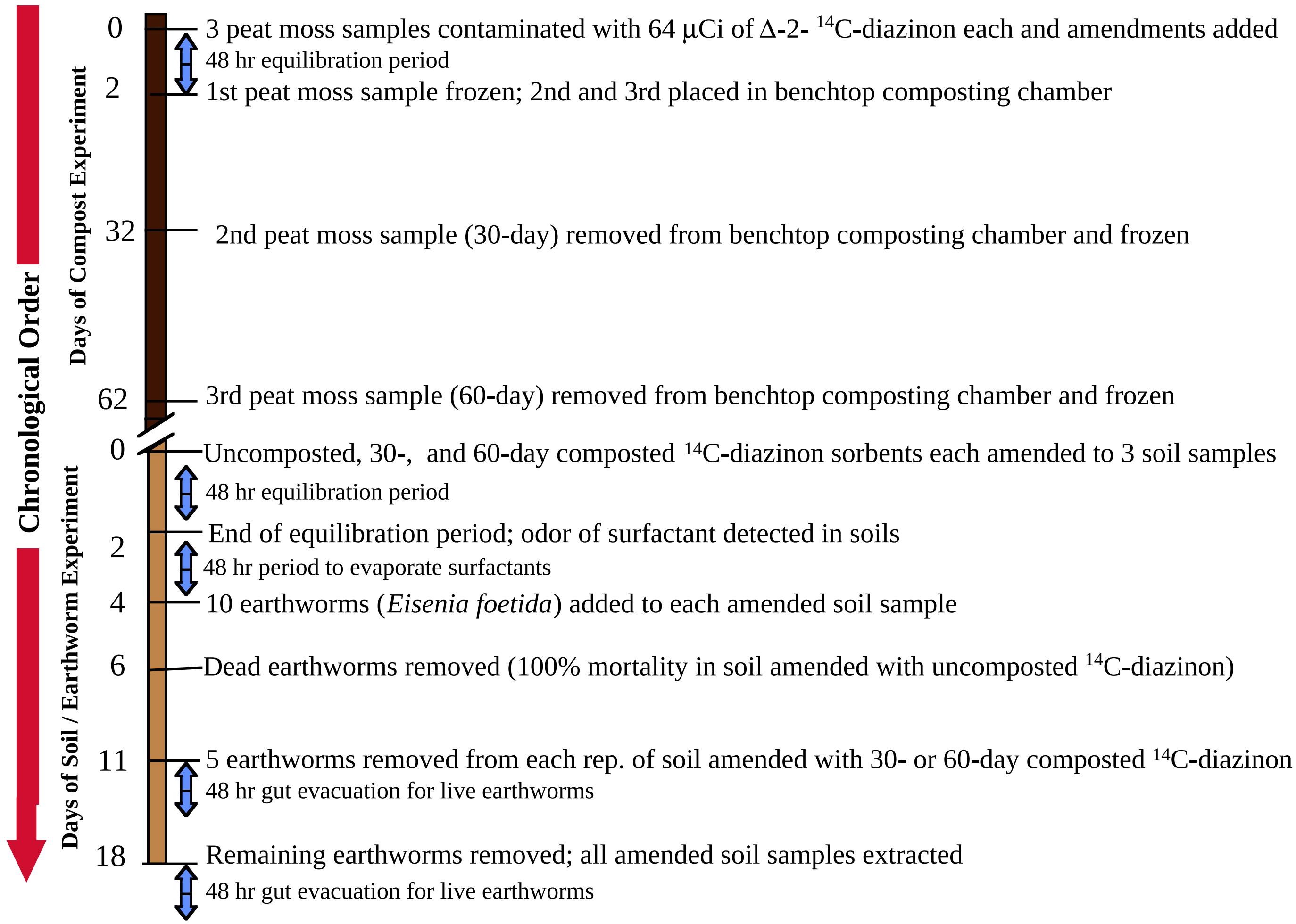


Figure 28. Timetable for ^{14}C-diazinon radiolabeled experiment: Contamination of peat moss with ^{14}C-diazinon and composting.

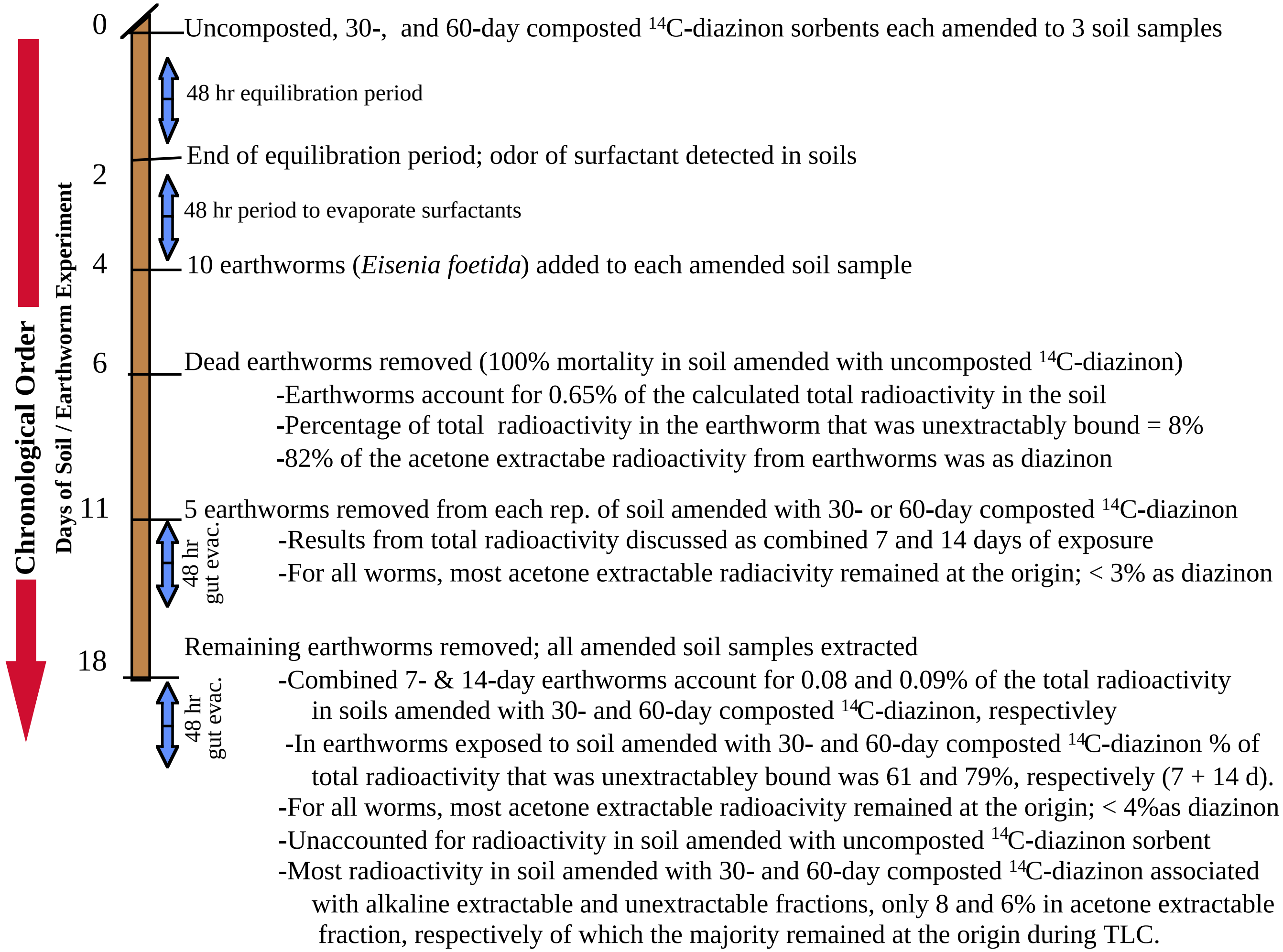


Figure 29. Timetable for ^{14}C-diazinon radiolabeled experiment: Amendment of composted ^{14}C-diazinon material to Groseclose topsoil and earthworm exposure.

4.5.1 Fate of Diazinon in Compost

4.5.1.1 CO_2 and Volatiles from Composting of Δ-2-^{14}C-diazinon

Results from the composting of ^{14}C-diazinon were similar to those of Petruska et al. (1985), with one exception. In their study, 22% of the radioactivity applied as diazinon was recovered as volatiles. In this study less than 0.05% of the radioactivity was recovered as volatiles. This dramatic difference can most likely be explained by a major change in the air sampling system. In the earlier study, air was drawn through compost during the sampling process; whereas, in this study air samples were taken from the head space above the compost. The air sampling system used in this study provides a better model for composting methods used by the Pesticide Waste Disposal Group. However, the former (Petruska et al., 1985) air sampling system better models forced air composting.

Only small amounts (0.17 and 0.14%) of the initially applied ^{14}C-diazinon were recovered as $^{14}CO_2$, following 30 and 60 days of composting, respectively (see Table 3). Similar results were obtained by Petruska et al. (1985), who reported recoveries of 0.2% of the applied ^{14}C-diazinon as $^{14}CO_2$ after 3 weeks of composting. Reported mineralization rates for diazinon in soil are much higher than results obtained in our composting studies. For example, a study by Getzin (1968) found that 35% of radioactivity, applied to soil as diazinon, was recovered as $^{14}CO_2$, after 20 weeks. In another study, approximately 50% of the radioactivity from ^{14}C-diazinon was accounted for as $^{14}CO_2$ following 180 days of incubation in soil (unpublished date, Sumner et al., 1988). However, in submerged soil only 0.4 to 0.8% of the applied radioactivity as diazinon was recovered as $^{14}CO_2$ after 50 days of incubation (Sethunathan and MacRae, 1969). This was likely due to the anoxic conditions in the submerged soils. It is generally accepted that the mineralization of diazinon requires the action of microorganisms in an aerobic environment (Gunner and Zuckerman, 1968; Sethunathan, 1982; Getzin, 1968). It may have been that sorption to organic matter limits the bioavailability of diazinon and its metabolites to microorganisms. Micropore entrapment or adsorption to organic matter surfaces would reduce the bioavailability of compounds to microorganisms. Alternatively, the composting environment may not have been appropriate for microbial species that are capable of diazinon mineralization or, even if conditions were appropriate, the species may simply not have been present. It should also be noted that enhanced degradation of diazinon in soils may result in higher mineralization rates because microorganisms have been selected for diazinon degradation. In addition, availability of carbon sources other than diazinon or its metabolites are more limited in soil than in the composting environment, which may also help to stimulate microbial metabolism of diazinon or its metabolites.

Another consideration is the microbial toxicity of diazinon. Although sorption will reduce the concentration of diazinon bioavailable to microorganisms, the diazinon concentration in the compost was very high. Somasundaram et al. (1990) determined the EC_{50} of diazinon and IMHP to *Photobacterium phosphoreum* to be 10 and 886 μg/g, respectively. Additional evidence for this microbial toxicity effect comes from the results of Fredrick et al. (1996). In this study on composted yard waste, approximately 11% of the ^{14}C-ring-labeled diazinon was recovered as $^{14}CO_2$, following 50 days of composting. The higher mineralization rates may have been due to composting a less humified material resulting in greater bioavailability. It also may have been the result of lower initial diazinon concentrations (10 μg/g) allowing for more reactive sites within the compost matrix or decreased microbial toxicity (Robson and Gunner, 1980; Somasundaram et al., 1990).

4.5.1.2 Exhaustive Extraction and Combustion

Comparisons of the uncomposted with the 60-day composted ^{14}C-diazinon indicate that there was a significant shift from the acetone extractable fraction into the alkaline extractable, and unextractable fractions, during the composting process. The acetone extractable fraction decreased by 46%; corresponding to an increase of 25% in the alkaline extractable and 11 % in the unextractable fraction. The alkaline extractable fraction includes the humic and fulvic acids. Fulvic acid has a molecular weight ranging from 280 to 2100 daltons (Bollag and Loll, 1983 review Schnitzer, 1988) and the molecular weights of humic acids range from 1400 to 1,000,000 daltons (Bollag and Loll, 1983 review Stevenson, 1982). Considering the molecular weights of humic and fulvic acids it is unlikely that intermolecular diffusion is the limiting factor in the release of contaminants associated with these fractions. Rather, it is likely that radioactivity associated with this alkaline soluble fraction was strongly adsorbed or chemically bound to the surface of the humic and fulvic acids. It is unlikely that the radioactivity associated with the alkaline soluble fraction was diazinon. Saint-Fort and Visser (1988) reported that the sorption of diazinon by humic acids with molecular weight greater than 300 kdaltons was limited by interparticle diffusion. They found no interaction between diazinon and the humic acid fraction of less than 300 kdaltons, suggesting that adsorption is not critical factor affecting sorption. Further work might involve conducting a more rigorous organic solvent extraction regime on the alkaline extracts to determine if additional radiolabeled compounds may be released.

The unextractable radioactivity was primarily associated with the humin fraction. Radiolabeled compounds may be physically entrapped within the large molecular sieve of this fraction (Khan, 1991) or bound to its internal and external surfaces by the various bonding mechanisms reviewed previously (Bollag, 1991; Bollag and Loll, 1983; Khan, 1991) (see Section 2.13.3.1). Since the NaOH extraction of the compost was not conducted under nitrogen some oxidative coupling may have occurred during this extraction process. As a result of this oxidative coupling, some of the unextractably bound radioactivity may represent compounds that would have been NaOH extractable but became bound during the extraction process. Because the radiolabeled compounds that are associated with the alkaline soluble and insoluble fractions cannot be extracted with organic solvents, they cannot be identified by routine chemical analysis. The nature of this interaction, therefore, requires further elucidation.

As shown in Figure 13, exhaustive extraction of 30- and 60-day composted diazinon with acetone required seven extractions to reduce radioactivity below to background, whereas, exhaustive extraction of uncomposted diazinon required only four. These results indicate that the radiolabeled compounds were further incorporated into the 30- and 60-day compost organic matrix than the uncomposted. Nonetheless, for uncomposted, 30-day composted and 60-day composted ^{14}C-diazinon sorbent, the majority of the radioactivity was extracted in the first three extractions and the greatest recovery in the first extract. Saint-Fort and Visser (1988) found that adsorption of diazinon in a humic acid fraction of greater than 300 kdaltons was complete within 3 min.

Exhaustive extraction of 30- and 60-day composted diazinon with NaOH required fifteen extractions, whereas, exhaustive extraction of uncomposted diazinon required only five. For all three sorbent groups, the majority of the radioactivity was extracted in the first three extractions, with the largest amount of radioactivity recovery in the first extract. It is likely that the NaOH was, to some extent, degrading the humin to soluble humic and fulvic acids during the long extraction process (Dr. Duane Berry, Personal Communication). The degradation of covalent bonds by NaOH may have released radioactive compounds by two potential processes. As the larger molecular weight humin was degraded by NaOH, radiolabeled compounds may have been released that were either bound to the humin or physically entrapped in the humin. Analogous to these results, is a study that used isothermal High Temperature Distillation to release bound residues of prometryn and degradation products (Khan, 1991 reviews Khan and Hamilton, 1980). In this study, approximately 1% of the bound residues of prometryn and its bound hydroxy and N-dealkylated analogs were released at 150 °C; about half was released at 325 to 350 °C, and the rest was released by 800 °C. In the humic fraction, COOH and OH groups are eliminated between 200 and 400 °C; decomposition of the humic nuclei occurs at 450 to 550°C. This data may be interpreted in two ways. The pesticide residues may have been linked to with phenolic-OH and -COOH groups of the SOM. Alternatively, the thermal decomposition of the organic matter's structure may also result in the release of these bound residues.

4.5.1.3 Chromatography of Acetone Extractable Fraction of Compost

The majority of the acetone extractable radioactivity in the uncomposted ^{14}C-diazinon sorbent was as diazinon 85% (Table 4). Following 30 and 60 days of composting, diazinon in the acetone extractable fraction of compost had degraded almost entirely to IMHP (Table 4). This represents a much more rapid half life than the 30-day half-life reported for soils (Wauchope et al., 1992). The increased degradation rate may have been the result of adsorption catalyzed degradation (Saint-Fort and Visser, 1988); pH catalyzed (Gomma et al., 1969); or microbial degradation (Sethunathan and MacRae; 1969; Bro-Rasmussen et al., 1968). There was also an increase in radioactivity that did not leave the origin during TLC. This may represent radioactive compounds associated with acetone soluble organic matter.

4.5.2 Fate of Composted ^{14}C-diazinon in Amended Soil

It is highly unlikely that this disposal process would include the land application of uncomposted pesticide wastes, and a minimum endpoint would likely be sixty days of composting. The results from soil amended with 30- and 60-day composted ^{14}C-diazinon are, therefore, more critical to the purposes of this experiment than results from soil amended with uncomposted ^{14}C-diazinon diazinon. Before the fate of composted ^{14}C-

diazinon in amended soil can be discussed, however, difficulties with soil amended with uncomposted ^{14}C-diazinon must be addressed. A large amount (88%) of the radioactivity from soil amended with uncomposted ^{14}C-diazinon could not be accounted for in the mass balance of applied radioactivity (Table 5). There are two possibilities that may explain the inability to account for this radioactivity. First radiolabel may have been lost due to volatility. It may be that this diazinon was lost as volatiles when the surfactant was being evaporated from the soil (48 hours at 60 °C). However, it is unlikely that this would account for such a high percentage of the radioactivity because 30- and 60-day composted diazinon sorbents were exposed to similar temperatures (approximately 60 °C) for 30 and 60 days with minimal loss due to volatility (0.02 and 0.03%, respectively; Table 3).

The second possible explanation for the inability to account for a major amount of radioactivity may involve inefficient combustion due to interparticle diffusion into Groseclose soil (clay / mineral fraction). Combustion recoveries for spiked soil samples were very low (56 ± 5%); whereas, recoveries for spiked peat moss samples were much higher (89 ± 10%; see Sections 4.3.4.2.1 and 4.3.4.1.1, respectively). In order to examine the possibility of lowered radioactivity recovery from spiked soils, I spiked a series of soil masses with identical volumes and concentrations of ^{14}C-diazinon standard. Visually the lower soil masses did not provide enough surface area to sorb all of the ^{14}C-tiazinon standard. I observed a linear relationship between soil mass and percent recovery that became asymptotic to approximately 50%; which was when the mass of soil was great enough to sorb all of the standard. Therefore, it appears that the combustion process is less efficient for soils than purely organic matrices.

The acetone extractable fraction in the compost matrix would be expected to be more available for soil sorption during soil amendment than the alkaline extractable and residue combustion fractions of the compost. The percentage of radioactivity associated with the acetone extractable fraction was 72% in the uncomposted ^{14}C-diazinon sorbent; whereas, it represented only 22 and 25% of the radioactivity in 30- and 60-day composted ^{14}C-diazinon sorbents. Therefore, a greater percentage of the radioactivity from the uncomposted ^{14}C-diazinon sorbent would become associated with the Groseclose soil fraction of the amended soil. Since the ratio of Groseclose soil to peat moss in the amended soil was nine to one, this may represent a considerable percentage of soil-sorbed radioactivity. In addition, soil amended with uncomposted ^{14}C-diazinon still contained surfactant and was heated at 60 °C for 48 hours, and then incubated for 2 weeks. Whereas, ^{14}C-diazinon was applied to the combustion standard in acetone less than 24 hours before combustion at room temperature. Greater interparticle diffusion of diazinon in to the Groseclose soil matrix would, therefore, be expected for soil amended with uncomposted ^{14}C-diazinon than for the combustion standard to which it was compared. The incomplete combustion was likely not as significant for soil samples amended with 30- and 60-day composted ^{14}C-diazinon, because the radioactive compounds were more incorporated within the humus, and, therefore, not as available for sorption by the Groseclose soil.

4.5.2.1 CO_2 and Volatiles from Incubation of Earthworms in Amended Soil

The values for $^{14}CO_2$ evolution from this study cannot be compared statistically, because the $^{14}CO_2$ trapping system combined air samples from the replicates in each test group. The greatest production of $^{14}CO_2$, however, appears to have been in the soil amended with uncomposted ^{14}C-diazinon sorbent (1.3%) versus 0.8 and 0.7% in soil amended with 30- and 60-day composted ^{14}C-diazinon (Table 5). The larger percentage of radioactivity released as $^{14}CO_2$ from the soil amended with uncomposted ^{14}C-diazinon, may be because the acetone extractable fraction is more bioavailable for mineralization (70% of the uncomposted ^{14}C-diazinon sorbent was acetone extractable before soil amendment).

In addition, it appears that during the 2-week earthworm incubation, the production rate of $^{14}CO_2$ from soils amended with 60-day composted ^{14}C-diazinon sorbent (0.13%; Table 3) was approximately three times that generated during 60 days of composting (0.7%; Table 5). The higher mineralization rates in soil, as compared to compost, may be the result of increased bioavailability. Alternatively, the composting environment may have degraded diazinon to products that were more susceptible to mineralization and these products were then mineralized during soil incubation. In addition, the presence of earthworms in the amended soil may also have increased mineralization rates. Meharg (1996) found that the presence of earthworms increased the mineralization rates of atrazine in soil. The presence of mucilage secretions and change in soil structure where thought to increase the bioavailability of atrazine. These workers proposed that increased bioavailability may have acted synergistically with increased microbial metabolic activity to increase mineralization rates.

4.5.2.2 Exhaustive Extraction and Combustion of Amended Soil

The changes in radioactivity distribution from the compost to the amended soil can be calculated by subtracting the percentage of total radioactivity present in compost fractions (Table 3) from the percentage of total radioactivity present in amended soil fractions (Table 5). By comparing the 30- and 60-composted ^{14}C-diazinon to their respective amended soils, there appeared to be a shift from the acetone extractable fraction to the alkaline extractable, and unextractable fractions, during the 14-day soil incubation (see Tables 3 and 5). The percentage of radioactivity accounted for by acetone extractable fraction decreased by 18 and 19%, respectively, when comparing the compost to the amended soil. There was a corresponding increase in the alkaline extractable fraction of 4 and 1%, respectively and a corresponding increase of in the unextractable fraction of 19 and 15%, respectively. Such comparisons are difficult for soil amended with uncomposted ^{14}C-diazinon sorbent because of unaccounted-for radioactivity. NaOH extraction of the amended soil was not conducted under nitrogen. Therefore, some oxidative coupling may have occurred during this extraction process. As a result of this oxidative coupling, some of the unextractably bound radioactivity may represent compounds that would have been NaOH extractable but became bound during the extraction process. Based on the results from this laboratory study, it appears the land farming of composted diazinon may result in an overall increase in soil bound residues.

Exhaustive extraction of the each soil type only required three extractions (Figure 20); whereas four extractions were required for uncomposted ^{14}C-diazinon sorbent and seven extractions for 30- and 60-day composted diazinon sorbents. The specific activity of the soil, however, was less than that of the compost. Soils amended with 30- and 60-day composted ^{14}C-diazinon required eight and ten extractions, respectively, and the uncomposted sorbent amended soil requiring only four extractions (Figure 20). For all three soil groups, the majority of the radioactivity was able to be extracted in the first three extractions and the greatest recovery in the first extract. As mentioned in Section 4.5.1.2, it is likely that the NaOH was, to some extent, degrading the humin to soluble humic and fulvic acids during the long extraction process.

4.5.2.3 Chromatography of Acetone Extractable Fraction from Amended Soil

A considerable percentage of the acetone extractable radioactivity from the soil amended with 30- and 60-day composted ^{14}C-diazinon remained at the origin on the HPTLC and TLC plates. These extracts may have contained transformation products that were associated with acetone soluble organic matter or clay. Getzin and Rosefield (1966) described a procedure to free diazinon associated with soluble clay prior to chromatography. They found maximum extractability resulted from extracting with a mixture of water and water miscible organic solvents. Therefore, a solution of 1:1, 0.05 M $CaCl_2$ and acetone, was used. The $CaCl_2$ was used to facilitate the precipitation of colloidal clay during centrifugation following extraction. They then extracted diazinon from the water-soluble hydrolysis products using chloroform. Precipitation of the colloidal clay may have been useful prior to chromatography. The radioactivity, however, may have precipitated with the clay colloids. An alternative explanation is that the radioactivity at the origin is associated with a conjugate or other metabolic products of earthworms. In soil amended with 30- and 60-day composted diazinon, earthworms were incubated for a longer period of time (up to 14 days) and were in better health than those in soil amended with uncomposted diazinon (100% mortality within 2 days). These earthworms may have had more opportunity to metabolically transform or conjugate and excrete the radiolabeled compounds into the soil.

4.5.3 Uptake of Radioactivity by Earthworms

4.5.3.1 Toxicity of Amended Soil to Earthworms

In the radiolabeled study, diazinon represented 85%, 0.9% and 0.6% of the acetone extractable fraction of uncomposted, 30- and 60 day composted sorbents, respectively. Based on relative concentration of diazinon in the acetone extractable fraction, uncomposted diazinon would be expected to much more toxic to *E. foetida* than the 30- and 60-day composted diazinon. However, the uncomposted diazinon was considerably more toxic than the 30- and 60-day composted diazinon (Figure 27). An LC_{50} for uncomposted diazinon was not determined because the only mortality data was obtained following 2 days of exposure when it was 100%. The 30-day composted diazinon appeared to be more toxic than the 60-day composted diazinon. A comparison, however, was difficult because of the low number of replicates and high variability among earthworms exposed to the 30-day composted diazinon. This experiment was not designed as a toxicity test. Because of the significant cost of radioactive material it would be impractical to provide the replications needed for a more comprehensive toxicity test.

4.5.3.2 Total Uptake of Radioactivity by Earthworms

Earthworms exposed to soil amended with uncomposted ^{14}C-diazinon accumulated approximately seven times the amount of radioactivity in 2 days as compared to earthworms exposed to soil amended with 30- and 60-day composted diazinon sorbent for 7 and 14 days. The amount of radioactivity that became unextractably bound within the earthworms increased with increasing composting time. As noted in Section 4.4.2.4, the percentage of total radioactivity associated with the earthworms that was unextractably bound was 8% for soil amended with uncomposted ^{14}C-diazinon, 61% for soil amended with 30-day composted ^{14}C-diazinon, and 79% for soil amended with 60-day composted ^{14}C-diazinon. These results are consistent with those reported by Fuhremann and Lichtenstein (1988) who found that, in earthworms exposed to fresh residues of methyl [^{14}C] parathion, only 18-33% of the residues became bound in the earthworm tissue. However, in earthworms that had accumulated radioactivity from soil-bound residues, 58-66% of these residues became again bound in the earthworm. In this study, earthworms incubated in freshly deposited m-parathion soil had thirteen times more radioactivity than those incubated in bound m-parathion soil.

The radioactivity obtained in sequential gut evacuations was evaluated to determine if a longer time was needed to purge additional intestinal contents. A 24-h gut evacuation appeared to be adequate, because there was little radioactivity in the second or third 24-h gut evacuations. Using this gut evacuation technique, it is not possible to distinguish between radioactivity that passes through the earthworm intestine unabsorbed from radioactivity that was initially absorbed by the intestine and then either was excreted into the hind gut or through nephridopores or passively diffused out of the earthworm's epidermis. Total body burdens of radiolabeled compounds when earthworms were in the soil may, therefore, be underestimated.

4.5.3.3 Chromatography of the Acetone Extractable Fraction from Earthworms

Lord et al. (1980) described the uptake of diazinon by earthworms as being an equilibrium between diazinon in the worm, diazinon in the soil water, and diazinon in the soil organic matter. The distribution of radioactive metabolites in earthworm exposed to soil amended with uncomposted ^{14}C-diazinon sorbent corresponded to the distribution of radioactive metabolites in uncomposted ^{14}C-diazinon sorbents. In uncomposted ^{14}C-diazinon sorbent, diazinon and IMHP accounted for 85 and 20% of the radioactivity, respectively. In earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent, diazinon and IMHP accounted for 82 and 23% of the radioactivity, respectively.

The distribution of ^{14}C-diazinon metabolites in earthworms exposed to soil amended with 30- and 60-day composted diazinon does not appear to reflect the passive diffusion equilibrium described by Lord et al. (1980). It appears that radioactive compounds were in some way becoming incorporated into the acetone soluble and insoluble fraction of the earthworm tissue. This may represent radioactive compounds that have been conjugated by some mechanism (Stenersen, 1984). This conclusion is supported by two results: 1) the amount of radioactivity that became unextractably bound within the earthworms increased with increasing composting time; and 2) most of the radioactivity from acetone extracts of these earthworms of did not migrate from the origin during TLC and, therefore, were likely associated with larger molecular weight acetone soluble compounds. These earthworms were healthier than those exposed to soil amended with uncomposted ^{14}C-diazinon sorbent and, therefore, may have been more able to conjugate the xenobiotics. Alternatively, radioactivity may have been converted into a form that was more readily assimilated into earthworm tissue, during the composting process.

Earthworms exposed to soil amended with 30- and 60-day composted ^{14}C-diazinon contained very little diazinon. None of the earthworm groups presented in Table 7 contained greater than 4 % diazinon in the acetone extractable fraction of their tissue. In addition, these earthworms contained approximately seven times less radioactivity than earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent (Table 5). This experiment, therefore, demonstrates that the composting of ^{14}C-diazinon and subsequent amendment to soil greatly reduces the hazard that diazinon waste presents to a representative beneficial soil invertebrate (*Eisenia foetida*).

5. Conclusions

The purpose of this study was to evaluate the hazard of land applying composted diazinon waste. Earthworms (*Eisenia foetida*) were used as a model beneficial soil macroinvertebrate in this hazard evaluation. The ecotoxicity of composted diazinon waste to *E. foetida* was evaluated in the field experiment (Chapter 3). The fate of composted diazinon in amended soil and uptake by earthworms exposed to this soil was evaluated in the radiolabeled laboratory experiment (Chapter 4). Although these two experiments were designed to answer different questions, comparisons between the experiments are useful in evaluating the relevance of the radiolabeled laboratory experiment to field conditions.

5.1 Comparisons Between Field and Lab Conditions

Because of the cost and hazards associated with using radioisotopes in this study, compromises were made when modeling field studies in the laboratory. The differences between laboratory and field conditions may, in part, explain differences in results between these two studies. In the field experiment, diazinon was sorbed under batch mixing and flow-through column conditions. The emulsion was stripped from the formulation by increasing the pH. Whereas, in the laboratory experiment, diazinon was added as an emulsion directly to the peat moss / $Ca(OH)_2$ mixture. In the field study, the higher solution to sorbent ratio and longer sorption times may have been more ideal for the sorption of diazinon. Saint-Fort and Visser (1988), however, found that adsorption of diazinon in a humic acid fraction of greater than 300 kdaltons was complete within 3 min. Assuming that humic acids in this study had similar characteristics to Saint-Fort and Visser (1988), the difference in sorption times between field and laboratory studies may not have had an effect of diazinon sorption and desorption. The high pH solution in field conditions may have been more effective at disrupting the emulsion and also increased the sorption of diazinon to humus. If diazinon remained as an emulsion under laboratory conditions, it may have been more bioavailable to earthworms.

In the field experiment, the sorbent dried for 38 to 80 days before composting. During this time, the concentration of extractable diazinon decreased by approximately 97%. Abiotic hydrolysis was likely the most significant degradation pathway (Getzin, 1968; 1968; Getzin and Rosefield, 1968; Lichtenstein, 1966; Rosefield, 1966; Sethunathan and MacRae, 1969; Suett, 1981). In the laboratory experiment, uncomposted sorbents were frozen immediately following peat moss contamination. After the sorbents were incorporated into the soil, the surfactants were evaporated from the soil for 48 h at 60 °C. Based on the diazinon concentration in the compost, the initial diazinon concentrations in amended soils from the field study were 5.8, 0.8, and 0.5 μg/g, for soil amended with uncomposted, 30-day composted and 60-day composted diazinon sorbent, respectively. The same values from the laboratory study were 740, 2.7, and 0.6 μg/g , respectively. After the two week earthworm-incubation period in the laboratory study, the resulting values were 18.4, 4.8, and 3.0 μg/g, respectively. The large decrease in diazinon recovery, in the soil amended with uncomposted ^{14}C-diazinon sorbent, may be from interparticle diffusion into the Groseclose soil. The concentrations of extractable diazinon in the thirty- and sixty-day composted diazinon sorbents, however, are comparable between the field and laboratory studies.

In the field experiment, the compost reached a maximum temperature of 58 °C and elevated temperatures were sustained for approximately fifteen days. After the second series of amendments to the field composting bins, the temperatures did not increase greatly above ambient temperatures. The second series of amendments did not include grass clipping or peanut hulls; this combined with a general lowering of the nutrient concentrations may explain the lower generation of metabolic heat. A second explanation is that the second series of amendments were added in the first week of December. At this time, ambient temperatures were quite cold. Microbial populations may have had difficulty becoming established at these low temperatures. Under laboratory conditions the maximum temperature was 63 °C and elevated temperatures were maintained for 45 days. The concentration of diazinon in the 60-day composted diazinon sorbent was similar between the laboratory and field studies. However, the concentration of diazinon in the uncomposted diazinon sorbent just prior to composting was much higher in the laboratory study than in the field study. Therefore, diazinon degradation occurred more rapidly in the laboratory study than in the field study. This may in part be explaned by the fact that elevated temperatures were maintained for a longer period of time in the laboratory study than in the field study. Alternatively degradation rates may be rapid for higher concentrations of diazinon and may slow as the concentration decreases. Incubation temperatures for earthworms in the laboratory study were approximately 5 °C cooler than in the field study.

5.2. Comparison of Results Between Field and Laboratory Studies

The field study and the laboratory study were conducted to answer two different questions. The objective of the field study was to evaluate the toxicity of composted diazinon to earthworms, in amended soil. The objective of the laboratory study was to evaluate the fate of composted diazinon in amended soil and earthworms. However, for both of these studies, the most important results were from soil amended with 60-day composted diazinon sorbent. Treated pesticides would not be land applied until they had undergone extensive degradation in the composting environment. In the case of diazinon, 60 days of composting would likely represent a minimum end point.

In the field study, the toxicity of soil amended with uncomposted diazinon sorbent was correlated with the toxicity of soil amended with 30-day composted diazinon sorbent. Both, mortality (LT_{50}) and the sublethal effect of curling (RT_{50}) were significantly correlated ($\alpha = 0.05$). However, toxicity was not detected in earthworms exposed to soil amended with 60-day composted diazinon sorbent. In the laboratory study, soil amended with uncomposted diazinon sorbent was highly toxic to *E. foetida*; much more toxic than any of the other sorbent materials in either the field or laboratory studies. The toxicity of the soil amended with 30-day composted diazinon sorbent from the radiolabeled study was intermediate between the toxicity of soil amended with uncomposted and 60-day composted sorbent. No toxicity was found to be associated with soil amended with 60-day composted sorbent from the radiolabeled study. Sublethal effects, however, were not measured in the laboratory radiolabeled study. The disparity in results for soils amended with uncomposted and 30-day composted diazinon sorbents, between field and laboratory studies, may have been due to the extent of diazinon incorporation or degradation. Greater degradation of diazinon occurred prior to composting in the field study because of the long residence time before composting . In any case, the most important toxicity information was that soil amended with 60-day composted diazinon was not toxic to *E. foetida*; this result was consistent between studies.

The sublethal effects of coiling and rigidity, observed in the field study, suggest involvement of the central nervous system (Edwards and Bohlen, 1992). Similar sublethal effects have been observed in *Pheretima posthuma* exposed to aldrin (Hans et al., 1990), *E. andrei* and *E. fetida* exposed carbaryl (Stenersen et al., 1992), *L. terrestris* exposed to carbaryl (Fisher, 1984), *E. foetida*, *A. chlorotica*, *L. rubellus* and *A. caliginosa* exposed to various cholinesterase-inhibiting insecticides (Stenersen, 1989a), *E. foetida* exposed to *N*-methyl carbamates (Roberts, 1984), and was the most common reaction of *L. terrestris* exposed to 24 different pesticides (Haque and Ebing, 1983). Curling effect could, therefore, be a very useful bioassay metric, particularly when assaying for the presence of cholinesterase inhibiting insecticides.

Earthworm tissues from the field toxicity study were not extracted for diazinon. However, approximately 82% of the acetone extractable radioactivity was diazinon from earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent for 2 days; whereas, less than 4% of the acetone extractable radioactivity was diazinon from earthworms exposed to soil amended with 30- and 60-day composted ^{14}C-diazinon sorbent for up to 14 days. As discussed previously, approximately 97% of diazinon degraded before samples were taken for uncomposted diazinon sorbent in the field study. Therefore, it is unlikely that earthworms in the field study accumulated as high a concentration of diazinon as earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent for 2 days. This explains the higher mortality of earthworms exposed to soil amended with uncomposted ^{14}C-diazinon sorbent for 2 days relative to corresponding earthworms in the field study. The concentrations of diazinon in soil amended with 30- and 60-day composted ^{14}C-diazinon, however, were similar between the field and laboratory studies. In addition, mortality data was similar between the field and laboratory studies. Earthworms exposed to soil amended with 30-day composted ^{14}C-diazinon sorbent experienced approximately 50% mortality after 14 days of exposure in both the field and laboratory radiolabeled experiments (Figures 8 and 27, respectively); whereas, earthworms exposed to soil amended with 60-day composted ^{14}C-diazinon sorbent did not experience any mortality in either experiment after 14 days of exposure (Figures 8 and 27, respectively). The difference in mortality between these to test groups (30- and 60- day composted) is not as clearly explained by the diazinon concentrations in their tissues. First, comparisons between the low percentages of diazinon present in earthworm tissues are difficult to interpret because they are at the threshold of detectability. Small differences in percentage of spotted radioactivity for these earthworm extracts may be artifacts of accounting for low radioactivity. Nonetheless, diazinon accounted for less than 4% of the acetone extractable radioactivity in all of the earthworm groups that were exposed to soil amended with uncomposted ^{14}C-diazinon sorbent. The low toxicity and low diazinon concentrations in the tissues of earthworms exposed to 60-day composted ^{14}C-diazinon sorbent provides additional evidence that composted diazinon waste presents little hazard to *E. foetida*.

On the basis of field toxicity data and laboratory fate data, 60 days of composting may be considered a minimal end point to bioremediation of sorbed diazinon waste. This, of course does not preclude chemical testing of the composted material before land application, particularly in cases where higher initial diazinon concentrations are present on the sorbent matrix. In addition, this information was generated for diazinon. Additional testing should be conducted for other pesticides that are to be treated by this disposal system. These experiments have established methods for such additional testing in addition to providing information specific to diazinon.

5.3. Errors and Lessons for Future Research

Local agricultural soil was used for both the field toxicity tests and the laboratory bioavailability tests in this study. When these tests were conducted we were aware of the artificial soil used by the European Economic Community (EEC), the Organization for Economic Co-operation and Development (OECD), the U.S. EPA, and the American Society for Testing and Materials (ASTM) (E.E..C., 1985; O. E. C. D., 1984, Greene et al., 1989; A.S.T.M, 1995). We decided, however, that it would better represent field conditions to use a local agriculture soil. Our logic was similar to that of Haque and Ebing (1983) where we wanted to use a more realistic soil media found in agriculture. However, unlike Haque and Ebing (1983) the addition of organic matter was as compost amendments. After further evaluating the literature, I believe that it is more important to use a universal-standardized soil than to represent local soil conditions. More recent studies on the toxicity of chlorpyrifos and trichloropyrimidine have been conducted using the standard artificial soil. In addition the toxicity of these two compounds has been evaluated using the standard contact filter paper test. Future studies should also include the use of chloroacetimide or benomyl as a positive control. Chloroacetimide would be a more ideal positive control because the toxicity of benomyl is derived from a metabolite (Haque and Ebing, 1983).

The activity that was not accounted for in soil amended with uncomposted ^{14}C-diazinon sorbent is not necessarily an error in the experiment. This may be a limitation of the combustion techniques used and future exhaustive extractions may need to include supercritical fluid extraction as a more affective extraction technique. It would have been appropriate, however, to scrub the air leaving soil containers for volatiles while the surfactants were being evaporated. This would clarify the fate of the unaccounted for radioactivity. It was assumed that a large percentage of radioactivity would not have been lost as volatiles, because so little was lost as volatiles during the composting period. The most important information, however, relates to the 60 day composted diazinon because this would be a minimum composting time recommended before land application.

Alkaline extractions during the radiolabel study should have been conducted under nitrogen to reduce the possibility of oxidative coupling. Because this was not done some of the radioactivity associated with the combustion fraction may have become oxidatively coupled during the alkaline extraction process. However, this error should not effect the concentration of radioactive metabolites associated with the acetone extractable fractions. The amount of radioactivity associated with the combustion fractions may, however, be overestimated and the radioactivity associated with the alkaline extractable fractions underestimated.

5.4. Challenges Regarding Future Use of Biologically Based Sorption and Degradation to Dispose of Pesticides.

In some cases, the incorporation of pesticide residues into organic matter may protect the pesticide residue from biological and chemical degradation. Depending on the degradation pathway, association with organic matter may enhance or inhibit degradation. Soil organic matter is typically negatively charged. Reactions that require cationic species may be accelerated; whereas, those that require anionic species (e.g. base catalyzed hydrolysis of chlorpyrifos, the octyl ester of 2,4-D and other organic solutes) may be inhibited by humic substances (Barbash and Resek, 1996). Microorganisms are generally excluded from soil micropores and sorbed contaminants are generally not bioavailable to microorganisms (Barbash and Resek, 1996 review Ogram et al., 1985; Loch, 1991; Miller and Alexander, 1991; Alvarez-Cohen et al., 1993). Studies designed to establish models for the biodegradation of compounds sorbed to particle surfaces or sorbed within micropores have demonstrated that biodegradation is dependent on desorption rates (Miller and Pedit, 1992; Miller and Alexander, 1991; Scow and Hutson, 1992; Scow and Alexander, 1992; and Harms and Zehnder 1995) These factors that limit the bioavailability of sorbed xenobiotics may also be advantageous. High concentrations of many pesticides are toxic to microorganisms, and therefore may inhibit degradation rates. Examples of this include methyl parathion (Barbash and Resek, 1996 review Butler et al., 1981), chlorpyrifos (Barbash and Resek, 1996 review Cink and Coats, 1993; Racke et al., 1993 and Racke et al., 1988), and metolachlor (Barbash and Resek, 1996 review Zheng et al., 1993). If, however, the concentration in soil water is decreased due to sorption effects, microorganisms may remain active in soils containing relatively high pesticide concentrations. In studies conducted by the Pesticide Waste Disposal Group, pesticide concentrations on uncomposted sorbents are typically in the 10,000 μg/g range. Microbial toxicity, therefore, becomes a serious consideration. Many pesticides may be initially degraded by abiotic processes, but further degradation of the transformation products requires biotic factors. The degradation of transformation products during the composting process should be more closely scrutinized by this research team. These highly concentrated pesticides and their transformation products may be diluted by incorporating the sorbents into soil. If sorbents were incorporated into soils at label rates, based on the initial pesticide concentration, degradation rates of transformation products may be similar to "normal" field application conditions. Alternatively, because the pesticide distribution in the soil may be more heterogeneous than under "normal" field application conditions, microbial degradation in these high concentration areas may still be inhibited. Microbial degradation would likely be limited to the borders of the high concentration areas, where the pesticide was in lower concentration (Alexander, 1991).   Transport of the pesticide away from the highly concentrated microsites would serve to decrease the pesticide concentration to levels that may not be toxic to microorganism. Studies that compare pesticide degradation in a highly contaminated soil or organic matter after land farming to degradation under "normal" field application conditions should be conducted. Information from such studies would be useful in evaluating disposal options for contaminated soils resulting from spills, improper disposal, or from evaporation degradation tanks, as well as our rinsate treatment technology. Studies, similar to that of Felsot et al. (1992) should be conducted by our group to compare the degradation and transport of sorbed pesticides or transformation products versus pesticides applied under label-approved conditions.

It has been found in previous radiolabel studies (Petruska et al., 1985; Judge, 1996; Xiao, 1998), as well as in this study, that pesticides or their transformation products become associated with humic and fulvic acids during the composting process. Pesticides associated with water soluble organic matter (WSOM) may have increased soil mobility due to facilitated transport (Barbash and Resek, 1996). In addition the sorption of pesticides to the organic matter carrier may protect the pesticide from degradation (Barbash and Resek, 1996). It would, therefore, be prudent to further investigate the facilitated transport of pesticides associated with water soluble organic matter.

Bioassays should continue to be used in evaluating the hazard of composted pesticides for two main reasons. Routine chemical analysis only provides an estimate of the extractable residue present in the composting environment. Depending on several factors, such as the nature of incorporation (physical binding, chemical binding, micropore entrapment, desorption kinetics) and ability for soil microorganisms to dislodge incorporated residues, routine chemical analysis may under or overestimate the bioavailability residues. Bioassays are also important because the toxicity of pesticide degradation or synergistic effects may not be available in the literature. Although the transformation of parent compound generally results in less toxic products, in some cases, transformation may result in more toxic products (Alexander, 1981). Bioassays should, therefore, be conducted with appropriate representative species. The earthworm, *E. foetida*, may be used for animal bioassays, but other assays should be considered as well. Examples of other bioassays used to evaluate waste from pesticide bioremediation technologies include; oat (*Avina sativa* L.) and corn root borer larvae (*Diabrotica virgifera vergifer* Laconte) (Berry et al., 1993), tomatoes (Bugbee and Sareceno, 1994), microtox system (Seiber, 1992 reviews Bulick et al.), the Ames Assay (Seiber, 1992 reviews Somich et al., 1990; DeMarini et al., 1991; Wong et al., 1991), fish (Seiber, 1992) and crops and weeds under field conditions (Felsot et al., 1992), In addition, the mobility and toxicity of the parent compound and degradation products of concern would be evaluated in the field using soil core profiles and biomonitoring. Considering the long list of pesticides that need to be evaluated by this process such an evaluation may only be possible by making some assumptions. There is already a wealth of information gathered during the labeling process regarding the mobility and toxicity of pesticides applied at label rates. A few directed studies, comparing the mobility and degradation pesticides that are sorbed and soil incorporated versus “normally” applied, would help to determine if the behavior of sorbed-soil incorporated xenobiotics may be predicted by information obtained during the labeling process. Pesticides would be selected that represent various classes, degradation pathways and K_{om} values. Although this would not be as ideal as studying the environmental fate of every pesticide treated by this system, it may be the only practical option.

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Vita

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