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Microbial Transformation of DDT in Pine River Sediments

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Major professor

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MICROBIAL TRANSFORMATION OF DDT IN PINE RIVER SEDIMENTS

By

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ABSTRACT

MICROBIAL TRANSFORMATION OF DDT IN PINE RIVER SEDIMENTS

By

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This study examined the fate of DDT in sediments at five sampling locations near the Pine River Superfund site. River sediments were analyzed for DDT and its dechlorination products, DDD, DDE, and DDMU (collectively referred to as DDX). Both *p, p'*- and *o, p'*-isomers for each compound were detected at all sites at concentrations ranging from 0.05 ppm to 20,000 ppm. Compositional analysis revealed an increase in *p, p'*- vs *o, p'*- isomers and higher proportions of DDD relative to technical grade DDT at 3 of the sites. In addition to analyzing for DDX compounds, we conducted microcosms studies to determine the abilities of the sediments to support anaerobic dechlorination and to clarify specific degradation pathways. We compared the extent of anaerobic transformations of ^{14}C- radio-labeled DDT, DDD, and DDE amended to live and autoclaved anaerobic sediments slurries. Although DDT was converted primarily to DDD in both live and autoclaved treatments, no transformation of DDD or DDE occurred. Our results indicate that the majority of DDD formation in the Pine River sediment microcosms was due to abiotic processes, but there was also a substantial biotic contribution (19 % to 41 %) during the 40 week incubations.

This work is dedicated to my mother, Annie Mae Roberts, who always sacrificed for my education, and to my wife, Jennifer, whose support and patience has made this goal achievable.

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CHAPTER 1

INTRODUCTION

Historical Information

In 1874, Othmar Zeidler, a young German chemist at the University of Strassburg, was the first to synthesize 1,1,1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane, commonly known as DDT, and examine its chemical and physical properties. While working on the synthesis of aromatic compounds through dehydration, he published a report on a series of compounds resulting from the condensation of chloral with mono-chlorobenzene (Zimmerman et al., 1946). Unknown to Zeidler, DDT would later save millions of lives by killing disease carrying insects (Zimmerman et al., 1946); unfortunately, due to its persistence and toxicity, it would also become a global environmental problem.

In 1936, a Swiss chemist, Paul Müller, discovered the insecticidal properties of DDT (Cruz, 1946). DDT was patented as an insecticide by the Geigy Company, Inc. for commercial production in 1943 in Switzerland, and later by Du Pont, Merck, and Hercules Powder Co. in the United States (Zimmerman et al., 1946). By the early months of 1944, the United States Army was using DDT to protect troops against pests. Specifically, DDT was first used to stop the epidemic of typhus fever in Naples, Italy. DDT also proved effective for curbing vectors of other diseases such as malaria, yellow fever and bubonic plague.

Shortly after DDT was used by the military in 1944, the general public began using it extensively to control many pests. Aside from transmitting diseases, insects destroy property and cause discomfort. To reduce diseases and to maintain relatively pest-free homes, DDT was sprayed directly onto beds, walls, and floors, and dusted onto the body and hairs of humans, livestock and pets.

Between 1946, when the toxicity of DDT to humans was first reported (Zimmerman et al., 1946), and the 1960s, evidence started surfacing that DDT and its metabolites were persistent in the environment and bioaccumulate in higher animals. During this period it began to be realized that exposure to DDT threatened the survival of many species (Heberer and Dunnbier, 1999). Some studies (Stine, et al., 1996) pointed out that fish and birds of prey suffered most from effects of DDT. DDT and its metabolites are responsible for causing a decrease in the eggshell thickness of birds (Korte and Stuttgart, 1992). A direct negative correlation has been found between 2,2-bis (*p*-chlorophenyl) 1,1-dichloroethylene (DDE) levels in Alaskan hawks and eggshell thickness (Cade et al., 1971; Peakall, 1974).

It has been estimated that 1.2 billion pounds of DDT were used in the United States between 1945 and 1972 (Chem. Eng. News, 1988a). The U.S. Environmental Protection Agency (EPA) (EPA, 1988; Stine, et al., 1996) banned DDT in U.S for agricultural purposes in 1972 and for all other purposes in 1988. Today, in the United States, DDT is no longer produced; however, Mexico, China, Russia, and India, the last known producers of DDT, continue to manufacture it for use against mosquitoes (C&EN, 1998). The World Health Organization (WHO) reports that roughly two million people

die annually from malaria, and therefore, DDT remains in use for controlling mosquito-borne malaria in many nations in Africa, Asia and Latin America.

DDT is inexpensive to produce and has been accepted as among the best man-made compounds for controlling potential outbreak of insect-borne diseases. However, because of its toxicity and environmental effects, many environmental groups have advocated that DDT be phased out of use worldwide by 2007.

Environmental Fate

Pathways for the degradation of DDT suggest sequential dechlorination of the three aliphatic chlorine atoms through a combination of two types of dechlorination reactions: reductive dechlorination and dehydrochlorination. Additionally, the carbon skeleton of DDT can be degraded through oxidative reactions, presumable during any stage of dechlorination. The following sections will discuss these general reactions.

Dechlorination Reactions

Reductive dechlorination (or reductive dehalogenation) proceeds by replacing a chlorine (or halogen) atom with a hydrogen atom (Hill and McCarty, 1967; Morrill et al., 1982). For DDT and analogues this occurs on the non-aromatic chlorine bearing carbon. Reductive dechlorination is a reduction reaction as the two-electron transfer reduces the oxidation-state of the carbon atom by 2 and also adds H to the molecule. This is a thermodynamically favorable reaction that has been shown to support the growth of a variety of anaerobic microorganisms through the process of halorespiration (Mohn and Tiedje, 1992; Wohlfarth and Diekert, 1997; Janssen, Oppentocht and Peolarends, 2001).

Reductive dechlorination typically occurs under anaerobic conditions and is commonly observed in the degradation pathway of DDT.

Dehydrochlorination involves the simultaneous removal of hydrogen and chlorine producing a double bond. This reaction typically takes place between a saturated chlorinated carbon and the adjacent hydrogen on the neighboring carbon (Lal and Saxena 1982). Overall, this is a redox-neutral reaction as the carbon losing the hydrogen atom is oxidized and the carbon atom losing the chlorine is reduced. Dehydrochlorination is a common abiotic transformation and also occurs frequently as an aerobic microbial reaction. The details of this process for abiotic dehydrochlorination of chlorohydrocarbons are found in Lal and Saxena (1982).

DDT and its partial transformation products are persistent and toxic compounds that threaten ecosystems and humans. Many studies have examined the fate of DDT in the environment (Morrill et al., 1982; Gibson, 1984; Bitton and Gerba, 1984; Ward et al., 1985). DDT can be transformed in soils and sediments by reductive dechlorination, dehydrochlorination, and oxidation through both abiotic and biotic processes. The relative contribution from the different reactions varies among soils and microorganisms. The types of transformations of DDT in aerobic and anaerobic environments are summarized in Figure 1.1.

Microorganisms play a major role in the breakdown of a wide array of pesticides, including DDT, in environments such as soils, river and lake sediments, and in sewage sludges. Studies with a variety of pure cultures have shown the microbially mediated transformation of DDT into several different products (Lal and Saxena 1982). In a laboratory experiment with sewage sludge, evidence of dechlorination of DDT was also

obtained (Marei et al., 1978 and Ware et al., 1980). Miskus et al. (1965), and Essac and Matsumura (1980) also have established that degradation of DDT can be mediated by iron porphyrins under anaerobic conditions. Despite the potential of microorganism to transform DDT, it is evident from the literature that DDT often persists in soil and water for long periods of time. Thus, studies on transformation of DDT in specific well defined environments remain important in order to understand why DDT persists.

Laboratory studies have shown that anaerobic reductive dechlorination by microorganism is an important step in DDT degradation. Dechlorination of DDT may result in products with different biological activities than the parent compound. For example, the *p, p'*-isomer of DDT was found to be weakly anti-androgenic while its major metabolite *p, p'*-DDE was a strong anti- androgen (Kelce et al., 1995). In the case of other persistent chlorinated aromatic compounds, anaerobic dechlorination may reduce toxicity. For example, microbially dechlorinated polychlorinated biphenyls (PCB) mixtures (Aroclors) showed substantially lower "dioxin-like" activity as compared to unaltered Aroclors (Quensen et al., 1998). Generally, dechlorination makes the resultant compounds more easily degradable in aerobic environments. Recently, considerable interest has developed in utilization of anaerobic reductive dechlorination for environmental remediation because it has the potential to reduce toxicity and increase the likelihood of further microbial degradation. The use of sequential anaerobic/ aerobic microbial processes to degrade PCBs, DDT and their metabolites has often been considered for contaminated sites.

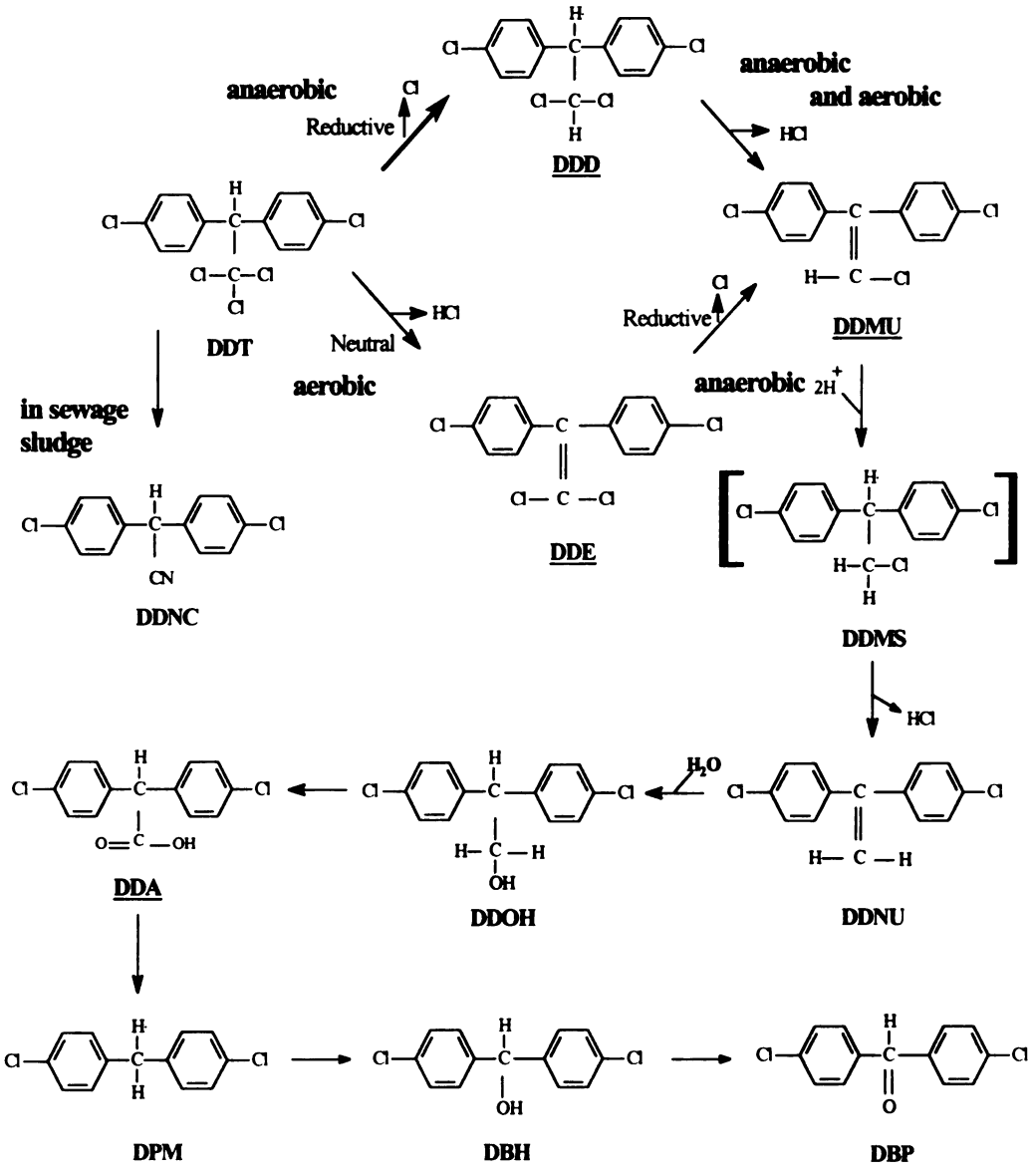


Figure 1.1. Metabolic pathway of DDT biotransformation (Adapted from Rochkind and Blackburn, 1986) with additions of (Jensen et al.1971; Marei et al., 1978; Quensen et al. 1998). The steps observed most common in DDT the degradation pathway are indicated by underlined letters, and the step most commonly found in Pine River sediment microcosms are indicated by a thick arrow. In addition to DDD, DDE and DDMU, there are several other degradation products that are less commonly observed.

Table 1.1. Abbreviations, chemical formulae and names of DDT and its degradation products given in the text.

DDT= 1,1,1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane;

DDD= 1,1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane

DDMU= 1-chloro-2, 2-bis (*p*-chlorophenyl) ethylene;

DDMS= 1-chloro-2, 2-bis (*p*-chlorophenyl) ethane;

DDNU= 1,1-bis (*p*-chlorophenyl) ethylene;

DDOH= 2,2-bis (*p*-chlorophenyl) ethanol;

DDA= dichlorodiphenylacetate, or bis (*p*-chlorophenyl) acetic acid;

DBH= dichlorobenzhydrol;

DBP= dichlorobenzophenone;

DDE= 2,2-bis (*p*-chlorophenyl) 1,1-dichloroethylene

DDNC= bis (*p*-chlorophenyl)-acetonitrile

DPM= *p*, *p*'-dichlordiphenylmethane

Oxidative Reactions

In addition to dechlorination reactions, the carbon structure of DDT and its metabolites can be degraded through oxidative reactions. DDT is degraded by a variety of soil microorganisms in aerobic environments. Focht and Alexander (1970) reported that certain microorganisms have the ability to cometabolize DDT and its metabolites. The term "cometabolism" refers to the ability of a microorganism to metabolize a substrate that it cannot use as a source of energy (Alexander et al., 1967). Currently, there is no evidence that DDT can be used as a carbon source for growth by microbes in the degradation of DDT.

In pure culture studies, Hay and Focht (1998) have proposed that *Pseudomonas acidovorans* M3GY cometabolically transformed DDE and its unchlorinated analog, 1,1-diphenylethylene (DPE) (Figure 1.2). DDE was cometabolized by a dioxygenase at the *ortho* and *meta* positions. Megharaj et al. (1997) reported transformation of DDT by pure cultures of 4,4'-dichlorobiphenyl and DPE-degrading bacteria through oxidative cometabolism. DDE is resistant to reductive cometabolism by pure obligately anaerobic microorganism (Strompl and Thiele, 1997; Megharaj et al., 1997). In general, cometabolic processes are slow, and the transformations of DDT are controlled by environmental conditions (Alexander et al., 1967).

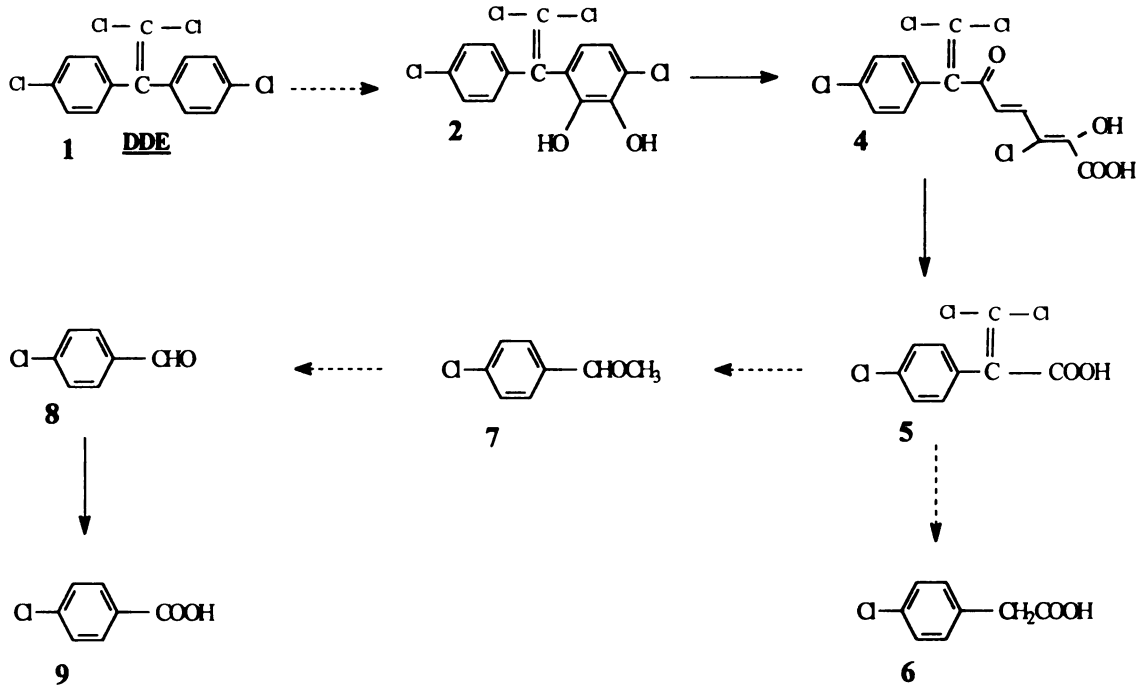


Figure 1.2. Cometabolic, meta-fission products of DDE as proposed for biphenyl-grown *Pseudomonas acidovorans* M3GY (Adapted from Hay and Focht, 1998).
(1) DDE; (2) 1,1-dichloro-2-(dihydroxy-4- chlorophenyl)-2-(4-chlorophenyl)ethylene; (4) 4, 6-oxo-2-hydrooxy-7-(4-chlorophenyl)-4,8,8-trichloroocta-2,4-dienoic acid; (5) 5, 2-(4- chlorophenyl)-3,3-dichloropropenoic acid; (6) 6, 4-chlorophenylacetic acid; (7) 7, 4-chloroacetophenone; (8) 8, 4-chlorobenzaldehyde; (9) 9, 4-chlorobenzoic acid.

Compounds detected at DDT contaminated sites

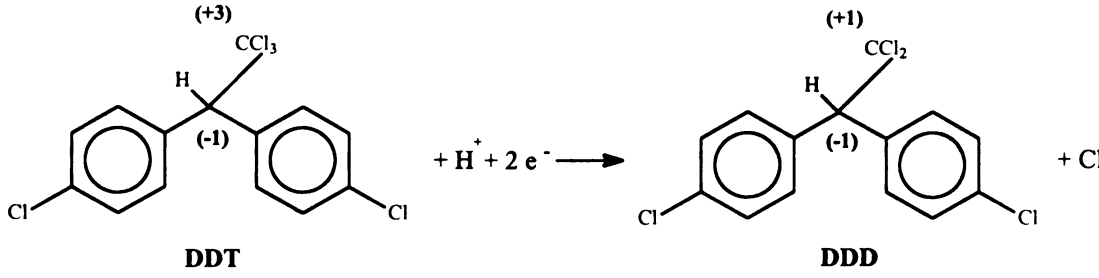
Several studies have proposed pathways for the degradation of DDT, and Figure 1.1 represents a generalized scheme (Rochkind and Black 1986). The degradation reactions are reductive dechlorination, dehydrochlorination and oxidation. DDT can be degraded under both aerobic and anaerobic conditions and it degradation can be catalyzed by either microbial or abiotic reactions. The sequential order of these reactions determines the degradation pathway.

An initial reductive dechlorination of DDT produces 1,1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane (DDD). This is a commonly observed reaction by both abiotic and anaerobic microbial systems. An initial dehydrochlorination of DDT produces DDE. This is most commonly observed as an aerobic microbially mediated process. Traditionally DDE has been considered a dead-end product. Recently, however, Quensen et al. (1998) showed that DDE is further converted to 1-chloro-2, 2-bis (*p*-chlorophenyl) ethylene (DDMU) by a reductive dechlorination reaction. DDD has been shown to also be converted to DDMU by a dehydrochlorination reaction (Wedemeyer et al., 1967). Further reduction of DDMU has not been studied in as much detail, but is believed to form 1,1-bis (*p*-chlorophenyl) ethylene (DDNU) either directly from a reductive dechlorination reaction, or by a dehydrochlorination reaction following a hydrogenation reaction. DDNU has a completely dechlorinated aliphatic carbon and further degradation proceeds through sequential oxidation of this carbon to form 2,2-bis (*p*-chlorophenyl) ethanol (DDOH), then bis (*p*-chlorophenyl) acetic acid (DDA), before decarboxylation to form *p*, *p*'-dichlordiphenylmethane (DPM). There is evidence that DPM may also be oxidized to dichlorobenzhydrol (DBH) and then dichlorobenzophenone (DBP) (Perry et

al., 1963 and Pinto et al., 1965). The complete mineralization of DDT beyond this point has also been suggested (Hay and Focht, 1998).

Specific Transformations

DDT to DDD There have been two central studies describing research on DDT transformations in sediments (Matsumura et al., 1971; Jensen et al., 1972). Matsumura et al. (1971) examined the fate of DDT in Lake Michigan sediments, and observed that DDT was reductively dechlorinated to DDD (Figure 1.3). This is consistent with other studies of isolated bacteria (and other microorganisms), as well as in anaerobic soil conditions, where the major pathway of DDT involves reductive dechlorination (Guenzi, and Beard 1967; Johnsen, 1976; Essac and Matsummura, 1980; Lal and Sexena, 1982; Rochkind and Blackburn, 1986). Jensen et al. (1972) showed that DDT was rapidly transformed to DDD within several hours of incubation in anaerobic sewage sludge and lake sediments. Often DDD is the first and sole transformation product of DDT under anaerobic conditions (Figure 1.3). The extent of this transformation can be significant; Wedemeyer (1967) and Guenzi et al. (1967) showed that less than 1% of original DDT remained in the soil after 12 weeks of incubation. In the reductive dechlorination of DDT to DDD, DDT accepts 2 electrons and the oxidation state of the aliphatic carbon atom changes from +3 to +1, while the oxidation state of all other atoms remain the same (Guenzi and Beard, 1968; Parr et al., 1970; Parr and Smith, 1974).

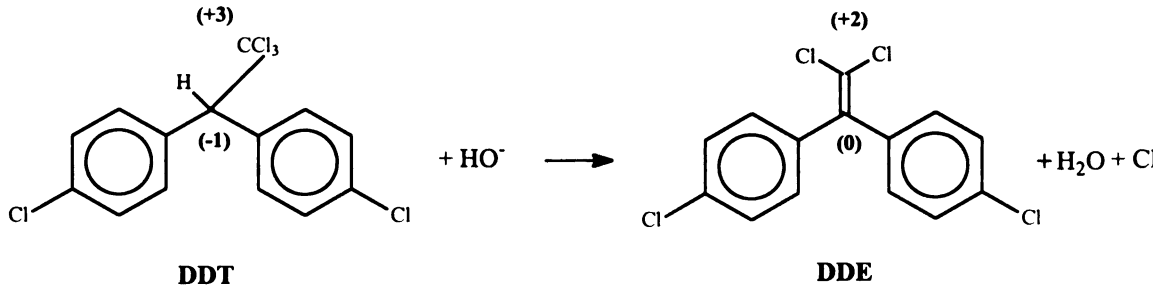


DDT **DDD**

Figure 1.3 Proposed pathway for reductive dechlorination reaction of DDT (Schwarzenbach et al., 1993) in anaerobic systems. The oxidation state for aliphatic carbon atom for each compound is shown in parenthesis.

In addition to microbially mediated reactions, the addition of some chemical agents (e.g., Fe (II) porphyrin, heamatin, detergent) in anaerobic systems may accelerate the transformation of DDT to DDD (Quirke et al., 1979). It has been shown that the extent of DDT degradation to DDD is negatively correlated with redox potential (Burge, 1971; Guenzi et al., 1971; Parr and Smith, 1974). Furthermore, Glass (1972) showed that DDD formation is related to the levels of ferrous (Fe^{2+}) iron present in the anaerobic environment.

DDT to DDE DDT can be transformed to DDE by microorganisms through dehydrochlorination (Figure 1.4). Dehydrochlorination is the dominant pathway by which microorganisms degrade DDT in aerobic environments. Aerobic dehydrochlorination of DDT is a process whereby hydrogen and chlorine are removed simultaneously from the aliphatic side-chain portion of the molecule, reducing the number of chlorines and generating a carbon-carbon double bond.



DDT **DDE**

Figure 1.4. Proposed pathway for dehydrochlorination of DDT in aerobic environments (Schwarzenbach et al., 1993).

A number of studies have shown that microorganisms can dehydrochlorinate DDT to DDE (Ott and Gunther, 1965; O'Brien, 1967; Menzie, 1969; and Metcalf, 1971). This is consistent with other studies in aerobic soil conditions, where DDE is the primary metabolite of DDT transformations (Wedemeyer,1967), but other compounds such as dichlorobenzophenone (DBP) and DDMU have been reported (Marei et al.,1978). Quirke et al., (1979) observed that the transformation of DDT to DDE involves a reduction and subsequently oxidative step via a free radical mechanism. As discussed below, DDT can be transformed to DDE, and subsequently to DDMU (Quensen et al., 1998).

DDD to DDMU Several studies examined the fate of DDD and proposed a pathway for degradation of DDD (Quirke et al., 1979; Quensen et al., 1998). DDD can be transformed to DDMU by microbes in aerobic and anaerobic environments. Aerobic dehydrochlorination of aliphatic side carbon is the main reaction for the degradation of DDD, however, this reaction is typically slow (Engst and Kujawa, 1967; Quirke et al., 1979). Quirke et al. (1979) have proposed that DDD can be dehydrochlorinated to DDMU via a free radical process involving a hydrogen radical. Pereira et al (1996) also

described finding DDMU in environmental samples. Evidence of anaerobic degradation of DDD has previously been established for microbial cultures (Wedemeyer, 1967). The findings indicated that DDD was converted to *p, p'* dichlorobenzophenone (DBP) via DDMU and bis (*p*-chlorophenyl) acetic acid (DDA).

DDE to DDMU Traditionally, DDE, a toxic breakdown product, has been viewed a dead-end metabolite in the environment (Spencer et al., 1996; Hay and Focht, 1998). DDE is extremely persistent in the environment, and degradation is largely controlled by the environmental conditions. Despite the persistence of DDE in soils, studies with a variety of pure cultures have shown degradation of DDE by microbes (Hay and Focht, 1998). In sediments, the presence of DDMU among the anaerobic degradation products of DDT has been attributed to reductive dechlorination of DDE (Quensen et al., 1998). Laboratory assays have shown that DDE can be reductively dechlorinated to DDMU by microbes under methanogenic and sulfidogenic conditions (Quensen et al., 1998).

DDMU and beyond In addition to DDD and DDE, intermediate compounds such as DDMU, 1-chloro-2, 2-bis (*p*-chlorophenyl) ethane (DDMS), DDNU, DDA, and DBP can occur from anaerobic degradation of DDT. There is an evidence for the degradation of DDT beyond DDMU. Jensen et al. (1971) proposed that bis (*p*-chlorophenyl)-acetonitrile (DDNC) is formed from DDT by reduced hematin and ammonia. Furthermore, it has been shown that DDNC can be oxidized to DBP in aerobic environments by dehydrochlorination reactions (Marei et al., 1978; Quirke et al., 1979).

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CHAPTER 2

INTRODUCTION

Sampling and Chemical Analysis of Pine River sediments

Between 1936 and 1978, Velsicol Chemical Corporation, formerly Michigan Chemical, manufactured a wide array of compounds such as 1,1,1-trichloro-2, 2-bis (*p*'-chlorophenyl) ethane (DDT), polybrominated biphenyls (PBBs), hexabromobenzene (HBBs), and tris (2,3-dibromopropyl) phosphate (Tris). These synthetic compounds entered Pine River sediments through the discharge of wastewater and sludge. The Velsicol Chemical manufacturing site ("Site") is an approximately 50-acre parcel located on the Pine River. The Site has been viewed as a threat to general public health, welfare, and the environment. The Site, including the adjoining Pine River, has been subject to considerable attention from regulatory agencies and ultimately did not withstand regulatory scrutiny for PBBs and DDT. The State of Michigan and U.S. Environmental Protection Agency (U.S. EPA) closed the Velsicol Chemical facility in 1976 after forty years of intense activities with production of 11 million pounds of PBBs. This action resulted from contamination of dairy cattle throughout Michigan by PBB- tainted feed and operating violations of the company's landfill in 1974. The former incident occurred when Velsicol mistakenly shipped PBB rather then the feed additive magnesium oxide (MgO) to operators.

As part of a five-year remediation plan, the Velsicol Chemical Company and the State of Michigan began work to treat the contaminated site by dismantling the

manufacturing facility, and placing a slurry wall around it and a five-foot thick clay cap over it to stabilize contaminants. However, in negotiations between Velsicol and the State of Michigan regarding clean up, a no action alternative regarding the contaminated sediments of the Pine River was agreed to. The idea was that with time the contaminated sediments would become physically isolated by the deposition of non-contaminated sediments. A no consumption advisory was established for fish in the Pine River (St. Louis) to its confluence with the Chippewa River (a 20 mile stretch) in 1977 and by 1982, the site was identified as a Superfund site at the National Level (Forba et al., 1982)

For the past three decades EPA has monitored contamination of the river by DDT, PBB and other chemicals. This began in the early 1980's when Forba et al. (1980 and 1982) reported that sediments in Pine River were heavily contaminated with DDT, petroleum products and brominated compounds such as HBBs and PBBs. Analysis of sediments in the Pine River reservoir adjoining the Velsicol manufacturing site indicated that the average concentration of DDT was 3,500 μg/g (ppm) and the maximum concentration detected was 44,000 μg/g (Forba et al., 1982).

Analysis of fish in Pine River showed that DDT levels were extremely high in 1985 and exceeded the Federal Department of Agriculture (FDA) tolerance level of 5 ppm for DDT (Forba et al., 1982). Subsequent analysis of DDT levels in fish (e.g. carp and black crappie) taken in 1989, 1994, 1995 and 1997 from the Pine River within and below the St. Louis impoundment show that concentrations have remained high. During this period the average DDT concentrations in fish appear to have increased about 2X but the statistical significance of this is difficult to establish because of the wide variability of concentrations and limited number of fish taken. The average DDT concentration in the

1995 samples was highest at 15.6 μg/g, but concentrations of up to 200 μg/g were observed in individual fish. These findings indicate that the no action alternative taken for sediment remediation in this region of the river had failed. The high levels of DDT in fish tissue indicate continual exposure of the fish to contaminated sediments within the St. Louis impoundment. Concern for human exposure to DDT via consumption of contaminated fish has driven a $ 40,000,000 Superfund clean-up which is currently underway.

In 1998 the U.S.EPA approved a dredging plan for the removal of the contaminated sediments from the impoundment. The first stage of this intensive cleanup program was to install sheet piling around the most contaminated sediments near the old plant outfall. The overlying water was then pumped out and the sediments were solidified then excavated down to the underlying peat. The excavated sediments were dewatered before they were landfilled offsite. The water from the sediments was treated on site before being returned to the river. The process has now been expanded to the rest of the impoundment and should be complete by the end of 2001.

In this study, we measured the total concentration of DDT and related compounds in sediments from Pine River Reservoir. Similar to previous analyses, we found that sediments of the Pine River in close vicinity to former manufacturing site contain high concentrations of *p*, *p*'-DDT, *o*, *p*'-DDT, *p*, *p*'-DDE, *o*, *p*'-DDE, *p*, *p*'-DDD, *o*, *p*'-DDD, and *p*, *p*'-DDMU (collectively referred to as DDX). Of the DDX compounds present in Pine River sediments, the components comprising the greatest amounts of DDX were DDT and DDD while DDE and DDMU represented smaller portions.

MATERIALS AND METHODS

Sampling and Chemical Analysis

DDT contaminated sediments were collected in September 1998 from the Pine River (PR) at Velsicol Chemical Company Superfund Site in St. Louis, Michigan. Sections of polyvinyl chloride pipes with inner diameter of 7.62 cm and approximately 90 cm in length were used as sampling devices for withdrawing sediments. Pre-drilled rubber stoppers fitted with a rubber flapper were inserted at one end of each pipe for releasing water and gas as the pipes were pushed into sediments to a depth of approximately 90 cm. The pipes were withdrawn, capped and sealed with tape to reduce exposure to oxygen, and the cores were then transported to the laboratory and stored at 4°C. Samples were taken in triplicate from five locations.

Prior to analysis, the sediments were extruded from the PVC pipes, air-dried at room temperature for 3 to 5 days, and passed through a 2 mm sieve. Sediments from each core were homogenized over depth and duplicate ten-gram samples were extracted with 250 ml of hexane and acetone (1:1, vol: vol) in a Soxhlet apparatus for 24 hours. The solvent extract was then transferred to a separatory funnel and back-extracted with approximately 50 mls of 2% aqueous NaCl. After draining off the aqueous portion, 2 to 4 mls of concentrated sulfuric acid (H_2SO_4) was added to the remaining hexane extract, and the whole was shaken by hand then allowed to separate. The acid was drained off and the hexane residues were then washed 2-3 times with 25 mls of 2% NaCl solution to remove residual acidity. The hexane extract was then filtered through anhydrous Na_2SO_4 to remove water. To remove polar compounds and sulfur, the sample was eluted through

a 160 mm sample clean-up column (Supelco, Bellefonte, PA Cat. No. 58099) packed in layers with 5 mm anhydrous sodium sulfate, 60mm of a Florisil-acid rinsed copper powder mixture (4:1 ratio, vol: vol), and 5 mm more of sodium sulfate.

The solvent extracts were then analyzed for DDT analogs (*p, p'*-DDT, *o, p'*-DDT, *p, p'*-DDE, *o, p'*-DDE, *p, p'*-DDD, *o, p'*-DDD, and *p, p'*-DDMU) by high-resolution gas chromatography. The retention times of authentic standards were matched to the DDX components in the extracts. An internal standard (octachloronapthalene) method was used to quantify the amount of DDX in each sample. The analysis was performed on a HP 5890 gas chromatograph equipped with an electron capture detector and a capillary column (HP Ultra 2, 50m long 0.22 mm id and 0.33 μm film thickness). The inlet temperature was 220° C, and the detector temperature was 325° C. The column temperature program began at 140 ° C for 1min and was increased to 240 ° C at the rate of 2 ° C/min, then held at 240 ° C for 4 min. A 5μl sample was injected using a split ratio of approximately 10:1.

RESULTS AND DISSCUSION

Site Description

Sediments from five locations in the Pine River between the Washington Street and Mill Street bridges (Figure 2.1) were analyzed for DDX components. Sediments at the five sites contained varying concentrations of DDX. The highest concentrations of DDX were found in the middle basin (at Site 2) between the industrial site jetty and the Mill Street bridge (Figure 2.1), with the total concentration of DDX in sediments being ~20,000 μg/g (Table 2.1). The high levels of DDX were not surprising in this region of the river because it lies adjacent to and slightly downstream from the former Vesicol Chemical Company manufacturing site. Previously, Forba (1980) reported a maximum DDX concentration of 44,000 μg/g in sediments collected from this region of the Pine River. Similar results by National Enforcement Investigations Center (NEIC) and the EPA (1997) indicated that maximum concentrations of total DDX were 26,000 μg/g and 44,000 μg/g, respectively for this portion of reservoir, consistent with our results.

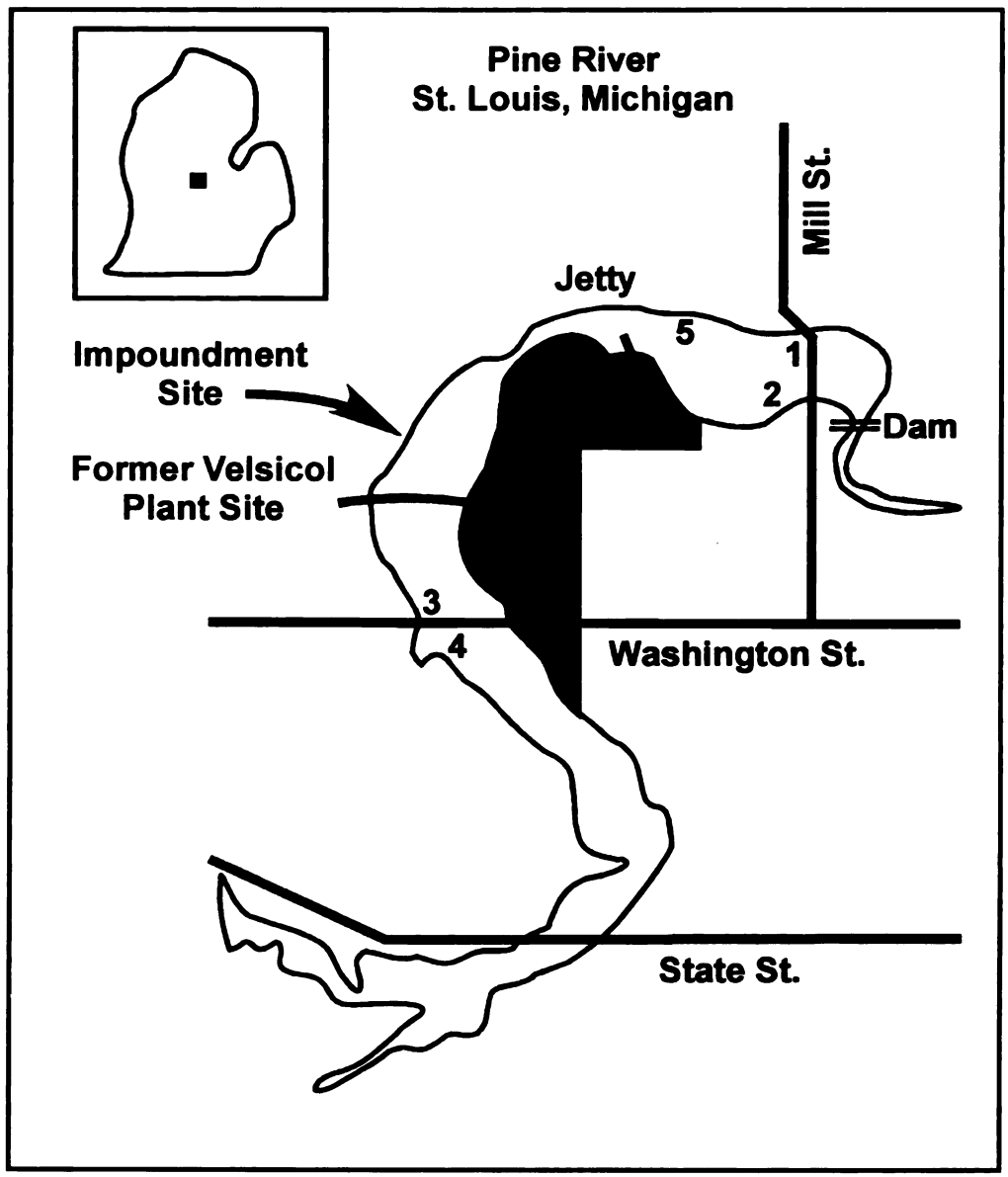

Figure 2.1. Map of sampling locations at the Pine River.

The five sites sampled grouped naturally into one site with high (site 2), two sites with medium (sites 1 and 5), and two sites with low (sites 3, 4) levels of DDX (Table 2.1). The total concentration of DDX was obtained by summing the concentrations of *o*, *p*' and *p*, *p*'-isomers of all DDX compounds for each sediment. Total concentrations of DDX for sediment collected near the Mill Street bridge and across from the Velsicol

Plant site (site 1 and site 5, Figure 2.1) were intermediate among the samples taken with DDX concentrations of approximately 13 μg/g for site 5 and 5.5 μg/g for site 1. In the upper basin (sites 3 and 4), located upstream of the Velsicol Plant site, the DDX levels were relatively low at approximately 0.5 μg/g. DDX levels were high (approximately 20,000 μg/g) at site 2, located adjacent to the former Velsicol manufacturing site. The field study indicated differences in total concentrations of DDX as well as the relative distribution of *p, p'*- and *o, p'* - DDX isomers at the five sediment sampling location (Table 2.2). *p, p'*-DDT was the predominant isomer at site 1 whereas for other sites a more even distribution of the four DDT and DDD isomers was observed. In general, concentrations of DDE and DDMU were considerably less that those of DDT and DDD.

Table 2.1. Mean concentrations (μg/g) of DDT and metabolites and total DDX in sediments collected from the Pine River. Values, in μgrams/grams (calculated from measurements of estimated concentration in sample extract), are the average of two replicates. The component percentage of the total DDX concentration is shown in parentheses.

	Mean Concentration (μg/g)				
Analyte	Site 1	Site 2	Site 3	Site 4	Site 5
p, p'-DDT	4.29 (77.4)	7020 (35.1)	0.04 (27.8)	0.10 (25.9)	5.43 (42.0)
o, p'-DDT	0.20 (3.61)	5380 (26.9)	0.04 (27.1)	0.06 (15.06)	0.59 (4.54)
p, p'-DDD	0.05 (0.96)	2910 (14.5)	0.03 (18.2)	0.11 (27.8)	2.89 (22.4)
o, p'-DDD	0.01 (0.12)	3930 (19.7)	0.03 (18.4)	0.10 (26.01)	1.99 (15.43)
p, p'-DDE	0.42 (7.52)	301 (1.51)	0.01 (5.5)	0.01 (1.83)	1.0 (4.75)
o, p'-DDE	0.06 (1.17)	200 (1.0)	0.001 (0.7)	0.001 (0.43)	0.39 (3.05)
p, p'-DDMU	0.51 (9.23)	252 (1.3)	0.003 (2.4)	0.01 (2.95)	1.01 (7.82)
Total DDX	5.54	19993	0.154	0.391	13.3

In general technical grade DDT is composed of several compounds listed in (Table 2.2) and the mixture typically contains from 73% to 77% *p*, *p*'- DDT and up to 20% *o*, *p*'-DDT (West and Campbell, 1950). The exact composition of DDT products that were originally discharged into the Pine River reservoir is unknown. It is likely, however, that the DDT composition was relatively similar to technical grade DDT. The DDT analogs *p*, *p*'-DDT, *o*, *p*'-DDT, *p*, *p*'-DDE, *o*, *p*'-DDE, *p*, *p*'-DDD, *o*, *p*'-DDD, and *p*, *p*'-DDMU were detected in all sediments sampled. The presence of these compounds may be due to the original mixtures of DDT that were discharged into the river or the result of microbial and chemical transformation of DDT. Of the DDX compounds found in PR sediments, DDT and DDD were consistently higher than other components. DDE and DDMU were detected in sediments at comparatively low levels; these compounds are not normally detectable in technical DDT mixtures (West and Campbell, 1950). Because it is unknown what exact DDT mixtures were discharged into the PR, and because the composition of the DDT products may vary over time, it remains uncertain whether the presence of DDE and DDMU at low concentrations is an indication of DDT transformation. However, these are known environmental transformation products of DDT so this is their likely origin in the Pine River Sediments.

Table 2.2. Percent of Total DDT and metabolites in Technical DDT (Adapted from West and Campbell, 1950).

Composition of Technical DDT			
Compound	Constitution (%) of sample of given setting point		
	88.6°C	91.2°C	91.4°C
p, p'-DDT	70.5	66.7	72.7
o, p'-DDT	20.9	19.0	11.9
o, o'-DDT	-	-	0.01
p, p'-DDD	4.0	0.3	0.17
o, p'-DDD	-	-	0.04
2,2,2,-Trichloro-1-*o*-chlorophenyl ethyl-*p*-chlorobenzenesulfonate	1.8	0.4	0.57
1-*p*-Chlorophenyl-2, 2, 2-trichloroethanol	-	0.2	-
1,1,1,2-tetrachloro-2-*p*-chlorophenylethane	-	-	-
Bis (*p*-chlorophenyl)-sulfone	0.1	0.6	0.03
o-chlorophenyl chloroacetamide	0.007	-	-
p-chlorophenyl chloroacetamide	0.01	-	0.006
Sodium *p*-chlorobenzenesulfonate	-	0.02	-
Ammonum *p*-chlorobenzenesulfonate	-	-	0.005

In examining the DDX component distributions (Table 2.1), it is apparent that site 1 has a much higher percent component of *p*, *p*'-DDT as compared to the other four sites. Interestingly, the *p*, *p*'-DDT component percentage (of total DDX) at site1 (77.4%) is similar to the typical composition of technical grade DDT. Thus, among the sites evaluated, the DDX composition at site 1 may be most representative (i. e. least transformed) of what was originally discharged. However, the component percentage of *o*, *p*'-DDT at site 1 (3.6%) is considerably lower than that typically found in technical grade DDT. In the other four sites the *p*, *p*'-DDT component percentage ranges from 26 to 42% of total DDX. This is considerably lower than would typically be found in technical DDT indicating significant transformation of *p*, *p*'-DDT. Transformation of *p*, *p*'-DDT presumably yields predominately *p*, *p*'-DDD. In fact, for sites 2-5 the component percentage of *p*, *p*'-DDD are significantly elevated compared to that found in technical DDT. For sites 2-4 the component percentage of *o*, *p*'-DDT are similar to those found in technical DDT whereas for sites 1 and 5 they are significantly lower. The component percentages of *o*, *p*'-DDD appear to be elevated, especially for sites 2-5, as compared to technical DDT. Overall, this presents somewhat confusing picture regarding the composition of technical DDT originally discharged into the river, and regarding what environmental transformations may have occurred. It does seem clear however that a significant amount of *p*, *p*'-DDT has been transformed into *p*, *p*'-DDD in four of the five sites assessed.

In a further attempt to identify differences in DDX component distributions among sites, the components were grouped according to *p*, *p*'- versus *o*, *p*'- substitution (Table 2.3 and Figure 2.2). Again, the major component at site 1 is DDT and there is

surprisingly little DDD. This is true for both *p, p'*- and *o, p'*-substitution. At the other four sites, the component percentages of DDT are lower and those of DDD are higher. This indicates significant environmental transformation of DDT to DDD for both *p, p'*- and *o, p'*-substitution. Significant component percentages of DDE are also observed at each site. Since DDE is not typically found in technical DDT products, its presence likely originates from in-situ transformation of DDT. Both *p, p'*-DDE and *o, p'*-DDE are present at somewhat similar component percentages indicating no obvious preference for transformation of the *p, p'*- versus *o, p'*-isomers.

Table 2.3. Mass concentrations (μg /g) and percentage of DDT and its derivatives in sediments collected from the Pine River. Values, in μgrams/grams (calculated from measurements of estimated concentration in sample extract), are means of two replicates except for three replicates from site 4 sediments. The component percentage of the total concentration is shown in parentheses.

p, p' - Substituted Compound, Mean Concentration (μg/g)					
Compound	Site 1	Site 2	Site 3	Site 4	Site 5
DDT	4.29 (81.40)	7020 (66.97)	0.04 (48.19)	0.10 (43.48)	5.43 (54.63)
DDD	0.05 (0.95)	2910 (27.76)	0.03 (36.14)	0.11 (47.83)	2.89 (29.07)
DDE	0.42 (7.97)	301 (2.87)	0.01 (12.05)	0.01 (4.35)	1.00 (6.14)
DDMU	0.51 (9.70)	252 (2.40)	0.003 (3.61)	0.01 (4.35)	1.01 (10.16)
Total	5.27	10483	0.083	0.23	10.33
o, p' - Substituted Compound, Mean Concentration (μg/g)					
DDT	0.20 (74.07)	5380 (56.57)	0.04 (56.34)	0.06 (37.27)	0.59 (19.87)
DDD	0.01 (3.70)	3930 (41.32)	0.03 (42.25)	0.10 (62.11)	1.99 (67.00)
DDE	0.06 (22.22)	200 (2.10)	0.001 (1.41)	0.001 (0.62)	0.39 (13.13)
Total	0.27	9510	0.071	0.161	2.97

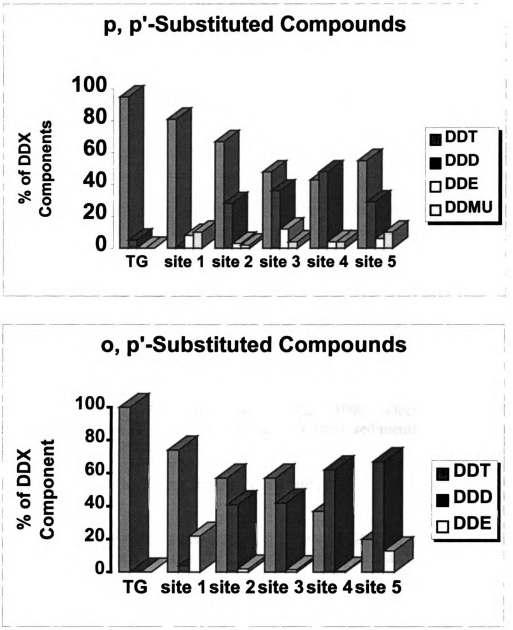

Figure 2.2. Percentage of DDX component distributions among sites, the components were grouped according to *p, p'*- versus *o, p'*- substitution.

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CHAPTER 3

INTRODUCTION

From analysis of DDX concentrations in environmental samples, such as data presented in chapter 2, it is often difficult to decipher degradation pathways unequivocally. Laboratory studies using proposed intermediates, preferably radio-labeled, as substrates are needed to clarify specific degradation pathways. In chapter 2, several DDT analogs were quantified in Pine River sediments, mainly DDD, DDE and to a lesser extent DDMU. Although these intermediates are clearly present, several questions remain including whether DDD is produced chemically or biologically, and whether DDMU is being formed from DDT via DDD, as is traditionally believed, or via DDE as was recently shown by Quensen et al. (1998) in marine sediments. It is also unclear whether DDMU, or any other metabolites, are dead-end products.

OBJECTIVE

In this study we examined the fate of DDX in Pine River sediments contaminated with DDT ranging from 0.05 to 20,000μg/g sediment. We compared the extent of transformations over a 40 week period. The objective of this study was to determine the pathway of DDT degradation and distinguish between abiotic and microbial processes.

MATERIALS AND METHODS

Materials

Chemicals 1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane (99% purity); 1,1-dichloro-2, 2-bis (p-chlorophenyl) ethane (99%purity) ; 2,2-bis (p-chlorophenyl) 1,1-dichloroethylene (99.9% purity) were obtained from Ultra Scientific, Co., North Kingstown, RI. ^{14}C-DDT (12.7 mCi/mmol, 97% radiochemical purity) ^{14}C-DDD (2.6 mCi/mmol, 100 % radiochemical purity) and ^{14}C-DDE (13 mCi/mmol, 97.7% radiochemical purity) were ring labelled and provided by the Sigma Chemical Co. St. Louis, MO. The chemical purity for ^{14}C-DDT, DDD and DDE were 97, 99.4 and 98.2% respectively.

Sediments Sampling and Analyses

Three sediment cores from each of five sites were collected in September 1998 from the Pine River (PR) at the Velsicol Chemical Company Superfund Site in St. Louis, Michigan (Figure 2.1). Sections of polyvinyl chloride pipe with inner diameter of 7.62 cm and approximately 90 cm in length were used as sampling devices for withdrawing sediments. Pre-drilled rubber stoppers with an attached rubber flapper were placed at one end of the pipes for releasing water and gas as the pipes were inserted (by hand) into the sediment as deep as possible (up to 90 cm). After withdrawal, the pipes were capped and sealed with tape to reduce the exposure of oxygen, and cores (in vertical positions) were then transported to the laboratory. The samples were stored at 4°C until they were used in this study.

Methods

Sediment Slurry Preparation

Five sediment samples (one from each site) were selected from the fifteen sediment cores for further study. Sediments were extruded from the PVC pipes then homogenized by mixing with an equal volume of Reduced Anaerobic Mineral Medium (RAMM) as described by Quensen et al. (1998). The RAMM was prepared as previously described in the general method for determining anaerobic biodegradation potential (Shelton and Tiedje, 1984). The autoclaved RAMM (600 ml) was added to a 2000 ml Erlenmeyer flask that had been pre-flushed with oxygen-free nitrogen and carbon dioxide (80:20, vol/vol). While continuously sparging with the N_2/CO_2 gas mixture, an equal amount of contaminated sediments was then added to the flask with a sterile spatula and mixed on a magnetic stirrer.

Microcosms Setup

^{14}C-radiolabelled-DDT, DDD and DDE were used as substrates separate dehalogenation assays. A mixture containing 2.8, 12.3 or 2.4 mg of ^{14}C- DDT, DDD or DDE, respectively, and also including 54.2, 44.7 or 54.6 mg of unlabelled-DDT, DDD or DDE was diluted with acetone to 2 ml. This gave a solution of each compound with a concentration of 28.5 μg/μl and an activity of approximately $1.07X10^5$ dpm/μl, as verified by liquid scintillation counting (Beckman LS 6500). A volume of 7 mls of a sediment slurry (containing about 2 g of sediments on a dry weight basis) was placed in each sample vial (20ml) with a sterile Pasteur pipette. The entire contents of each vial were spiked with 7μl ^{14}C-labeled DDT, DDD or DDE. This gave an activity of

approximately $7.5X10^5$ dpm per microcosm and a DDX concentration of approximately 100μg/g sediment (dry weight). The microcosms were then stored in the dark at room temperature, approximately 25°C. Both biologically active and sterile (abiotic) samples were set up in parallel. The abiotic treatments were autoclaved on three consecutive days for one hour prior to addition of ^{14}C-labeled compounds to vials. Four replicate samples were sacrificed and evaluated after 0, 4, 8, 12, 16, 24 and 40 week intervals for live, and 0, 8, 16, and 40 week intervals for abiotic treatments.

Methane Analysis

After predetermined periods of incubation, sediment microcosms were analyzed for methane using a gas chromatograph fitted with a flame ionization detector. Methane production in headspace of anaerobic sediment microcosms was used to establish the existence of anaerobic conditions, and as a relative index or the biologically activity of each microcosm. Methane activity was determined as the percent of headspace gas in microcosms. The mean of three or four samples at each time interval was considered as one observation.

Dechlorination Assays (Extraction and Analysis)

After incubation for various time intervals, the entire contents of each sediment microcosm were extracted three times by shaking for 10 minutes with 7 mls of a petroleum ether-acetone mixture (5:2, vol: vol). The solvent extracts were combined and evaporated to a final volume of 0.5 mls under a stream of dry nitrogen. Samples of the reduced volume extracts (20μl) were spotted on activated silica gel plates that were

subsequently developed to 15 cm with a petroleum ether-hexane mixture (5:95) in a lined thin layer chromatography (TLC) chamber at room temperature. Autoradiography was used to determine the locations of the parent compound and metabolites on the TLC plates. Kodak Scientific Imaging Film (X-OMAT AR) was exposed to the TLC plates for 7 days at minus 20° C and then developed. Autoradiography films and TLC plates were aligned on a back-lighted box, and the parent compound and metabolite zones were visualized and marked for scraping. These zones corresponded to DDT, DDE, DDD, DDMU, and the origin where polar metabolites would remain. The ^{14}C-labeled activity in the scraping was determined by liquid scintillation counting.

The results for DDT degradation and dechlorination are presented as the percent of ^{14}C recovered. The results for DDT degradation were determined by dividing the amount of ^{14}C-DDT recovered by the amount of ^{14}C-DDT added (X100) to get the percent of total recovery. The percent of radioactivity recovered for each compound, thus, was not normalized to total radioactivity recovered.

RESULTS AND DISSCUSION

DDT Transformations

The Pine River sediments in St. Louis, Michigan are heavily contaminated with DDT, polybrominated biphenyls (PBB), hexabromobenze (HBB), petroleum, chlordane, and heavy metals. Previous studies on Pine River sediments sought to determine the degradation potential for PBBs at this site. Morris et al. (1993) found evidence for limited in situ anaerobic biodegradation of PBBs based on the distribution of congener patterns in sediment. Morris et al. (1993) indicated that the Pine River sediments partially debrominated the commercial PBB mixture Firemaster BP6 by removal of meta and para bromide. Although microorganisms capable of PBB debromination were found in the sediments, high concentrations of co-contaminants were believed to inhibit in situ debromination.

Previous studies on the Pine River Reservoir revealed the presence of DDT in high concentrations (up to 40,000 ppm) (Forba et al. 1980; 1982). In addition to detecting up to 20,000 ppm DDT, our sediment analysis also identified the proposed DDT metabolites DDD, DDE, and DDMU (Chapter 2). Quensen et al. (1998) recently demonstrated DDMU formation from the reductive dechlorination of DDE. This conclusion differed from previous studies that have reported DDE as a dead end product from DDT biodegradation, and DDMU formation from DDD via dehydrochlorination (Quirke et al., 1979; Spencer et al., 1996; Hay and Focht, 1998). The present study was undertaken to examine the degradation pathway of DDT in Pine River sediments in order to determine the origin of DDMU detected in the sediments as well as the origin and fate of DDD and DDE under anaerobic conditions.

Sediments from five locations in the Pine River Reservoir were used to study their ability to support anaerobic dechlorination. Thin layer chromatography and autoradiography of solvent extracts were used to analyze transformations of DDT, DDE and DDD in sediment microcosms under anaerobic conditions. In addition to live treatments, replicate samples were autoclaved to account for abiotic transformations. Biologically mediated transformations were estimated by examining the difference between live and autoclaved samples. When statistically valid, comparisons were made between the contribution of biotic and abiotic transformations at a given site. The Pine River sediments supported microbial dechlorination of DDT, but did not support anaerobic reductive dechlorination of DDD or DDE. Therefore, this discussion will focus on the transformations of DDT to DDD in microcosms.

Transformations in live microcosms

In the active microcosms, the anaerobic sediments supported anaerobic reductive dechlorination of DDT to DDD. As the counts for DDT declined over time, there were comparable increases for DDD (Table 3.1), but not for DDE or DDMU (data not shown). Also, there was no significant temporal increase of counts at the origin (putative polar metabolite) (Table 3.1). Thus dechlorination of DDT to DDD was only significant reaction observed in the live microcosms. The greatest dechlorination of DDT to DDD occurred by 8 weeks, and gradually increased thereafter (Table 3.1). For the live samples at 8 weeks DDT recovery ranged from 38 to 53%. Furthermore, most DDT dechlorination had occurred in microcosms by 16 weeks of incubation. After 40 weeks of incubation, the average recovery of DDT for four sites (1,3,4 and 5) was 28% of the applied DDT and the average recovery of DDT at site 2 was 39% (Table 3.1). A low

percentage of radioactivity in microcosms was recovered at time zero as DDD and polar metabolites (Table 3.1); DDD amounts ranged from 3 to 14% (of added ^{14}C-DDT) among the five sampling sites. Two possible explanations for this are: (1) DDT was transformed rapidly within several hours before they were transferred to the freezer, or (2) DDT may have been transformed to DDD while microcosms were stored in the freezer. For the live treatments, the average of radioactivity as DDD at weeks 8 and 16 in live samples was 33% and 36%, respectively, among all sites (Table 3.1). After 24 weeks average DDD was 41% of the added DDT (data not shown), and after 40 weeks of incubation DDD ranged from 40% to 44%. The general trends observed in Pine River sediments for conversion of DDT to DDD under anaerobic conditions are shown in (Figure 3.1).

Table 3.1. Comparison of observed percent ^{14}C-radioactivity recovered as various analytes from Pine River sediments (5 sites) incubated under anaerobic conditions. The standard deviation is reported in parentheses. The t-Test was applied to determine the statistical significance ($P<0.05$) for differences in DDT transformations among the biologically active and abiotic controls.

Incubation time (wk)	Site 1			Site 2			Site 3			Site 4			Site 5		
	Live	Auto-claved	Diff.	Live	Auto-claved	Diff.	Live	Auto-claved	Diff.	Live	Auto-claved	Diff.	Live	Auto-claved	Diff.
	% ^{14}C recovered as DDT														
0	74.19	80.83	6.64	81.59	83.26	1.67	77.55	79.89	2.33	87.59	87.62	0.03	71.77	77.94	6.17
	(9.57)	(2.08)		(8.53)	(4.47)		(3.55)	(1.68)		(3.00)	(3.69)		(2.42)	(5.56)	
8	42.66	60.27	17.60	52.92	61.16	8.25	39.51	47.08	7.57	38.30	53.37	15.07	40.79	39.28	-1.52
	(3.89)	(15.18)		(8.03)	(13.56)		(5.33)	(9.13)		(12.69)	(11.21)		(4.85)	(7.84)	
16	38.81	67.10	28.29	54.87	53.23	-1.60	37.26	45.40	8.14	31.44	44.92	13.48	30.38	51.05	20.67
	(16.11)	(15.18)		(1.62)	(6.94)		(1.63)	(4.62)		(6.73)	(8.42)		(7.58)	(7.58)	
40	28.58	51.98	23.40	39.24	45.88	6.64	29.06	47.90	18.83	27.07	51.30	24.23	28.68	34.23	5.55
	(3.80)	(2.76)		(7.11)	(9.43)		(1.97)	(7.85)		(15.99)	(15.99)		(5.63)	(2.30)	
	% ^{14}C recovered as DDD														
0	7.83	4.13	3.69	5.03	4.02	1.01	6.67	5.12	1.64	2.95	2.71	0.24	13.85(	8.66	5.19
	(0.31)	(0.15)		(2.17)	(1.47)		(1.75)	(0.49)		(0.76)	(0.53)		0.62)	(3.53)	
8	30.75	11.83	18.92	28.71	17.29	11.42	33.86	21.05	12.08	34.81	24.78	10.03	34.72	31.60	3.12
	(5.88)	(NA)		(6.56)	(9.85)		(2.22)	(4.32)		(8.01)	(5.48)		(5.58)	(4.98)	
16	36.09	20.30	15.79	26.21	22.93	3.28	32.51	24.79	7.72	39.26	26.38	12.88	41.43	24.45	16.98
	(8.16)	(5.38)		(0.73)	(5.73)		(2.81)	(4.26)		(3.84)	(4.62)	***	(4.31)	(4.08)	***
40	40.47	25.85	14.62	40.67	29.88	10.79	43.60	25.82	17.78	44.48	27.65	16.83	42.05	34.15	7.90
	(3.38)	(3.48)	***	(4.86	(5.63)	**	(3.34)	(3.63)	***	(10.49)	(9.05)	NA	(3.47)	(1.57)	**
	% ^{14}C recovered as Polar Metabolites														
0	2.07	1.79	0.28	1.64	1.98	0.34	2.29	2.19	0.09	1.64	1.66	-0.02	2.00	1.61	0.38
	(0.21)	(0.11)		(0.35)	(0.36)		(0.19)	(0.32)		(0.21)	(0.27)		(0.06)	(0.57)	
8	3.74	2.76	0.98	2.72	3.43	0.70	3.96	3.99	-0.03	2.87	3.06	-0.19	2.98	3.79	-0.81
	(0.15)	(NA)		(0.45)	(0.91)		(0.27)	(0.63)		(0.37)	(1.01)		(0.39)	(0.32)	
16	4.15	4.22	-0.08	2.46	3.55	1.08	3.25	5.10	-1.86	2.23	3.97	-1.74	3.87	2.97	0.90
	(0.50)	(0.70)		(0.19)	(0.51)		(0.49)	(0.57)		(0.18)	(0.17)		(0.77)	(0.53)	
40	3.57	2.95	0.62	2.65	3.73	1.08	3.40	3.29	0.11	2.89	4.57	-1.68	4.71	5.62	-0.91
	(0.29)	(0.26)		(0.33)	(0.58)		(0.24)	(0.52)		(0.07)	(1.00)		(0.80)	(1.15)	

*, Not significant at the 0.05 **, *** Significant at the 0.01, and 0.001 probability levels, respectively. NA, not applicable.

Of the ^{14}C-DDT added in microcosms, between 2 and 5 % was recovered as polar metabolites; similar amounts occurred in all treatments (live and sterile) and at all sampling points. Because they represented a small stable fraction of analytes in all treatments, no attempt was made to identify the polar metabolites.

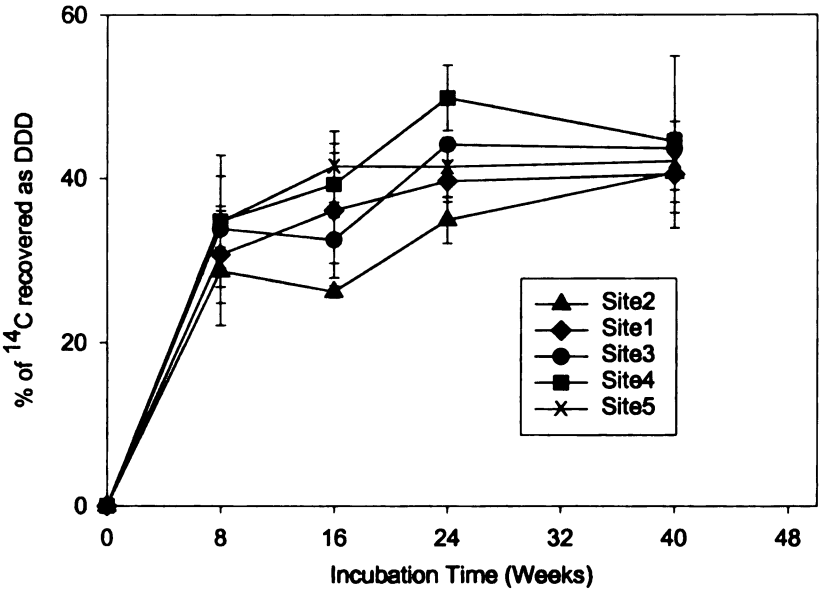

Figure 3.1. Dechlorination of DDT to DDD in sediment microcosms by microorganisms under anaerobic conditions during 40 weeks of incubation. There were no significant differences in DDD formation among sites at the 8 and 40 week sample intervals.

Sediments from site 2, which had the highest of DDX levels, were collected close to sites that were reported to contain high PBB levels (~125µg/g) and other co-contaminants (Morris et al., 1993). Morris et al. (1993) suggested that the presence of organic co-contaminants, petroleum products, and heavy metals in sediments such as

those found at site 2 can inhibit *in situ* dehalogenation. Furthermore, Morris et al. (1993) found that Pine River sediments containing the highest PBB did not support microbial dechlorination of PCBs during a 32-week incubation. High sulfate levels in sediments from this region of the Pine River probable may also inhibit dehalogenation (Morris et al., 1993), but Quensen et al. (1998) showed biodegradation of DDE to DDMU in sediments under sulfidogenic conditions. Our results show even the sediments from site 2 which contained the highest concentration DDX compounds supported dechlorination of added ^{14}C-DDT.

Transformations in abiotic microcosms

Since methanogens are ubiquitous in anaerobic inhabitants, methane production was used as an indicator microbial activity in the microcosms. At zero time, methane production was below detection limits. In the live sediment microcosms, methane was detected within 4 weeks of incubation and gradually increased during the 40 weeks incubation (Figures 3.2, 3.3, 3.4, 3.5, 3.6). There was no correlation between the extent of DDT dechlorination and the initial or cumulative methane production in the sediments. Unlike live treatments that contained up to 25% methane content in the headspace by 40 weeks, methane in the autoclaved samples was less than 0.01% or not detected. This suggests that there was no methanogensis in autoclaved microcosms, and the transformations in the autoclaved treatments are likely due to abiotic processes.

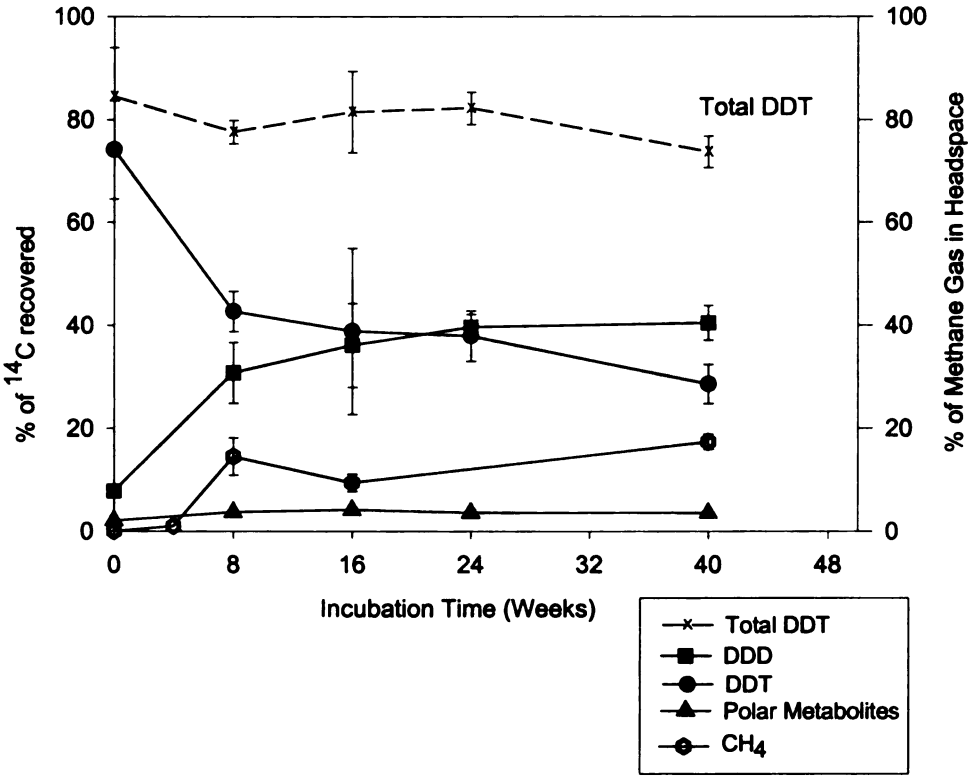

Figure 3.2. Microbial anaerobic dechlorination of DDT to DDD in sediment microcosms prepared from site 1 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts. Percent of Methane produce at time of analysis by GC.

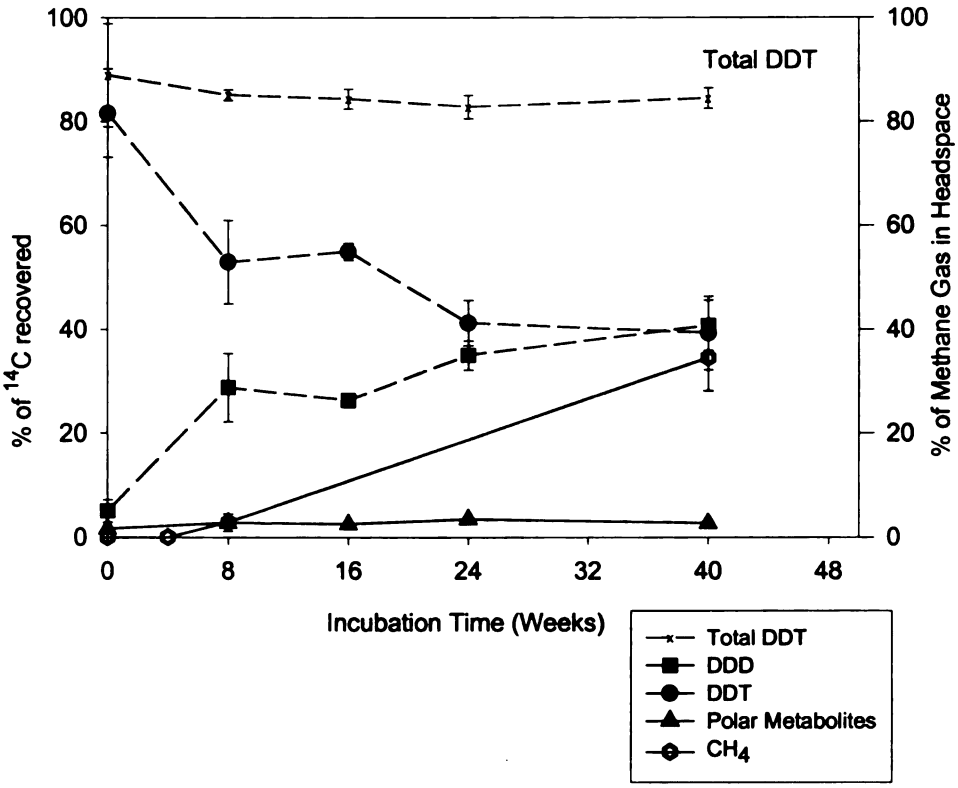

Figure 3.3. Microbial anaerobic dechlorination of DDT to DDD in sediment microcosms prepared from site 2 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts. Percent of Methane produce at time of analysis by GC.

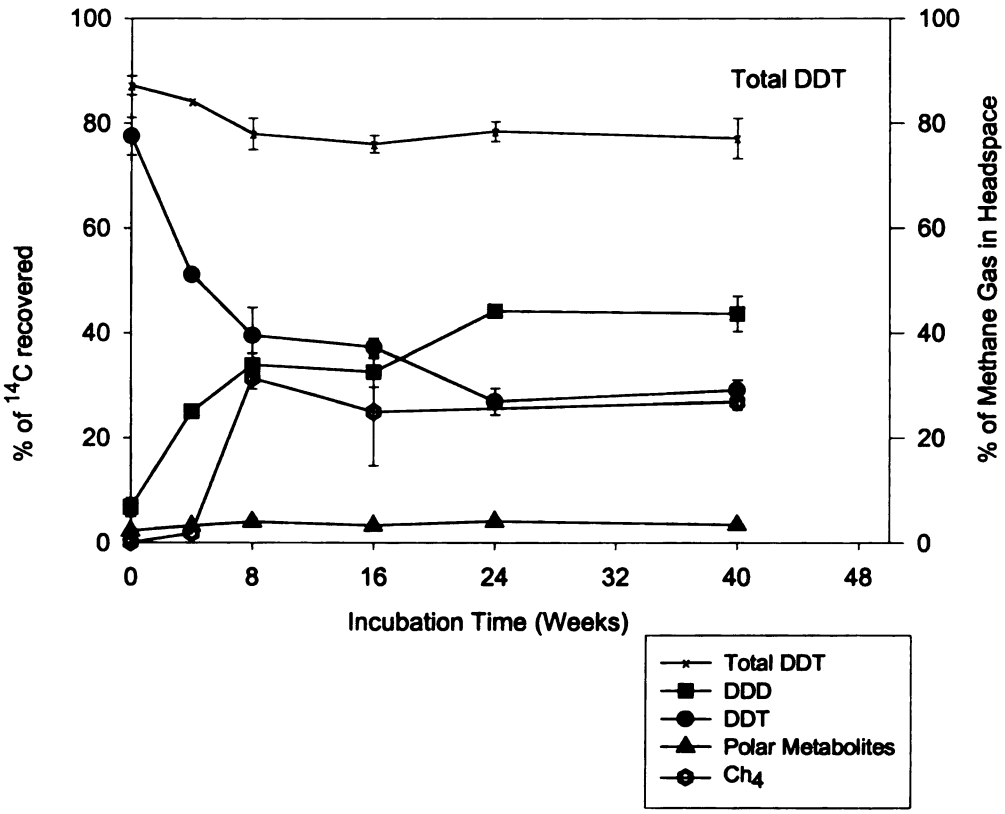

Figure 3.4. Microbial anaerobic dechlorination of DDT to DDD in sediment microcosms prepared from site 3 sediments (refer to Figure 2.1for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts. Percent of Methane produce at time of analysis by GC.

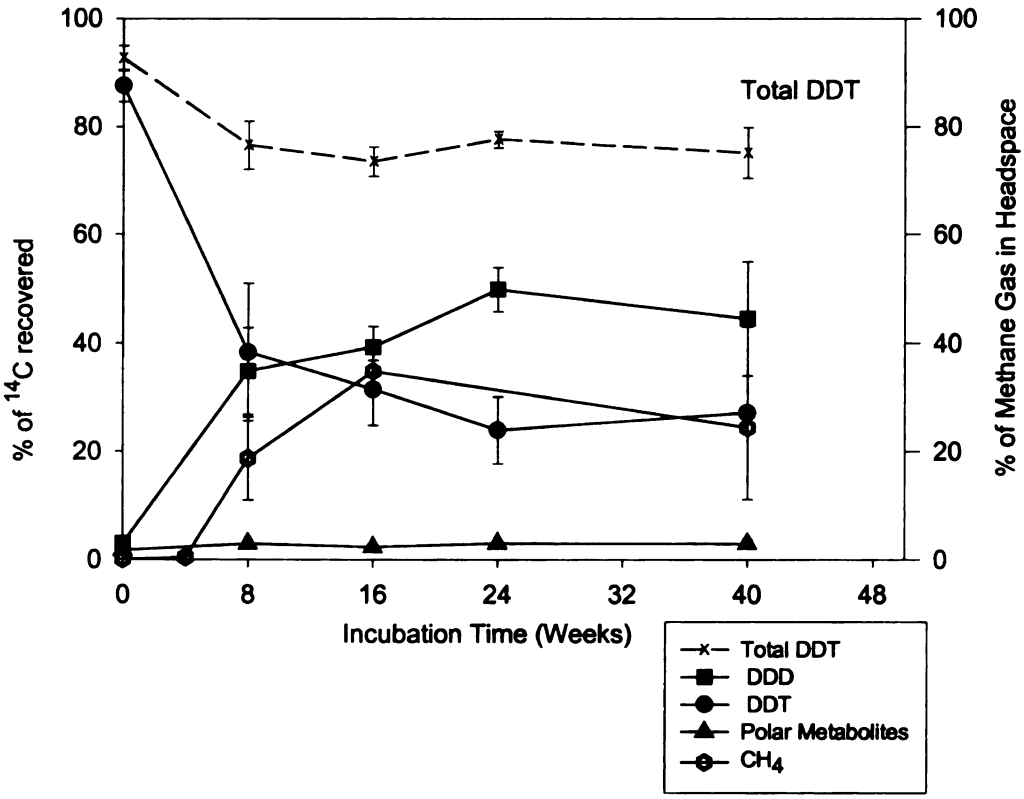

Figure 3.5. Microbial anaerobic dechlorination of DDT to DDD in sediment microcosms prepared from site 4 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts. Percent of Methane produce at time of analysis by GC.

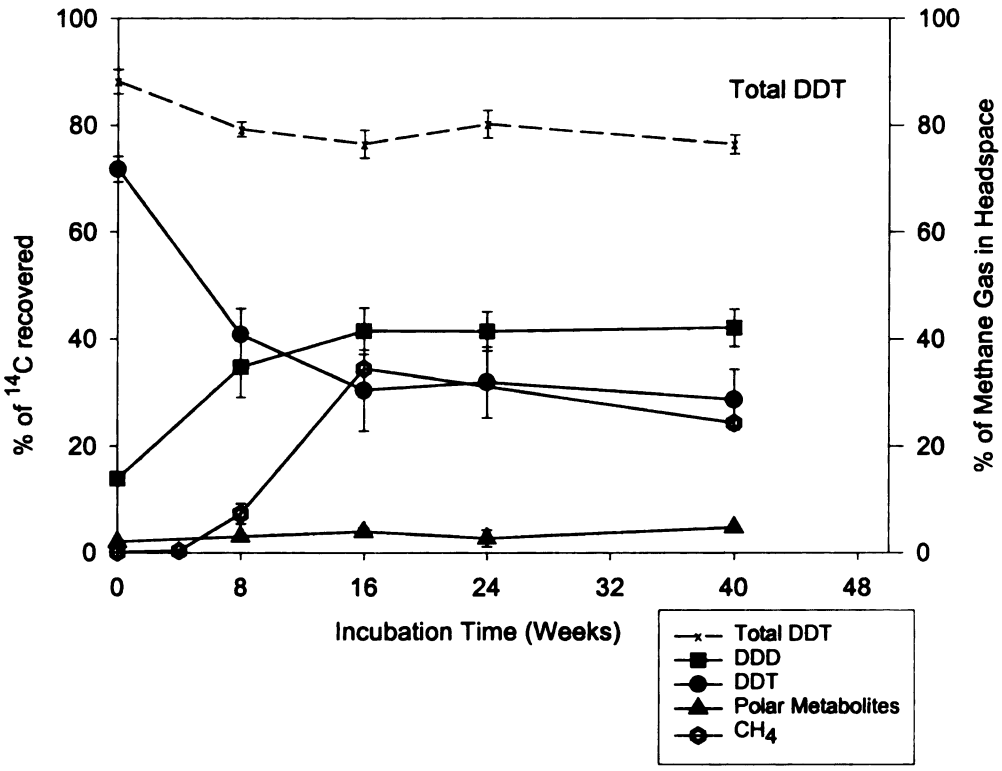

Figure 3.6. Microbial anaerobic dechlorination of DDT to DDD in sediment microcosms prepared from site 5 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts. Percent of Methane produce at time of analysis by GC.

Transformation of DDT to DDD was observed in both live and autoclaved treatments, however it was higher for the live treatments than for autoclaved treatments. After 8 weeks of incubations, the recovery of DDT ranged from 39 to 61% for autoclaved samples (Table 3.1). In general there was a more gradual decline in DDT levels between 8 and 40 weeks, similar to the trends observed in the biologically active incubations (Table 3.1). After 40 weeks of incubation under sterile conditions, DDT recovered ranged from 34 to 51% for the autoclaved samples. As in the live samples, DDD was recovered in autoclaved samples at week zero with concentrations ranging from 3 to 9 %. By 40 weeks of incubation DDD formation ranged from 26 to 34 %.

Estimated (calculated) biotic transformation

Since abiotic transformations were likely to also occur in live treatments, the difference between autoclaved and live treatments was used to estimate biologically mediated (biotic) transformations. Before computing differences, however, the t-Test was performed to compare the statistical significance ($P<0.05$) for differences in DDT transformations between biologically active and corresponding abiotic controls (Table 3.1). In general, the relatively large standard deviations for each time point resulted in varied levels of significance. There were, however, significant differences between DDD recovery in live and autoclaved treatments at sites 4 and 5 at week 16 and at sites 1, 2, 3, and 5 at 40 weeks. The estimated recovered radioactivity that resulted from biotic transformation of DDT to DDD at 16 weeks was 12.88 % at site 4 and 16.98 % at site 5. At 40 weeks, the biotic contribution was 14.62 % at site 1, 10.79 % at site 2, 17.78 % at site 3, and 7.90 % at site 5.

Abiotic and biotic formation of DDD

The results discussed above demonstrate DDD was formed from DDT by both abiotic and biotic transformations. The relative contributions of each of these were calculated for each statistically valid time point (Figure 3.7). At 16 weeks, 67 % of DDD formation was abiotic and 33 % was biotic at site 4; whereas, 59% was abiotic and 41% was biotic at site 5. At 40 weeks the abiotic fraction of DDD formation was 64, 73, 59, and 81 % for sites 1, 2, 3, and 5 respectively. The biotic contribution was 36, 27, 41, and 19% for sites 1, 2, 3, and 5 respectively. These results demonstrate that the majority of DDD formation in the Pine River sediment microcosms is due to abiotic processes, but there is also a substantial biotic contribution.

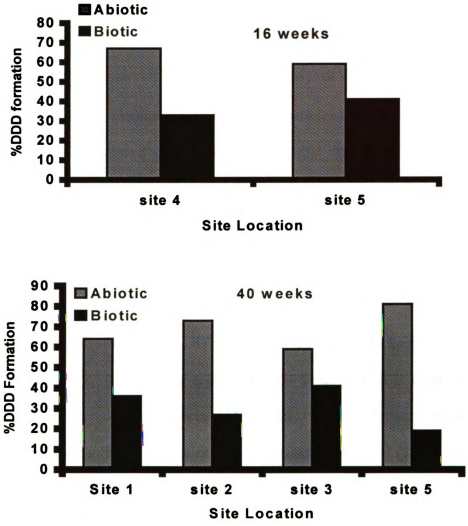

Figure 3.7. The fraction of DDD recovered resulting from biotic and abiotic transformations of DDT in Pine River sediment microcosms. The abiotic fraction was determined by dividing the DDD recovered in the autoclaved treatments by the DDD recovered in the live treatments (X 100 %). Similarly the biotic fraction was determined by dividing the difference of the live and autoclaved treatments by the live treatment (X 100 %). Only sites that had a significant difference between live and autoclaved are shown.

^{14}C Total Recoveries

There was DDT degradation in both live and autoclaved samples during 40 weeks of incubation. At time zero, the ^{14}C recovered as total DDT ranged from 84% to 93 % in biologically active microcosms, and similar recoveries of total DDT ranged between 87% and 93% for autoclaved microcosms (Table 3.2). The total recovery decreased at 8 weeks in live treatments and remained stable thereafter. A similar trend was observed with the autoclaved treatments, however with the live samples higher amounts of radioactive materials were recovered at intermediate time points. After 40 weeks of incubation, the ^{14}C recovered as total DDT ranged from 73% to 84% for live treatments, and similar recoveries of total DDT were detected for the sterile samples across the sites 1-5 (Table 3.2). In both live and autoclaved treatments, it is clear that a portion of radioactive materials were unaccounted for. The reason for failure to recover a portion of the added radioactivity DDT is not clear. Guenzi and Beard (1967) have shown DDT recoveries ~ 79% for soils incubated for 12 weeks under anaerobic conditions. This is similar to recoveries of total DDT in our experiment for sediments incubated for 40 weeks. They also concluded that between 15% and 20% of the added ^{14}C-DDT was not accounted for in autoclaved treatments. The observed loss of DDT in microcosms may be due to several factors including losses during extraction, cleanup and concentrating steps as well as volatilization.

Table 3.2. Total ^{14}C activity recovered at 0 and after 40 weeks for various analytes with live and autoclaved microcosms prepared with biologically active (live) and autoclaved sediments amended with either ^{14}C-DDT.

	Site1		Site2		Site3		Site4		Site5	
Analyte	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved
					% ^{14}C recovered at 0 week					
DDT	84.46	87.15	88.93	90.01	88.31	87.21	92.66	93.17	88.16	88.21
Polar compounds	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total DDT	84.46	87.15	88.93	90.01	88.31	87.21	92.66	93.17	88.16	88.21
					% ^{14}C recovered after 40 weeks					
DDT	28.58	51.98	39.24	45.88	29.06	47.89	27.07	51.31	28.68	34.22
DDD	40.47	25.85	40.67	29.88	43.59	25.82	44.50	27.65	42.05	34.14
Polar compounds	3.57	2.95	2.65	3.73	3.40	3.29	2.89	4.57	4.71	5.62
Aqueous phase	0.70	0.69	1.11	0.87	0.77	0.65	0.64	0.55	0.94	0.354
Total DDT	73.32	81.47	83.67	80.36	76.82	77.65	75.10	84.08	76.38	74.33
Methane (%)	17.36	0.00	34.43	0.00	26.81	0.01	24.34	0.00	24.26	0.00

DDD and DDE Transformation in sediment microcosms

After 40 weeks of incubation, DDD and DDE were not degraded in sediment samples that were sterilized prior to incubation, and no degradation occurred for any of the non-sterile sediment microcosms. After completion of the incubations the average of radioactivity recovered as DDD in live treatments was 87% of the added DDD. Trace amounts of unidentified polar metabolites or impurities that always were recovered also occurred in controls. For the sterile control microcosms the recovery ranged from 83.5 to 93.07 % as DDD across the five sites (Table 3.3). The results for DDE amended sediment samples closely resemble those of DDD. By 40 weeks of incubation, the radioactivity recovered as DDE ranged from 88% to 94% for biologically active microcosms across all five sites sampling (Table 3.3). These results indicate that the Pine River sediments do not support dechlorination of DDD or DDE in laboratory microcosms after 40 weeks incubation.

Table 3.3. Total ^{14}C activity recovered after 40 weeks for various analytes with live and autoclaved microcosms prepared with biologically active (live) and autoclaved sediments amended with either ^{14}C-DDD or ^{14}C-DDE.

	Site1		Site2		Site3		Site4		Site5	
Analyte	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved	Live	Autoclaved
					% ^{14}C recovered					
DDD	86.27	84.38	87.51	92.61	88.79	91.50	89.63	93.07	88.38	83.45
Polar compounds	1.03	1.62	0.87	NA	1.61	4.13	0.59	1.16	1.97	4.78
Aqueous phase	0.18	0.57	0.51	NA	0.19	0.15	0.11	0.08	0.24	0.13
Total DDD	87.48	86.57	88.20	NA	90.59	95.78	90.33	94.31	90.59	88.36
Methane (%)	22.60	0.00	31.90	0.00	28.20	0.00	23.40	0.00	23.40	0.00
					% ^{14}C recovered					
DDE	89.65	88.23	89.18	90.82	91.25	92.19	91.42	109.73	87.71.	97.62
Polar compounds	2.80	3.22	3.05	3.29	2.55	2.63	2.24	3.55	1.67	1.06
Aqueous phase	0.47	0.91	0.91	0.34	0.29	0.40	0.27	0.34	0.40	0.24
Total DDE	92.92	92.35	93.14	94.45	94.09	95.22	93.93	113.62	89.78	98.92
Methane (%)	22.34	0.00	35.59	0.00	27.37	0.00	23.36	0.00	25.90	0.00

SUMMARY

DDT was converted primarily to DDD in both live and autoclaved treatments. Microbial dechlorination accounted for 19 to 41 % of DDD formation during 40 weeks of incubation in live treatments (Figure 3.7). There was no evidence for the transformation of DDT to DDE in laboratory microcosms. This was not surprising since DDE is a major degradation product of aerobic DDT degradation and considering that our conditions were strictly anaerobic. No dechlorination of DDD or DDE was observed in either live or autoclaved treatments. No significant differences were found among sites despite the fact that endogenous DDT concentrations ranged from 0.05μg/g to 20,000μg/g sediment. The results from this laboratory experiment provide no evidence for DDMU formation, although DDMU was observed at sites 2, 3 and 4.

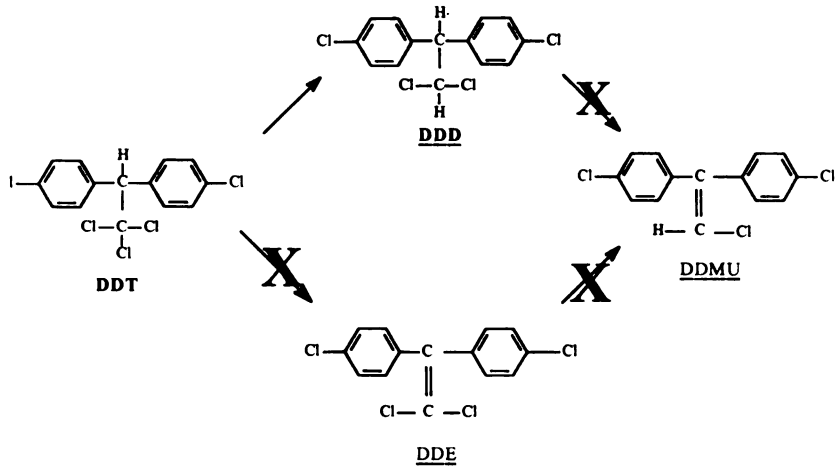

Figure 3.8. Summary of metabolites detected in Pine River sediments and proposed reactions leading to formation. Transformation marked with X were not observed in laboratory microcosms containing river sediments and added ^{14}C-DDT, -DDD or DDE.

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APPENDIX

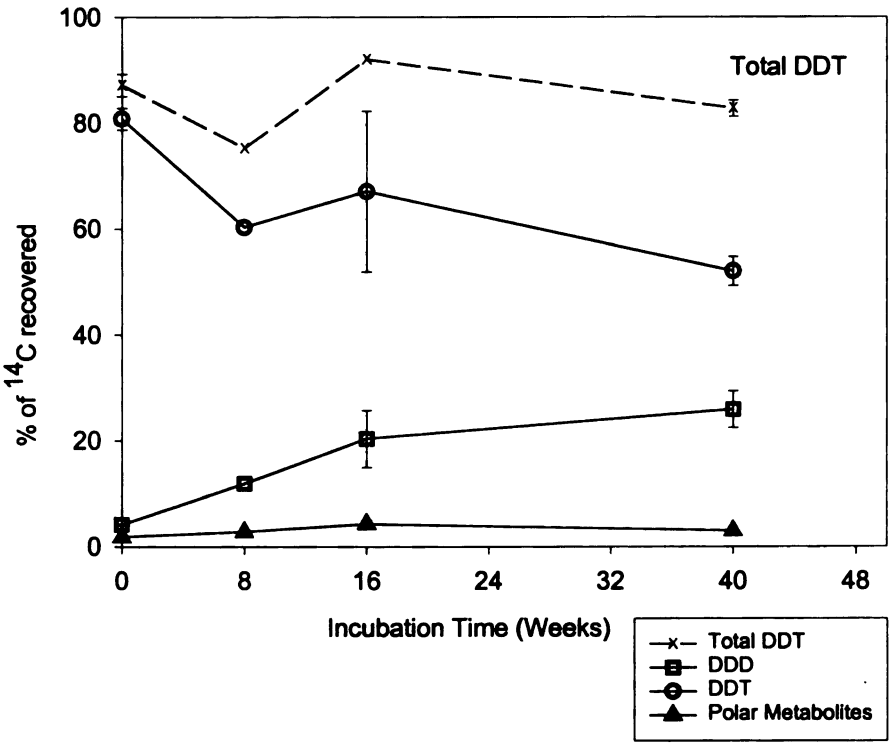

Figure A.1. Dechlorination of DDT to DDD by non-biological processes in sediment microcosms prepared from site 1 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts

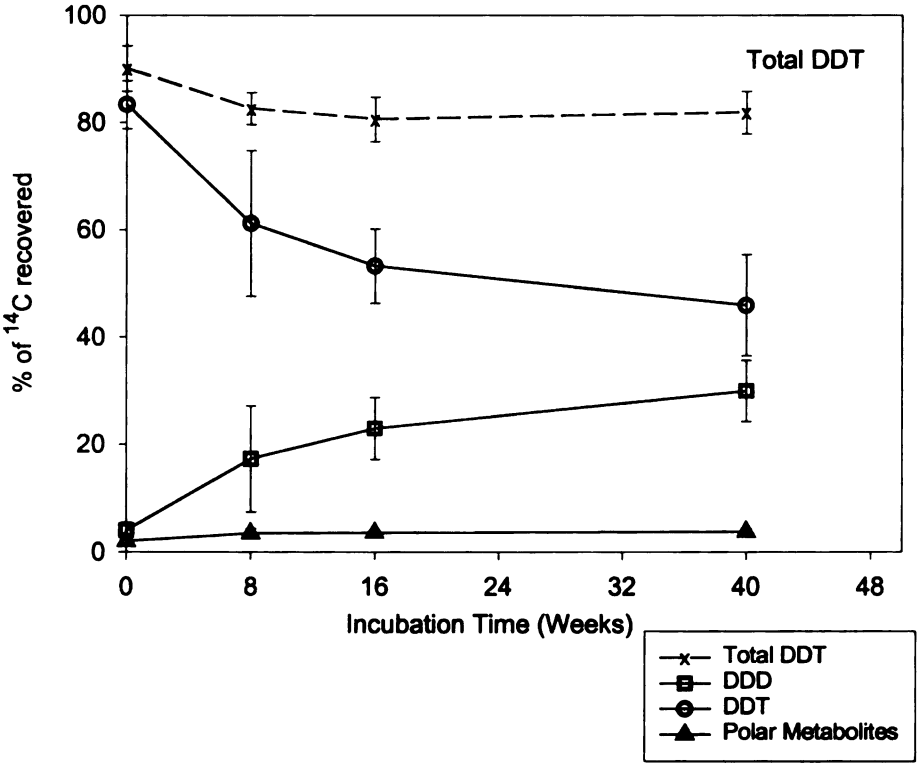

Figure A.2. Dechlorination of DDT to DDD by non-biological processes in sediment microcosms prepared from site 2 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts

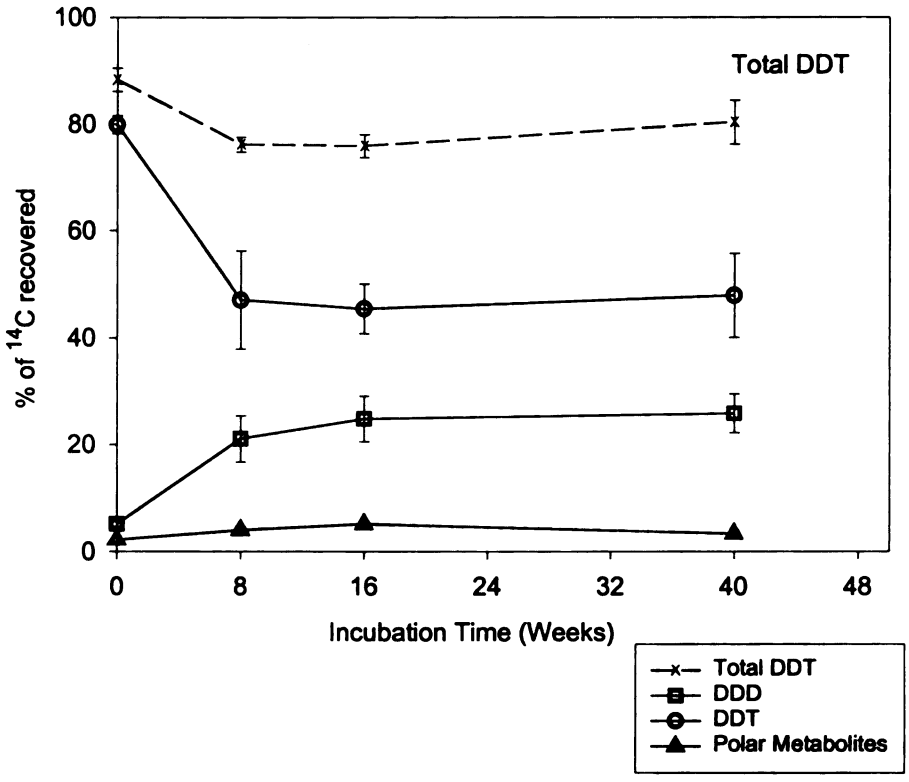

Figure A.3. Dechlorination of DDT to DDD by non-biological processes in sediment microcosms prepared from site 3 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts

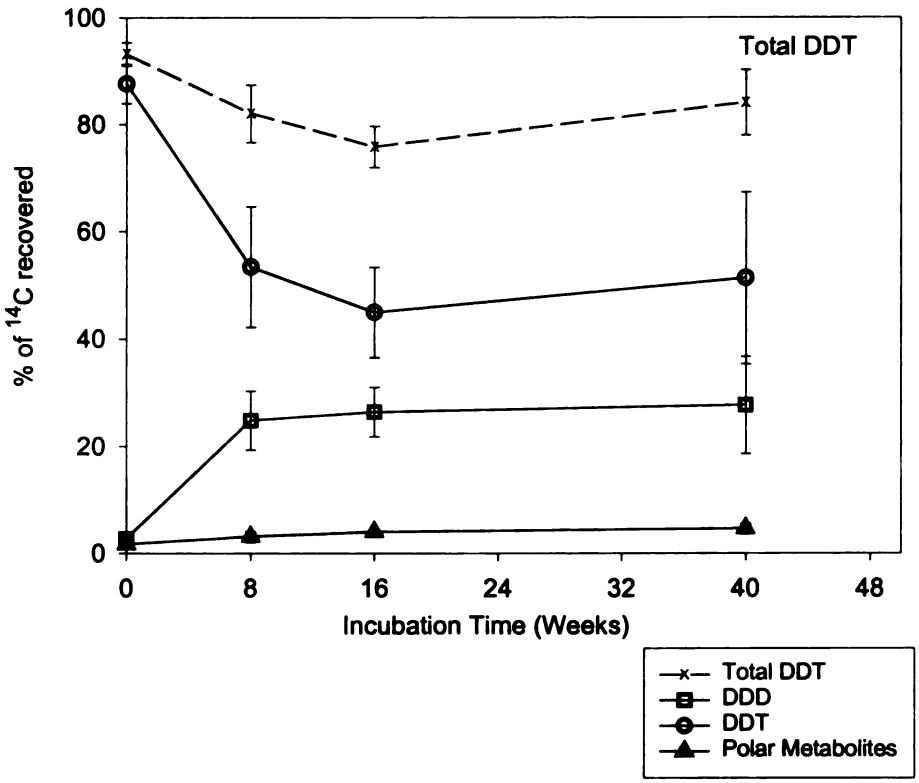

Figure A.4. Dechlorination of DDT to DDD by non-biological processes in sediment microcosms prepared from site 4 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts

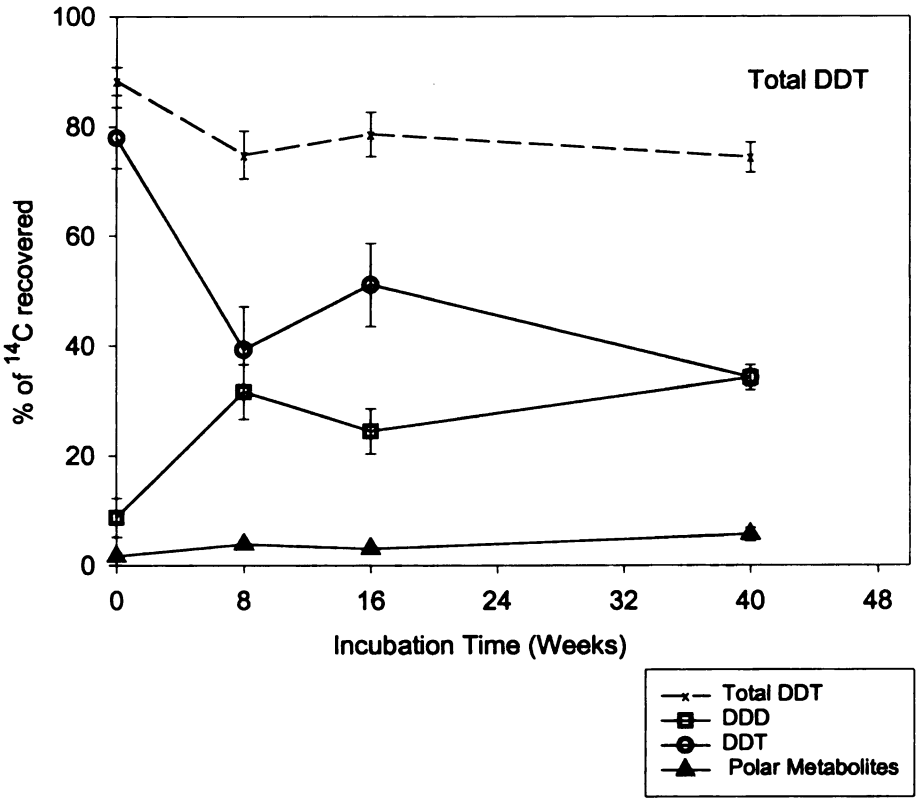

Figure A.5. Dechlorination of DDT to DDD by non-biological processes in sediment microcosms prepared from site 5 sediments (refer to Figure 2.1 for sampling location) during 40 weeks incubations. Dashed line indicates Recovery Total added as DDT. These results were obtained by liquid scintillation counting of scrapings from the TLC plates. Recovery Total represents the amount of ^{14}C-labeled radioactivity DDT recovered in the solvent and aqueous extracts